

# Post-release evaluation of *Eretmocerus hayati* Zolnerowich and Rose in Australia

P.J. De Barro\* and M.T. Coombs

CSIRO Entomology, 120 Meiers Road, Indooroopilly,  
Queensland 4068, Australia

## Abstract

*Bemisia tabaci* biotype B is a significant pest of agriculture world-wide. It was first detected in Australia in 1994. Assessments of the potential of parasitoids already present in Australia to control this pest indicated that two species of *Eretmocerus* and 11 species of *Encarsia* were present, but they did not exert sufficient control with a combined average of  $5.0 \pm 0.3\%$  apparent parasitism of 4th instars. Further, only 25% of samples containing biotype B had parasitised individuals present. The surveys also identified that fewer B biotype were being parasitised compared with the Australian indigenous biotype. Overall, *Er. mundus* was the most abundant parasitoid prior to the introduction. Previous research indicated that *Er. hayati* offered the best prospects for Australia and, in October 2004, the first releases were made. Since then, levels of apparent parasitism have averaged  $29.3 \pm 0.1\%$  of 4th instars with only 24% of collections having no parasitism present. *Eretmocerus hayati* contributed 85% of the overall apparent parasitism. In addition, host plants of the whitefly with low or no parasitism prior to the release have had an order of magnitude increase in levels of parasitism. This study covers the establishment of the case to introduce *Er. hayati* and the post-release establishment period November 2004–March 2008.

**Keywords:** biological control, *Eretmocerus mundus*, efficacy

(Accepted 4 June 2008)

## Introduction

A significant pest of agriculture, the silverleaf whitefly (SLW) *Bemisia tabaci* biotype B (Gennadius, also known as *Bemisia argentifolii*), was first detected in Australia in late 1994 (Gunning *et al.*, 1995); it is likely to have first entered the country some time between mid-1992 and mid-1993. Since its arrival in northern New South Wales, it spread through the wholesale commercial ornamental nursery network to Queensland and Northern Territory and from there to the retail nurseries across Australia. Over the past 13 years, it has become an economic problem primarily in Queensland and, to a lesser extent, in coastal northern New South Wales, Northern Territory and Carnarvon in Western Australia.

Crops most frequently affected include Brassicaceae, Cucurbitaceae, Solanaceae and Fabaceae vegetables, especially melons, cotton and soybean, as well as commercial ornamental species. Losses occur as a result of reduced yield and reductions in quality as a consequence of physiological changes in colour, loss of even maturation and contamination with honeydew and sooty mould. At present, begomoviruses have yet to cause any serious concerns.

Goolsby *et al.* (2005) undertook a review of the USDA biological control program in terms of which of the released species had established in the southwestern USA with a view to identifying potential candidates for introduction into Australia. The USDA program released a total of seven species of *Encarsia* and five species of *Eretmocerus*, of which all species of *Eretmocerus* and one species of *Encarsia* established (Goolsby *et al.*, 2005). The study used the climate-matching software CLIMEX to produce an index of suitability between the climates of the locations of origin for each of the established *Eretmocerus* species with climates in

---

\*Author for correspondence  
Fax: +61 7 3214 2885  
E-mail: paul.debarro@csiro.au

Table 1. Examples of successful biological control introductions against different species of Aleyrodidae.

Species	Parasitoid	Location	References
<i>Aleurocanthus spiniferus</i> Quaintance	<i>Encarsia smithi</i> (Silvestri)	South Africa	Van den Berg <i>et al.</i> (2000), Van den Berg & Greenland (2001)
<i>Aleurocanthus woglumi</i> Ashby	<i>Eretmocerus serius</i> Silvestri, <i>Encarsia opulenta</i> (Silvestri), <i>Amitus hesperidum</i> Silvestri	Cuba; Mexico; Trinidad; South Africa; USA, Florida, Texas	Vail <i>et al.</i> (2001), Van den Berg & Greenland (2001), White <i>et al.</i> (2005)
<i>Aleurodicus dispersus</i> Russell	<i>Encarsia dispersa</i> Polaszek	Australia, Queensland; Pacific Island Countries,	Waterhouse & Norris (1989)
<i>Aleurodicus dugesii</i> Cockerell	<i>Entedononecremnus krauteri</i> Zolnerowich and Rose, <i>Encarsiella noyesi</i> Hayat	USA, Florida	Nguyen & Hamon (2008)
<i>Aleurothrix floccosus</i> Maskell	<i>Cales noacki</i> Howard	USA, California; Sicily; Tunisia	Liotta <i>et al.</i> (2003), Chermiti <i>et al.</i> (1993), Miklasiewicz & Walker (1990)
<i>Aleurotrachelus atratus</i> Hempel	<i>Eretmocerus</i> n. sp.	Comoros Island	Borowiec <i>et al.</i> (2007)
<i>Aleurotuberculatus takahashi</i> David & Subramanian	<i>Eretmocerus longipes</i> Compere	China, Fuzhou	Sengonca & Liu (1998)
<i>Bemisia tabaci</i> Gennadius	<i>Eretmocerus hayati</i> Zolnerowich and Rose	USA, Texas	Gould <i>et al.</i> (2008)
<i>Dialeurodes citri</i> Ashmead	<i>Encarsia lahorensis</i> (Howard)	Italy, Sicily	Liotta <i>et al.</i> (2003)
<i>Parabemisia myricae</i> (Kuwana)	<i>Eretmocerus debachi</i> Rose & Rosen	USA, California; Turkey	Rose & Rosen (1992), Rose & DeBach (1992) Sengonca <i>et al.</i> (1993, 1995)
<i>Siphoninus phillyreae</i> (Haliday)	<i>Encarsia inaron</i> (Walker), <i>Eretmocerus siphonini</i> Viggiani & Battaglia	USA, California; Israel	Jetter (2000), Abd-Rabou (2002), Pickett & Wall (2003), Gerling <i>et al.</i> (2004)
<i>Tetraleurodes perseae</i> Nakahara	<i>Cales noacki</i> Howard	USA, California	Rose & Woolly (1984a,b)
<i>Trialeurodes vaporariorum</i> Westwood	<i>Encarsia formosa</i> Gahan	cosmopolitan	Hodde <i>et al.</i> (1998)

each of the four areas in the southwestern USA where releases took place. The resultant index was then used to rank the species. CLIMEX was then used to compare the regions in the USA where the releases took place to Australia. The Lower Rio Grande Valley was identified as having a climate that was most similar to those parts of Australia most affected by the invasion of SLW. Based on the CLIMEX indices and observations on establishment, *Eretmocerus hayati* Zolnerowich and Rose (Hymenoptera: Aphelinidae) was selected as the only candidate species for introduction.

Biological control of whiteflies has had a long history with numerous examples of success (table 1). While most of the examples involve perennial cropping systems, some success has been achieved against whitefly pests of annual crops. This study considers the case for introduction and the results from the first 3.3 years since releases of *Er. hayati* began and compares them to the period prior to release.

## Materials and methods

### Host specificity testing

The process of importation, evaluation and release of *Er. hayati* was undertaken in accordance with the requirements under Australian legislation governing the importation and release of exotic biological control agents.

### Culturing of *E. hayati*

*Eretmocerus hayati* was imported into quarantine at the CSIRO Long Pocket Laboratories, Indooroopilly during September and October 2002 as parasitised mummies of *B. tabaci* (biotype B) from the Lower Rio Grande Valley,

Texas, USA. Parasitoids were identified as *E. hayati* following Zolnerowich & Rose (1998) and comparison of ribosomal ITS1 with material previously obtained and identified at USDA-APHIS Mission, Texas. Cultures of *E. hayati* were maintained in 3.5-l plastic containers on *Hibiscus rosa-sinensis* L. var Mrs George Davis 'plants' (two 'plants' per container). Each 'plant' consisted of a single stem and leaf rooted in agar in a 45-ml plastic tube. Each plant had previously been infested with *B. tabaci* (biotype B) eggs to achieve a density of 20–30 nymphs cm<sup>-2</sup>. Following egg hatch, the first instars were allowed to settle before parasitoids were added to the cage. The first and second instars are the preferred stages for oviposition by *Eretmocerus* parasitising *B. tabaci* (Jones & Greenberg, 1998). Parasitism of *B. tabaci* by *E. hayati* typically averaged 80–98%.

### Culturing of non-target test species

Sustained cultures of each of the test species were maintained under glasshouse conditions on appropriate host plants (potted) (see table 2) and held in mesh screened cages. All whitefly cultures were initiated from field-collected adults. Species identifications were made using morphological characters of 4th instar nymphs following Martin (1999). Voucher material for each test species are held at the ANIC as both slide-mounted and alcohol-preserved nymphs.

### No-choice experiments

*Eretmocerus hayati* was assessed for non-target attack using no-choice experiments. All *Er. hayati* adults were naïve (no prior egg lay) and had been cultured as above. For each test, single age cohorts of settled 1st–2nd instar nymphs of a

Table 2. Results of no-choice host specificity tests for *Eretmocerus hayati* against selected Australian native or exotic whitefly species and *Bemisia tabaci*.

