Output 4: Global IPM networks (Integrated Whitefly Management Technology) and knowledge systems developed.

Introduction

Phase III of the Tropical Whitefly IPM Project (TWFP) was approved on February 9th, 2005, following modifications made to the original Concept Note submitted to DFID in September 2004. The revised Concept Note included new partners from NRI and new activities, which required a re-organization of the TWFP and its activities, as well as a re-distribution of funds. The modifications introduced responded to:

1. The need to expand activities to the original number of target countries surveyed during Phase I, and include some new countries recently affected by whitefly pests and whitefly-transmitted viruses.

2. The need to maintain the basic composition of the team and research activities pursued since Phase I, in view of the termination of two satellite subprojects of the TWFP in 2004, financed by NZAID (Host Plant Resistance/CIAT) and ACIAR (Begomoviruses affecting tomato in S.E. Asia/AVRDC).

3. The opportunity to bring into the TWFP another international centre (CIP) that had participated in Phase I both with the TWFP (sub-Saharan Africa sub-project) and one of the Crop Protection Programme (CPP) projects (R6617) also working on sweetpotato viruses in SSA.

4. The opportunity to secure the participation of the Farmer Participatory Research (FPR) Working Group (led by CABI, UK), and the Impact Assessment Working Group (led by CIP) of the Systemwide IPM Programme.

5. The proposal advanced by NRI and NRInternational to assure the continuity (beyond the requested one year extension) of two of the CPP projects (R6617 and R6627(working in India (whitefly-borne tomato viruses) and SSA (sweetpotato viruses), that had previously collaborated with the TWFP since Phase I.

To this end, a Planning Meeting was organised by the Coordinator of the TWFP, with the collaboration of Dr. Frances Kimmins of NRI, in London, on 28 February and 1 March, 2005. The participants in this Task Force were: Frances Kimmins, NRI; Tim Chancellor, NRI; Richard Gibson, NRI; John Colvin, NRI; Barbara Adolph, NRI; James Legg, IITA/NRI; Pamela K. Anderson (CIP); Peter Hanson (AVRDC); Janny Vos, CABI-UK; Anthony Bellotti, CIAT; Cesar Cardona, CIAT; and Francisco J. Morales, CIAT. The primary objective of this meeting was to discuss future collaborative activities, and to produce a final Project Framework for Phase III.

This working group defined goal and objectives of Phase III as follows:

Goal: To promote sustainable agriculture and socio-economic growth in resource-poor farming communities possessing mixed cropping systems affected by whitefly pests and whitefly-transmitted viruses in Sub-Saharan Africa (SSA), southern Asia and tropical Latin America.

Objectives

1. To provide reliable information and technical assistance to small- (< 3 ha) and medium-scale (3-8 ha) farmers, on the biology, dissemination, and integrated management of whiteflies and whitefly-transmitted viruses affecting major food and cash crops in the Tropics.

2. To instruct farmers about the diverse negative consequences of misusing insecticides to control whiteflies, emphasizing the need to reduce yield losses, production costs, environmental and food contamination, human health risks, and the gradual development of resistance to insecticides in whitefly pests.

3. To establish sustainable mixed cropping systems in order to promote food security and economic growth in small- and medium-scale farming communities seeking to diversify their traditional food staples with high-value horticultural crops.

Achievements

All sub-projects have initiated activities according to the Logframe and proposed Workplan. The major achievement for Phase III has been the tangible results obtained by the TWFP and related CPP projects in the previous two phases. For instance, sub-project 1 has been extremely successful in diagnosing the nature of the severe outbreaks of Cassava Mosaic Disease (CMD) in SSAfrica, and, more important, in containing these outbreaks by deploying CMD-resistant germplasm. This experience and the complementary IPM measures validated, will be the base for the successful dissemination of this work throughout SSAfrica. The work on sweet potato in this region is regarded as a very complementary effort to safeguard food security in SSA and improve the nutritional value of sweet potato, by linking these activities with the promotion CIP is doing of its orange-fleshed (VitA-rich) sweet potato germplasm.

In Central America, the new begomovirus-resistant common bean cultivar EAP-951077, has been accepted as a superior variety by 92% of 60 farmers selected in Phase II to multiply the seed. Phase III will see a significant increase of the area planted with this material in Central America. During this semester, farmers in the Valley of Zapotitan, El Salvador (Pilot Site of the TWFP) harvested over 60 tons of seed to distribute in the country. Similar activities are now reported from Honduras and Nicaragua. The use of physical barriers to protect horticultural crops against whitefly- and other insect-borne viruses, has been promoted by different projects in Central America and Mexico. In Colombia, this strategy is also being promoted at present. Some farmers claim that the adoption of this technique reduces the cost in pesticide inputs by 60%, and triples the yield of uncovered controls.

The profit made by tomato farmers in India has been increased up to 10 times following the adoption of the virus-resistant tomato varieties promoted by the CPP project led by Dr. John Colvin with the collaboration of AVRDC and the University of Agricultural Sciences of Bangalore. The promotion of these varieties has continued this semester as a joint effort of the CPP and TWFP. More virus-resistant materials are currently being selected by AVRDC for India and Latin America.

The Andean subproject has successfully tested and validated the IPM technologies based on the identification of an ‘action threshold’, at which point, farmers have to control the population of *Trialeurodes vaporariorum*. This recommendation drastically reduces unnecessary insecticide applications on a preventive basis, the leading cause of pesticide abuse. This subproject has produced substantial data on the socioeconomic situation of small-scale farmers affected by the whitefly *T. vaporariorum*, and the benefits accrued from the adoption of IPM strategies. These data and

conclusions will be published shortly, and the experience accumulated will be transferred to the Bolivian highlands.

The search for host plant resistance in cassava against whitefly pests, continues to yield positive results both against the main whitefly pest in South America, Aleurotrachelus socialis, and B. tabaci in Africa. Current efforts are directed towards increasing the resistance to B. tabaci in CMD-resistant cassava cultivars.

Activity 4.1. Monitoring of whitefly populations in the Andean zone

Contributors: I. Rodríguez, C. Cardona, and H. Morales

Highlight:

- Detected important changes in whitefly species composition in the target area

Rationale

Continuous monitoring of changes in whitefly populations and species composition in target areas is one of the most important objectives of the DFID-funded project on Sustainable Management of Whiteflies. This is needed to develop appropriate management systems and, if necessary, to modify existing systems so as to be able to cope with new situations.

Materials and Methods

In 2005 we processed a total of 53 whitefly samples (adults and pupae) collected in 20 locations of the Cauca Valley region of Colombia, at altitudes ranging from 950 to 2200 meters. Samples were taken from beans, snap beans, squash, pepper, cucumber, tomatoes, melons, and several other annual crops. When possible, identification was initially based on morphological characteristics of pupae. To differentiate between biotypes (which are impossible to differentiate by morphology), we used RAPD techniques (primer OPA-04). RAPD patterns of pupae and adults collected in the field were compared with those of existing mass rearings of different whiteflies maintained at CIAT (Figure 4.1.1).

Results and Discussion

Analysis of the 53 samples taken in the Cauca Valley (Colombia) showed that 43.4% of the whiteflies collected belonged to the B biotype of *Bemisia tabaci*, the most aggressive form of whitefly known to date. The B biotype was found affecting pepper, cucumber, tomato, melon, snap beans and several other vegetables, as well as soybeans, cotton, and other crops. As shown in Figure 4.1.2, species composition in the Cauca Valley has changed drastically in the past eight years and the trend continues: since its introduction to Colombia (Quintero *et al*., 1998, Rev. Col. Entomol. 24: 23-28.) the B biotype is occupying niches previously reserved to the A biotype or to *T. vaporariorum* even in areas located above 1000 meters. *T. vaporariorum,* the predominant species in 1997 is now the second most important species (24.5% of samples) in areas located below 1000 meters above sea level. In many locations, *T. vaporariorum* is associated with *B. tabaci* (biotypes A and B). The B biotype is an aggressive form of whitefly that is causing all the serious problems described in our 2003 and 2004 Reports. These include physiological disorders in several different crops (pod chlorosis in snap beans, silvering of leaves in squash, uneven ripening of tomatoes), and the ability to transmit a geminivirus that has devastated snap beans in areas below 1200 meters.

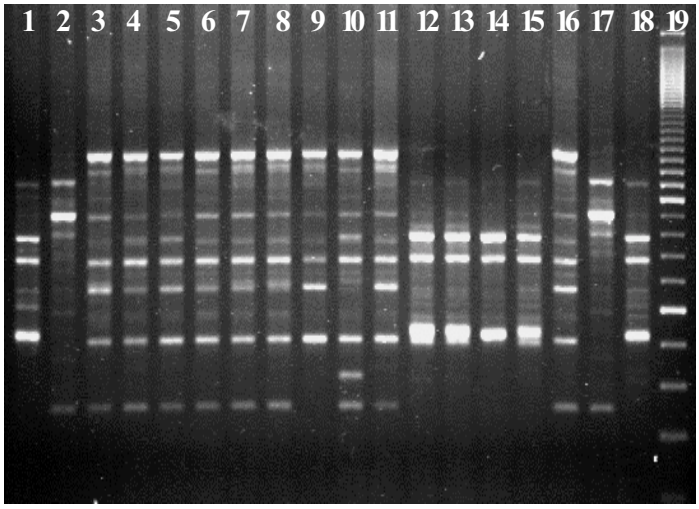


Figure 4.1.1. RAPD's for whitefly adults and pupae collected in the Cauca Valley region of Colombia. Amplification using the OPA-04 primer: 1, 2, 3, *T. vaporariorum, B. tabaci* biotype A, and *B. tabaci* biotype B adults, respectively, from reference rearings maintained at CIAT; 4-5, *B. tabaci* biotype B adults collected on squash in Quintero, Roldanillo (934 masl); 6-7, *B. tabaci* biotype B pupae collected on squash in Turin, Candelaria (999 masl); 8-9, *B. tabaci* biotype B adults collected on snap beans in Villagorgona, Candelaria (998 masl); 10-11, *B. tabaci* biotype B pupae collected on tomato in La Regina, Candelaria (1015 masl); 12-13, *T. vaporariorum* adults collected on snap beans in El Pedregal, Florida (1109 masl); 14-15, *T. vaporariorum* pupae collected on snap beans in El Pedregal, Florida (1096 masl); 16, 17, 18, *B. tabaci* biotype B, *B. tabaci* biotype A and *T. vaporariorum* adults, respectively, from reference rearings maintained at CIAT; 19, Molecular marker (100 pb).

