CHAPTER 5.2

Biological Control of Whiteflies by Indigenous Natural Enemies for Major Food Crops in the Neotropics

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Introduction

Whiteflies as direct feeding pests and virus vectors constitute a major problem in cassava (*Manihot esculenta* Crantz) and associated crops in Central and South America and the Caribbean region. There is a large complex in the neotropics, where 11 species are reported on cassava alone (Bellotti et al., 1999). In the northern region of South America (Colombia, Venezuela, and Ecuador) the major species is *Aleurotrachelus socialis* (Bondar) (Castillo, 1996). Two additional species, of lesser importance, but frequently found on cassava, are *Bemisia tuberculata* (Bondar) and *Trialeurodes variabilis* (Quaintance). High populations of *A. socialis*, frequently observed in the region, can cause serious yield reductions in cassava. There is a correlation between duration of attack and yield loss: 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).

Until recently, the *Bemisia tabaci* (Gennadius) biotypes found in the Americas did not feed on cassava. It has been speculated that the absence of African cassava mosaic disease (ACMD) in the Americas may be related to the inability of its vector, *B. tabaci*, to colonize cassava (Costa and Russell, 1975). Since the early 1990s, a new biotype (B) of *B. tabaci*, considered by some to be a separate species (*Bemisia argentifolii* Bellows & Perring) (Perring et al., 1993) has been found feeding on cassava in the neotropics (França et al., 1996; Quintero et al., 1998). More recently, in greenhouse studies done at the Centro Internacional de Agricultura Tropical (CIAT), it has been shown that *B. tabaci* females will oviposit on cassava, and immatures will feed and pupate; however, few adults emerged (CIAT, 1999). It is considered that ACMD now poses a more serious threat to cassava production, because most traditional varieties in the neotropics are highly susceptible to the disease. In addition, the B biotype of *B. tabaci* as a virus vector causes heavy crop losses on numerous other crops in the neotropics and these often are grown in association with cassava, or in the same area. The possibility of virus diseases moving between these crops, or the appearance of previously unrecorded viruses, has become a potential threat.

Host plant resistance and biological control agents (e.g., parasitoids, predators and entomopathogens) increasingly are accepted as a means of pest control that reduces environmental

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contamination and other disadvantages arising from excessive use of chemical pesticides. Many natural enemies are found associated with species of whiteflies on cassava in the neotropics, especially in Colombia and Venezuela (Gerling, 1986; Gold et al., 1989; Castillo, 1996; Evans and Castillo, 1998). Although a large complex of parasitoids have been collected from cassava whiteflies, little is known about the levels of parasitism they impose on the whitefly population, either as individual species or collectively.

For instance, during 1994 and 1995, CIAT personnel carried out surveys for cassava whitefly natural enemies in three regions of Colombia: the Andes, the Atlantic Coast and the Eastern Plains (Castillo, 1996). One hundred sites were visited in 20 departments (states). The three regions differ in altitude, temperature and precipitation, as well as in their cassava cultivation patterns. The Andean region contains many small plantations dispersed over a wide area; in the Atlantic Coast, cassava cultivation is generally in extensive and uniform areas; while in the Eastern Plains, cassava is cultivated in small "patchy" areas near the foot of the easternmost range of the Andes.

The whitefly species collected on cassava during these surveys were *A. socialis*, *B. tuberculata*, *T. variabilis*, *Tetraleurodes* sp. and *Aleuroglandulus malangae* Russell. The latter two species were collected for the first time in very low numbers in the Atlantic Coast and Andean region. *A. socialis* represented 64.5% of the adults and 86% of the immature (eggs, nymphs, and pupae) collected across the three regions. *T. variabilis* was represented by 27.5% of the adults and 11.0% of the immatures, while *B. tuberculata* represented 8.0% of the adults and 3.0% of the immatures (Castillo, 1996).

