SECTION FIVE

Special Topics on Pest and Disease Management

BLANCA 302

CHAPTER 5.1

Sustainable Integrated Management of Whiteflies through Host Plant Resistance

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Introduction

As direct feeding pests and vectors of plant viruses, whiteflies constitute a major problem in the production of cassava (*Manihot esculenta* Crantz) in Africa, the neotropics and, to a lesser degree, Asia. The largest complex in the neotropics includes *Bemisia tabaci* (Gennadius)[1], *B. tuberculata* (Bondar) and *Trialeurodes variabilis* (Quaintance). *B. tabaci* has a pantropical distribution, feeding on cassava throughout Africa, where it transmits African Cassava Mosaic Disease, and several countries in Asia, including India and Malaysia.

Whitefly feeding affects cassava in three ways. Direct damage is caused by feeding in the phloem of the leaves, inducing chlorosis and leaf fall, which results in considerable reduction in root yield if prolonged feeding occurs. Yield losses of this type are common in the neotropics. Whiteflies also produce a honeydew, which provides a medium for sooty mould growth that can reduce yields. Most importantly, *B. tabaci* is a major vector of cassava viruses.

Research on whitefly control in the neotropics has emphasized host plant resistant (HPR) and biological control. The bulk of this report will cover HPR research on whiteflies (*Aleurotrachelus socialis* Bondar) as a component of the Cassava Whitefly Integrated Pest Management (IPM) Project.

In traditional production systems, resource-limited farmers have few options available for controlling pests. The cassava germplasm bank at the Centro Internacional de Agricultura Tropical (CIAT) has nearly 6000 accessions and locally selected cultivars (land races) collected primarily in the neotropics. These traditional cultivars represent centuries of cassava cultivation in diverse habitats, having been selected by farmers over a long period in the presence of a high diversity of herbivores. These land races often possess traits that confer low to moderate levels of resistance to multiple pests. This germplasm bank is constantly being evaluated for resistance to several arthropod pests that can cause yield losses in cassava. Evaluations often are done in more than one ecosystem.

The general purpose of this project is "to reduce crop losses due to whitefly feeding damage and whitefly-transmitted viruses, and

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1. Evidence suggests that *Bemisia tabaci* represents a species complex with numerous biotypes.

prevent further environmental degradation and food contamination due to excessive pesticide use, leading to more productive and sustainable agricultural systems".

The project has three major objectives, which are to:

(1) Identify and access exotic or novel genes and gene combinations that can contribute to germplasm enhancement for whitefly resistance in cassava;
(2) Study the genetics of resistance and to map genes for whitefly resistance in cassava and develop molecular markers for their incorporation into improved African, Latin American and Asian germplasm; and
(3) Develop crop management options for reducing whitefly populations and the transmission of whitefly-transmitted viruses.

The research presented in this chapter is being funded by the Ministry of Foreign Affairs and Trade (MFAT) Host Plant Resistance (HPR) Project and consists of four major areas of activity:

(1) Cassava germplasm evaluation to identify sources of whitefly resistance in land race varieties in the CIAT cassava germplasm collection;
(2) Identification of genomic regions responsible for the determination of whitefly resistance in cassava;
(3) Identification of resistance mechanisms in cassava; and
(4) Tritrophic relationships to determine the effect of HPR on whitefly parasitism.

Cassava Germplasm Evaluation

Field screening of cassava germplasm can be done at several sites in Colombia. Ideally, field populations of whiteflies should be high and damage levels significant so as to distinguish susceptible cultivars. Field evaluations of cassava germplasm use a population (nymphs) scale combined with a leaf damage scale (Table 1). Evaluations are made periodically throughout the growing cycle; four to five evaluations are done, 1.5 to 2.0 months apart. During the 1998 growing cycle, cassava clones were evaluated at CIAT in the Cauca Valley and Nataima in the Tolima valley of Colombia.

