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

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## 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

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## 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

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

| Species | Parasitoid | Location | References |
|---|---|---|---|
| *Aleurocanthus spiniferus* Quaintance | *Encarsia smithi* (Silvestri) | South Africa | Van den Berg *et al*. (2000), Van den Berg & Greenland (2001) |
| *Aleurocanthus woglumi* Ashby | *Eretmocerus serius Silvestri, Encarsia opulenta (Silvestri), Amitus hesperidum Silvestri* | Cuba; Mexico; Trinidad; South Africa; USA, Florida, Texas | Vail *et al*. (2001), Van den Berg & Greenland (2001), White *et al*. (2005) |
| *Aleurodicus dispersus* Russell | *Encarsia dispersa* Polaszek | Australia, Queensland; Pacific Island Countries, | Waterhouse & Norris (1989) |
| *Aleurodicus dugesii* Cockerell | *Entedononecremnus krauteri* Zolnerowich and Rose, *Encarsiella noyesi* Hayat | USA, Florida | Nguyen & Hamon (2008) |
| *Aleurothrixus floccosus* Maskell | *Cales noacki* Howard | USA, California; Sicily; Tunisia | Liotta *et al*. (2003), Chermiti *et al*. (1993), Miklasiewicz & Walker (1990) |
| *Aleurotrachelus atratus* Hempel | *Eretmocerus* n. sp. | Comoros Island | Borowiec *et al*. (2007) |
| *Aleurotuberculatus takahashi* David & Subramanian | *Eretmocerus longipes* Compere | China, Fuzhou | Sengonca & Liu (1998) |
| *Bemisia tabaci* Gennadius | *Eretmocerus hayati* Zolnerowich and Rose | USA, Texas | Gould *et al*. (2008) |
| *Dialeurodes citri* Ashmead | *Encarsia lahorensis* (Howard) | Italy, Sicily | Liotta *et al*. (2003) |
| *Parabemisia myricae* (Kuwana) | *Eretmocerus debachi* Rose & Rosen | USA, California; Turkey | Rose & Rosen (1992), Rose & DeBach (1992) Sengonca *et al*. (1993, 1995) |
| *Siphoninus phillyreae* (Haliday) | *Encarsia inaron* (Walker), *Eretmocerus siphonini* Viggiani & Battaglia | USA, California; Israel | Jetter (2000), Abd-Rabou (2002), Pickett & Wall (2003), Gerling *et al*. (2004) |
| *Tetraleurodes perseae* Nakahara | *Cales noacki* Howard | USA, California | Rose & Woolly (1984a,b) |
| *Trialeurodes vaporariorum* Westwood | *Encarsia formosa* Gahan | cosmopolitan | Hoddle *et al*. (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 $cm^{-2}$. 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

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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 (±SE) | Mean percent parasitism (±SE) |
|---|---|---|---|
| *B. afer* | *Breynia nivosa* | 41.9±4.9 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 49.0±5.8 | 93.8±2.3 |
| *B. gigantia* | *Elaeocarpus angustifolius* | 5.3±0.8 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 32.8±3.1 | 91.7±2.4 |
| *B. giffardi* | *Citrus limon* | 3.4±0.4 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 28.9±2.4 | 89.9±3.6 |
| *L. atriplex* | *Rhagodia spinescens* | 47.9±10.5 | 6.0±4.0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 61.1±8.6 | 92.6±2.2 |
| *L. atriplex* | *Einadia trigonos* | 36.4±5.7 | 15.6±4.3 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 47.9±3.5 | 89.7±4.0 |
| *L. euphorbiae* | *Euphorbia hirta* | 19.7±3.9 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 31.1±3.4 | 80.4±3.6 |
| *D. eucalypti* | *Corymbia citriodora* | 20.4±3.7 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 34.8±3.3 | 88.8±3.2 |
| *A. spiniferus* | *Cupaniopsis anacardioides* | 39.3±3.7 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 37.1±3.0 | 86.9±4.5 |
| *D. decempuncta* | *Callistemon viminalis* | 21.8±3.5 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 30.3±2.9 | 93.2±3.0 |
| *D. citri* | *Citrus limon* | 11.3±0.4 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 34.3±2.4 | 84.2±3.6 |
| *Dialeurodes sp.* | *Hymenosporum flavum* | 19.2±2.3 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 36.4±2.0 | 89.2±3.5 |
| *T. vaporariorum* | *Euphorbia peplis* | 32.3±2.8 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 25.4±2.3 | 97.7±1.2 |
| *A. proletella* | *Brassica* spp. | 39.7±5.8 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 42.3±2.3 | 92.9±2.9 |
| *X. eucalypti* | *Eucalyptus acmenoides* | 27.1±2.9 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 39.4±1.9 | 92.2±2.8 |
| *V. incomptus* | *Acacia aulacocarpa* | 14.9±4.2 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 31.2±2.3 | 91.3±2.9 |
| *Aleuroplatus sp.* | *Syzigium paniculatum* | 20.2±1.0 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 29.1±2.2 | 96.3±1.6 |
| *Pseudoaleuroplatus* sp. | *S. paniculatum* | 14.0±1.2 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 48.6±2.2 | 89.6±3.0 |
| *O. citri* | *Citrus limon* | 24.4±2.2 | 0 |
| *B. tabaci* | *Hibiscus rosa-sinensis* | 39.1±2.4 | 92.8±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 ($\leqslant$3 cm in length) host plants or as the number parasitised per 2.27 $cm^2$ 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′GACTCAACACGGGAAACCTC3′, 18sR 5′TCCTTCCGCAGGTTCACC3′ 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 proletella* (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), *Dialeuropora decempuncta* (Quaintance & Baker), *Dumbletoniella eucalypti* (Dumbleton), *Orchamoplatus citri* (Takahashi), *Pseudaleuroplatus* n. sp. (ex *Syzigium 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*