A. socialis and *B. tuberculata* had a wide distribution, both being collected in almost all sites surveyed. *T. variabilis* was collected from the Atlantic Coast and the Andean region, but not from the Eastern Plains. *A. socialis* populations were highest in the Eastern Plains and lowest in the Andean region. *B. tuberculata* numbers were highest on the Atlantic Coast and Eastern Plains, and almost all *T. variabilis* were collected from the Andean region. The data from this study showed that *A. socialis* and *B. tuberculata* populations decreased with increasing altitude, and were most abundant below 900 m. *T. variabilis* was most abundant at higher elevations, above 1400 m. *A. socialis* populations were highest when temperatures were above 23 °C, while *T. variabilis* populations were higher at lower temperature (<19 °C). *B. tuberculata* were most abundant in those areas with higher temperatures. Annual precipitation did not have a significant effect on distribution of any one of the three species.

A species richness of whitefly parasitoids was collected during these surveys. Ten species were collected from *A. socialis*, *B. tuberculata* and *T. variabilis*. Several of these were unrecorded species (Castillo, 1996; Evans and Castillo, 1998). The parasitoids collected were represented by three genera, *Encarsia* (four species), *Eretmocerus* (four species) and *Amitus* (two species). Three of the *Encarsia* species were *E. hispida* DeSantis, *E. pergandiella* Howard and *E. bellotti*. None of the *Eretmocerus* genus was identified to species and one *Amitus* was identified to species, *A. macgowni* Evans and Castillo. *Eretmocerus* sp. "b", *E. hispida*, *A. macgowni* and *E. bellotti* were collected parasitizing *A. socialis*. *E. bellotti*, *E. hispida*, *E. pergandiella* and *Eretmocerus* sp. "a" were collected from *T. variabilis*. Only *Eretmocerus* sp. "c" was collected parasitizing *B. tuberculata* (Castillo, 1996).

The highest level of parasitism was around 15% measured on *A. socialis* in the Eastern Plains; almost 14% was measured on *B. tuberculata* and 12% on *T. variabilis*, both in the Andean region. *E. hispida* represented the greatest number of individuals collected, 1845 or about 64% of all parasitoids collected. *Eretmocerus* sp. "b" was the second most commonly collected (485 individuals), followed by *A. macgowni* with 159 individuals. *E. hispida* and *Eretmocerus* sp. "b" were collected in all three regions. The greatest complex of species was found in the Andean region, whereas only *E. hispida* and *Eretmocerus* sp. "b" were observed in the Coastal and Eastern Plains regions.

The results of this initial phase of the surveys indicated a rich complex of parasitoids associated with cassava whiteflies, many of which are unrecorded species that could prove useful in biological control programs. It was further realized that an expanded survey would prove beneficial and increase our knowledge of the natural enemy complex associated with whiteflies.

A second phase to these studies was undertaken with the objectives to:

(1) Further define the complex of natural enemies, especially parasitoids, and quantify their association with whitefly species;
(2) Expand these surveys to include other crops in addition to cassava;
(3) Determine which natural enemies regulate whitefly populations by evaluating parasitoid species under controlled conditions (laboratory studies with *E. hispida*, a parasite of *A. socialis*, were initiated); and
(4) Determine if the B. biotype of *B. tabaci* is infesting cassava fields.

Methods

Survey methodology

Three geographic areas were selected for exploration for whitefly species and their parasitoid natural enemies. These were the Atlantic Coast (the Departments of Atlántico, Córdoba, Bolívar and Magdalena), and the mid altitude Central Highlands (Departments of Cauca, Valle del Cauca, Caldas, Quindío and Risaralda) of Colombia. The surveyed Ecuadorian regions were the coastal area and the highlands (Sierra).

The Colombian departments surveyed represented two distinct ecological zones. The Atlantic Coast, which is hot and fairly dry, has temperatures ranging from 27 to 36 °C and an average relative humidity (RH) from 25% to 70%. The Andean region is much cooler, with temperatures ranging from 22 to 33 °C, and RH ranging from 7% to 100%. The Atlantic Coast is also characterized by a 4- to 6-month dry season. Dry periods in the Andean region are usually 2 to 3 months. Altitude was 0 to 200 m for the collection sites on the Atlantic Coast, and 25 to 1750 m in the Andean region.