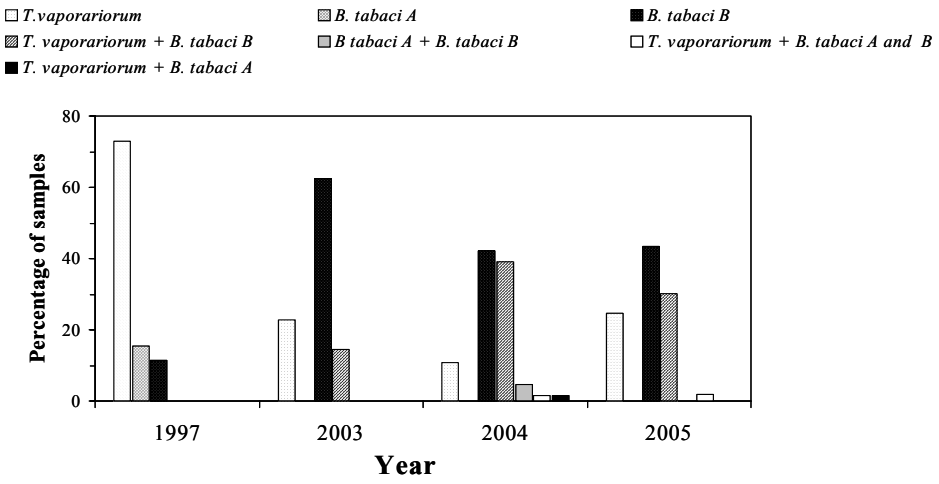


Figure 4.1.2. Changes in whitefly species composition in the Cauca Valley region of Colombia (1997-2005).

Activity 4.2. Monitoring of insecticide resistance in whitefly populations

Contributors: I. Rodríguez, H. Morales, and C. Cardona

Highlight:

- Detected varying levels of resistance or susceptibility to some of the insecticides commonly used for whitefly control in the target region

Rationale

Monitoring of insecticide resistance is another major objective of the DFID-funded project on Management of Whiteflies in the Tropics. Both major whitefly species and their biotypes in the Andean zone are the targets of excessive use of insecticides. This is reflected in ever increasing levels of resistance to insecticides (Cardona *et al*., 2001, Rev. Col. Ent 27: 33-38.) and difficulties in control. The main purpose of a continuous monitoring of insecticide resistance is to develop alternative management strategies that will help to overcome resistance or delay the onset of this phenomenon.

Materials and Methods

Using previously established diagnostic dosages for nymphs, and adults, we tested populations of whiteflies in the Cauca Valley in Colombia. Adult resistance levels were monitored under field conditions by means of the insecticide-coated glass vial technique. Resistance of first instar nymphs was measured using the foliage dipping technique. Systemic novel insecticides (mostly neonicotinoids) were tested using the petri dish technique (see 2003 and 2004 PE-1 Annual Reports).

Results and Discussion

No major changes were detected. In general, the response of *T. vaporariorum* adults (Table 4.2.1) and nymphs (Table 4.2.2) is still that of susceptibility to the insecticides that were tested. A reduced response to the neonicotinoids imidacloprid and thiamethoxam and to the insect growth regulators buprofezin and diafenthurion in host spots like Pradera deserves continuous monitoring.

Table 4.2.1. Response (percentage corrected mortality) of adults of *Trialeurodes vaporariorum* to six insecticides in three areas of the Cauca Valley region of Colombia.

Race	Percentage corrected mortality	
	methomyl (2.5ε g/vial)	imidacloprid SC (40 ppm)
'CIAT'[a]	95.3a[b]	91.2a
Tenerife	96.0a	92.4a
El Dovio	95.1a	87.9a
Pradera	88.2b	87.6a
C.V.(%)[c]:	4.7	7.3
	thiamethoxam (200 ppm)	imidacloprid WG (0.5 g/l)
'CIAT'	91.3bc	94.4b
Tenerife	95.0ab	96.0ab
El Dovio	98.5a	98.5 a
Pradera	88.0c	96.3ab
C.V.(%):	5.3	4.3
	thioxyclam hydrogen oxalate (0.5 g/l)	imidacloprid + ϐ cyfluthrin (2.5 cc/l)
'CIAT'	89.3b	98.4a
Tenerife	98.5a	99.0a
El Dovio	92.5b	100.0a
Pradera	98.5a	99.5a
C.V.(%):	5.4	2.3

[a] Susceptible strain maintained at CIAT

[b] For each product, means within a column followed by the same letter are not significantly different at the 5% level by LSD. Each product was analyzed separately

[c] Coefficient of variation.

Table 4.2.2. Response (percentage corrected mortality) of nymphs of *Trialeurodes vaporariorum* to six insecticides in three areas of the Cauca Valley region of Colombia.

Race	Percentage corrected mortality	
	buprofezin 9.2 ppm	diafenthiuron (60.1 ppm)
'CIAT'	100.0a[a]	90.0ab
Tenerife	90.3a	97.4[a]
El Dovio	100.0a	98.9[a]
Pradera	76.1b	77.9b
C.V.(%)	8.7	12.2
	imidacloprid SC (171.5 ppm)	thiamethoxam (0.5 g/l)
'CIAT'	100.0a	100.0a
Tenerife	99.1ab	99.8a
El Dovio	100.0a	99.0a
Pradera	93.6b	78.7b
C.V.(%)	4.0	3.7
	imidacloprid + ƀ cyfluthrin (2.5 cc/l)	imidacloprid WG (0.5 g/l)
'CIAT'	100.0a	100.0a
Tenerife	100.0a	99.3a
El Dovio	100.0a	100.0a
Pradera	94.0b	85.0b
C.V.(%)	2.1	4.7

[a] Susceptible strain maintained at CIAT
[b] For each product, means within a column followed by the same letter are not significantly different at the 5% level by LSD. Each product was analyzed separately
[c] Coefficient of variation.

Activity 4.3. The development of "rapid selection" method to determine whitefly resistance in cassava genotypes.

Contributors: A. Carabali and A. C. Bellotti.

Highlight:

- The ovipositional rate (N_o. eggs/female) of the cassava whitefly *Aleurotrachelus socialis* on a given genotype is a good indication of the level of resistance in that genotype.

Rationale

The cassava whitefly, *A. socialis*, causes direct damage to cassava in the neotropics by feeding on the pholoem of leaves, inducing leaf chlorosis and abscission which can result in considerable reduction in root yield during prolonged attack. Because of cassava's long growth cycle (8 to 24 months), the continued use of chemical pesticides for whitefly control increases production costs and is uneconomical for the small farmer (Holguin and Bellotti, 2004, Revista Colombiana de Entomología

30: 37-42.). Stable host plant resistance (HPR) offers a practical, low cost, long-term solution for maintaining reduced whitefly populations. Whitefly resistance is rare in cultivated crops; however, good sources of resistance to *A. socialis* have been detected in several *M. esculenta* genotypes and high yielding hybrids with moderate levels of whitefly resistance are being developed (Bellotti and Arias, 2001, Crop. Prot. 20: 813-823). In addition, a preliminary evaluation of wild *Manihot species* has revealed high levels of resistance to *Aleurotrachelus socialis* in accessions of *M. flabellifolia* and *M. esculenta* subsp *peruviana*.

The development of cassava hybrids with whitefly resistance will involve screening a considerable number of F1 progeny from crosses between two or more *M. esculenta* genotypes and/or the progeny from interspecific crosses between *M. esculenta* and wild *Manihot* species such as M. *flabellifolia.* Projects to accomplish this goal are already in progress. A mechanism is needed that can speed up the evaluation of a large number of genotypes for whitefly resistance. Previous evaluations of whitefly resistance in *M. esculenta* genotypes had detected a possible correlation between resistance and low oviposition rates (Bellotti and Arias, 2001, Crop. Prot. 20:813-823.). Evaluations of the interrelations between ovipositional preference by females for specific genotypes and the development, survival and reproduction of immature offspring constitutes a crucial point in theoretical host plant/insect relationships (Thompson, 1988, Entomol. Exp. Appl. 47: 3-14).

The objective of this present study is to evaluate ovipositional preference/non-preference in *A. socialis* for specific cassava genotypes as a possible "rapid selection" process for resistant/susceptible genotypes.

Materials and Methods

Ten plants, progenies of an interspecific cross between *M. esculenta* and M. *flabellifolia* (CW 235-72, CW 259-3, CW 259-43, CW 257-10, CW 258-17 and CW 259-10) and the commercial variety CMC-40, were planted in plastic pots at a depth of 15 cm. Five forty day old plants of each genotype were placed in wooden/nylon mesh cages (1 m x 1 m x 1 m) for whitefly infestation. Adult *A. socialis* individuals were harvested from the CIAT established colony being maintained in the greenhouse (25 $\pm$ 5°C, 70 $\pm$ 5% RH and 12 hr photoperiod). Studies on ovipositional preference and nymphal development were carried out in the greenhouse. Ten pair (10 males 10 females) of recently emerged *A. socialis* adults (sexed according to the technique described by Eichelkrant and Cardona, 1989) were harvested from the CIAT established colony on CMC-40. Each adult pair was placed in a small leaf cage (2.5 cm diameter x 2.0 cm depth) and attached to the underside of leaves of each genotype (Figure 4.3.1a). Adults were removed after five days and the number of eggs oviposited were recorded (Figure 4.3.1b). Oviposited eggs were allowed to develop for 10 additional days in order to determine the number that will develop to the third nymphal instar.

