

## RELEASE, ESTABLISHMENT AND MONITORING OF *BEMISIA TABACI* NATURAL ENEMIES IN THE UNITED STATES

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### INTRODUCTION

Severe infestations of *Bemisia tabaci* (Gennadius) [Biotype 'B'] (Homoptera: Aleyrodidae) (= *Bemisia argentifolii* Bellows and Perring) occurred following its introduction into the United States in the mid-to-late 1980s. *Bemisia tabaci*'s broad host range and its multivoltine development overwhelmed the then-current management practices and the ability of native natural enemies to limit whitefly populations. In response, a multi-agency national research and action plan was developed with participation by U.S. Department of Agriculture agencies (Agricultural Research Service, Animal and Plant Health Inspection Service, and Cooperative State Research, Education and Extension Service), State departments of agriculture, universities, and private industry (Faust, 1992; Henneberry *et al.*, 1998). Biological control was a major component of the plan.

Colonization of new, non-indigenous natural enemies of *B. tabaci* in the United States was complicated by these various factors: (1) the host range of *B. tabaci* included a large number of species of crops, ornamentals, weeds, and native plants that permitted the whitefly to have nearly continuous development throughout the year; (2) the severity of the problem varied widely in different areas according to local cropping cycles and whitefly reservoirs; (3) the most severely affected crops were of short duration and often heavily treated with insecticides; (4) the most severely affected region was the desert southwest, where reservoirs of non-crop hosts were patchy and ephemeral; (5) whitefly parasitoids have short life-spans and relatively limited dispersal capability; (6) surveys demonstrated that there were a number of native enemies that were likely to compete with introduced species; and (7) the taxonomy and biologies of most exotic and indigenous species of *B. tabaci* parasitoids were not well known.

Foreign exploration for the discovery of new natural enemies was conducted in climatically varied regions throughout the world to match the varied climates in target regions of the United States. Collections were sent to quarantine facilities in the United States for evaluation (Lacey *et al.*, 1993; Goolsby *et al.*, 1999; Kirk *et al.*, 2001); the resulting collections contained extensive natural enemy diversity. Initially, taxonomic knowledge of species of *Eretmocerus* and *Encarsia*, the genera with the majority of *B. tabaci* parasitoids collected, was inadequate to permit conclusive identifications of exotic and indigenous species using morphological methods. To ensure purity of cultures and to differentiate among the populations and species of parasitoids in the laboratory and in the field following eventual releases, individuals from each accession were characterized using RAPD-PCR (Legaspi *et al.*, 1996; Goolsby *et al.*, 1999). RAPD markers were obtained that discriminated among apparent species and, in some cases, geographic populations that were not distinguishable morphologically. Specimens of original parental material were also sent to cooperating systematists for taxonomic study. As a result, laboratory and field evaluations proceeded while taxonomic study of the imported material was conducted. A high degree of correspondence was obtained between the populations that were successfully distinguished by PCR and the named species that were eventually described using morphometrics (Rose and Zolnerowich, 1997; Zolnerowich and Rose, 1998).

## Quarantine Culture and Evaluation

From the foreign collections and surveys of indigenous *B. tabaci* parasitoids in the United States, 11 species or distinct populations of *Eretmocerus* and 12 species of *Encarsia* were identified by a combination of RAPD-PCR banding patterns and classical taxonomy. Cultures of these parasitoids and selected predators were maintained at the USDA-APHIS Mission Biological Control Center, Mission, Texas, to support evaluations and releases at various locations in the United States. The exotic species cultured are given in Table 1. After review of environmental assessments, APHIS authorized the field release of any *Eretmocerus* or *Encarsia* species derived from original field collections of any *Bemisia* species (including *Encarsia* species with autoparasitic males whose females were primary parasitoids of *Bemisia* species).

**Table 1.** Exotic parasitoids of *Bemisia* species maintained in culture from 1992 to 1998 for evaluation by the USDA-APHIS, Mission Biological Control Center, Mission, Texas, U.S.A.