Several crop hosts, including cassava, common bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.), cotton (*Gossypium hirsutum* L.), cucumber (*Cucumis sativus* L. var. *sativus*) and snap bean (*P. vulgaris*) were sampled. From each collection site, 100 leaves were collected randomly and 1 square inch leaf area was examined to determine the whitefly species present and their respective densities.

The rate of parasitism was determined by collecting 40 leaves randomly from the field and removing a 1-inch square from each leaf sampled. Only one whitefly species was allowed to remain on each leaf square and the emergence of parasitoids is recorded for each whitefly species. This methodology allowed us to accurately determine the parasitoid species associated with each whitefly species. Identifications remain pending for some whitefly and parasitoid species.

Methodology for parasitism studies with Encarsia hispida

Field surveys carried out over several years have shown that the parasitoid *E. hispida* is frequently found parasitizing cassava whiteflies. A colony of this species was established on *A. socialis* on cassava in the greenhouse, and activities were initiated to study *E. hispida* biology and behaviour.

To ensure successful rearing of whitefly parasitoids, a colony of the whitefly A. *socialis* was maintained on cassava (var. CMC-40) in the greenhouse. Potted 5-week old cassava plants were infested weekly by exposing them to *A. socialis* adults for oviposition for 48 hours. The parasitoid colony was initiated by collecting cassava leaves with parasitized *A. socialis* pupae from the field. These were placed in a black plastic box (emergence chambers) with a clear glass bottle attached to an opening in the lid where emerging parasites are drawn to the light and collected. The parasitoids were removed from the bottle with an aspirator and identified.

The desired parasitoid species (*E. hispida*) was collected in sufficient numbers and released into nylon-mesh cages (50 x 50 x 90 cm) in the greenhouse (25 to 32 °C, 60%-80% RH). Each cage contained potted cassava plants infested with second to third instar *A. socialis* nymphs; 16 to 18 days after release, adult parasitoids began to emerge. Once emergence was detected, cassava leaves (now with parasitized pupae) were collected and placed in the "emergence chamber" where parasitoids were collected and species identification confirmed. Collected parasitoids were used to maintain the parasite colony, or utilized in the various studies described in this report.

E. hispida parasitism studies were carried out in the greenhouse (25 to 32 °C; 60%-80% RH), using *A. socialis* as host species. Three methodologies were evaluated to determine parasitism efficiency:

(1) Four nylon-mesh cages (50 x 50 x 90 cm) were used, containing two potted cassava plants infested with whitefly (*A. socialis*) nymphs. Sixty parasitoids were released into each cage with four repetitions for each instar.
(2) Four whitefly-infested cassava plants were used; four infested leaves were placed in a petri dish (100 x 15 mm), and 30 parasitoids were released into each petri dish with 16 repetitions for each instar.
(3) A muslin "bag" was placed over an entire cassava leaf and sealed at the petiole. Each leaf was infested with 80 nymphs, and 20 parasitoids were released into each bag with four repetitions per instar.

Results

Surveys on the Colombian Atlantic Coast and the departments of Cauca and Valle del Cauca resulted in four species of whiteflies being confirmed as feeding on cassava: *Aleurotrachelus socialis*, *Bemisia tuberculata*, *Trialeurodes* sp. (probably *T. variabilis*) and *Tetraleurodes* sp. (Table 1). *A. socialis* was the predominant species

Table 1. Whitefly species and their associated parasitoid complex collected on cassava from three geographical regions of Colombia.