Figure 4.3.1. a) A small leaf cage for confining whitefly females in oviposition studies; b) *A. sociales* eggs oviposited on cassava leaf undersurface

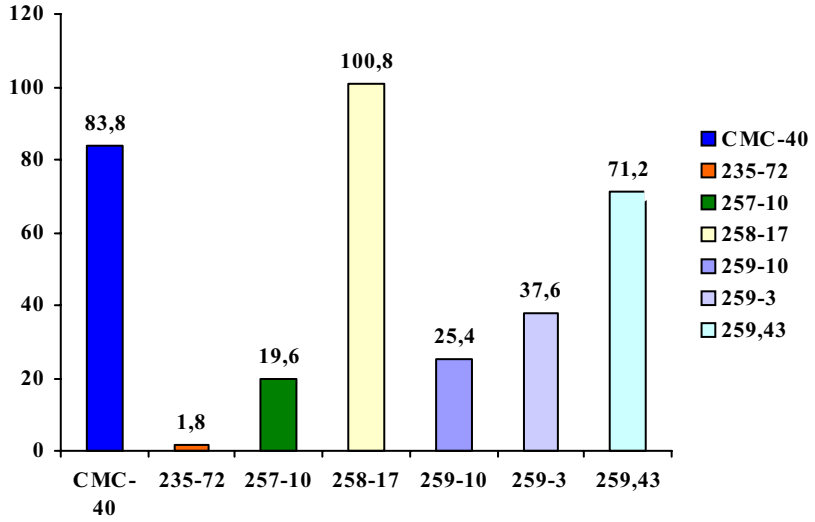


Figure 4.3.2. Genotypes Oviposicional rates (No. eggs/female/5days) of *Aleurotrachelus socialis* on cassava genotypes.

Statistical analysis was carried out using the Stat View, version 5.0.1 (SAS Institute, 1999) program. Differences between average values for oviposition and nymphal development were analyzed using ANOVA. Multiple comparisons were preformed by the Fisher test.

Results and Discussion

Development of *A. socialis* nymphs on cassava (interspecific progeny) genotypes displayed a behavior similar to the rate of oviposition on these genotypes. The genotype CW 235-72 had the lowest ovipositional value (1.8 eggs/10 females) when compared to other genotypes such as CMC-40 (83.8 eggs), CW 258-17 (100.8), CW 259-43 (71.2) (Figure 4.3.2)

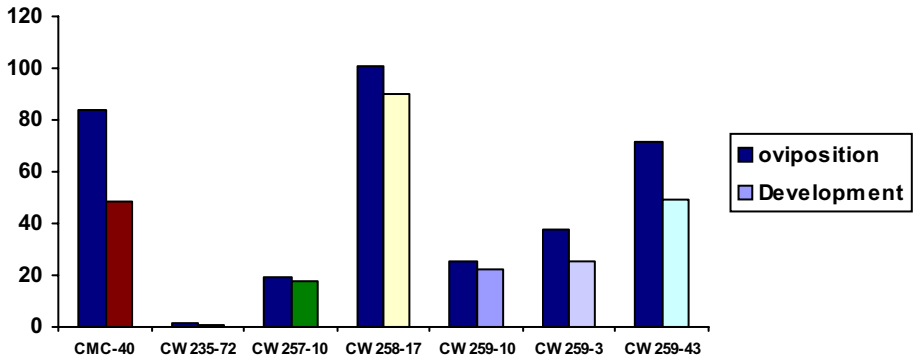


Figure 4.3.3. *Aleurotrachelus socialis* oviposition and nymphal development to the third instar on cassava genotypes

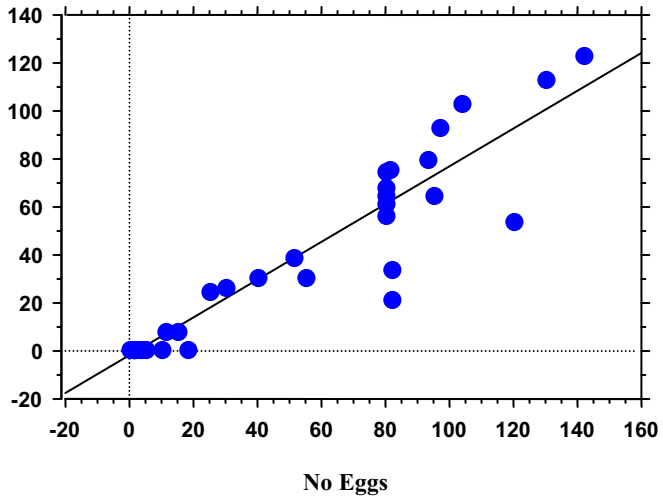
(ANOVA-Fisher's $P < 0.05$). The differences in oviposition expressed in this test is an indication that this variable can be used to distinguished between resistant and susceptible genotypes. This was a no-choice test so the ovipositional rates expressed indicate the ability of *A. socialis* females to oviposit on that genotype.

It is not yet understood why females will oviposit so few eggs on genotypes such as CW 235-72, CW 257-10 (19.6) and CW 259-10 (25.4) when no other choice (genotype) is available. A free-choice test among these genotypes might provide additional information on the mechanism involved in ovipositional preference.

The genotype CW 235-72 had the least development of nymphal stages, owing to the very low ovipositional rates. CMC-40 and CW 259-43 displayed the highest percentage difference (42% and 30% respectively) in nymphal development when compared to the initial ovipositional rates (Fisher's $P < 0.05$). (Figure 4.3.3).

The genotypes CW 235-72 and CW 257-10 showed the least difference (0% and 17% respectively) between oviposition and nymphal development.

A regression analysis was conducted to obtain a correlation between oviposition and nymphal development (3rd. instar) of *A. socialis* on progeny of *M. esculenta* x M. *flabellifolia* (Figure 4.3.4). Results show an 87% correlation in development of the nymphal stages and number of eggs oviposited. It can therefore be concluded that the number of eggs oviposited on a given genotype can be used as an indicator of the preference/resistance of *Aleurotrachelus socialis* for that genotype.



Y = -1.875 + .786 * X; R^2 = .873

Figure 4.3.4. Correlation between oviposition and nymphal survival of the whitefly *Aleurotrachelus socialis* on cassava genotypes (from interspecific crosses of *M. esculenta* x *M. flabellifolia*).

Activity 4.4. Determining the plant metabolites involved in whitefly (*Aleurotrachelus socialis*) resistant cassava varieties, MEcu 64, MEcu 72 and MPer 334

Contributors*:* D, F. Múnera, S. Lapointe, A.Valencia, P. Calatayud and A. C. Bellotti.

Highlights:

- Research on the biochemistry of whitefly resistance in cassava indicates that a relationship may exist between proteins and the presence of resistance to the species *Aleurotrachelus socialis*.

- Research indicates that a relationship may exist between proteins in the cassava leaf and resistance to the whitefly, *Aleurotrachelus socialis*.

Rationale

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 [Conjunto audiotutorial], CIAT). 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. Additional yield reduction can be caused by the growth of a "sooty-mold" on whitefly exudates deposited on cassava leaves that deters photosynthesis.

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 and 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. Greenhouse and field studies show that *A. socialis*feeding on resistant clones have less oviposition, longer development period reduced size and higher mortality than those feeding on susceptible ones (Arias, 1995, MSc thesis, Univ. Nacional de Colombia, Palmira, Colombia, pp 164). *A. socialis* nymphal instars feeding on MEcu 72 suffered a 72.5% mortality, mostly in the early instars (Bellotti and Arias, 2001, Crop Protection 20:813-823).

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 334 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, Memorias XXX Congreso SOCOLEN, Colombia).

Plant strategies for resisting insect attack often involve 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

Whiteflies have piercing-sucking feeding habits; this has made it difficult to develop an artificial liquid diet that would allow testing the biological activity of protein extracts for each of the resistant and susceptible genotypes to determine the relationships between the protein and resistance to the whitefly.

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. The resistant genotypes evaluated were MEcu 72, MEcu 64 and MPer 334. The susceptible control was the genotype CMC40. This process will be carried out using healthy plants (non-infested), and plants infested with *A. socialis*, for each of the genotypes, to detect 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.

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 immunodetection which consists of the identification and characterization of a fixed antigen by means of antibody tests (Timmons and Dunbar, 1990, Methods in Enzymology 182: 678-688; Garfin, 1990, Methods in Enzymology 182: 459-477).

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.

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 4ºC 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 4ºC, 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, Methods in enzymology 182: 663-670).

To produce polyclonal antibodies the following steps were developed:

- ü 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.
- ü 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.
- ü 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.
- ü Ten days after the last injections, the animals were bled, obtaining 15-20 ml of blood from each.
- ü The collected blood was left at room temperature for 24 hours, than 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:

- ü One milligram of each of the proteins was dissolved with 200 ε1 of Tris Glycine (pH 6.8) buffer. On each nitrocellulose membrane 5 ε1 of the stock solution was applied to each of the proteins.
- ü Blockage of the nitrocellulose membrane with the sample in TBS containing 1% gelatin.
- ü Exposure of the membrane to 30 ε1 of the first antibody dissolved in 30 ml of blockage solution.
- ü Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.
- ü Exposure of the membrane in 30 ε1 of the second antibody (Bound to PER) dissolved in 30 ml of the blockage solution.
- ü Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.

ü Addition of 5 ml of revealed solution (40 ml of TBS, 3 ε1 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:

ü 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.
ü 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.
ü 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 an 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.

First immunization tests were with healthy plants (no whitefly infestation) using the Western Blot Technique. This test seeks to determine the specificity of each antibody in the genotypes being evaluated. An SDS-PAGE electrophoresis of the proteic extracts was carried out for each of the genotypes, using the previously determined conditions. Subsequently a transfer of the bands obtained during electrophoresis was made to a nitrocellulose membrane using the Western Blot Technique. Antibody recognition was determined using the Dot Blot Technique, with the exception of the first two steps.