From 1994 through to 1996, whitefly (*A. socialis)* populations at Nataima, Tolima, were lower than normal. Low whitefly populations provide inadequate selection pressure to ensure reliable resistance evaluation. All evaluations done at Nataima are with natural field populations of whiteflies.

During 1997-98, whitefly populations increased significantly, allowing for more accurate germplasm evaluation. During 1999, some 1651 cassava clones were planted in observational fields, and 1418 were evaluated. These clones were the F_1 progeny from crosses of cassava clones previously selected for their resistance to whiteflies, with clones with desirable agronomic characteristics. These crosses resulted in 17 families (Table 1). Four field evaluations were done over a period of several months, using a whitefly damage and populations scale, as previously described.

Results indicate that whitefly populations were high and selection

Table 1. Families of cassava clones formed from crosses of whitefly (*Aleurotrachelus socialis*)-resistant and -susceptible cultivars.

Family	Crosses[a]		Observation
	Female	Male	
CM 3317	MBra 12 (T)	MCol 1468 (S)	MCol 1468 = CMC-40
CM 5438	MBra 12 (T)	MCol 1505 (S)	
CM 7559	MNGua 2	MBra 12 (T)	
CM 8884	CG 489-4 (R)	MCol 1468 (S)	CG 489-4 = MEcu 72 X MBra 12
CM 8885	CG 489-4 (R)	MCol 1505 (S)	CG 489-4 = MEcu 72 X MBra 12
CM 8887	CG 489-4 (R)	MCol 2256	CG 489-4 = MEcu 72 X MBra 12
CM 8889	CG 489-23 (R)	MCol 1468 (S)	CG 489-23 = MEcu 72 X MBra 12
CM 8891	MCol 1468 (S)	CG 489-34 (R)	CG 489-34 = MEcu 72 X MBra 12
CM 8892	MCol 2246	CG 489-34 (R)	CG 489-34 = MEcu 72 X MBra 12
CM 8893	MCol 2256	CG 489-34 (R)	CG 489-34 = MEcu 72 X MBra 12
CM 8960	MCol 2246	MBra 12 (T)	
CM 8961	MCol 2256	MBra 12 (T)	
CM 8984	MCol 1505 (S)	CG 489-34 (R)	CG 489-34 = MEcu 72 X MBra 12
CM 8990	MCol 2026	CG 489-34 (R)	CG 489-34 = MEcu 72 X MBra 12
CM 8991	MCol 2026	MBra 12 (T)	
CM 8995	MEcu 72 (R)	MCol 1468 (S)	
CM 8996	MEcu 72 (R)	MCol 2246	

a. R, whitefly resistant cultivar; T, whitefly tolerant cultivar; and S, whitefly susceptible cultivar.

pressure was good. Of the 1418 clones evaluated, 938 (66%) had damage ratings above 3.6 and were eliminated as susceptible (Figure 1); 308 (22%) clones had an intermediate damage evaluation (2.6 to 3.5). The remaining 172 (12%) clones had damage ratings below 2.6 and are considered promising for resistance; 56 (3.9%) clones had damage ratings of 1 to 1.5, indicating possible high levels of resistance. However, one cycle (year) of field evaluation is not adequate to identify resistance confidently and these clones will continue to be evaluated for at least 3 cycles (years). A 1.0 to 1.5 rating signifies no damage symptoms and low whitefly population levels. The families that most frequently occurred in this group were CM 8984, with nine selections, and CM 8893, with six. In both these families, the resistant parent was CG 489-34, indicating that this clone may contain good heritable resistant traits.