Region	Whitefly species	Parasitoid species
Atlantic Coast	*Aleurotrachelus socialis*	*Encarsia* sp.
		Eretmocerus sp.
	Bemisia tuberculata	*Encarsia* sp.
		Eretmocerus sp.
		Metaphycus sp.
	Trialeurodes sp.	*Encarsia* sp.
		Eretmocerus sp.
	Tetraleurodes sp.	
Valle del Cauca	*A. socialis*	*Encarsia* sp.
		Eretmocerus sp.
	B. tuberculata	
Cauca	*A. socialis*	*Encarsia bellotti*
		Eretmocerus sp.
		Signiphora aleyrodis
	B. tuberculata	*Encarsia pergandiella*
		Eretmocerus sp.
		Euderomphale sp.
		Signiphora aleyrodis
	Trialeurodes sp.	*Encarsia hispida*
		Encarsia pergandiella
		Eretmocerus sp.

found in all three areas; however, its population was considerably higher in Valle del Cauca and lowest in Cauca (Figure 1). All four species were collected from the Atlantic Coast, whereas *Tetraleurodes* sp. was not collected in either Cauca or Valle del Cauca.

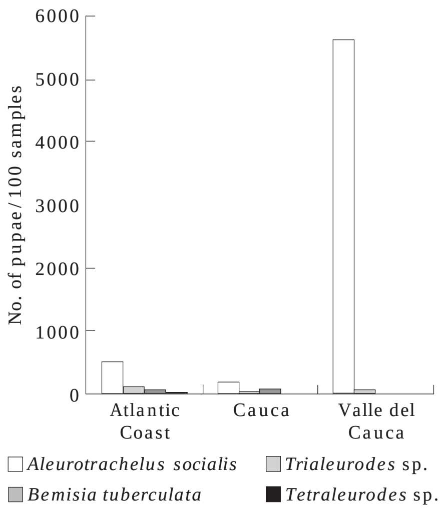

Figure 1. Whitefly population densities on cassava in three geographic zones of Colombia.

Pupal populations of *A. socialis* ranged from an average of about 55 per square inch on leaf samples from Valle del Cauca to about 5 on the Atlantic Coast and 2 in Cauca (Figure 1). The data also showed that whenever there are high whitefly densities or populations, *A. socialis* is the predominant species regardless of geographic area. However, when densities are low at sampling sites, other species may predominate (Figure 2). On the Atlantic Coast, under low population densities, *B. tuberculata* and *A. socialis* had similar populations, whereas in Valle del Cauca, *B. tuberculata* predominated. In Cauca, *Trialeurodes* had the highest population.

Numerous whitefly parasitoids were collected from the three areas (Table 1). These parasitoids belong to the genera *Encarsia*, *Eretmocerus*, *Metaphycus* and *Euderomphale*, and indications are that the collection contains several unrecorded species. The hyperparasitoid

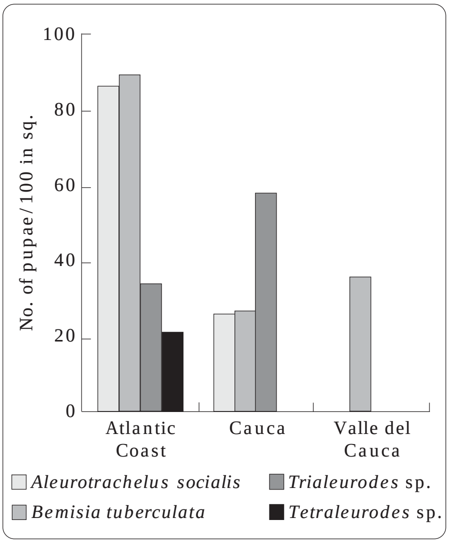

Figure 2. The whitefly species complex at collecting sites with low population densities in three geographic zones of Colombia.