Results and Discussion

Tests for antibody recognition using Dot Blot: By using 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 4.4.1).

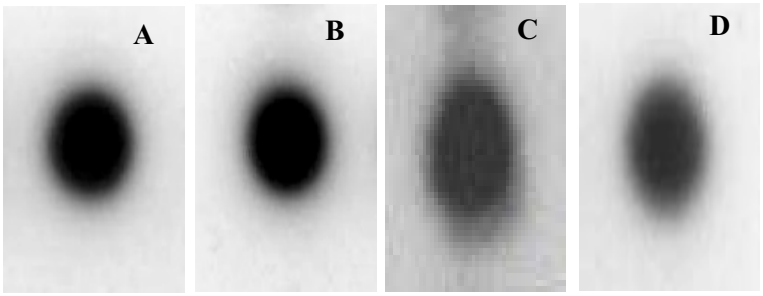


Figure 4.4.1. Test for antibody recognition using the Dot Blot technique. A: antibodies against MEcu 72, B: antibodies against MEcu 64, C: antibodies against MPer 334, D: antibodies against CMC 40.

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 4.4.2).

The protein concentration that gave adequate results for the resolving phase by providing good visualization of the protein bands was 14% (Figure 4.4.2). 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 4.4.2, 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 arrows), although it is less intense for MPer 334. The genotype MEcu 64 shows a high polymorphism as well as an additional band that is absent in the other genotypes (yellow arrow). The yellow circle on Figure 4.4.2 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 are a good indication that a relationship may exist between these proteins and the presence of resistance to *A. socialis*.

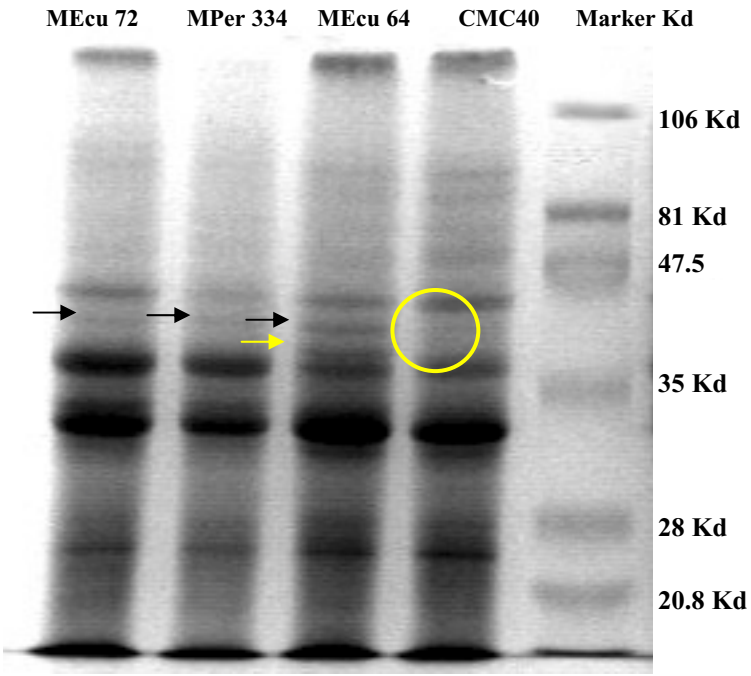


Figure 4.4.2. SDS-Page. Phase resolving concentration of 14%, Sample concentration of 2 mg/ml. The black arrow indicates the polymorphic band commonly present in the resistant genotypes and absent in the susceptible, CMC 40, indicated by the yellow circle. The yellow arrow shows an additional polymorphic band that is only evident in the resistant genotype MEcu 64.

First immunization test with healthy plants (no whitefly infestation) using the Western Blot Technique: Antibody specificity obtained from MEcu 64 with proteins originating from the same genotypes (B pool 3) can be observed in Figure 4.4.3. The four bands located in different positions, as indicated by the arrows, can only be observed in this genotype and are absent in the control (CMC 40) and the other resistant genotypes. These results support those obtained from SDS-PAGE electrophoresis, indicating that this genotype is markedly different than the other genotypes and this could be related to whitefly resistance.

In general, common bands, for all of the genotypes combined with all of the antibodies can be observed. This indicates common proteins, be they structural or functional, in the genotypes.

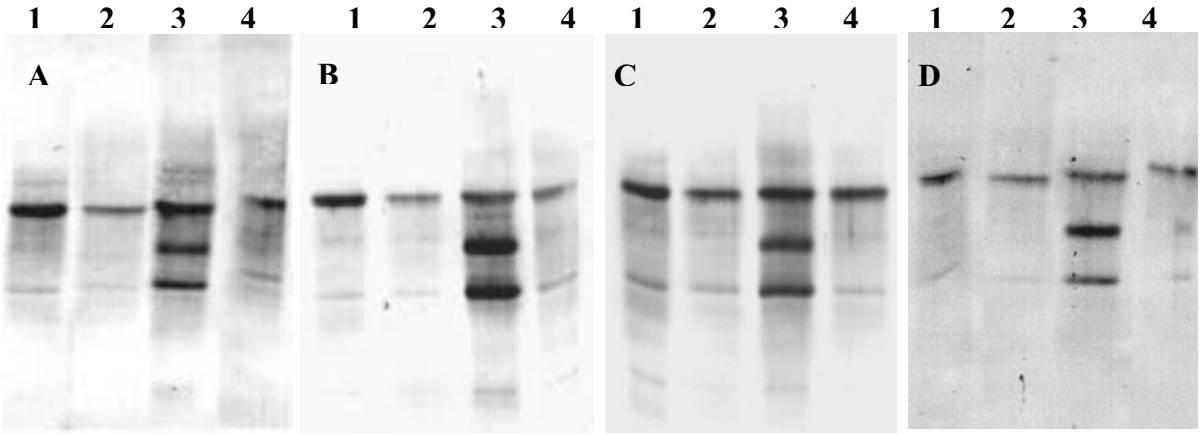


Figure 4.4.3. Immunodetection of healthy genotypes (non whitefly infested) using the Western Blot Technique. 1: MEcu 72, 2: MPer 334, 3: MEcu 64, 4: CMC 40. A) developed with antibodies of CMC 40; B) developed with antibodies from MEcu 64; C) developed with antibodies of MEcu 72; D) developed with antibodies from MPer 334. The black arrows signal the polymorphic bands of MEcu 64 that are absent in the other genotypes.

Projections

Present results indicate a distinct difference in the proteic behavior of at least one of the genotypes when free of whitefly (*A. socialis*) infestation. Therefore the following processes and activities are suggested:

- Development of a well defined proteic profile for each of the genotypes using more sensitive techniques, such as silver staining. This profile would be used as a reference for the protein behavior of each genotype in the absence of whitefly infestation and utilized for future comparisons.
- Obtain a proteic profile for whiteflies feeding on susceptible plants using more sensitive techniques. This profile would also be used as a protein behavior reference for whiteflies feeding on non-resistant genotypes.
- Determine the proteic profiles for resistant genotypes infested with feeding whiteflies, and compare these to the previously obtained profiles. Differences in the proteic activity would be established for the presence of the whitefly as well as the interaction with genotype resistance.

- Determine, through immunodetection, what proteins associated with each genotypes are found in the whiteflies feeding on them. This would provide a direct relationship with the proteic activity in whitefly resistant genotypes.
- Perform SDS-PAGE preparations of total proteins of the genotypes and whiteflies, especially where differences in the initial proteic profiles have been detected and corroborated through immunodetection; thereby extracting different bands, concentrating them in a gel and carry out an electro blot on the membrane.
- Once the electro blot is conducted on the membrane, digestion of the fixed bands in the membrane will be done, with the objective of obtaining internal fragments from the membrane.
- The next step will consist of high resolution electrophoresis of the eluted digestion from the membrane. From here, sequenciation of the amino acids blocked on the N terminal will be carried out and a search for analogues in the amino acid data bank will be performed to determine the protein group, or the type of protein, of selected bands.
- Lastly, a genetic sequence of the protein bands will be done to determine the codifying gene(s).

Activity 4.5. Wild Manihot species as a source of resistance to the cassava whitefly (A*leurotrachelus socialis)*.

Contributors: B. Arias, G. Pérez, C. Ñañez and A. C. Bellotti.

Highlight:

- Numerous genotypes from interspecific crosses with *Manihot esculenta* and wild*Manihot* species, indicating the possible presence of resistance of these pests *in M. flabellifolia*, *M. peruvians*, *M. tristis* and others.

Rationale

Moderate levels of resistance to the cassava whitefly, A*leurotrachelus socialis,* have been identified in *Manihot esculenta* germplasm. More than 90% of the *M. esculenta* accessions in the CIAT cassava germplasm bank have know been evaluated and it is estimated that only about 1% of these have low to moderate levels of resistance to *A. socialis.* These studies have led to the development of a whitefly resistant commercial cultivar, Natiama-31, that has been released to cassava producers by the Colombian Ministry of Agriculture.

The rapid increase and spread of whitefly populations and damage (reduction in cassava yields) necessitates a continued effort to identify additional and better sources of resistance to whiteflies. Recent evaluations of the *Wild Manihot* species in the CIAT collection indicate that high levels of resistance to *A. socialis* may be available in certain species (CIAT Project IP-3, 2003 Annual Report).