Species	Collection Localities	Biology
<i>Encarsia bimaculata</i> Heraty & Polaszek	India	autoparasitic
<i>Encarsia formosa</i> Gahan	Greece, Egypt, Italy, Thailand	uniparental
<i>Encarsia sophia</i> (Girault & Dodd) [= <i>E. transvena</i> (Timberlake)]	Taiwan, Philippines, Thailand, Malaysia, Pakistan, Spain	autoparasitic
<i>Encarsia lutea</i> (Masi)	Israel, Cyprus, Sicily, Italy, Spain	autoparasitic
<i>Encarsia</i> nr. <i>pergandiella</i> Howard	Brazil	uniparental
<i>Encarsia</i> nr. <i>hispida</i> De Santis	Brazil	uniparental
<i>Encarsia</i> sp. (parvella group)	Dominican Republic	autoparasitic
<i>Eretmocerus mundus</i> Mercet	Spain, Italy, Sicily, Cyprus, Israel, Egypt, India, Taiwan	biparental
<i>Eretmocerus hayati</i> Zolnerowich & Rose	India, Pakistan	biparental
<i>Eretmocerus melanoscutus</i> Zolnerowich & Rose	Thailand, Taiwan	biparental
<i>Eretmocerus staufferi</i> Rose & Zolnerowich	USA (Texas); but believed to be adventitiously introduced	uniparental
<i>Eretmocerus furuhashii</i> Rose & Zolnerowich / nr. <i>furuhashii</i>	Taiwan	biparental
<i>Eretmocerus emiratus</i> Zolnerowich & Rose	United Arab Emirates	biparental
<i>Eretmocerus emiratus</i> / nr. <i>emiratus</i>	Ethiopia	biparental

Four non-indigenous whitefly predators were imported as a result of foreign exploration. These included three coccinellids, *Serangium parcesetosum* Sicard, *Serangium* sp. nov., and *Clitostethus arcuatus* (Rossi), and the drosophilid *Acletoxenus formosus* (Loew). The *Serangium* species were studied in the laboratory. *Serangium parcesetosum* was eventually released in Arizona and California, but it has not been recovered in the field. None of the other three exotic predators were released in the United States against *B. tabaci*.

Fungal pathogens were also found in many foreign collections; isolates were deposited at the U.S. Department of Agriculture ARS entomopathogen repository in Ithaca, New York. Research and development of exotic fungal pathogens of *B. tabaci* have been reviewed by Kirk *et al.* (2001); however, pathogens were not a focus for classical biological control release and establishment. Further discussion will deal exclusively with parasitoids.

While maintained in quarantine culture, parasitoids were screened against *B. tabaci* on cotton, melons, broccoli, and sowthistle (*Sonchus oleraceus* L.) (Goolsby *et al.*, 1996, 1998). More extensive field evaluations in these crops were then conducted with those cultures that produced the most progeny in the laboratory screenings (Goolsby *et al.*, 1998; Hoelmer, 1998; Roltsch and Goolsby, 1998). The best-performing *Eretmocerus* species included *Eretmocerus emiratus* Zolnerowich and Rose, *Eretmocerus hayati* Zolnerowich and Rose, and several geographic populations of *Eretmocerus mundus* Mercet (Hoelmer, 1998). Although populations of *Encarsia sophia* (Girault and Dodd) could not be distinguished using PCR or morphological methods, there were significant biological differences in field evaluations among geographic populations on different crop hosts. A population of *E. sophia* from Pakistan was identified as highly effective in cotton (Roltsch and Goolsby, 1998). The best performing species in these trials were mass-reared at insectaries in Texas, Arizona and California to provide parasitoids and predators for larger-scale field evaluations and releases.

## METHODS OF COLONIZATION

To increase the likelihood of successful introductions, releases were conducted using a variety of colonization techniques. These methods included releases made as follows: (1) within agricultural demonstration projects (“biointensive” mixed-crop farms, seasonal augmentation of parasitoids in spring melons and “banker plant” seedling melons); (2) in urban settings on trees and shrubs that were hosts of *B. tabaci*, on ornamentals at selected farmsites containing garden and ornamental hosts, and in wholesale and retail nurseries with a variety of whitefly hosts; and (3) in managed and unmanaged refugia consisting of unsprayed crop hosts and annuals or perennial plants suitable for desert climates.

### Agricultural Demonstration Projects

**Seasonal augmentation in melons.** *Bemisia tabaci* populations increase rapidly on spring melons in southwestern desert valleys, leading to large migrations to other crops later in the season. To determine whether parasitoid populations could be augmented early in the season, field-scale evaluations in commercial melons were conducted in the Imperial Valley, California, with large field releases of *Eretmocerus mundus*, *E. hayati* and *E. emiratus* / *nr. emiratus*. An insectary was constructed in the Imperial Valley to produce large numbers of *Eretmocerus* spp., which were distributed as pupae. During the first year, paired releases demonstrated that the exotic *E. mundus* was superior to the native *Eretmocerus eremicus* Rose and Zolnerowich, producing much higher levels of parasitism, and confirming the results of previous cage evaluations. In the largest trials, 40,000 to 100,000 *Eretmocerus* spp. pupae per acre were distributed over 50 acres. Four years of augmentation field trials with exotic *Eretmocerus* species demonstrated that parasitism could be increased significantly and *B. tabaci* suppressed, with open-field inoculative releases. Releases of the parasitoids were also shown to be compatible with the use of selective pesticides in melon crops (Simmons *et al.*, 1997, 1998).