Test species	Host Plant	Mean no. nymphs per leaf or leaf disk ( $\pm$ SE)	Mean percent parasitism ( $\pm$ SE)
<i>B. afer</i>	<i>Breynia nivosa</i>	41.9 $\pm$ 4.9	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	49.0 $\pm$ 5.8	93.8 $\pm$ 2.3
<i>B. gigantia</i>	<i>Elaeocarpus angustifolius</i>	5.3 $\pm$ 0.8	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	32.8 $\pm$ 3.1	91.7 $\pm$ 2.4
<i>B. giffardi</i>	<i>Citrus limon</i>	3.4 $\pm$ 0.4	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	28.9 $\pm$ 2.4	89.9 $\pm$ 3.6
<i>L. atriplex</i>	<i>Rhagodia spinescens</i>	47.9 $\pm$ 10.5	6.0 $\pm$ 4.0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	61.1 $\pm$ 8.6	92.6 $\pm$ 2.2
<i>L. atriplex</i>	<i>Einadia trigonos</i>	36.4 $\pm$ 5.7	15.6 $\pm$ 4.3
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	47.9 $\pm$ 3.5	89.7 $\pm$ 4.0
<i>L. euphorbiae</i>	<i>Euphorbia hirta</i>	19.7 $\pm$ 3.9	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	31.1 $\pm$ 3.4	80.4 $\pm$ 3.6
<i>D. eucalypti</i>	<i>Corymbia citriodora</i>	20.4 $\pm$ 3.7	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	34.8 $\pm$ 3.3	88.8 $\pm$ 3.2
<i>A. spiniferus</i>	<i>Cupaniopsis anacardioides</i>	39.3 $\pm$ 3.7	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	37.1 $\pm$ 3.0	86.9 $\pm$ 4.5
<i>D. decempuncta</i>	<i>Callistemon viminalis</i>	21.8 $\pm$ 3.5	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	30.3 $\pm$ 2.9	93.2 $\pm$ 3.0
<i>D. citri</i>	<i>Citrus limon</i>	11.3 $\pm$ 0.4	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	34.3 $\pm$ 2.4	84.2 $\pm$ 3.6
<i>Dialeurodes</i> sp.	<i>Hymenosporum flavum</i>	19.2 $\pm$ 2.3	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	36.4 $\pm$ 2.0	89.2 $\pm$ 3.5
<i>T. vaporariorum</i>	<i>Euphorbia peplis</i>	32.3 $\pm$ 2.8	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	25.4 $\pm$ 2.3	97.7 $\pm$ 1.2
<i>A. prolella</i>	<i>Brassica</i> spp.	39.7 $\pm$ 5.8	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	42.3 $\pm$ 2.3	92.9 $\pm$ 2.9
<i>X. eucalypti</i>	<i>Eucalyptus acmenoides</i>	27.1 $\pm$ 2.9	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	39.4 $\pm$ 1.9	92.2 $\pm$ 2.8
<i>V. incomptus</i>	<i>Acacia aulacocarpa</i>	14.9 $\pm$ 4.2	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	31.2 $\pm$ 2.3	91.3 $\pm$ 2.9
<i>Aleuroplatus</i> sp.	<i>Syzygium paniculatum</i>	20.2 $\pm$ 1.0	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	29.1 $\pm$ 2.2	96.3 $\pm$ 1.6
<i>Pseudoaleuroplatus</i> sp.	<i>S. paniculatum</i>	14.0 $\pm$ 1.2	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	48.6 $\pm$ 2.2	89.6 $\pm$ 3.0
<i>O. citri</i>	<i>Citrus limon</i>	24.4 $\pm$ 2.2	0
<i>B. tabaci</i>	<i>Hibiscus rosa-sinensis</i>	39.1 $\pm$ 2.4	92.8 $\pm$ 2.3

given non-target species and *B. tabaci* were exposed separately to *Er. hayati* adults ( $n=30$  females for each replicate). Nine replicates of the non-target species/host plant combination and nine replicates of *B. tabaci* (biotype B) on hibiscus were used in each pair test. Parasitoids were 2–3 days post emergence, with females having been held with males to enable mating. In each experiment, parasitoids remained with the test species for the duration of their (parasitoids) lifespan. All tests were carried out in mesh-screened cages. Parasitism rates were assessed by recording either numbers of parasitised nymphs per leaf for small leaved ( $\leq 3$  cm in length) host plants or as the number parasitised per 2.27 cm<sup>2</sup> leaf disk for plants with leaves longer than 3 cm in length. Development of *Er. hayati* could be discerned directly through the host cuticle for pale-bodied nymphs. For whitefly species with dark-bodied nymphs, nymphs were allowed to develop either to emergence of the adult whitefly or the adult parasitoid. All observations were made using a stereo dissecting microscope.

#### Analysis of ribosomal 18s

The 3' end of the ribosomal DNA 18s gene (Campbell *et al.*, 1996) was used to determine the genetic relatedness of

*B. tabaci* (biotype B) to the non-target species chosen for testing for host specificity testing. A 762–782 bp fragment was obtained using the primers 18sF 5'GACTCAACACGG-GAAACCTC3', 18sR 5'TCCTTCCGCGAGTTCACC3' and the protocol of Campbell *et al.* (1994).

A total of four outgroup species from the Aleyrodidae subfamily Aleurodicinae, *Aleurodicus destructor* Mackie, *A. dugesii* Cockerell, *Lecanoideus floccissimus* Martin and *Paraleyrodes bondari* Peracchi were used to root the analysis. The species *Aleurocanthus spiniferus* (Quaintance), *Aleuroplatus* n. sp. (ex *Syzygium paniculatum*, Brisbane, Indooroopilly) *Aleyrodes prolella* (L.), *Bemisia afer* (Priesner & Hosny), *B. decipiens* (Maskell), *B. giffardi* (Kotinsky), *B. gigantea* Martin, *Lipaleyrodes atriplex* (Froggatt), *L. euphorbiae* David & Subramaniam, *Dialeurodes citri* (Ashmead), *Dialeurodes* n. sp. (ex *Hymenosporum flavum*, Brisbane, Brookfield), *Dialeurodes decempuncta* (Quaintance & Baker), *Dumbletoniella eucalypti* (Dumbleton), *Orchamoplatus citri* (Takahashi), *Pseudaleuroplatus* n. sp. (ex *Syzygium paniculatum*, Brisbane, Indooroopilly), *Trialeurodes vaporariorum* (Westwood), *Viennotaleyrodes incomptus* Martin and *Xenaleyrodes eucalypti* (Dumbleton) formed the ingroup. Species were chosen for host specificity testing based on the information in Martin (1999). Two species of *Bemisia*, *B. decipiens* and *B. subdecipiens*

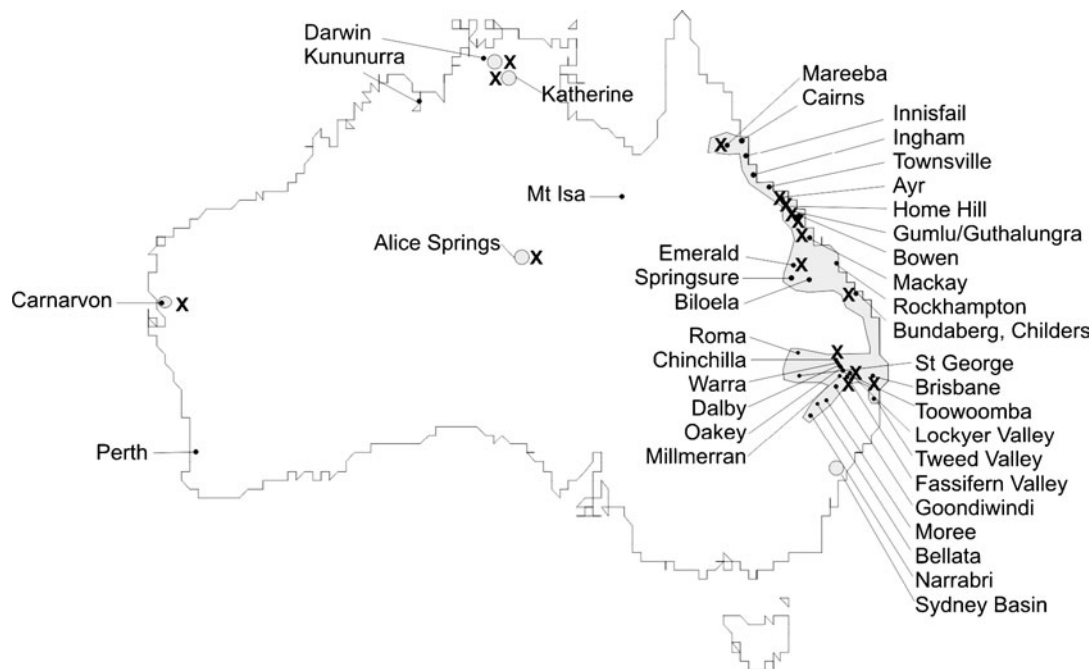


Fig. 1. Map showing location of pre-release surveys, release sites and the area where establishment has been confirmed through post-release surveys (•, pre-release surveys; ×, releases; ⊗, establishment confirmed).

Martin, were not found during the time of testing; however, subsequent to the release of *Er. hayati*, *B. decipiens* was collected.

The ingroup and outgroup sequences were aligned using clustal w (Thompson *et al.*, 1994) and required no adjustment by hand. Evolutionary trees were estimated using the distance method with the Kimura 2 parameter model. Analyses incorporating 1000 bootstrap replicates were undertaken using Phylip (Felsenstein, 1993).

#### Pre-release surveys

Surveys to determine the level of apparent parasitism of *B. tabaci* by Hymenoptera already established in Australia were undertaken between 1995 and 1999. The different parasitoids, all members of the genera *Encarsia* and *Eretmocerus* (Hymenoptera: Aphelinidae), found in Australia have been described in De Barro *et al.* (2000a) and Schmidt *et al.* (2001). A total of 2974 collections were made, 1228 from infestations of B and 1746 from AN. At the time of the surveys, both B (Mediterranean/Africa/Asia Minor genetic group) and the indigenous Australia genetic group (AN) co-occurred (genetic groups based on Boykin *et al.* (2007)). For each sample, RAPD-PCR was used to determine which genetic group the *B. tabaci* belonged to (De Barro & Driver, 1997). Co-infestations of both genetic groups that persist are uncommon, as B displaces individuals from the indigenous group (Liu *et al.*, 2007), and any samples where co-infestations occurred were excluded from this study as it is not possible to determine the genetic group of parasitised 4th instars.