CIAT cassava geneticists are producing a considerable number of interspecific crosses between Wild *Manihot* species and *Manihot esculenta*. The cassava entomology section evaluates the progeny from these interspecific crosses for resistance/susceptibility to certain pests, especially mites and whiteflies. The following is a condensed report on some of these evaluations for resistance to the whitefly, *A. socialis.*

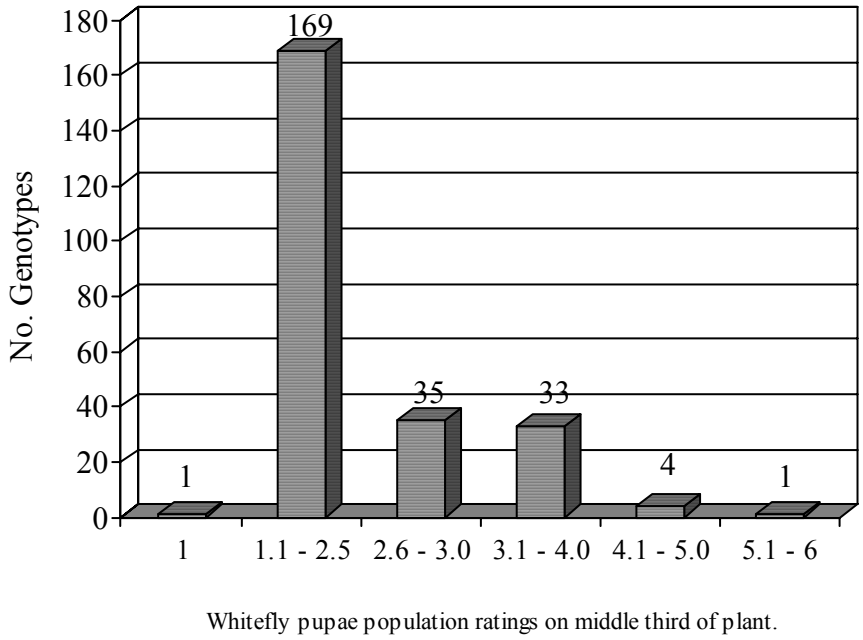


Figure 4.5.1. Whitefly (*A. socialis)* pupae population ratings (1 to 6 scale) on progeny from interspecific crosses (CW) (Dry matter content) at CIAT, Palmira, 2005.

Materials and Methods

Whitefly (*A. socialis)* population and damage evaluations were carried out in seven trials, consisting of interspecific crosses at the CIAT, Palmira (Valle) farm. Numerous *Wild Manihot* species were used in producing the progenyevaluated in these trials. These included *Manihot flabellifolia, M. peruvianus, M. tristis, M.crassesepala* and *M. walkerii.* These wild species were crossed with several *M. esculenta* genotypes, producing more than 1000 interspecific progeny. Each group of crosses designed by the cassava geneticist was being evaluated for specific agronomic traits. These included dry matter content, yellow (high beta-carotene) roots, mite resistance, and polycrosses.

Whitefly population evaluations carried out using a 1 (no whitefly life stages present) to 6 (high populations, often exceeding 5000 individuals per leaf) scale. Plant damage was also based on a 1 (no damage symptoms) to 6 (severe damage; leaf curling, mottled, considerable leaf necrosis and defoliation, sooty mold present) scale. Plant populations and experimental design varied between trials (for details see genetics section).

Results and Discussion

Trial 1. Evaluation of 243 progeny of interspecific crosses designed for high dry matter content. 170 (70%) of the 243 genotypes had a population rating of 1.0 to 2.5 (Figure 4.5.1). Population data was tabulated based on the pupae population of *A. socialis* on the leaves of the middle third of the plant. 23 genotypes had pupae populations rated between 1.0 (CW-14-11) and 1.5 (CW 120-41, CW 121-2, CW 150-30, CW 159-1, CW 164-12 and others). 29% (68) of the genotypes had pupae population ratings

between 2.6 and 4 and only five genotypes had a rating above 4.0 (Figure 4.5.1). Plant damage ratings were correspondingly low as 164 (67.5%) had a leaf damage rating of 1.0. These results indicate either very low whitefly populations, and therefore very low selection pressure, or there is considerably high levels of whitefly resistance in these interspecific genotypes.

Trial 2. Evaluation of 120 progeny of intespecific crosses developed for high dry matter and yellow (high beta-carotene) roots. In this trial 120 CW genotypes were planted in 2 to 4 replications of 10 plant plots (total of 400 plots). *A. socialis* populations were recorded on all the genotypes (Figure 4.5.2). Forty-seven (39.2%) of the genotypes had a low population rating of 1.5 to 2.5 and 61 (51%) had a rating of 2.6 to 3.0, while the remaining 12 genotypes were between 3.1 and 4.0. *M. peruvianus* and *M. tristis* were the *Manihot* species included in many of these crosses. Leaf damage symptoms were absent in 53% (64) of the genotypes, again indicating either high levels of whitefly resistance or low populations.

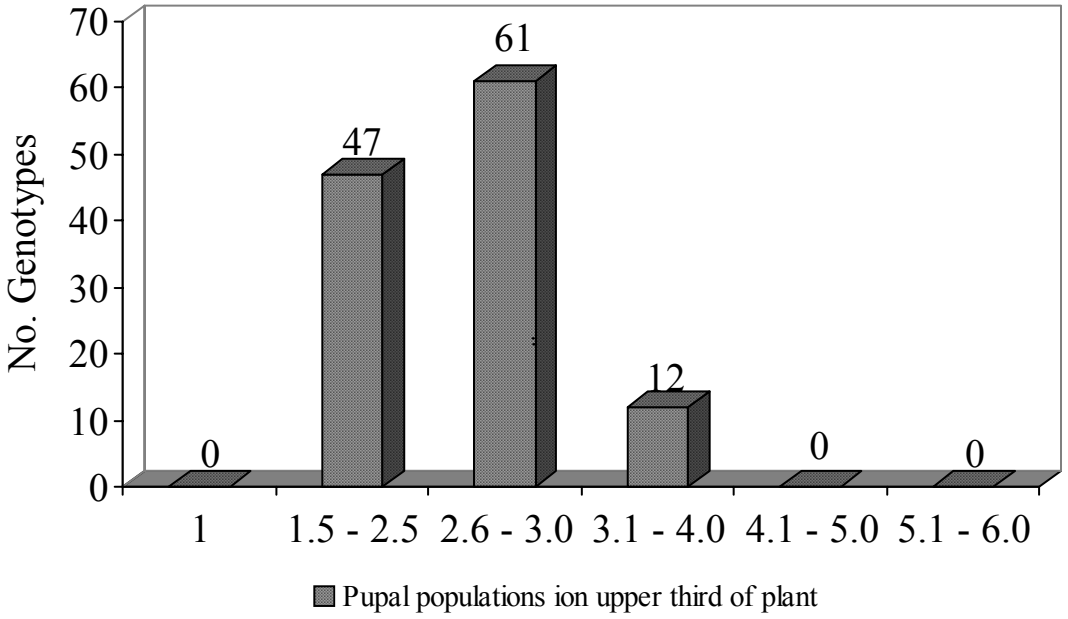


Figure 4.5.2. Whitefly (*A. socialis)* pupae population ratings (1 to 6 scale) on progeny from interspecific crosses (CW: Dry matter content and yellow roots) at CIAT, Palmira, 2005.

Trial 3. Evaluation of 137 progeny of interspecific crosses WWW-PTR-WWW designed for dry matter content. This trial consisted of 137 WWW genotypes sown in 324 plots in three replications. High pupal populations occurred on the upper and middle thirds of the plants, with few lower leaves observed (Figure 4.5.3). Pupae populations were similar for these two levels of the genotypes. In general, whitefly (*A. socialis)* populations were low and only one genotype had a population rating above 4.0. Seven genotypes had no pupae stages on the upper nor middle leaves; these include the genotypes WW 11-18, WW 11-13, WW 11-45, WW 11-6, WW 25-71, WW 30-38 and WW 32-241. In an additional 29 genotypes (21.2%) the pupae population rating did not exceed 1.5 on either the upper or mid leaves. In total 117 genotypes (85.4%) had a maximum pupae rating of 2.5 or lower on the upper or mid leaves. These results indicate high levels of *A. socialis* resistance or very low selection pressure.

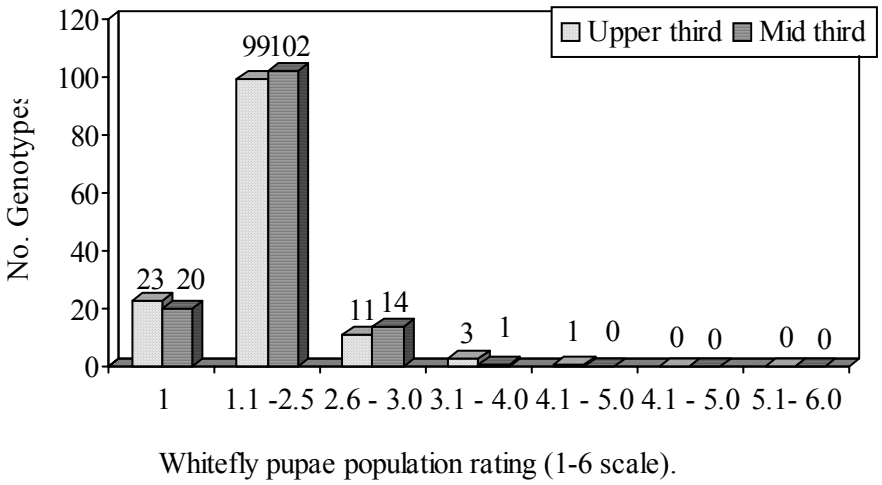


Figure 4.5.3. Whitefly (*A. socialis*) pupae population ratings (1 to 6 scale) on progeny from interspecific crosses (WWW-PRT-WWW) at CIAT, Palmira, 2005.

Trial 4. Evaluation of 330 progeny from interspecific crosses with *M. esculenta* x *Manihot flabelifolia* designed for mite (*Mononychellus tanajoa*) resistance. The 330 genotypes were sown in 10 plant plots with three replicates. Whitefly populations in this trial were low as 35 progeny (10.6%) had no (1.0) pupae on leaves and 287 genotypes (87%) were rated between 1.1 and 2.5 (Figure 4.5.4). Genotypes with no pupae included CW 213-1, CW 217-12, CW 218-7, CW 224-7, CW 225-29, CW 229-25, CW 232-12 and CW 235-2. Leaf damage was absent on 99% of the genotypes, with only one genotype with a 3.0 damage rating.