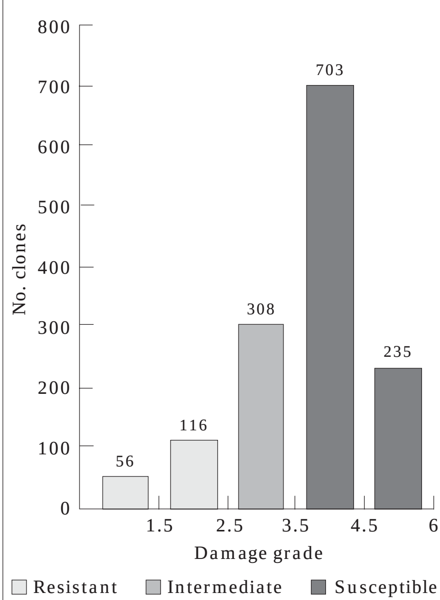


Figure 1. Evaluations of F_1 cassava clones from crosses of whitefly (*Aleurotrachelus socialis*)-resistant and -susceptible cultivars at the Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Nataima, Tolima (1997-98).

Whitefly populations at CIAT were moderate to high for the fourth consecutive year. Evaluations at CIAT during 1998 concentrated on cultivars that had been selected as promising for resistance during previous years at the Instituto Colombiano Agropecuário (ICA)-Nataima.

Thirty-two clones sown in 50-plant plots were evaluated using a 1 to 6 (low to severe damage or low to high whitefly population level) scale. Whitefly population scales are based on counts of nymphs, pupae and adults. The 32 clones evaluated consisted of land race varieties, hybrids and backcrosses, and previously had shown good levels of resistance in screening trials in Tolima. Five regional or farmer varieties from the Tolima area also were included.

Results showed that nine cultivars, or 26.5%, presented very low damage levels (1.0 to 1.5) and three cultivars had damage levels between 1.6 and 2.5. The remaining 20 cultivars had damage levels between 2.6 and 5. The regional cultivars from Tolima, Azucena, Ceiba Blanca, Almidona, Llanera Precoz and Cuero de Marrano had damage and population ratings between 4 and 4.5, indicating that farmer varieties in the regions are susceptible to whiteflies and probably experiencing significant yield losses.

The nine best cultivars were MEcu 64, MPer 335, MPer 415 and MEcu 72 (all land race varieties), and CM 8424-6, CM 8424-33, CM 8424-4, CG 489-34 and CG 489-4 (all hybrids). All had damage ratings of 1.0, except MPer 415 and CG 489-34 with ratings of 1.5. MEcu 72 and CG 489-34 had maintained low damage ratings consistently, over several years. The varieties MEcu 64 and MPer 335 had excellent growth habits as well as low damage ratings and low whitefly populations. These two varieties will continue to be evaluated at CIAT and Tolima and should enter into breeding schemes for improved whitefly-resistant clones.

Identification of Genomic Regions Responsible for the Determination of Whitefly Resistance in Cassava

Previous research at CIAT determined the existence of different sources of resistance to whitefly. The most important genotypes are MBra 12 and MEcu 72, which were used as parentals in the generation of new genotypes. CG489-34 has shown the best resistance behaviour. The more susceptible genotypes, MCol 2026 and MCol 1505, also were selected and evaluated.

The goal of this project is to study and screen plants with resistance to whitefly in cassava, using molecular techniques.

Different breeding populations were obtained from the resistant and susceptible genotypes as parentals:

CG 489-34 X MCol 2026 = 131 individuals
CG 489-34 X MCol 1505 = 108 individuals
MBra 12 X MCol 2026 = 135 individuals

We selected the more contrasting individuals (resistant and susceptible) in the field for each family. DNA was extracted from the different individuals of each group and, with the parental, was mixed in a bulk. We used molecular screening (DNA restriction fragment length polymorphisms [RFLPs] and random amplified polymorphic DNA [RAPDs]) intended to find markers associated to resistance, and later it is intended to isolate the responsible genes. The parental lines and the bulk will be screened with amplified fragment length polymorphism (AFLP) markers.

Parentals from each family were evaluated with RFLP markers from the cassava map (Fregene et al., 1997). Seventy-two polymorphic probes were obtained. Sixty-two RAPD markers have been screened with the bulks of the same families; the primer OP.P3 showed a clear polymorphism between the susceptible and resistant group. This marker is being evaluated with the whole population of each cross to confirm its association with the resistance (Figure 2).