The area covered by the surveys is shown in fig. 1. Leaves infested with 4th instars were collected from *Atriplex rhagodioides*, *Convolvulus arvensis*, *Cucumis melo* (*cantalupensis* & *inodorus*), *Citrullus lanatus*, *Datura* sp., *Emilia sonchifolia*,

*Euphorbia cyathophora*, *E. pulcherrima*, *Gossypium hirsutum*, *Helianthus annuus*, *Hibiscus rosa-sinensis*, *Lactuca seriola*, *Lantana camara*, *Lycopersicon esculentum*, *Malva parviflora*, *Malvastrum coromandellum*, *Sida cordifolia* and *Sonchus oleraceus*. Numbers of leaves collected varied and ranged from 20 to 100 and were based on the numbers of whitefly-infested leaves collected from plants at a given location in 15 min. The leaves were collected from the part of the plant where a preliminary assessment identified the presence of 4th instars. Throughout the study, only the 4th instar was assessed for parasitism as visual detection of parasitism in younger instars is unreliable and these individuals seldom survive on the collected leaves sufficiently long to enable the parasitoid to complete development. Nymphs were counted within 2.2-cm diameter (3.8 cm<sup>2</sup>) leaf discs. In the 4th instar, the shape of the parasitoid larva can be clearly seen through the integument as can the presence of meconium pellets in *Encarsia*; nymphs were determined as being parasitised or unparasitised with the aid of a stereo dissecting microscope. If parasitised, the parasitoid larvae were identified to genus on the basis of its shape and the presence of meconium. The leaves were then placed in emergence chambers until all the parasitoids had emerged. Parasitoids were identified to species using the descriptions in De Barro *et al.* (2000a) and Schmidt *et al.* (2001). Mean parasitism per collection was calculated by dividing the number of 4th instars present by the number that was parasitised.

#### Post-release surveys

A total of approximately 637,000 *Er. hayati* were released between October 2004 and March 2005 in the Bundaberg-Childers, Lockyer Valley and Emerald production areas; 390,000 between July 2005 and April 2007 in Alice Springs, Ayr, Bowen, Darwin, Gumlu, Guthalungra, Home Hill,

Table 3. A total of 1,067,000 *Er. hayati* were released between 29 October 2004 and 30 March 2008. The numbers of releases at any location ranged from 1500 to 130,000 individuals.

Date	Locality	Lat Long	Crop type	No. released (1000s)
29 Oct 2004	Lowood	27° 26' 38 S; 152° 36' 25 E	soybean	5
29 Oct 2004	Gatton	27° 32' 55 S; 152° 17' 39 E	pumpkin	14
5 Nov 2004	Bundaberg	24° 51' 6 S; 152° 26' 12 E	melon/zucchini	30
9 Nov 2004	Gatton	27° 34' 8 S; 152° 16' 17 E	eggplant	5
10 Nov 2004	Gatton	27° 29' 16 S; 152° 26' 2 E	pumpkin	10
17 Nov 2004	Childers	25° 3' 55 S; 152° 15' 9 E	tomato	5
17 Nov 2004	Bundaberg	24° 51' 6 S; 152° 26' 12 E	melon	5
17 Nov 2004	Bundaberg	24° 47' 3 S; 152° 13' 9 E	eggplant	5
24 Nov 2004	Gatton	27° 39' 21 S; 152° 22' 44 E	soybean	2
24 Nov 2004	Gatton	27° 40' 4 S; 152° 22' 14 E	pumpkin	2
25 Nov 2004	Helidon	27° 34' 32 S; 152° 09' 13 E	tomato	5
28 Nov 2004	Childers	25° 3' 55 S; 152° 15' 9 E	tomato	2.5
28 Nov 2004	Bundaberg	24° 51' 6 S; 152° 26' 12 E	melon	7
3 Dec 2004	Helidon	27° 33' 30 S; 152° 7' 46 E	tomato	10
3 Dec 2004	Grantham	27° 34' 13 S; 152° 10' 38 E	pumpkin	1.5
14 Dec 2004	Bundaberg	24° 51' 06 S; 152° 26' 12 E	melon	10
14 Dec 2004	Childers	25° 3' 55 S; 152° 15' 9 E	melon	5
21 Dec 2004	Helidon	27° 33' 24 S; 152° 8' 7 E	tomato	40
23 Dec 2004	Logan Village	27° 46' 0 S; 153° 5' 60 E	herbs	50
2 Feb 2005	UQ Gatton	27° 32' 31 S; 152° 21' 35 E	soybean	60
4 Feb 2005	UQ Gatton	27° 32' 31 S; 152° 21' 35 E	soybean	50
8 Feb 2005	UQ Gatton	27° 32' 31 S; 152° 21' 35 E	soybean	60
9 Feb 2005	Bundaberg	24° 51' 23 S; 152° 25' 6 E	soybean	10
13 Feb 2005	Gatton	27° 36' 40 S; 152° 16' 34 E	soybean	50
21 Feb 2005	Gatton	27° 39' 34 S; 152° 22' 40 E	broccoli	3
10 Mar 2005	Aratula	27° 56' 45 S; 152° 34' 57 E	green bean	130
17 Mar 2005	Forest Hill	27° 35' 20 S; 152° 22' 30 E	green bean	20
17 Mar 2005	Emerald	24° 7' 10 S; 148° 5' 26 E	sunflower	20
17 Mar 2005	Emerald	23° 32' 27 S; 148° 9' 18 E	cotton	20
22 Oct 2005	Ayr	19° 36' 10 S; 147° 24' 27 E	melon	25
22 Oct 2005	Ayr	19° 46' 13 S; 147° 13' 2 E	sow thistle	25
22 Oct 2005	Gumlu	19° 53' 11 S; 147° 41' 39 E	eggplant	20
22 Oct 2005	Guthalungra	19° 55' 49 S; 147° 50' 12 E	melon	20
22 Oct 2005	Gumlu	19° 52' 28 S; 147° 43' 08 E	eggplant	20
23 Oct 2005	Home Hill	19° 41' 14 S; 147° 26' 20 E	melon	25
23 Oct 2005	Home Hill	19° 40' 13 S; 147° 27' 10 E	melon	25
23 Oct 2005	Bowen	19° 59' 15 S; 148° 12' 59 E	tomato	20
23 Oct 2005	Bowen	19° 58' 38 S; 148° 11' 53 E	tomato	20
23 Oct 2005	Bowen	19° 59' 26 S; 148° 11' 32 E	tomato	20
23 Oct 2005	Bowen	19° 59' 21 S; 148° 12' 38 E	tomato	20
31 Oct 2005	Mareeba	16° 59' 18 S; 145° 31' 27 E	tomato	25
10 Mar 2007	Bowen	19° 59' 20 S; 148° 12' 28 E	bean	10
9 Mar 2007	Ayr	19° 35' 11 S; 147° 24' 18 E	weeds	15
9 Mar 2007	Home Hill	19° 40' 42 S; 147° 24' 19 E	weeds	15
2/3 Apr 2007	Mareeba	17° 2' 18 S; 145° 25' 27 E	tomato	40
29 Apr 2007	Katherine	14° 32' 02 S; 132° 27' 29 E	melons	15
29 Apr 2007	Darwin	12° 34' 37 S; 131° 15' 14 E	melons	15
29 Apr 2007	Alice Springs	23° 44' 12 S; 133° 51' 24 E	melons	15
30 Mar 2008	Carnarvon	24° 53' 16 S; 113° 40' 29 E	melons	40
			TOTAL	1067

Katherine and Mareeba; and 40,000 on 30 March 2008 in Carnarvon (fig. 1, table 3). By the time the releases of *Er. hayati* commenced, the indigenous genetic group of *B. tabaci* (biotype AN) had been displaced from across much of the range of the invader (Liu *et al.*, 2007), and all *B. tabaci* collected were assumed to belong to B. A total of 722 collections were made between November 2004 and March 2008. Collections were made primarily from commercial crops of *Phaseolus vulgaris*, *Brassica oleracea* (broccoli, cabbage, cauliflower), *Gossypium hirsutum*, *Cucumis sativus*, *Solanum melongena*, *Dolichos lablab*, *Solanum tuberosum*, *Ipomea batatas*, *Lycopersicon esculentum*, *Cucumis melo* (*cantalupensis*

& *inodorus*), *Citrullus lanatus*, *Cucurbita maxima*, *Cucurbita moschata*, *Glycine max*, the ornamentals *Duranta repens*, *E. pulcherrima*, *Gerbera* sp. and *Hibiscus rosa-sinensis* and the weeds *Sonchus oleraceus*, *Emilia sonchifolia* and *Lantana camara*. Leaves were collected and assessed as in the pre-release surveys.

#### Bundaberg 1997–2006

The survey data for the Bundaberg production area spans the period prior to the first outbreaks of *B. tabaci* (biotype B) through to the post-release evaluation period. It provides

a good opportunity to compare the changes in whitefly density and parasitism. A total of 372 collections were made prior to the release of *Er. hayati* and 151 post-release. Collections consisted of 20–100 leaves and followed the protocol outlined above.

#### Statistical analysis

The results are presented as means  $\pm$  standard error, and all percentage data were arcsine transformed before analysis. Pre-release data were analysed using two sample *t*-tests, as were the host specificity testing data. The data for the Bundaberg 1997–2006 surveys were analysed using ANOVA, and significant differences between means were identified using LSD.

### Results

#### Host specificity testing

Analysis of rDNA 18s revealed that the genus *Bemisia* was paraphyletic with two species of *Lipaleyrodes* falling within the group containing both *B. tabaci* and *B. afer*, indicating that they are likely to be congeneric and more closely related to the target *B. tabaci* than any other of the species tested (fig. 2). In no-choice experiments, *E. hayati* consistently parasitised 80–98% of *B. tabaci* (biotype B) nymphs (table 2). Only one non-target species (*Lipaleyrodes atriplex*) supported development of *E. hayati*. Parasitism of *L. atriplex* averaged 5.9% on *Rhagodia spinescens* and 15.6% on *Einadia trigonos*; and, in both cases, parasitism was significantly less (two-sample *t*-test:  $t=12.72$ ,  $P<0.001$ ) than that observed for *B. tabaci*. All parasitoids successfully emerged from *L. atriplex* on *R. spinescens*. However, parasitoid adults emerging from *L. atriplex* on *E. trigonos* became immobilized in the waxy coating of the parasitised nymph. Adult parasitoids were observed to groom their body repeatedly, resulting in additional wax particles accumulating on their legs, wings and antennae. All adults eventually died either on the leaf surface or fell to the cage floor and died. None of the other species tested supported the development of *E. hayati*. Whether eggs were laid or whether larvae failed to penetrate the nymphs is not known.