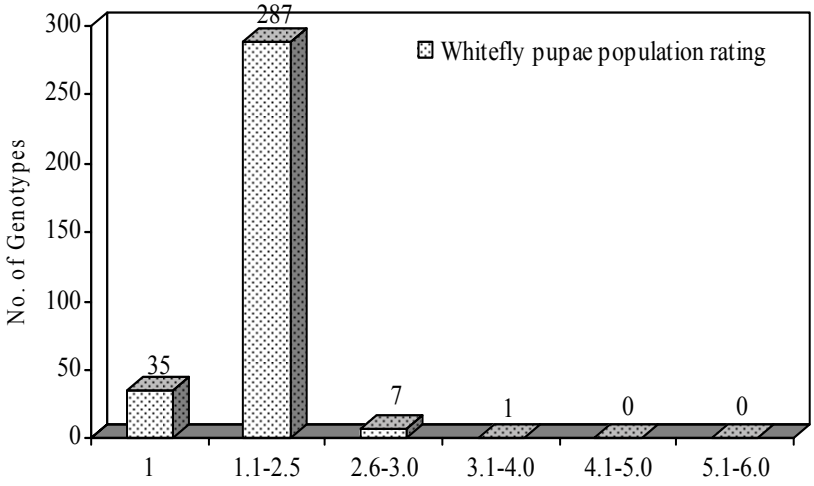


Figure 4.5.4. Whitefly (*A. socialis*) pupae population rating (1 to 6 scale) on progeny from interpsecific cross with *M. flabelifolia* (mite resistente) at CIAT, Palmira, 2005.

Trial 5. Evaluation of 50 progeny from polycrosses for whitefly (*A. socialis)* populations and damage. The polycrosses in this trial involved several *Wild Manihot* species, including *M. flabelifolia, M. tristis, M. peruviana, M. crassisepala and M. walkerii.* The 50 genotypes consisted of 37 CW, 10 OW, 1 CRA, 1 CWR and 1 not identified. *A. socialis* pressure was higher in this trial and whitefly populations and damage ranged from 1.0 to 5.5 on the 1 to 6 rating scales (Figure 4.5.5). Fifteen of the 50 genotypes (30%) had no leaf damage, a 1.0 rating, while 11 genotypes (22%) were rated 1.1 to 2.5 and 20 genotypes (40%) had a susceptible damage rating above 3.0. Whitefly populations were high with a 3.1 to 5.5 rating on 28 genotypes (56%).

Two genotypes, CRA-013 and CW 184-4, had no whiteflies present and 14 additional genotypes had a rating of 1.1 to 2.5. Additional genotypes with low pupae populations and low damage (1.0) ratings include CW 67-89, CW 67-142, CW 67-130, CW 67-44 and CW 67-33. Given the increased selection pressure in this trial, those genotypes with low whitefly populations and low damage ratings should be further evaluated.

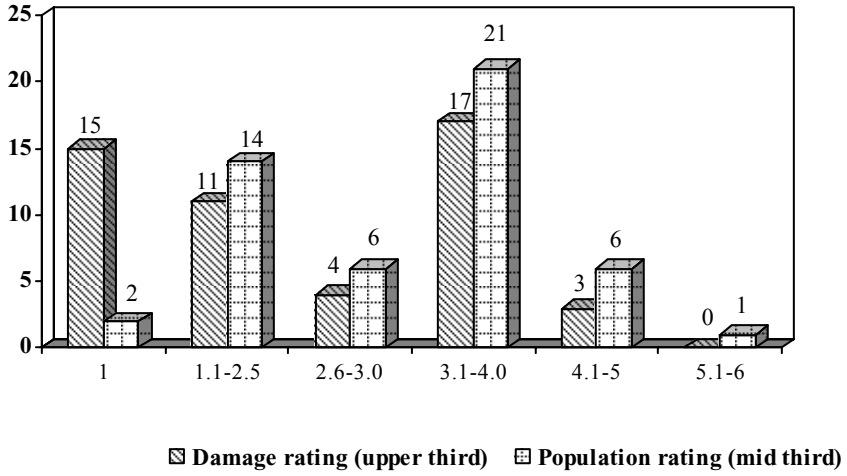


Figure 4.5.5. Whitefly (*A. socialis*) population and damage ratings (1 to 6 scales) on CW and OW polycrosses with several Wild Manihot species (CIAT,

Complete whitefly (*A. socialis)* population and damage evaluations for every genotype in the five trials cited in this report are available in the cassava entomology data files.

Activity 4.6. Establishment of an IPM strategy for whiteflies on cassava in the Colombian Departments of Quindio and Risaralda

Contributors: C. Ma. Holguín, C. J. Herrera and A. C. Bellotti.

Highlight:

- Cassava farmer surveys in Quindío Department of Colombia identify whiteflies as the major pest effecting production. More than 50% of farmers apply chemical pesticides for whitefly control. A whitefly 1PM Project has been initiated.

Rationale

Cassava is traditionally grown on small scale farming systems using few purchased inputs such as fertilizers or pesticides and where cassava is usually one of several crops grown. Distances between cassava fields may be considerable and this can contribute to the sporadic occurrence of some cassava pests. However, in Latin America there are indications of a shift towards larger-scale production units where cassava is grown as a plantation crop. In these situations where cassava is utilized more as an industrial crop, it is advantageous for farmers to employ a multiple planting, multiple harvesting production system in order to meet the constant market demands of the processing industries.

In this type of production system, the cassava crop can be observed at several different growth stages in the same or surrounding farmer fields. Evidence now indicates that pest problems will be compounded in these overlapping production systems. Populations of certain pests, such whiteflies and hornworms, and possibly mealybugs, tend to increase when a constant food supply, e.g. young cassava foliage is available.

The continual food supply prevents or deters a break in the reproductive cycle of the insect, altering its population dynamics that could have been adversely affected by the lack of optimal foliage for feeding and reproduction. This situation is more apt to occur where environmental conditions such as an evenly dispersed rainfall pattern favors or provides for several planting dates throughout a one-year cycle.

In addition, crop management alternatives can be influenced where the availability of irrigation provides for more frequent plantings, especially in semi-arid or seasonally dry agroecosystems.

The aforementioned described conditions occur in certain cassava growing regions of Colombia, Venezuela and Brazil. In the coffee growing region of Colombia, rainfall is dispersed throughout the year and a prolonged dry period of three or more months is not common. In Northeast Brazil (e.g. Bahia State) the availability of irrigation is resulting in large cassava plantations, some more than 3000 ha. In this seasonally dry region cassava was seldom planted more than twice during the year. The availability of irrigation had led to more frequent plantings (Osmar Lorenzi, personnel communication, 2005). A similar situation also occurs in the plains region of Venezuela. The Colombian coffee growing region (CCGR) and Northeast Brasil are reporting more frequent outbreaks of hornworm and increasing whitefly populations.

Whitefly populations in the CCGR have increased dramatically in recent years. Two whitefly species predominate, A*leurotrachelus socialis* and *Trialeurodes variabilis*. This senario has led to the implementation of an IPM project to generate alternatives for whitefly control. Chemical pesticide applications in the region are on the rise and this is increasing production costs as well as the potential to cause outbreaks of secondary pests. Whiteflies are difficult to control with agrochemicals and this

often leads to more frequent applications (on a calendar basis), a build up of pest resistance to the chemical insecticides, and eventually environmental contamination.

The general objective of this project is to establish a whitefly pest management system that will provide cassava producers with an adequate, opportune, economical and sustainable alternative that will reduce pesticide applications and provide good yields.

Specific objectives include:

1) Obtain information on current farmer practices being used by cassava producers.
2) Provide cassava producers with information and training in integrated pest management and provide alternative techniques, such as biological control, reducing pesticide use.

Materials and Methods

A diagnostic survey was conducted with cassava farmers in the target region, in order to obtain information on pest distribution, crop damage levels and present farmer practices being employed to control whiteflies. Thirty cassava farmers were visited and interviewed in the region, especially in the Quindio Department. A survey questionnaire was designed for use during farm visits. These visits and interviews were designed with the purpose of obtaining information on the actual situation confronting cassava producers in the field, the severity of phytosanitary problems and farmer needs and priorities. Posterior to these surveys, random sampling was carried out in cassava fields to confirm the presence of the different insect pests and diseases and to try to determine pest populations or crop damage severity. This information is being tabulated and analyzed.

At the time of this survey meetings were held with farmer groups, students, technicians and agronomists in the region, providing them with information on pest biology and behavior and introducing some whitefly management practices.

Results and Discussion

Surveys were carried out with the assistance of entities such as the Federación Nacional de Cafeteros (The Colombian Coffee Federation) and directly with the Coffee Growers Committee's in each municipality) and ICA Regional Quindio. Cassava producers from six municipalities in the Departments of Quindio (Armenia, Montenegro, Calarcá, Buena Vista, La Tebaida, Quimbaya and Circacia, where cassava is produced at altitudes varying from 1100 to 2900 masl (Figure 4.6.1).

Several pest species were detected feeding on cassava in this region. Whiteflies were the most important and predominant pest found damaging cassava production. Whiteflies were the main pest on 52% of the farms surveyed followed by fruitflies (*Anastrepha sp*.) (20%) and mites (16%) (Figure 4.6.2).

Additional arthropod pests detected in cassava fields included the cassava hornworm (*Erinnyis ello*), whitegrubs, thrips (*Frankliniella williamsi*), the burrower bug (*Cyrtomenus bergi*) and mites (*Mononychelus tanajoa*).