We intend to isolate and sequence the polymorphic bands and generate a sequence characterized amplified region (SCAR). This can be used to make a diagnosis of resistant materials and determine the most promising genotypes for breeding programs and farmers.

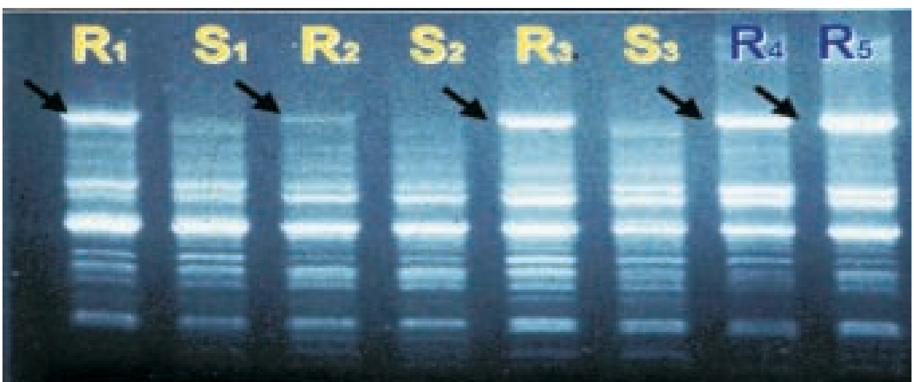
With the sequence of the genes of resistance, homologies will be established with those reported on other crops, to understand expression patterns. The RFLP, RAPD, microsatellites and AFLP markers will be used to generate a framework map and for the quantitative trait locus (QTL) analysis.

Identification of Resistance Mechanisms

Whitefly feeding behaviour

Cassava genotypes with resistance to whiteflies have been identified at CIAT. Resistant clone MEcu 72 shows high mortality for both adult and immature whiteflies, which may suggest less feeding on this genotype under natural conditions. It is necessary to identify whitefly feeding behaviour on susceptible genotypes and to make comparisons with MEcu 72 in order to better understand the mechanisms of resistance.

Electronic monitoring of insect feeding (EMIF) is a technique that permits the identification and quantification of the feeding behaviours



R_1 and S_1: CG 489-34 x MCol 2026 R_2 and S_2: MBra 12 x MCol 2026

R_3 and S_3: CG 489-34 x MCol 1505 R_4: Ecu 72; R_5: Ecu 72 progeny

Figure 2. Random amplified polymorphic DNA (RAPD) bulk analysis of whitefly (*Aleurotrachelus socialis*)-resistant and -susceptible cassava clones.

of hemipteran insects. By passing an electrical signal to a test plant, and tethering an insect with a fine gold wire, modifications caused by stylet movements and feeding behaviours can be observed as waveforms. The EMIF technique has been used extensively for the study of mechanisms of plant resistance to insects. CIAT presently owns two AC-Electronic feeding monitors, and has access to a DC version of the system (electrical penetration graph [EPG]). The AC systems have been used with leafhoppers (*Empoasca kraemeri* Ross & Moore) on common bean (*Phaseolus vulgaris* L.), and the DC systems with cassava mealybugs (*Phenacoccus manihoti* Matile-Ferrero). Preliminary observations with both systems suggested that an easy protocol could be developed for monitoring the whitefly. In addition, CIAT technicians needed to be trained in wiring techniques, operation of the system, computer display and data acquisition.

Electronic monitoring methodology

The methodology devised for electronic monitoring of whitefly feeding behaviour includes two major steps: a wiring technique to attach the thin wire to the mesonotum of an adult whitefly and the proper settings (ground voltages, signal frequency) of the electronic monitoring system.

The "normal" gold wire used with leafhoppers (12.7 µm) is too thick and stiff for the small whitefly adults. A thinner wire needed to be obtained. Since purchase of a thinner wire was difficult in the short period of time allowed for this project, thinning of the existing gold wire was necessary. To do this, a 10- to 20-cm piece of the thick wire is placed for 45 min in a solution of 3 mol nitric acid and 9 mol hydrochloric acid. This is a potent oxidizer that dissolves away part of the gold, leaving a thinner wire while conserving its electricity conducting properties. Pieces of this thin gold wire are attached with silver paint to a copper stub.