#### Pre-release surveys 1995–1999

Across all collections made during this period,  $9.1 \pm 0.3\%$  of 4th instars were observed to be parasitised. Further, 76% of those collections taken from B and 58% from AN infestations had no parasitism (fig. 3). When nymphs were partitioned in regards to their genetic group, there was a 2.4-fold difference in parasitism between B ( $5.0 \pm 0.3\%$ ) and AN ( $12.0 \pm 0.2\%$ ) (fig. 3; arcsine transformed, *t*-test:  $t=12.2$ ,  $P<0.001$ ). *Eretmocerus* contributed to 91.5% of the overall observed parasitism, to 83.9% of the parasitism of B and 93.7% of AN. Parasitism by *Encarsia* was a very minor component of the overall parasitism (fig. 3). In total, eight species contributed 8.5% of the total parasitism, 16.1% for B and 6.3% for AN. *Eretmocerus mundus* was by far the most abundant species observed (fig. 3), accounting for 87.7% of the total apparent parasitism, 67.3% for B and 93.7% for AN. *Eretmocerus queenslandensis* contributed 3.8% of the total parasitism and 16.6% for B, but no parasitism of AN was observed. Mean parasitism by *Er. mundus* on B was

$3.4 \pm 0.3\%$  and  $11.2 \pm 0.2\%$  on AN. Further, *Er. mundus* was represented in 18.1% of the collections.

There was a significant difference in the densities of B and AN, with B biotype densities averaging  $4.9 \pm 0.1$  4th instars  $\text{cm}^{-2}$  and AN  $3.4 \pm 0.1$  4th instars  $\text{cm}^{-2}$ ; and no AN densities exceeded 15 4th instars  $\text{cm}^{-2}$  (*t*-test:  $t=11.0$ ,  $P<0.001$ ), whereas the maximum B densities ranged between 30 and 40 4th instars  $\text{cm}^{-2}$ . The relationship between the percentage parasitism by *Er. mundus* and whitefly density for both B and AN is negatively correlated, suggesting a density dependent relationship (fig. 4).

#### Post-release surveys 2004–2008

There was no relationship between establishment and the number of individuals released as all releases resulted in establishment (table 3). The post-release surveys showed that *Er. hayati* had spread well beyond the immediate release areas (fig. 1). Mean parasitism across all collections was  $29.3 \pm 0.1\%$  of 4th instars and 76% of collections had parasitism (fig. 4). Of these, *Er. hayati* contributed to  $23.6 \pm 1.0\%$  of the apparent parasitism, or 85.0% of the overall parasitism. Of the collections made, *Er. hayati* was present in 71.2% while *Er. mundus* was in 9.8%. Mean parasitism by *Er. mundus* was  $1.2 \pm 0.2\%$  and contributed to 5.2% of the apparent parasitism. None of the remaining species contributed to more than 2% of the 4th instars parasitised (fig. 4); of them, *E. lutea* (Masi) 6.4% and *E. formosa* 3.7% were the next most commonly observed species. During the sampling period, the average whitefly density was  $1.2 \pm 0.2$  4th instars  $\text{cm}^{-2}$ , and there was no density dependent relationship between whitefly density and parasitism by *Er. hayati* (fig. 4). However, the nymph densities were only a quarter of those observed for B in the pre-release surveys with only three collections exceeding 20 4th instars  $\text{cm}^{-2}$ .

#### Bundaberg 1997–2006

The marked order of magnitude increase in whitefly densities between 1997 and 1998 marks the start of outbreaks in the Bundaberg production area (fig. 5). On 5 November 2004, a total of 30,000 *Er. hayati* were released in Bundaberg and, by 14 December 2004, a further 27,000 had been released. Prior to the release, levels of parasitism were consistently less than 5% over the period 1997–2000 and again in 2004 just prior to the first release (fig. 5). During the post-release monitoring period, January 2005–December 2006, 152 samples were collected. Over the 24 months following the releases, whitefly densities declined significantly to levels equivalent to that seen in 1997 (fig. 5) (ANOVA,  $F_{12,1702}=256.3$ ,  $P<0.001$ ,  $\text{LSD}=4.7$ ). Further, parasitism increased significantly from a maximum average of  $2.8 \pm 0.3\%$  prior to the first release to a minimum of  $33.1 \pm 4.2\%$  and an average over the period of  $43.7 \pm 2.3\%$  (fig. 5) (arcsine transformed, ANOVA,  $F_{12,1702}=107.5$ ,  $\text{LSD}=18.2$ ). *Eretmocerus hayati* accounted for 89.9% of the parasitism observed, *Er. mundus* 3.9%, *E. lutea* 2.9%, *E. formosa* 1.5% with *E. azimi*, *E. bimaculata*, *E. pergandiella* and *E. sophia* contributing 1.8% between them. In addition, only 4.6% of collections recorded no parasitism, whereas between 1997 and 2004 89.2% of the 461 samples from B infestations collected had no parasitism.

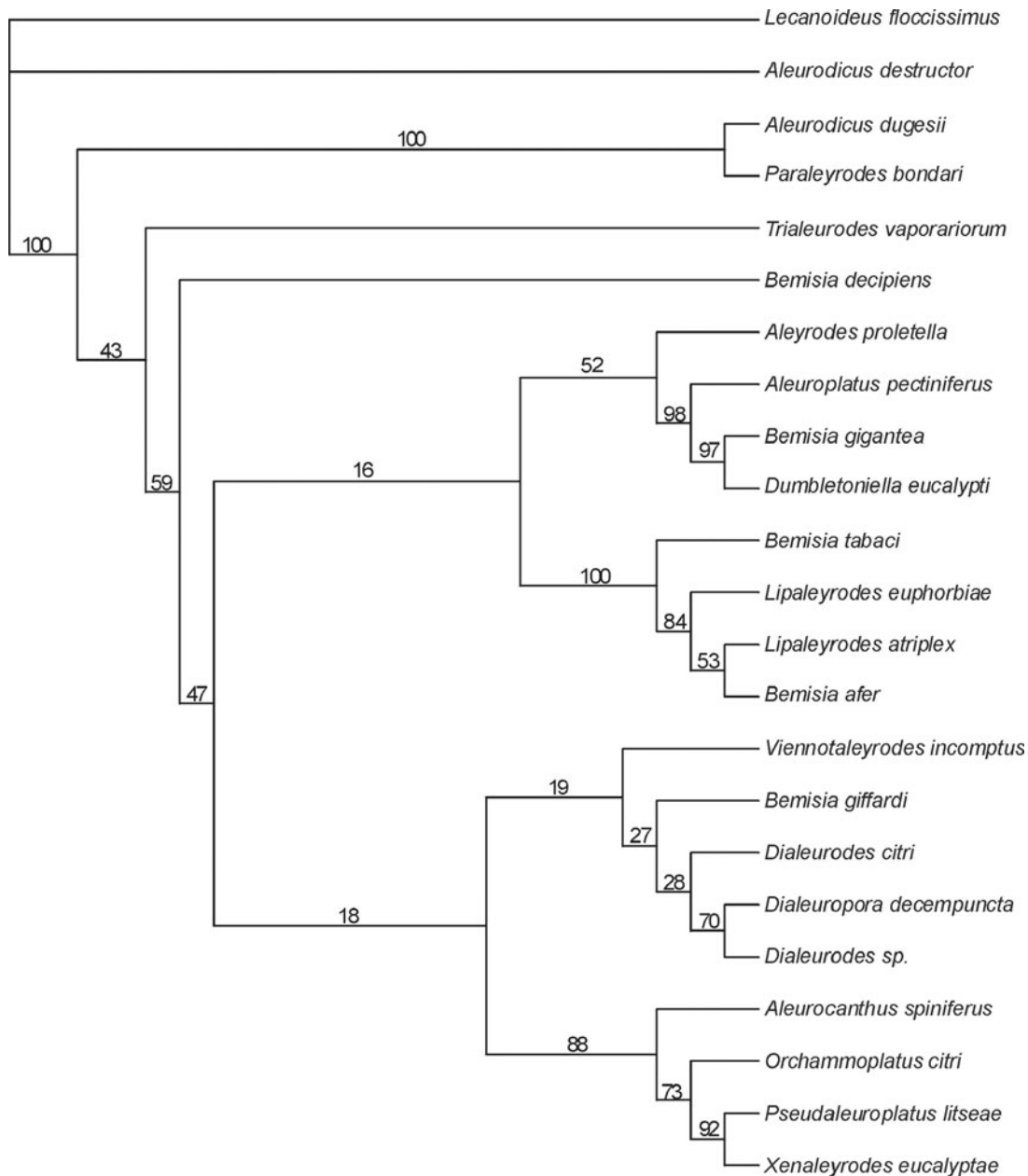


Fig. 2. Neighbour-joining phylogram inferred from a 762 to 782 bp portion of the ribosomal 18S gene. The subfamily Aleurodicinae species *Aleurodicus destructor*, *Lecanoideus floccissimus*, *A. dugesii* and *Paraleyrodes bondari* are outgroups. The remaining species belong to the subfamily Aleurodinae and form the ingroup. These species, with the exception of *B. decipiens*, were used to assess the host range of *Er. hayati* in no-choice tests against the target *B. tabaci* biotype B.