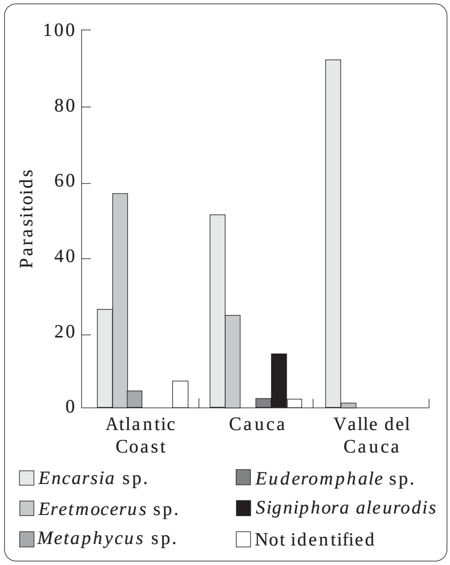

Figure 3. Parasitoid species (%) collected from whiteflies on cassava in three geographic zones of Colombia.

Signiphora aleyrodis was collected only in Cauca. *Encarsia* was the genera most frequently collected in Cauca and Valle del Cauca (Figure 3), with the highest percentage in the latter. On the Atlantic Coast, *Eretmocerus* was the most frequent genus. *Metaphycus* was only collected on the Atlantic Coast and *Euderomphale* only from Valle del Cauca.

Levels of infestations were high for *Aleurodicus* sp., *A. socialis*, *B. tuberculata* and *Tetraleurodes* sp. for the coastal region of Ecuador. *T. vaporariorum*, which is found infesting common bean, was found for the first time at high infestation levels in cassava in the coastal and highlands regions of Ecuador.

The association between whitefly species and parasitoid species was evaluated also. Results indicate a sizable parasitoid species complex associated with each whitefly species and that this can be influenced by geographic area. For example, with *A. socialis*, the predominant parasitoid genus in the Atlantic Coast was *Eretmocerus*, while in Cauca and Valle del Cauca, *Encarsia* predominated (Figure 4). In Valle del Cauca, 99.6% of the parasitism of *A. socialis* was by *Encarsia* and 0.4% by *Eretmocerus*. The most numerous complex of parasitoids was found associated with *B. tuberculata* (Table 1). *Eretmocerus* and *Metaphycus* were the predominant genera on the Atlantic Coast and *Eretmocerus* in Cauca. The hyperparasite *S. aleyrodis* was collected from both *A. socialis* and *B. tuberculata* pupae.

Four species of parasitoids were collected from Ecuador. The complex of parasitoids collected from whiteflies in cassava in Ecuador has been identified only to the generic level (Greg Evans, Mike Rose, personal communication, 1999). In the coastal region, *A. socialis* was parasitized by *Encarsia*, followed by *Amitus* and *Eretmocerus*; *Aleurodicus* was parasitized by *Euderomphale* sp. *Tetraleurodes* and *Trialeurodes* were mostly parasitized by *Eretmocerus* sp.

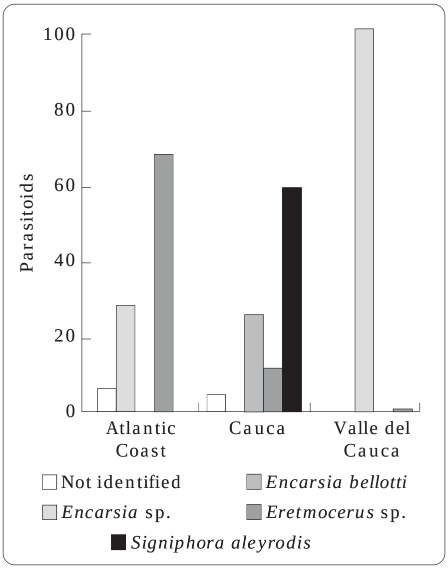

Figure 4. Parasitoid species (%) collected from the whitefly *Aleurotrachelus socialis* in three geographic zones of Colombia.

and *B. tuberculata* was parasitized by *Encarsia* sp. and *Euderomphale*. In the highland region, *T. vaporariorum* was the only whitefly species collected, with about 16 pupae per 2.54 cm^2. The dominant parasitoids were *Encarsia* spp., representing 98% of the sample.