Female whiteflies from a greenhouse colony are placed by mouth aspirator on a vacuum stand and held in place by a gentle vacuum. Several individuals can be placed simultaneously. With the help of the copper stub, the thin gold wire is placed on the mesonotum of a female, and a small drop of electrically conducting silver paint is used to attach the wire to the insect. Care needs to be taken to not get silver paint on the whitefly's eyes or wings. This affects their behaviour even more than the tethered condition. New insects need to be used should silver paint get on their wings or eyes.

Tethered whiteflies can be acclimated to the wire by placing them on a cassava leaf for 1 hour. Before the electronic monitoring session begins, whiteflies are starved for 30 minutes. The copper stub holding the tethered whitefly is attached to the alligator clip on one of the input electrodes of the electronic monitor.

A constant signal, 250 mV at 250 Hz, was established as the best setting for *A. socialis*. This signal is transmitted via an electrode inserted in the substrate of a potted plant (4-8 weeks old). Detached leaves placed on a container filled with water and sealed with the output electrode of the electronic monitor also worked and showed better conductivity than the potted plants. However, the effects of detached leaves vs. whole plants on whitefly feeding behaviour need to be evaluated further.

Feeding behaviour

During probing, stylet movements and other feeding behaviours induce changes to the constant signal. These modifications, known as waveforms, are captured in a computer using an analogue to digital converter board, displayed on the screen on a time scale, and stored for post-acquisition measurement and analysis. Once waveforms have been correlated with specific behaviours, quantification can be made on their frequency and duration.

Waveforms produced by probing *A. socialis* on CMC 40 are shown in Figure 3. Because of time limitations, waveforms cannot be correlated with specific behaviours for this whitefly species. However, it has been reported that several species of whiteflies share very stereotypical styles of feeding and that waveform patterns are very similar among them. Since waveforms shown are similar in appearance to those reported in the literature, we will describe them. All probes observed started with salivation waveforms corresponding to the intercellular path that the stylets follow in their way to the phloem. These waveforms are shown in Figures 3A and 3C as "intercellular stylet path". They have been correlated also with the deposition of a salivary sheath. In some instances, the stylets come across treachery elements in the xylem and the whitefly ingests from them. This particular waveform has not been correlated with completely, but there is evidence of ingestion. After reaching the phloem, normally whiteflies go into continued ingestion, producing a flat waveform whose beginning is illustrated toward the end of the trace (Figure 3).