#### Host plants and parasitism

In pre-release surveys, eight host plant species represented >98% of the collections made (table 4). Counts of the number of collections with and without nymphs parasitised by *Er. mundus* indicated that *E. sonchifolia*, *Eu. cyathophora*, *L. camara* and *S. oleraceus* all had lower than expected levels of parasitism of B (table 4 for significance values). When the numbers of collections of B for each of these hosts was compared against the same hosts in the post-release surveys,

*E. sonchifolia*, *Eu. cyathophora* and *S. oleraceus* all showed an increase in numbers of collections containing *Er. hayati* parasitised B, while *L. camara* showed no change (table 4 for significance levels). Further, the collection count for *G. hirsutum* also increased relative to the pre-release counts while remaining unchanged for *H. annuus* (table 4). The mean percentage overall parasitism by all sources combined showed significantly more parasitised AN with the exception of *Malvastrum coromandellum* (table 4). Counts for the numbers of collections with and without *B. tabaci* parasitised

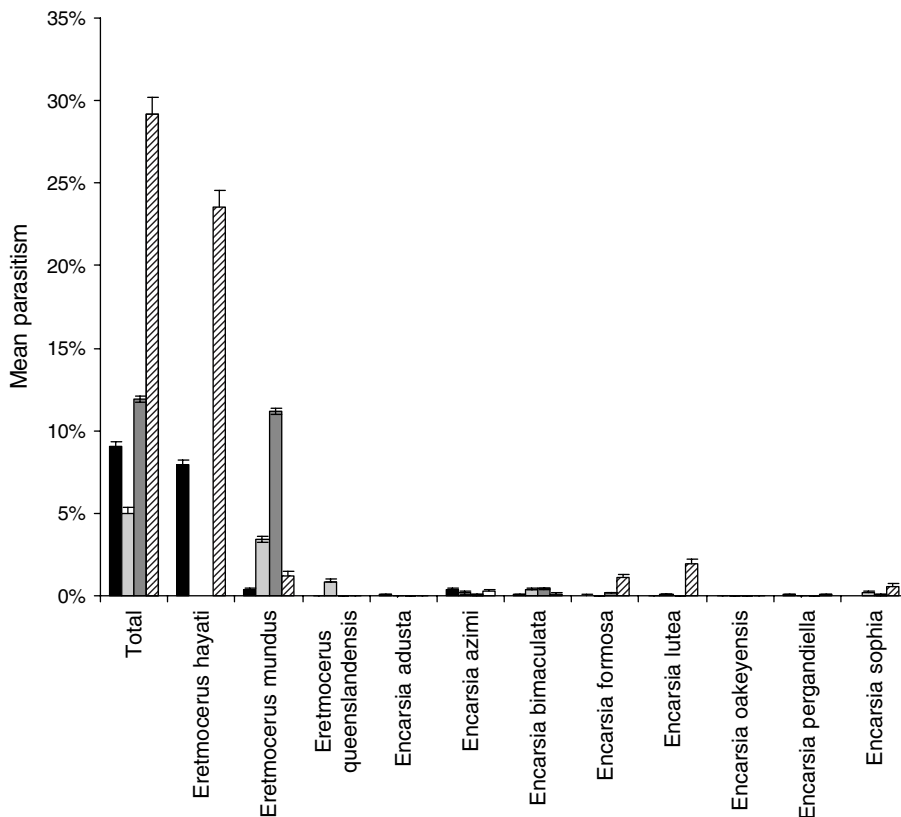


Fig. 3. Percent parasitism for each of the collections made during the pre-release (1995–1999) and post-release (2004–2008) surveys ranked from highest to lowest % parasitism. In the pre-release surveys and post-release, there were 937 and 173 collections, respectively, in which no parasitism was observed (■, pre-release total; □, pre-release B; ▒, pre-release AN; ▨, post-release).

by *Er. hayati* for a further ten species of host plant, which together with the previous five species made up 87.2% of the post-release collections, are also provided in table 4. All showed more collections with parasitism than without, the exception being *D. repens*. There was a significant increase in mean parasitism for all host plant species common to both pre- and post-release surveys (table 4).

### Discussion

A comparison of our pre-release survey data with those from Naranjo (2007) supported the conclusion that the pre-release guild of parasitoids in Australia was unlikely to provide the levels of parasitism required to provide useful reductions in whitefly numbers. Further, results from the Lower Rio Grande, Texas, USA (Goolsby *et al.*, 2005; Gould *et al.*, 2008) indicated that the introduction of *Er. hayati* would contribute to meaningful reductions in SLW abundance by substantially increasing the overall level of parasitism. The no-choice tests demonstrated that *Er. hayati* had an extremely narrow host range and that the level of non-target attack was considered too low to pose a threat to *L. atriplex*, and permission to release was granted in September 2004.

In the space of 3.3 years, *Er. hayati* has spread from 12 release areas along the east coast of Queensland to now covering much of the current distribution of SLW in eastern Australia. There has been a six-fold increase in the average

level of parasitism and an overall increase in the frequency of attack, such that 76% of all collections now contain parasitised whitefly whereas previously it was 25%. This suggests that *Er. hayati* has a superior host-finding capacity when compared with other parasitoids present in Australia. Further, whitefly host plants with either no or reduced levels of parasitism of B prior to the releases showed considerable increases in parasitism, and overall SLW densities have declined by 75% since the releases began. The rapid rate of spread may be due in part to the apparent intrinsic capacity of the parasitoid to disperse widely (N. Schellhorn & P. De Barro, unpublished data), but it is also due to the parasitoid being able to readily parasitise whiteflies infesting commercial ornamental nurseries (P. De Barro, data collected as part of this study). There is certain circularity here, as the same industry which so effectively spread SLW across Australia upon its introduction would now appear to be responsible for spreading its natural enemy.

The majority of parasitism, in the pre- and post-release surveys, was due to *Er. mundus* in the former and *Er. hayati* in the latter. Levels of parasitism by *Encarsia* spp. were only ever a minor contribution and so will not be discussed further. The pre-release surveys showed a marked difference in the levels of parasitism of B and AN with *Er. mundus* parasitising of a third fewer B. It is important, at this point, to note that while *Er. mundus* from Australia are morphologically identical to *Er. mundus*, elsewhere in the world they are



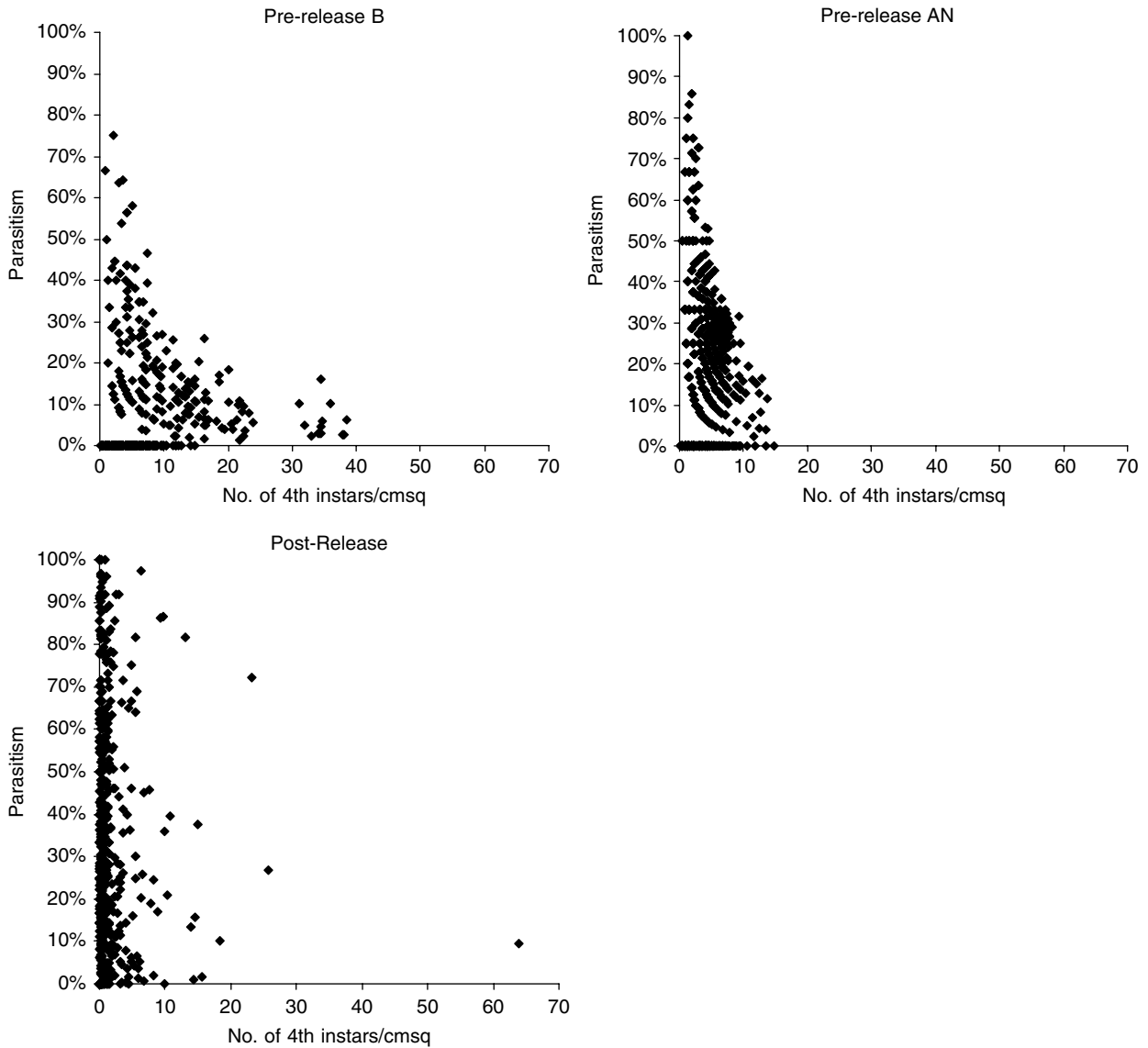


Fig. 4. The relationship between percentage parasitism by *Er. mundus* and B and AN *B. tabaci* densities and the relationship between percentage parasitism by *Er. hayati* and the B biotype.

genetically distinct (De Barro *et al.*, 2000a) from those in Europe and the USA. Further, *Er. mundus* has two modes of reproduction, arrhenotoky, where males are produced and thelytoky, where only females are produced. Elsewhere in the world, the arrhenokous population is the more common, although there are records of both occurring together in Egypt (Abd-Rabou & Ghahari, 2005), Iran (Ghahari *et al.*, 2005) and the USA (Powell & Bellows, 1992). In Australia, the population appears to be entirely thelytokous (De Barro & Hart, 2001; Ardeh *et al.*, 2005a,b).