In Colombia, *Trialeurodes* sp. was most frequently parasitized by *Encarsia*. Two species of *Encarsia* were identified, *E. hispida* and *E. pergandiella*, and a third species remains to be identified.

Encarsia sp. and *Eretmocerus* spp. were frequently collected from both high and low whitefly populations. *Metaphycus* sp. and *Euderomphale* sp. are observed only when whitefly populations are low. Parasitoids of the genera *Encarsia* and *Eretmocerus* were collected from sea level to about 2400 m. *E. pergandiella* was collected in high populations at all levels of whitefly infestation.

The whitefly species complex associated with cotton and legume and vegetable crops was distinct from that described on cassava. Two whitefly species were collected, *Bemisia tabaci* and *Trialeurodes vaporariorum*. During the period of field collections (January to June 1999), *B. tabaci* was the only species collected on the Atlantic Coast (departments of Atlántico and Córdoba), while in the Andean region (departments of Caldas, Quindío, Risaralda and Valle del Cauca), *T. vaporariorum* was the only species collected, with its highest populations observed in Valle del Cauca and Caldas.

B. tabaci was collected from cotton, eggplant and tomato, while *T. vaporariorum* was collected from common bean, snap bean, cucumber and tomato. Populations of *T. vaporariorum* in general were much higher than *B. tabaci* and this was especially the case on common bean and snap bean (Figure 5). These results indicate that *B. tabaci* may be adapted more to the warmer temperatures of the coastal region, whereas *T. vaporariorum* prefers the cooler temperatures of the Andean region.

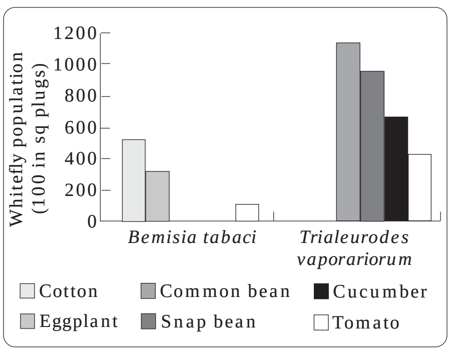

Figure 5. Whitefly nymph pupae populations on several crops in the departments of Atlántico, Córdoba, Caldas, Quindío, Risaralda and Valle del Cauca, Colombia.

Parasitoids from four genera, *Encarsia* and *Eretmocerus* (Aphelinidae), *Amitus* (Platygastridae) and *Metaphycus* (Encyrtidae) were collected. These collections have been sent to taxonomists and species identifications are pending. Therefore, in this report, they are referred to only by genera.

Encarsia was collected in all the departments except Risaralda (where only two sites were surveyed) but *Amitus* presented the highest populations, especially in Valle del Cauca and Caldas (Figure 6). This corresponded to the two departments with the highest whitefly populations. *Eretmocerus* was the predominant species in Córdoba. Parasitoid populations were highest in Valle del Cauca and lowest in Risaralda and Quindío. Only one specimen of *Metaphycus* was collected across the sites.

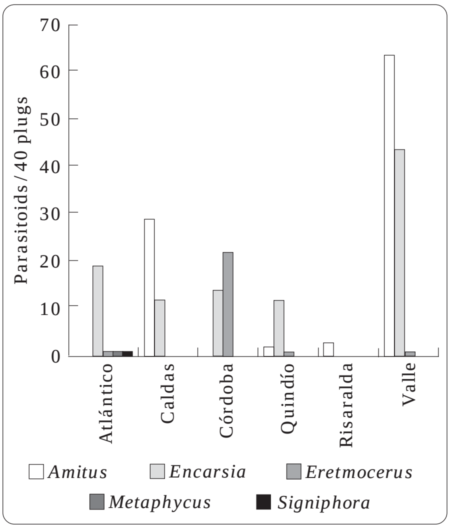

Figure 6. Whitefly parasitoids collected from several crops in the departments of Atlántico, Córdoba, Caldas, Quindío, Risaralda and Valle del Cauca, Colombia.