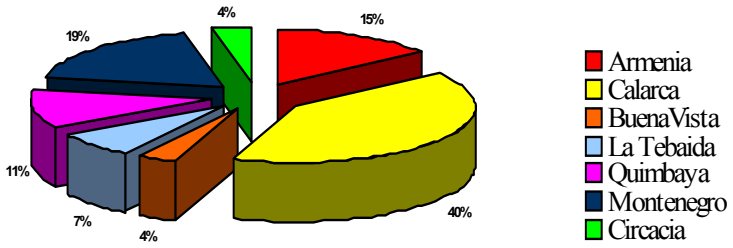


Figure 4.6.1. Municipalities in Quindio Department of Colombia where farmer surveys were conducted to determine cassava management practices. Figures indicate the percentage of the total number of farmers interviewed.

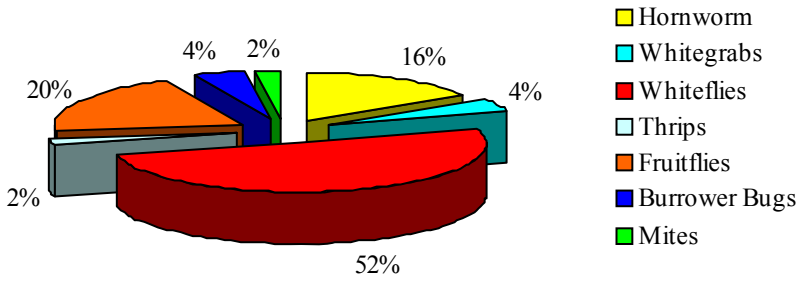


Figure 4.6.2. Arthropod pest populations on cassava farms in Quindío Municipality of Colombia. Figures indicate the percent of farms surveyed with each pest.

At the time of the survey, the municipality of Calarcá was most affected by whiteflies in cassava. This is reflected in the number of cassava farmers that was visited in this municipality (40% of the total). Two species of whiteflies were collected feeding on cassava in the region, A*leurotrachelus socialis* and *Trialeurodes variabilis* (Figure 4.6.3).

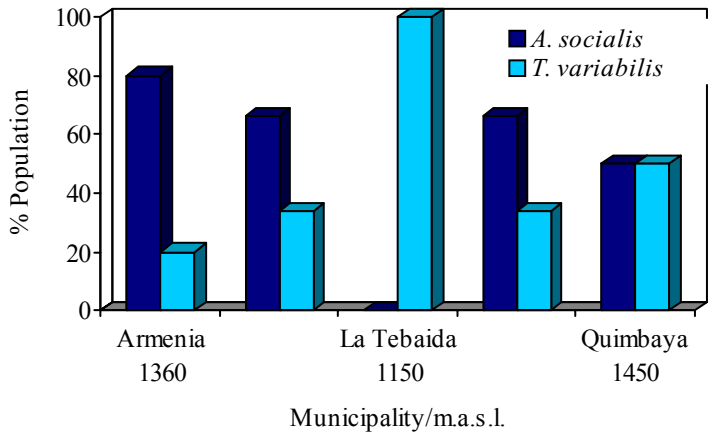


Figure 4.6.3. Percent whitefly species population found on cassava in five municipalities of Quindío Department in Colombia.

At the time of the survey, A. *socialis* was found in higher populations between 1360 and 1780 m.a.s.l., while *T. variabilis* was primarily found on cassava grown between 1100 and 1200 m.a.s.l. This is represented by the municipality of La Tebaida, where ambient temperatures are slightly higher than in the other municipalities in the survey.

The two most frequently grown cassava varieties in the region are Chirosa (MCol 2066) and ICA (HMC-1 and Catumare). Cassava is planted more frequently in monoculture (53.7%) than in association with other crop (46.3%) (Figure 4.6.4).

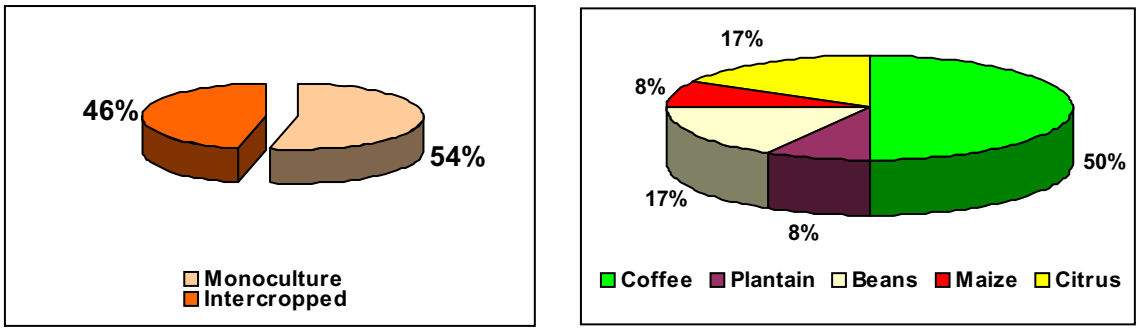


Figure 4.6.4. Cassava cropping systems being employed in Quindío Department of Colombia; a) monoculture vs. intercropping and b) percent of intercropped species.

The crops most frequently grown in association with cassava are coffee (50.0%), beans (17,0%), citrus (17.0%), plantain (8.0%) (Figure 4.6.4). It can also be noted that the whitefly species A. *socialis* predominated in the monoculture plantings of cassava while in the intercropping system the incidence of both species was nearly equal (Figure 4.6.5).

The damage or yield losses in cassava due to whitefly feeding in this region is being determined. Yield loses due to A. *socialis* feeding on cassava in other regions of Colombia have been recorded above 70% and averaging around 30 to 40%

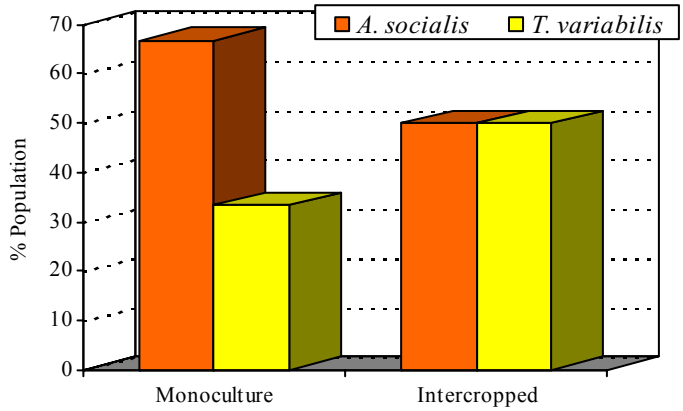


Figure 4.6.5. Percent whitefly species populations in two cassava cropping system, monoculture and intercropped, in Quindío Department of Colombia

The surveys also provided information on cassava farmer knowledge about pest biology, behavior, damage and ultimately about management of cassava pests. Results show that a great majority of producers, approximately 88% have little or no knowledge of whitefly biology, behavior and management. Only 12% of the farmers surveyed claimed knowledge of whiteflies as a cassava pest. These results support the need for farmer training in recognizing cassava pest problems.

Additional survey data shows that at present 50% of the cassava producers are applying chemical insecticides for whitefly control (Figure 4.6.6).

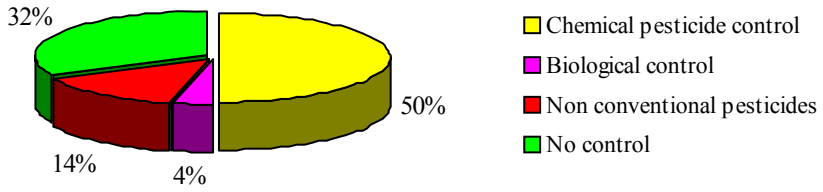


Figure 4.6.6. Whitefly control methods being used by cassava growers in Quindío Department of Colombia.

In most cases they receive no technical training or support. The continued high whitefly populations indicate that these pesticide applications are not effective in controlling the pest. Only 4% are presently using biological control, mostly entomopathogens such as *Beauveria bassiana* and *Lecanicillium lecanni* and predators like Crysopa. Approximately 14% of the farmers are using non-conventional pesticides; these are "home-remedy" types such as plant extracts and soap solutions. 32% of the farmers are not engaged in any whitefly control.

Cassava farmers are presently applying several different chemical pesticide products for whitefly control. The most popular is Sistemin (Dimethoate), being applied by nearly 48% of the farmers. Second is Actara (Tiametoxan) used by 19% of the growers, followed by Nodrin (9.5%) and Evisect (9.5%) (Table 4.6.1).

Products such Eviset (Tyocicam), Actara (Tiametoxan) and Trebon (Etoferprox) are reported as providing efficient control of whiteflies. However, farmers are not achieving adequate whitefly control, probably due to their lack of knowledge of whitefly biology and behavior. Pesticide applications are not timed to coincide with whitefly adult and 1st instar nymphal populations when these species are most susceptible to chemical treatment.

Similar surveys of cassava farmers in the Departments of Risaralda and Caldas have already been initiated as the project will be expanded into these two regions. Chemical pesticide and non-conventional pesticide products are being evaluated on farmer fields in the region to determine product efficiency and mode and timing of applications. Several biological control options will be evaluated. These include the use of entomopathogens and predator or parasite species. A whitefly resistant cassava variety is being compared to farmer varieties for acceptance as part of the varietal mixture.

The need to have farmers more knowledgeable about whitefly biology and behavior is obvious. Information sharing with growers has been initiated and courses in whitefly IPM have already been implemented. To date approximately 600 farmers and technicians have participated in these workshops and seminars.

Table 4.6.1. Percent usage by cassava growers of chemical pesticide products for cassava whitefly control in Quindío Department of Colombia.

Product/Activities Ingredient	% Utilization
Nodrin (Methomyl)	9.5
Sistemin (Dimetoato)	47.6
Evisect (Tyociclam)	9.5
Actara (Tiametoxam)	19.0
Thionil (Endosulfan)	4.8
Trebon (Etoferprox)	4.8

Activity 4.7. Intrinsic rate of increase the whitefly *Aleurotrachelus socialis* on the cassava (*Manihot esculenta)* varieties CMC-40 and MCol 2066 (Chirosa).