**Pre-plant inoculation of seedling melons.** Melons are a favored *B. tabaci* host that can be started commercially as transplanted seedlings. Trials were conducted by Goolsby and Ciomperlik (1999) in Texas using melon seedlings inoculated with both *B. tabaci* and parasitoids as “banker plants” to distribute parasitoids into commercial fields. By introducing seedlings containing *B. tabaci* parasitized by *E. hayati* at ratios ranging from 1:10 to 1:30 “banker” plants to clean seedlings, they showed that high rates of parasitism were possible and found that this method was an efficient method for introducing *Eretmocerus* spp. into crops.

Similar studies were carried out in Imperial Valley cantaloupe fields from 1998 to 2000 (Pickett *et al.*, 2001). These studies compared hand and transplant distribution of *Eretmocerus nr. emiratus* in imidacloprid and untreated “organic” culture. Higher parasitism was obtained in augmented fields, and transplant results were comparable to hand-releases. However, no measurable impact on whitefly numbers was seen, possibly due to continual influx of *B. tabaci* adults from other crops. Thus, the use of transplants to augment early season populations shows promise, but more work is needed to improve its predictability. However, transplant inoculation is a useful method for efficient and widespread distribution of parasitoids with reduced labor, which could be extended to other crops.

## MANAGED REFUGIA FOR PARASITOIDS

Seasonal desert vegetation and short-term crops are unstable environments for the survival of newly introduced natural enemies. To provide stable reservoirs and help to preserve parasitoid populations between cropping cycles, experiments were conducted with actively managed refuges consisting of mixtures of crops or native plant hosts that were situated adjacent to crops (Roltsch, 2001). For example, cole crops and sunflower were used as a winter (cool season) refuge planting; while basil and okra provided a summer (warm season) refuge mix. Marking studies demonstrated that parasitoids from refuges successfully dispersed into neighboring crops.

Total numbers of parasitoids and the proportion of exotic to native parasitoids increased annually for three consecutive years after releases into the refuges had ended. Exotic *Eretmocerus* spp. accounted for nearly all parasitism of *B. tabaci* in crucifer crops, where the native *E. eremicus* was not active, indicating that the introduced species will help to fill an important gap in parasitoid activity.

## RECOVERY SURVEY AND DETECTION METHODS

### Sentinel Plants

In addition to information obtained from routine samples collected for release projects, surveys to detect the establishment and spread of exotic parasitoids in Texas and California (Table 2) were implemented using sentinel plants. Sentinel plants were infested with immature whiteflies and placed in the field at widely separated locations to sample parasitoid species composition. After one week of field exposure, the plants were returned to the laboratory for rearing and identification of parasitoids. Cantaloupe seedlings were used as hosts in Texas; potted hibiscus plants (*Hibiscus rosa-sinensis* L.) were used in California.

The numbers of several introduced *Eretmocerus* spp. increased rapidly in Texas. At the beginning of the survey in June 1997, native *Eretmocerus tejanus* Rose and Zolnerowich represented more than 95% of all *Eretmocerus* sp. individuals recovered. Just three months later, during fall 1997, exotic species accounted for 85% (n = 700) of all the individuals of various *Eretmocerus* spp. recovered. Two exotic species, *E. mundus* and *E. hayati*, are now widely established across the Rio Grande River flood plain.

**Table 2.** Exotic parasitoids released in Texas, Arizona and California from 1993 to 1999 (including caged field evaluations).