There are several possible explanations for the lower level of parasitism in B. Firstly, as indicated earlier, AN and B belong to different genetic groups, and a comparison of mitochondrial CO1 sequences indicates an average divergence of 18% (based on the comparison of sequences used in Boykin *et al.*, 2007). Increasingly, using CO1, species level

divergence is associated with levels of divergence  $\leq 3\%$  (Hebert *et al.*, 2003). The level of divergence suggests that AN has been in Australia for a considerable period of time, and so it is likely that the Australian *Er. mundus* has had considerable opportunity to co-evolve with AN and may have become physiologically and behaviourally better adapted to AN than B. One difference between B and AN shown by this study is that B forms denser infestations than AN, and so it is possible that the Australian *Er. mundus* may simply be unable to adapt to the higher population densities of B. De Barro *et al.* (2000b) also assessed the capacity of *Er. mundus* to parasitise different densities of B on tomato and rockmelon. They showed no negative effect of density; but, as densities were below six nymphs  $\text{cm}^{-2}$ , it is not possible to predict the response to the higher densities that were often encountered in our surveys. Further research is,

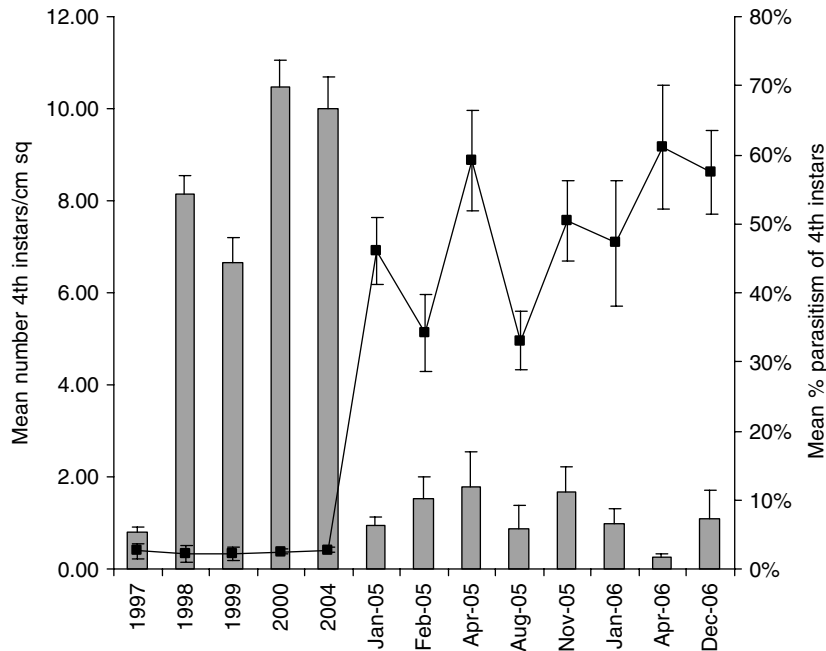


Fig. 5. The mean number of 4th instar *B. tabaci* between 1997 and 2006 together with the mean percentage parasitism over the same period. A total of 57,000 *Er. hayati* were released in Bundaberg between 5 November and 14 December 2004 (■, 4th instars/cmsq; -■-, % parasitism).

therefore, needed to resolve the role of host density in the performance of the two parasitoids.

Analysis of the post-release survey data showed no evidence for a density-dependent relationship between *Er. hayati* and *B. tabaci*. This is unusual as one would normally expect to find such a relationship. The most likely explanation would be that the population, having only recently been introduced, is still in a state of disequilibrium, which is contributing to the lack of evidence for such a relationship.

While physiological differences between AN and B may be contributing to the lower-than-expected levels of parasitism of B, it is more probable that host plant is exerting an effect on the parasitoid, either directly or via the nymph, and this is leading to the reduced parasitism. Three of the most frequently collected hosts, *E. sonchifolia*, *L. camara* and *S. oleraceus*, in the pre-release surveys all showed significantly reduced parasitism of B relative to AN. Of these three hosts, *S. oleraceus* made up 74% of the collections with only 18% having parasitised nymphs present, an observation which tends to support the role for a tritrophic interaction leading to reduced parasitism. Such tritrophic interactions are not unexpected in host-parasitoid interactions. Leaf hairs and leaf waxiness have previously been shown to significantly affect parasitism of whiteflies by aphelinids. McAuslane *et al.* (2000) observed reductions in leaf wax in collards were associated with increased levels of parasitism of B by both *Eretmocerus* sp. and *E. pergandiella*. Qui *et al.* (2005) demonstrated that performance of *Er. sp. nr furuhashii* declined as leaf hair density increased. Leaf hairs interfering with movement and host finding and resulting in reduced levels of parasitism have also been shown for *E. formosa*, *Er. eremicus* and *Er. rui* (Li *et al.*, 1987; Headrick *et al.*, 1996a,b;

McAuslane & Nguyen, 1996). However, *E. sonchifolia* and *S. oleraceus* are both glabrous and *L. camara* is weakly pubescent (<http://plantnet.rbgsyd.nsw.gov.au/floraonline.htm>). Furthermore, a fourth host from this study, *Eu. cyathophora*, which is also glabrous, showed no parasitism for *Er. mundus* and attempts to establish this parasitoid on both AN and B in the laboratory failed (P. De Barro, unpublished data). This suggests that while leaf hairs are responsible for reduced performance in other studies, they do not provide an adequate explanation for our results. In contrast, *Er. hayati* showed no such inability and readily parasitised B on *E. sonchifolia*, *Eu. cyathophora* and *S. oleraceus*. Further, it markedly increased the frequency of parasitism of B on *G. hirsutum*. In the post-release surveys, only two hosts, *L. camara* and *D. repens*, showed more collections without, than with, parasitism. It would, therefore, appear that part of the success of *Er. hayati* is its ability to attack B on host plants that *Er. mundus* was less able to utilize.

Plant community structure can have a significant influence on herbivore and parasitoid population dynamics. Goolsby *et al.* (1996, 1998) have shown that parasitism by *Er. hayati* varies considerably across different host plant species. Plant characteristics, such as shape, colour and structure, and other plant cues affect the capacity for parasitoids to search plant communities for infested plants and influence the time taken to find prey (Waage, 1979; Gingras *et al.*, 2002; Vos & Hemerik, 2003; Wang & Keller, 2004; Tentelier *et al.*, 2005). Factors such as these also influence the distribution of parasitoid attacks and can lead to the creation of enemy-free space for hosts on less attractive plants (Bukovinsky *et al.*, 2007). In this case, rather than changing the structure of plant communities, one explanation is that the introduction of a parasitoid that has

Table 4. The pre-release association between host plant and the numbers of collections with parasitism of either B or AN *B. tabaci* by *Er. mundus* and the mean percent parasitism from all sources using the eight most commonly collected host plants, which represent 98.2% of collections made during the surveys. The association between counts was analysed using Pearson's Chi Square except for \*, where samples sized required Fisher's exact test to be used; means were compared using the *t*-test. Also, the post-release comparison between host plant and the numbers of collections containing parasitism of B by *Er. hayati* and mean percent parasitism from all sources in the post-release surveys using the 15 most commonly collected host plants, which represent 87.2% of collections.

Host plant	AN parasitised	AN unparasitised	B parasitised	B unparasitised	<i>P</i>	AN % total parasitism	B % total parasitism	<i>P</i>
Pre-release counts ( <i>Er. mundus</i> )						Mean % Parasitism ± s.e.		
<i>Emilia sonchifolia</i> <sup>1</sup>	35	50	4	46	<i>P</i> < 0.001	11.9 ± 2.1	3.5 ± 1.9	<i>P</i> < 0.001
<i>Euphorbia cyathophora</i> <sup>1</sup>	0	67	0	5		7.5 ± 1.3		
<i>Gossypium hirsutum</i> <sup>1</sup>	52	220	30	125	<i>P</i> > 0.05	8.3 ± 1.1	3.3 ± 0.8	<i>P</i> < 0.01
<i>Helianthus annuus</i> <sup>1</sup>	35	46	13	33	<i>P</i> > 0.05	12.8 ± 1.9	5.4 ± 1.5	<i>P</i> < 0.01
<i>Lactuca seriole</i> *	5	7	2	5	<i>P</i> > 0.05	12.5 ± 6.0	5.7 ± 3.9	<i>P</i> < 0.01
<i>Lantana camara</i> <sup>1</sup>	14	11	1	10	<i>P</i> < 0.01	14.6 ± 3.0	2.0 ± 2.0	<i>P</i> < 0.001
<i>Malvastrum coromandellum</i> *	5	5	3	2	<i>P</i> > 0.05	15.9 ± 6.0	9.0 ± 5.4	<i>P</i> > 0.05
<i>Sonchus oleraceus</i> <sup>1</sup>	555	621	167	747	<i>P</i> < 0.001	12.9 ± 0.5	5.0 ± 0.4	<i>P</i> < 0.001
Post-release counts ( <i>Er. hayati</i> )								
<i>Brassica oleracea</i>			11	9			9.0 ± 4.8	
<i>Citrullus lanatus</i>			14	5			45.9 ± 7.6	
<i>Cucumis melo</i>			22	6			47.7 ± 6.7	
<i>Cucurbita maxima</i>			16	13			17.7 ± 5.0	
<i>Dolichos lablab</i>			11	0			37.9 ± 11.4	
<i>Duranta repens</i>			4	18			0.5 ± 0.5	
<i>Emilia sonchifolia</i> <sup>1</sup>			18	6	<i>P</i> < 0.001		39.3 ± 6.2	<i>P</i> < 0.001
<i>Euphorbia cyathophora</i> <sup>1</sup>			24	1	<i>P</i> < 0.001		30.7 ± 4.9	<i>P</i> < 0.001
<i>Euphorbia Pulcherrima</i>			18	1			9.0 ± 3.0	
<i>Glycine max</i>			39	3			40.8 ± 4.2	
<i>Gossypium hirsutum</i> <sup>1</sup>			85	20	<i>P</i> < 0.001		33.9 ± 2.9	<i>P</i> < 0.001
<i>Helianthus annuus</i> <sup>1</sup>			9	10	<i>P</i> > 0.05		51.3 ± 10.4	<i>P</i> < 0.001
<i>IPomea batatas</i>			27	5			39.1 ± 5.4	
<i>Lantana camara</i> <sup>1*</sup>			4	10	<i>P</i> > 0.05		9.9 ± 4.0	<i>P</i> < 0.001
<i>Lycopersicum esculentum</i>			24	10			21.1 ± 3.4	
<i>Sonchus oleraceus</i> <sup>1</sup>			140	38	<i>P</i> < 0.05		30.9 ± 2.0	<i>P</i> < 0.001