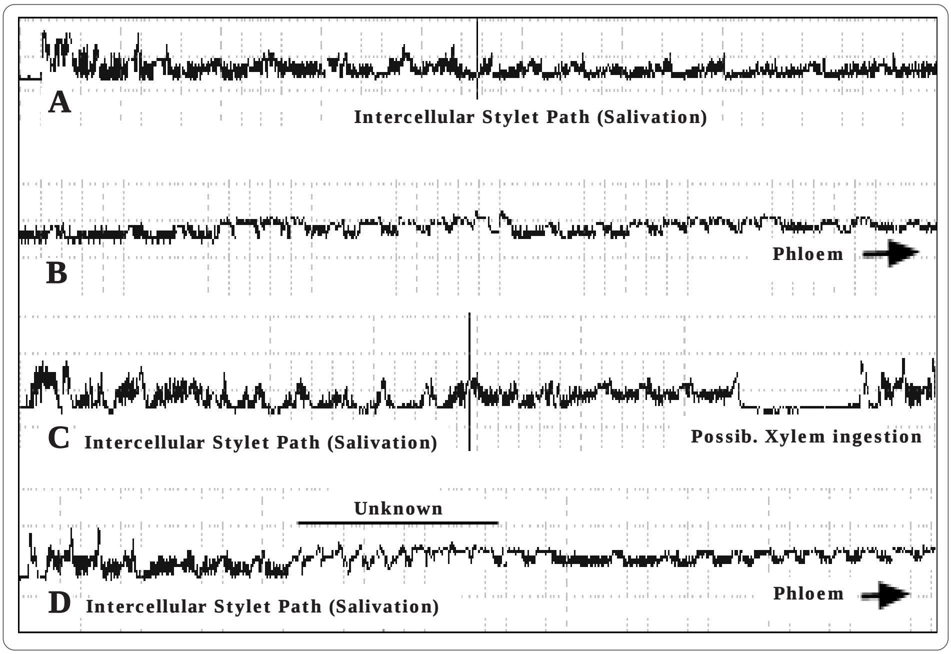


Figure 3. Waveforms produced by probing *Aleurotrachelus socialis* on cassava cv. CMC 40. A and B: Single probe (stylet penetration). B and C: Single probe forms a second whitefly. Note that waveform identified as possible xylem ingestion and an unknown waveform that has yet to be correlated with a feeding behaviour.

Figure 3D shows a new waveform that previously has not been identified or associated with any behaviour. It would be expected that, with more electronic monitoring of whitefly feeding, more patterns would be identified and more correlational work would be needed. Now that a methodology exists for the study of whitefly feeding behaviour at CIAT, comparisons can be made among resistant and susceptible genotypes.

Tritrophic Relationships: Determining Effect of HPR on Whitefly Parasitism

It has been observed that farmers will use pesticides to control high populations of cassava whiteflies. Pesticide applications will reduce the effectiveness of biological control as well as cause environmental contamination. Several years of research at CIAT has identified cultivars with varying levels of resistance to whiteflies, especially the species *A. socialis*.

Present research is investigating the compatibility between HPR and biological control. The combination of these two methods could reduce pest populations below economic injury levels or extend the usefulness of HPR. In addition, it is important to know the effect that plant resistance, especially antibiosis, might have on parasitoid populations.

The compatibility of the parasitoid *Encarsia hispida* DeSantis was evaluated on whitefly immatures feeding on whitefly resistance genotypes of cassava. The resistant varieties tested were MEcu 72, and CG 489-4; the susceptible check was CMC 40. Parasite development time, fecundity and survival were measured.

Plants of the abovementioned varieties at 1 month of age were infested with *A. socialis* eggs by exposing the leaf undersurface to ovipositing adults for 36 hours. After 15 days, when whitefly immatures were in the second instar, they were exposed to *E. hispida* parasites. *E. hispida* was obtained by collecting cassava leaves with parasitized whitefly pupae in the field at CIAT, placing the leaves in blackened plastic traps and capturing emerging parasites. The results at this point are preliminary.

Results to date indicate that the female longevity of *E. hispida* was similar on varieties CMC 40 (17.8 days) and CG 489-34 in experiments 1 and 2 (12.2 and 13.8 days, respectively) (Figure 4). *E. hispida* longevity on MEcu 72 was greatly reduced, only 7.7 days, indicating that this variety has factors or characteristics that affect the longevity of the parasite. These factors could be the number of tricomes or a chemical component that is interfering with parasitoid feeding (Table 2).

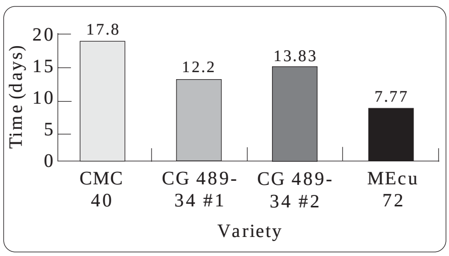


Figure 4. Longevity of the parasitoid *Encarsia hispida* on the whitefly *Aleurotrachelus socialis* on three cassava varieties, CMC 40, CG 489-34 (experiments #1 and #2) and MEcu72.