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Highlight:

- The high rate of survival and short generation time of the cassava whitefly, *Aleurotrachelus socialis* feeding on the commercial variety Chirosa, explain its rapid population increase in cassava plantations.

Rationale

Whiteflies as direct feeding pests constitute a major problem in cassava production in Central and South America and the Caribbean Region. There is a large complex in the neotropics where 11 species are reported. The major species causing yield losses in the northern region of South America (Colombia, Venezuela and Ecuador) and certain areas of Central America, is *Aleurotrachelus socialis.* On experimental fields, infestations of 1 month resulted in a 5% yield reduction of 6 months in a 42% reduction, and of 11 months in a 79% reduction (Vargas and Bellotti,1981, Revista Columbiana de Entomología 7: 13-20).

In recent years whitefly populations, especially of *A. socialis* have increased dramatically in certain cassava growing regions of Colombia. These include the Departments of Cauca, Valle del Cauca and the Colombian coffee growing region of Quindio, Risaralda and Caldas. In this latter region, prior to about three years ago, whitefly populations were relatively low and not suspected of causing yield losses. The rapid increase in whitefly populations, principally *A.socialis,* but also *Trialeurodes variabilis*, is presently causing severe crop damage.

The reason for this rapid buildup in populations is not fully understood. *A. socialis* is more trypically associated with lower altitudes and warmer temperatures as found in the Tolima Valley where the pest has been endemic for many years.

Studies have been initiated to try to better comprehend the possible changes in *A. socialis* biology and parameter population. These studies can provide information on the potential of *A. socialis* to invade

different cassava growing regions and will aid in developing effective pest management programs. The immediate objective of this research is to determine the biology of *A. socialis* and estimate population parameters on two cassava genotypes.

Materials and Methods

Two cassava genotypes were used in the experimental trials. The genotype CMC-40 is a vigorous cultivar that is susceptible to whiteflies; Chirosa (MCol 2066) is a high yielding commercial cultivar being planted throughout the coffee growing region of Colombia. Experiments are being carried out on 30 to 40 day plants being grown in plastic pots with sterile soil (1.0 kg). Plants are maintained in the screenhouse at 30 $\pm$ 2°C and RH of 50 to 60%.

A. *socialis* adults are obtained from the CIAT colony that is maintained in the greenhouse (27 $\pm$ °C temp. and 60 – 70% RH), on the cassava cultivar CMC-40.

Longevity and Fecundity Studies: Forty recently emerged A*leurotrachelus socialis* females, sexed using the Eichelkrant Cardona (1989, Turrialba 39:55-62) method, are selected from the CIAT colony. These females are placed individually in small leaf cages (2.5 cm diameter x 2.0 cm width) and placed on the undersides of the test genotypes, Chirosa and CMC-40. Every 48 hours adults were transferred to a new area of the leaf; this procedure is continued until the natural death of the whitefly females. Fecundity was estimated by recording the numbers of eggs oviposited by each female during these 48 hours intervals. Adult longevity was determined by the duration of survival of each female.

Development time, rate of survival and proportional females: Fifty two day old adult male and female, A. *socialis* were removed from the CIAT colony with the aid of a bucal aspirator. These were placed in the small leaf cages, as previously described, and attached to the undersides of CMC-40 and Chirosa leaves. Adults were removed after six hours and 200 eggs were randomly selected. Egg to adult development time was recorded, as well as the rate of survival of immature stages and the proportion if emerging females.

Demographic Parameters: Data on development time is combined with experimental data on reproduction (1xmx); generating life tables which are used to calculate the demographic parameters as defined by Price (1975, Insect Ecology, John Wiley & Sons, New York, 514 p): 1) Net reproduction rate ($_{RO}$), the average number of female descendents produced by one female per generation; 2) generation time (T), equivalent to the time period between parental birth and progeny birth and 3), the intrinsic rate of population increase (rm) estimated using the equation:

$$\times \exp(-rmX)1_x m_x = 1$$

Where X is the female age (days); 1 x is specific survival age and m_x represents the proportion of females from a female progeny at age X. To calculate the value of rm, the corrected age X + 0.5 and the equation In 2/rm were use to estimate the days required to double the population.

Results and Discussion

Longevity and Fecundity of Aleurotrachelus socialis: Longevity of A. *socialis* feeding on MCol 2066 (Chirosa) ranged from 2 to 8 days while the range on CMC-40 was 2 to 16 days; the average longevity of *A. socialis* on CMC-40 exceed that on MCol 2066 by 2.5 days (Table 4.7.1).

Table 4.7.1. Average longevity (days), average fecundity (eggs/female) and oviposition rate (eggs/females/2 days) of *Aleurotrachelus socialis* feeding on two cassava genotypes, CMC-40 and MCol 2066 (Chirosa).

Parameter	CMC-40	Chirosa (Mcol 2066)
Average Longevity (days)	6.4 a	3.9 b
Range	2 – 16	2 - 8
Average fecundity	33.8 a	7 b
Range	1 - 125	1 - 154
Average oviposition rate	5.29 a	1.8 b

Averages followed by different letters across the columns are significantly different λ ANOVA – one way P{ 0.0001, Tukey P{ 0.05.

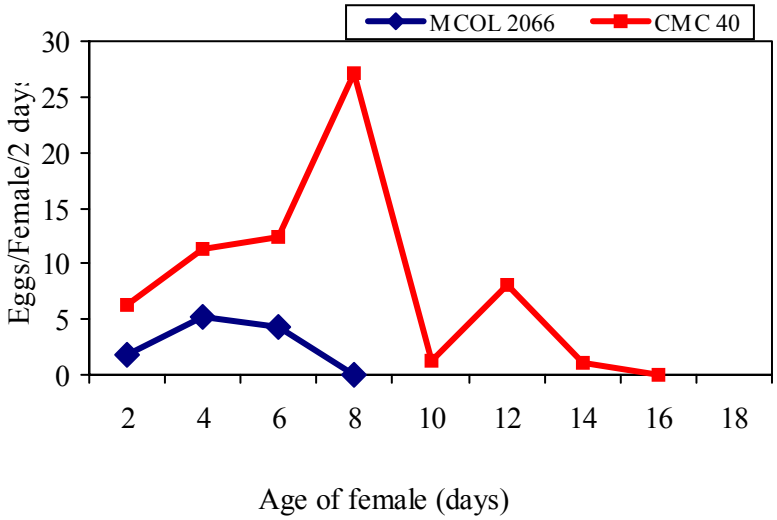

Figure 4.7.1. Fecundity of *A.socialis* feeding on cassava genotypes CMC-40 and MCol 2066 in the secreenhouse.

Ovipositional range per female was greater on CMC-40 (1-125) than that expressed on MCol 2006) 1-54) (Table 4.7.1). Average fecundity was significantly higher on CMC-40 than on MCol 2066. Peak oviposition per female on CMC-40 occurred on day 8, while oviposition on Mcol 2066 terminated during this period (Figure 4.7.1). Average fecundity on CMC-40 was 33.8 eggs per female and only 7.0 on MCol 2006. Ovipositional rate was higher on CMC-40, where each female oviposited 3.5 more eggs every two days, than on MCol 2066 (Table 4.7.1).

Development time, rate of survival of immature stages and proportion of females: The development time of *A. socialis* feeding on CMC-40 was significantly longer by 5 days than those feeding on MCol 2066 (Table 4.7.2).

Table 4.7.2. Development time, survival and proportions of female *Aleurotrachelus socialis* feeding on two cassava genotypes CMC-40 and MCol 2066 (Chirosa)

Parameter	CMC – 40	Chirosa MCol 2066
Development time (days)	39.7 b	34.7 a
Survival rate (%)	80 (160)	87 (174)
Proportional females (%)	50	50

Development time: different letters between columns are significantly different ANOVA Tukey test P{ 0.05. Rate of survival of immatures (χ^2=3.56, 1 df, P= 0.0593)

There was no significant difference in rate of survival between the two genotypes although survival was higher on MCol 2066 (174 vs. 160). These results indicate that *A.socialis* will successfully colonize both genotypes. The proportion of females emerging was the same on both genotypes, 1:1 (Table 4.7.2).

Demographic Parameters: The net reproductive rate (Rŭ allows us to estimate that, on average, at the end of a generation, *A. socialis* populations could multiply 33.7 times (individual/individual) on CMC-40, this being 27.5 times greater than on MCol 2066 (Table 4.7.3).

Table 4.7.3. Demographic parameters of *Aleurotrachelus socialis* feeding on CMC-40 and MCol 2066 (Chirosa)

Parameter	CMC-40	Chirosa (Mcol 2066)
Net Reproduction Rate (Ro)	33.74	6.2
Generation time (T)	44.23	36.71
Intrinsic rate of increase (r_m)	0.0296	0.0495
Days to duplicate population (DDP In $2r_m$	23.4	14

This considerable difference can be attributed to the low fecundity level of *A. socialis* on MCol 2066. *A. socialis* would complete one generation in 44.2 days on CMC-40 and in 36.7 days on Mcol 2066. This indicates that *A. socialis* would complete 10 generations per year on CMC-40 and 12 MCol 2066.

The intrinsic rate of increase (rm) was 40% greater on MCol 2066, when compared to CMC-40 (Table 4.7.3). These results demonstrate the biotic potential of *A. socialis* to develop high populations on Mcol 2066 (Chirosa), inspite of the lower fecundity that occurs on this host. *A. socialis* will double its population in 14 days feeding on MCol 2066, while it requires 23 days on CMC-40 (Table 4.7.3).

It can be conclude from the results of this research that both CMC-40 and Chirosa are favorable host genotypes for rapid population increases of *A. socialis.* The shorter development time and the high rate of survival of *A.socialis* on Chirosa (MCol 2066) are indicators for high whitefly populations found on this genotype. These results help explain the high populations and damage to the cassava crop being experienced in the Colombian coffee growing region (Figure 4.7.2).

Figure 4.7.2. High survival of populations of *Aleurotrachelus socialis* feeding on Chirosa (MCol 2066) (a) and CMC-40 (b).