Data associated with host plants common to both surveys denoted by <sup>1</sup>.

different searching abilities has greatly reduced the available enemy-free space, thereby exposing a greater portion of the population to attack.

In terms of non-target attack on whiteflies, current surveys have only detected field parasitism in *B. tabaci*. Whether *Er. hayati* has had any impact on the indigenous AN *B. tabaci* is difficult in part because the widespread presence of the invasive SLW has led to the displacement of the indigenous population (Liu *et al.*, 2007).

The introduction of *Er. hayati* appears to have had a considerable impact on SLW. Drought, which has affected much of Australia during the entire release and post-release period covered by this study, may have contributed to the decline in SLW abundance in some areas through the reduction in cropping. However, the decline in SLW numbers in places such as Bundaberg, which have so far escaped drought, are equivalent to those observed in drought-affected areas. In Bundaberg, a recent survey by Growcom (<http://www.growcom.com.au/home/default.asp>) has shown that growers have modified crop management practices so as to take advantage of the establishment of *Er. hayati*.

Given the successful establishment and spread of *Er. hayati*, the key question is whether the levels of parasitism now being achieved are sufficient to make an economic difference in regards to suppression of SLW populations. Naranjo & Ellsworth (2005) and Naranjo (2007)

considered the Arizona cotton system prior to the establishment of several introduced species, and Horowitz *et al.* (1984) concluded that in Maricopa, Arizona and Israel parasitoids contributed very little irreplaceable mortality. In the case of Arizona cotton, irreplaceable mortality contributed by parasitism was 1%, with a further 5% of irreplaceable mortality from all sources combined being required to achieve economic suppression, while a long-term key factor analysis of life table studies in the Arizona cotton system between 1997 and 2007 showed no appreciable increase in mortality due to parasitism as a result of the introductions (Naranjo, 2008). Our study was not a life table study, so the results are not directly comparable. However, in Australia parasitism in pre-release cotton averaged 3.3%; and, since the release, now averages 34%, a tenfold increase. Whether this increase is sufficient to deliver additional irreplaceable mortality is not known and what level of additional mortality is needed is also not known, but the initial results are promising.

#### Acknowledgements

The Australian nursery, melon, tomato and vegetable producers through Growcom and Horticulture Australia and the Grains and Cotton Research and Development Corporations are thanked for providing funds to support

this research. Veronica Brancatini, Shama Khan, Andrew Hulthen, Lynita Howie and Mark Wade are thanked for technical support at various times during the study. Dr John Goolsby, USDA-ARS and Dr Matt Ciomperlik, USDA-APHIS, are thanked for facilitating the export of parasitoids from the Lower Rio Grande Valley. Dr John Goolsby is thanked for sharing his extensive knowledge on *E. hayati* and on reading an early version of the manuscript. Drs John Goolsby, Nancy Schellhorn and Steve Naranjo are thanked for useful discussions during the drafting of this manuscript.

## References

- Abd-Rabou, S.** (2002) Biological control of two species of whiteflies by *Eretmocerus siphonini* (Hymenoptera: Aphelinidae) in Egypt. *Acta Phytopathologica et Entomologica Hungarica* **37**, 257–260.
- Abd-Rabou, S. & Ghahari, H.** (2005) Biological studies of two strains of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) on *Bemisia argentifolii* (Homoptera: Aleyrodidae) from Iran. *Egyptian Journal of Agricultural Research* **83**, 513–523.
- Ardeh, M.J., de Jong, P.W. & van Lenteren, J.C.** (2005a) Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp. *Biological Control* **33**, 74–80.
- Ardeh, M.J., de Jong, P.W. & van Lenteren, J.C.** (2005b) Selection of *Bemisia* nymphal stages for oviposition or feeding, and host-handling times of arrhenotokous and thelytokous *Eretmocerus mundus* and arrhenotokous *E. eremicus*. *BioControl* **50**, 449–463.
- Borowiec, N., Issimaila, M.A., Chadhouliati, A.C., Youssoufa, M.A., Quilici, S., Delvare, G., Reynaud, B. & Ollivier, L.** (2007) Programme de lutte biologique contre *Aleurotrachelus atratus* Hempel (Hemiptera: Aleyrodidae), principale espèce d'aleurode nuisible au cocotier aux Comores. Rapport technique en fin d'étape 3 et préconisations pour le contrôle durable d'*A. atratus* aux Comores, Déc. 2006–Sept. 2007. CIRAD-INRAPE-PRPV.
- Boykin, L.M., Shatters, R.G., Rosell, R.C., McKenzie, C.L., Bagnall, R.A., De Barro, P.J. & Frohlich, D.R.** (2007) Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial CO1 DNA sequence. *Molecular Phylogenetics and Evolution* **44**, 1306–1319.
- Bukovinszky, T., Gols, R., Hemerik, L., van Lenteren, J.C. & Vet, L.E.M.** (2007) Time allocation of a parasitoid foraging in heterogeneous vegetation: implications for host-parasitoid interactions. *Journal of Animal Ecology* **76**, 845–853.
- Campbell, B.C., Steffen-Campbell, J.D. & Gill, R.J.** (1994) Evolutionary origin of whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) inferred from 18S rDNA sequences. *Insect Molecular Biology* **3**, 73–88.
- Campbell, B.C., Steffen-Campbell, J.D. & Gill, R.J.** (1996) Origin and radiation of whiteflies. pp. 29–51 in Gerling, D. & Mayer, R.T. (Eds) *Bemisia: 1995 Taxonomy, Biology, Damage, Control and Management*. Andover, UK, Intercept.
- Chermitt, B., Onillon, J.C., Dali, M. & Messelmani, H.** (1993) Control of the woolly whitefly, *Aleurothrixus floccosus* (Hom., Aleyrodidae) by the parasitoid, *Cales noacki* (Hymenopt., Aphelinidae). *Bulletin OILB/SROP* **16**, 86–98.
- De Barro, P.J. & Driver, F.** (1997) Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Australian Journal of Entomology* **36**, 149–152.
- De Barro, P.J. & Hart, P.** (2001) Antibiotic curing of parthenogenesis in *Eretmocerus mundus* Mercet (Australian parthenogenetic form) (Hymenoptera, Aphelinidae). *Entomologia Experimentalis et Applicata* **99**, 225–230.
- De Barro, P.J., Driver, F., Naumann, I.D., Clarke, G.M., Schmidt, S. & Curran, J.** (2000a). Descriptions of three species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data. *Australian Journal of Entomology* **39**, 259–269.
- De Barro, P.J., Hart, P.J. & Morton, R.** (2000b) The biology of two *Eretmocerus* spp. (Haldeman) and three *Encarsia* spp. Forster and their potential as biological control agents of *Bemisia tabaci* biotype B in Australia. *Entomologia Experimentalis et Applicata* **94**, 93–102.
- Felsenstein, J.** (1993) Phylogeny Inference Package (PHYLIP). Version 3.5. Seattle, WA, University of Washington.
- Gerling, D., Rottenberg, O. & Bellows, T.S.** (2004) Role of natural enemies and other factors in the dynamics of field populations of the whitefly *Siphoninus phillyrae* (haliday) in introduced and native environments. *Biological Control* **31**, 199–209.
- Ghahari, H., Huang, J. & Wang, Z.H.** (2005) Biology of a thelytokous biotype of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). *Insect Science* **12**, 461–465.
- Gingras, D., Dutilleul, P. & Boivin, G.** (2002) Modeling the impact of plant structure on host-finding behaviour of parasitoids. *Oecologia* **130**, 396–402.
- Goolsby, J.A., Legaspi, J.C. & Legaspi, B.C.** (1996) Quarantine evaluation of exotic parasites of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). *Southwestern Entomologist* **21**, 13–21.
- Goolsby, J.A., Ciomperlik, M.A., Legaspi, B.C., Legaspi, J.C. & Wendel, L.E.** (1998) Laboratory and field evaluation of exotic parasitoids of *Bemisia tabaci* (Biotype 'B') in the Lower Rio Grande Valley of Texas. *Biological Control* **12**, 27–135.
- Goolsby, J.A., De Barro, P.J., Kirk, A.A., Sutherst, R., Canas, L., Ciomperlik, M., Ellsworth, P., Gould, J., Hartley, D., Hoelmer, K.A., Naranjo, S.J., Rose, M., Roltsch, B., Ruiz, R., Pickett, C. & Vacek, D.** (2005) Post-release evaluation of the biological control of *Bemisia tabaci* biotype 'B' in the USA and the development of predictive tools to guide introductions for other countries. *Biological Control* **32**, 70–77.
- Gould, J., Hoelmer, K. & Goolsby, J.** (2008) Classical Biological Control of *Bemisia tabaci* in the United States. A Review of Interagency Research and Implementation. *Progress in Biological Control* **4**, 343 pp.
- Gunning, R.V., Byrne, F.J., Conde, B.D., Connelly, M.I., Hergstrom, K. & Devonshire, A.L.** (1995) First report of B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *Journal of the Australian Entomological Society* **34**, 116.
- Headrick, D.H., Bellows, T.S. & Perring, T.M.** (1996a) Behaviors of female *Eretmocerus* sp. nr *californicus* (Hymenoptera: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on cotton, *Gossypium hirsutum* (Malvaceae) and melon *Cucumis melo* (Cucurbitaceae). *Biological Control* **6**, 64–75.