E. hispida emergence from *A. socialis* pupae was measured on the varieties CG 489-34 and MEcu 72. Results show a low rate of emergence of the parasitoid, 0.43 for CG 489-34 and 0.86 for MEcu 72. These

Table 2. Whitefly (*Aleurotrachelus socialis*) survival, development time and fecundity on four cassava varieties.

Variety	Percentage survival of immatures[a]	Development time/days[a]	Number eggs/female	Tricomes/cm² on second leaf
MBra 12	75.0 a	32.18 c	107	4.653
CMC 40	66.0 ab	32.07 c	64	189
CG 489-34	65.5 ab	33.13 b	46	15.290
MEcu 72	27.0 c	34.45 a	15	33.048

a. Means within a column followed by the same letter are not significantly different at the $P = 0.05$ level, using LSD test.

preliminary results indicate that host plant resistance in cassava may affect the development or emergence of parasitoids. Experiments using susceptible varieties (CMC 40) still need to be completed. Since *E. hispida* is the most frequently collected parasitoid in cassava fields, a much higher emergence is expected.

The survival of *E. hispida* when associated with *A. socialis* on the cultivar MEcu 72 decreased rapidly when compared to the other cultivars. One hundred percent mortality occurred within 19 days (Figure 5). Longest survival was on CMC 40, with individuals living for 40 days. Variety CG 489-34 was intermediate; in both experiments with this cultivar, individuals survived until 34 days. These data indicate that there may be varietal influence associated with *E. hispida* survival. Since MEcu 72 and CG 489-34 are resistant varieties, this resistance factor or mechanism may be affecting parasitoid survival.

Host plant resistance and biological control agents increasingly are accepted as complementary pest-control tactics that can reduce the use of chemical pesticide, especially with difficult-to-control pests such as whiteflies. Cassava is most often grown by resource-limited, small-scale farmers in the tropical and sub-tropical regions of the world. HPR and biological control offers a low-cost, practical, long-term solution for

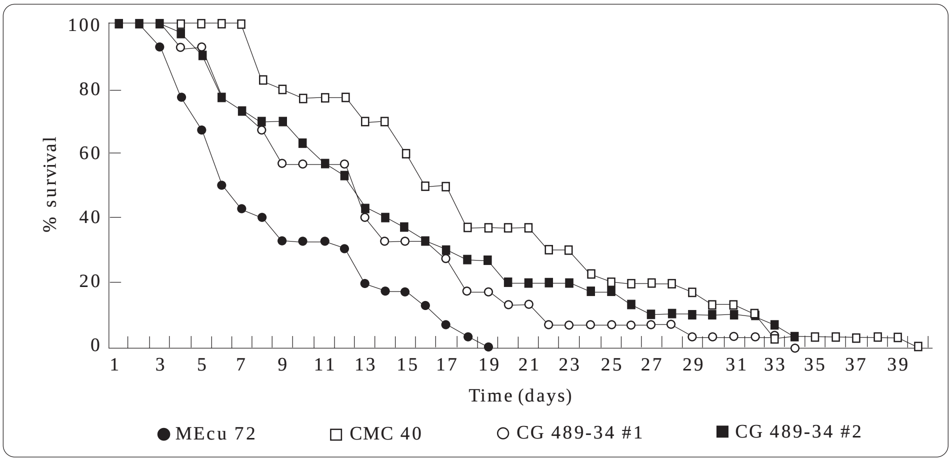


Figure 5. Survival of *Encarsia hispida*, parasitoid of *Aleurotrachelus socialis*, on three varieties of cassava, MEcu 72, CMC 40 and CG 489-34 (experiments #1 and #2).

maintaining lower whitefly populations, reducing crop losses, and limiting or eliminating the need for applications of costly insecticides. This is especially important in crops such as cassava, which has a long growing cycle (1 year or more) that would require numerous pesticide applications to achieve adequate whitefly control. Research on cassava whitefly control at CIAT initially emphasized HPR. More recently, a concentrated effort is being made to identify and use natural enemies in an IPM context (see Chapter 5.2).

Reference

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