Encarsia parasites were collected from all the plant hosts sampled and had the overall highest densities among all collected parasitoids (Figure 7). *Amitus*, although not collected from all hosts, also presented high populations, especially on common bean and snap bean. *Eretmocerus* was associated almost exclusively with cotton (only one individual was collected from tomato).

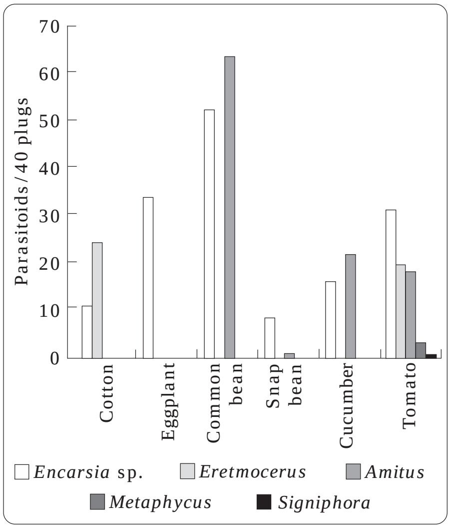

Figure 7. Whitefly parasitoid frequency collected from several crops in the departments of Atlántico, Córdoba, Caldas, Quindío, Risaralda and Valle del Cauca, Colombia.

Encarsia and *Eretmocerus* were collected from both whitefly species (Figure 8), while *Amitus* was collected only on *T. vaporariorum* and *Metaphycus* and *Signiphora* were collected only from *B. tabaci*.

During surveys, data on pesticide applications were recorded during farmer interviews. In general, parasitoid populations were higher in fields where pesticides were not applied, regardless of the whitefly species. Results show that populations of *Encarsia* are less affected by pesticide applications than populations

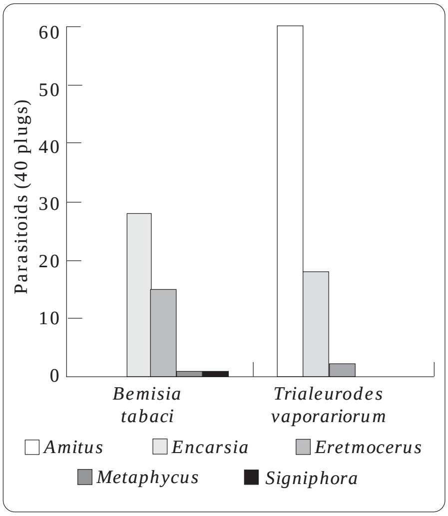

Figure 8. Relationship between parasitoid complex and whitefly species collected from several crops in the departments of Atlántico, Córdoba, Caldas, Quindío, Risaralda and Valle del Cauca, Colombia.

of *Amitus*, indicating that *Encarsia* may have acquired a degree of resistance to some of the pesticides applied.

Parasitism studies with E. hispida

Of the three methodologies evaluated, the highest rate of parasitism was obtained using the third method. Parasitism levels using the first two methods were low. The highest rate of parasitism with method 1, the use of nylon-mesh cages, only reached 9% in the third instar. This improved with the second method (the use of leaves enclosed in petri dishes) to 30% in the 2nd instar and to 20% in the 3rd.

The employment of muslin bags, as described in the third methodology, gave the best results. In the first of two experiments using this methodology, parasitism rates reached about 75% in the third instar and 16% in the 1st instar, 45% in the 2nd instar and 43% in the 4th instar (Figure 9). Results of a second experiment were similar, with about 75% parasitism in the third instar and 19% in the 1st instar, 61% in the 2nd instar and 25% in the 4th instar (Figure 9). The average parasitism rate for these two experiments were about 45%, whereas average parasitism rates were only 6% using methodology 1 and were 20% using methodology 2.