- Headrick, D.H., Bellows, T.S. & Perring, T.M. (1996b) Host plant effects on the behaviors of *Eretmoceris* sp. nr. *californicus* raised from melon. *Entomophaga* **4**, 15–26.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. & de Waard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society London, Series B* **270**, 313–321.
- Hoddle, M.S., van Driesche, R.G. & Sanderson, J.P. (1998) Biology and use of the whitefly parasitoid. *Annual Review of Entomology* **43**, 645–669.
- Horowitz, A.R., Podoler, H. & Gerling, D. (1984) Life table analysis of the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. *Acta Oecologica* **5**, 221–233.
- Jetter, K.M. (2000) The economics of biological control. pp. 5–11 in Hoddle, M. (Ed) *California Conference on Biological Control II*. University of California, Berkeley, CA, 11–12 July 2000, Riverside, CA.
- Jones, W.A. & Greenberg, S.M. (1998) Suitability of *Bemisia argentifolii* (Homoptera: Aleyrodidae) instars for the parasitoid *Eretmoceris mundus* (Hymenoptera: Aphelinidae). *Environmental Entomology* **27**, 1569–1573.
- Li, Z.H., Lammes, F., van Lenteren, J.C., Huisman, P.W.T., van Vianen, A. & de Ponti, O.M.B. (1987) The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera, Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera, Aleyrodidae). XXV. Influence of leaf structure on the searching activity of *Encarsia formosa*. *Journal of Applied Entomology* **104**, 297–304.
- Liotta, G., Agro, A. & Pinto, M. (2003) Biological control in citrus groves in the last 50 years: three successful cases in Western Sicily. 'Integrated control in citrus fruit crops'. *Proceedings of the IOBC/WPRS Working Group*, 6–8 November 2002, Valencia, Spain, *Bulletin OILB/SROP* **26**, 171–173.
- Liu, S.S., De Barro, P.J., Xu, J., Luan, J.B., Zang, L.S., Ruan, Y.M. & Wan, F.H. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* **318**, 1769–1772.
- Liu, T.X. (2007) Life history of *Eretmoceris melanoscutus* (Hymenoptera: Aphelinidae) parasitizing nymphs of *Bemisia tabaci* Biotype B (Homoptera: Aleyrodidae). *Biological Control* **42**, 77–85.
- Martin, J.H. (1999) The whitefly fauna of Australia. A taxonomic account and identification guide. *CSIRO Entomology Technical Paper* No. 38.
- McAuslane, H.J. & Nguyen, R. (1996) Reproductive biology and behavior of a thelytokous species of *Eretmoceris* (Hymenoptera; Aphelinidae) parasitizing *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Annals of the Entomological Society of America* **89**, 686–693.
- McAuslane, H.J., Simmons, A.M. & Jackson, D.M. (2000) Parasitism of *Bemisia argentifolii* on collard with reduced or normal leaf wax. *Florida Entomologist* **83**, 428–437.
- Miklasiewicz, T.J. & Walker, G.P. (1990) Population dynamics and biological control of the woolly whitefly (Homoptera: Aleyrodidae) on citrus. *Environmental Entomology* **19**, 1485–1490.
- Naranjo, S.E. (2007) Intraguild predation on *Eretmoceris* sp. nr. *emiratus*, a parasitoid of *Bemisia tabaci*, by three generalist predators with implications for estimating the level and impact of parasitism. *Biocontrol Science and Technology* **17**, 605–622.
- Naranjo, S.E. (2008) Establishment and impact of exotic Aphelinid parasitoids in Arizona: A life table approach. *Journal of Insect Science* **8**, 36.
- Naranjo, S.E. & Ellsworth, P.C. (2005) Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologia Experimentalis et Applicata* **116**, 93–108.
- Nguyen, R. & Hamon, A.B. (2008) Biological control of giant whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Florida Department of Agriculture & Consumer Services, <http://www.doacs.state.fl.us/pi/methods/giant-whitefly-bc.html>.
- Pickett, C.H. & Wall, R. (2003) Biological control of ash whitefly *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae) by *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) in Northern California: 1990–2000. *Pan-Pacific Entomologist* **79**, 156–158.
- Powell, D.A. & Bellows, T.S. (1992) Development and reproduction of two populations of *Eretmoceris* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environmental Entomology* **21**, 651–658.
- Qiu, B.L., De Barro, P.J. & Ren, S.X. (2005) Development, survivorship and reproduction of *Eretmoceris* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae) parasitizing *Bemisia tabaci* (Homoptera: Aleyrodidae) on glabrous and non-glabrous host plants. *Bulletin of Entomological Research* **95**, 313–319.
- Rose, M. & DeBach, P. (1992) Biological control of *Parabemisia myricae* (Kuwana) (Homoptera: Aleyrodidae) in California. *Israel Journal of Entomology* **25–26**, 73–95.
- Rose, M. & Rosen, D. (1992) *Eretmoceris debachi* n. sp. (Hymenoptera: Aphelinidae), an effective parasite of *Parabemisia myricae* (Homoptera: Aleyrodidae). *Israel Journal of Entomology* **25–26**, 199–207.
- Rose, M. & Woolley, J.B. (1984a). Previously imported parasite may control invading whitefly. *California Agriculture* **38**, 24–25.
- Rose, M. & Woolley, J.B. (1984b) Previously imported parasite may control invading whitefly. *California Avocado Society Yearbook* **68**, 127–131.
- Schmidt, S., Naumann, I.D. & De Barro, P.J. (2001) *Encarsia* (Hymenoptera: Aphelinidae) parasitoids of *Bemisia tabaci* and *Trialeurodes vaporariorum* in Australia and the Pacific. *Bulletin of Entomological Research* **91**, 369–387.
- Sengonca, C. & Liu, B. (1998) Release of *Eretmoceris longipes* Compere (Hymenoptera: Aphelinidae) against *Aleurotuberculatus takahashi* David & Subramaniam (Homoptera: Aleyrodidae) on jasmine plantations in the Fuzhou region of southeastern China. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **105**, 504–512.
- Sengonca, C., Uygun, N., Kersting, U. & Ulusoy, M.R. (1993) Successful colonization of *Eretmoceris debachi* (Hym.: Aphelinidae) in the Eastern Mediterranean Citrus region of Turkey. *Entomophaga* **38**, 383–390.
- Sengonca, C., Uygun, N., Kersting, U. & Ulusoy, M.R. (1995) Effectiveness of *Eretmoceris debachi* Rose and Rosen (Hym., Aphelinidae) in controlling the whitefly *Parabemisia myricae* (Kuwana) (Hom., Aleyrodidae). *Anzeiger für Schadlingskunde, Pflanzenschutz, Umweltschutz* **68**, 123–127.
- Tentelier, C., Wajnberg, E. & Fauvergue, X. (2005) Parasitoids use herbivore-induced information to adapt patch exploitation behaviour. *Ecological Entomology* **30**, 739–744.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.
- Vail, P.V., Coulson, J.R., Kauffman, W.C. & Dix, M.E. (2001) History of biological control programs in the United States

- Department of Agriculture. *American Entomologist* **47**, 24–50.
- Van den Berg, M.A., & Greenland, J.** (2001) Pest status of two blackfly species on citrus in South Africa and Swaziland. *African Plant Protection* **7**, 53–57.
- Van den Berg, M.A., Hoppner, G. & Greenland, J.** (2000) An economic study of the biological control of the spiny blackfly, *Aleurocanthus spiniferus* (Hemiptera: Aleyrodidae), in a citrus orchard in Swaziland. *Biocontrol Science and Technology* **10**, 27–32.
- Vos, M. & Hemerik, L.** (2003) Linking foraging behavior to lifetime reproductive success for an insect parasitoid: adaptation to host distributions. *Behavioral Ecology* **14**, 236–245.
- Waage, J.K.** (1979) Foraging for patchily distributed hosts by the parasitoid *Nemeritis canescens*. *Journal of Animal Ecology* **48**, 353–371.
- Wang, X.G. & Keller, M.A.** (2004) Patch time allocation by the parasitoid *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). III. Effects of kairomone sources and previous parasitism. *Journal of Insect Behavior* **17**, 761–776.
- Waterhouse, D. & Norris, K.** (1989). *Aleurodicus dispersus* Russell. pp 12–22 in Waterhouse, D. & Norris, K. (Eds) *Biological Control Pacific Prospects, Supplement 1*. Canberra, ACIAR.
- White, G.L., Kairo, M.T.K. & Lopez, V.** (2005) Classical Biological Control of the Citrus Blackfly *Aleurocanthus woglumi* by *Amitus hesperidum* in Trinidad. *BioControl* **50**, 751–759.
- Zolnerowich, G. & Rose, M.** (1998) *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) imported and released in the United States for control of *Bemisia (tabaci complex)* (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington* **100**, 310–323.