Species	Region of Origin
<i>Encarsia bimaculata</i> Heraty & Polaszek	India
<i>Encarsia sophia</i> (Girault & Dodd) <sup>a</sup>	Thailand, Malaysia, Pakistan <sup>b</sup> , Spain
<i>Encarsia lutea</i> (Masi)	Israel
<i>Encarsia</i> nr. <i>pergandiella</i> Howard	Brazil
<i>Encarsia</i> nr. <i>hispida</i> De Santis	Brazil
<i>Eretmocerus mundus</i> Mercet <sup>a</sup>	Spain and Israel (most releases <sup>b</sup> ); India, Italy, Egypt
<i>Eretmocerus hayati</i> Zolnerowich & Rose <sup>a</sup>	Pakistan
<i>Eretmocerus melanoscutus</i> Zolnerowich & Rose	Thailand, Taiwan
<i>Eretmocerus staufferi</i> Rose & Zolnerowich	unknown
<i>Eretmocerus</i> nr. <i>furuhashii</i> Rose & Zolnerowich	Taiwan
<i>Eretmocerus emiratus</i> Zolnerowich & Rose <sup>a</sup>	United Arab Emirates
<i>Eretmocerus</i> (nr) <i>emiratus</i> Zolnerowich & Rose <sup>a</sup>	Ethiopia
<i>Amitus bennetti</i> Viggiani & Evans (released by Univ. California Riverside)	Puerto Rico

<sup>a</sup> Species that are established at field sites in Texas, Arizona, or California.

<sup>b</sup> Origin of population established in the United States, where collections were made in more than one region.

Establishment of exotic parasitoids in California occurred somewhat more slowly, but recoveries have increased each year since the first recoveries were made in 1996. Large-scale rearing and releases were ended in 1998 and all releases stopped in 1999. By 1999 and 2000, exotic parasitoids had been recovered from a majority of sites surveyed in the Imperial Valley. The number of recovery sites, total numbers of exotic individuals per site, and proportion of exotic to native individuals has increased each year. In a separate survey of cotton fields in the Imperial Valley, exotic species were found in 74% of fields surveyed in 1999 (twice as many as 1998) and these represented one quarter of the parasitoid population in 1999 (Roltsch, 2001). *Eretmocerus emiratus* (ex U.A.E.), *E. emiratus* / nr. *emiratus* (ex Ethiopia), *E. mundus*, and *Encarsia sophia* (ex Pakistan) now appear to have established in this region.

Future identification of parasitized whiteflies will be assisted by the development and use of species-specific probes developed from satellite DNA (Heilmann, 1997). These probes will make it feasible to rapidly identify large numbers of *Eretmocerus* specimens collected from field evaluation studies without the need to hold samples for emergence of adult parasitoids.

## DESERT SURVEY

To document the spread and establishment of introduced parasitoids into the surrounding desert environment and to monitor for evidence of non-target impacts on native whitefly species, surveys were conducted from 1993 to 2002, with samples collected from 55 to 60 desert sites along a 160 km route around the perimeter of the Imperial Valley (Hoelmer and Culver, 1997). Many species of desert vegetation including introduced invasive weeds and native plants were examined for the presence of whiteflies. Various urban ornamentals were also examined during surveys for establishment at sites within the Imperial Valley. Because desert vegetation is dependent on brief periods of unpredictable rainfall that can occur at any time during the year, samples were collected at various times throughout the year. Eight species of whitefly in the genera *Trialeurodes*, *Tetraleurodes*, or *Aleuropleurocelus* were collected from 18 species of desert host plants and five species of urban ornamental trees and bushes. An undetermined number of parasitoid species, some of which are still undescribed, in the genera *Encarsia* and *Eretmocerus* (Aphelinidae), *Amitus* (Platygasteridae) and *Neopomphale* (Eulophidae) were reared from collections of these whiteflies. *Eretmocerus eremicus*, *Encarsia luteola* Howard, *Encarsia meritoria* Gahan and *Encarsia coquilletti* Howard were the only indigenous parasitoids reared from *B. tabaci* on desert vegetation that were also found on another whitefly species. These four species were reared from *Trialeurodes abutiloneus* (Haldeman), which like *B. tabaci* feeds on herbaceous plant hosts and not on woody perennials. By 2001, introduced *Eretmocerus* spp. had been recovered from *B. tabaci* on different occasions from several widely separated desert sites to the west of the Imperial Valley, documenting that establishment and spread of introduced species was occurring. Some of these sites were up to 50 kilometers from the nearest release location. However, no exotic *Eretmocerus* or *Encarsia* species have yet been found in rearing samples from any of the non-target species monitored.

In summary, millions of parasitoids were mass-reared during this interagency project and released as pupae, adults, or eggs or larvae within parasitized *B. tabaci* on live plants from 1993 to 1999 using these diverse methods. We believe that this varied approach utilizing a variety of distribution methods tailored specifically for different environments was a significant factor in successfully establishing new species of *Eretmocerus* (*E. mundus* in Texas and California, *E. hayati* in Texas, and *E. emiratus* and *E. sp. ex.* Ethiopia in California) in the United States on *B. tabaci*.

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