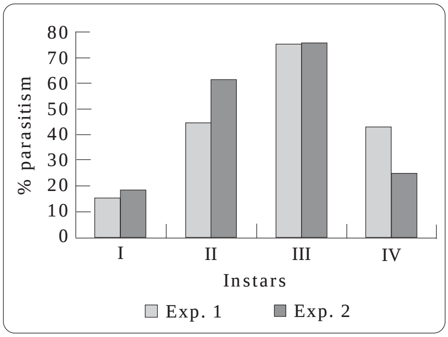

Figure 9. Cassava whitefly (*Aleurotrachelus socialis*) instar preference by the parasitoid *Encarsia hispida* in two greenhouse experiments.

These results also show that the third whitefly instar is preferred for parasitism by *E. hispida*. An average of all four experiments resulted in a parasitism rate of 21% for the 1st instar, 35% for the 2nd instar, 46% for the 3rd instar and 22% for the 4th instar. However, the average of the two experiments using methodology 3 (muslin bags) is 17% for the 1st instar, 53% for the 2nd, 75% for the 3rd and 34% for the 4th instar. The highest parasitism rate is in the 3rd instar, followed by the 2nd, 4th and 1st instar.

The time period for optimal parasitoid activity was evaluated using the third methodology described. Percent parasitism evaluations were made at 48, 72, 96 and 216 hours after parasitoid release. Peak parasitism activity occurred between 72 (35% parasitism) and 96 hours (33% parasitism) (Figure 10). However, even at 216 hours, parasitism rates still remained relatively high (nearly 29%) indicating a relatively lengthy parasitoid activity and the need to do further evaluations at time periods between 96 and 216 hours.

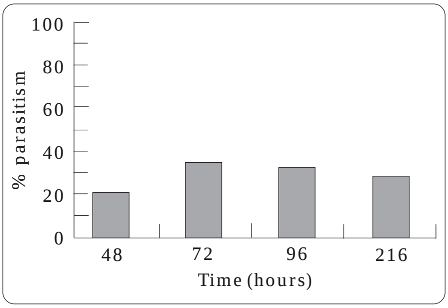

Figure 10. Parasitoid (*Encarsia hispida*) activity in relation to time of exposure to whitefly host *Aleurotrachelus socialis* on cassava in greenhouse studies.

The high rates of parasitism, especially using the muslin cage methodology, indicate that *E. hispida* could be an effective parasite in a biological control program for *A. socialis*. However, whitefly populations, especially of *A. socialis*, have been extremely high at CIAT for the past 4 or 5 years. During this period, *E. hispida* is the most frequently observed parasite. These previously described experiments were carried out under controlled conditions where the parasitoid had easy access to the whitefly prey and the parasitoid probably is not adversely influenced by environmental factors. Under natural field conditions, parasitoid activity may not be as efficient as indicated by these greenhouse studies.

Observations of pupal populations in the field at CIAT and other selected sites (i.e., Tolima) have shown that a high percentage of pupae are parasitized. However, whitefly populations remain high and cause considerable plant damage and yield loss, indicating that the actual activity of parasitoids is not sufficient to reduce whitefly populations below economic injury levels that reduce cassava yields.

Discussion

In future studies, additional parasites such as *Eretmocerus* and *Amitus* species will be evaluated in similar studies to those with *E. hispida*. Continued survey activities to identify additional natural enemies also are underway. During this phase of exploration, more emphasis will be given to regions or fields where there are low whitefly populations with the hope of identifying key parasitoids, predators or entomopathogens that are preventing eruptions of whitefly populations.

This is a collaborative project between CIAT and the University of Florida, and is funded by the United States Agency for International Development (USAID). This collaboration will provide training and improved in-country capacity for research, production, delivery and management of biological control agents. The University of Florida will provide expertise and input on parasitoid taxonomy, biology, behaviour, collecting, rearing, identification and data analysis. Since 1998, University of Florida researchers (Dr. Jorge Peña and R. Nguyen) have visited CIAT and collaborated in training CIAT personnel in some of the above areas. Whitefly parasitoids that are collected from sampling are sent to University of Florida taxonomists (G. Evans, M. Rose and A. Hammonds) for identification.

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