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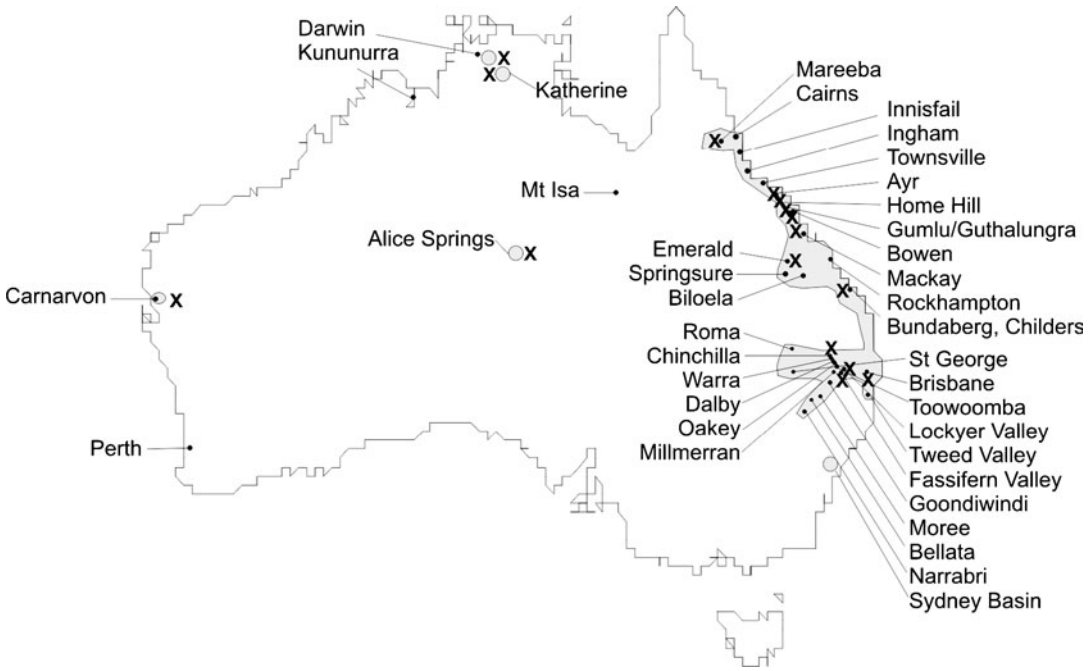


Fig. 1. Map showing location of pre-release surveys, release sites and the area where establishment has been confirmed through post-release surveys (•, pre-release surveys; ×, releases; ◯, establishment confirmed).

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*, *Lycopersicum 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 $cm^2$) 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,

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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*, *Lycopersicum 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

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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 ± 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*. Parasitisim 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 ± 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 ± 0.3%) and AN (12.0 ± 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 ± 0.3% and 11.2 ± 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 ± 0.1 4th instars $cm^{-2}$ and AN 3.4 ± 0.1 4th instars $cm^{-2}$; and no AN densities exceeded 15 4th instars $cm^{-2}$ (*t*-test: $t = 11.0$, $P < 0.001$), whereas the maximum B densities ranged between 30 and 40 4th instars $cm^{-2}$. 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 ± 0.1% of 4th instars and 76% of collections had parasitism (fig. 4). Of these, *Er. hayati* contributed to 23.6 ± 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 ± 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% ± 0.2 4th instars $cm^{-2}$, 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 $cm^{-2}$.

### *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, $F_{12,1702} = 256.3$, $P < 0.001$, LSD = 4.7). Further, parasitism increased significantly from a maximum average of 2.8 ± 0.3% prior to the first release to a minimum of 33.1 ± 4.2% and an average over the period of 43.7 ± 2.3% (fig. 5) (arcsine transformed, ANOVA, $F_{12,1702} = 107.5$, 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.

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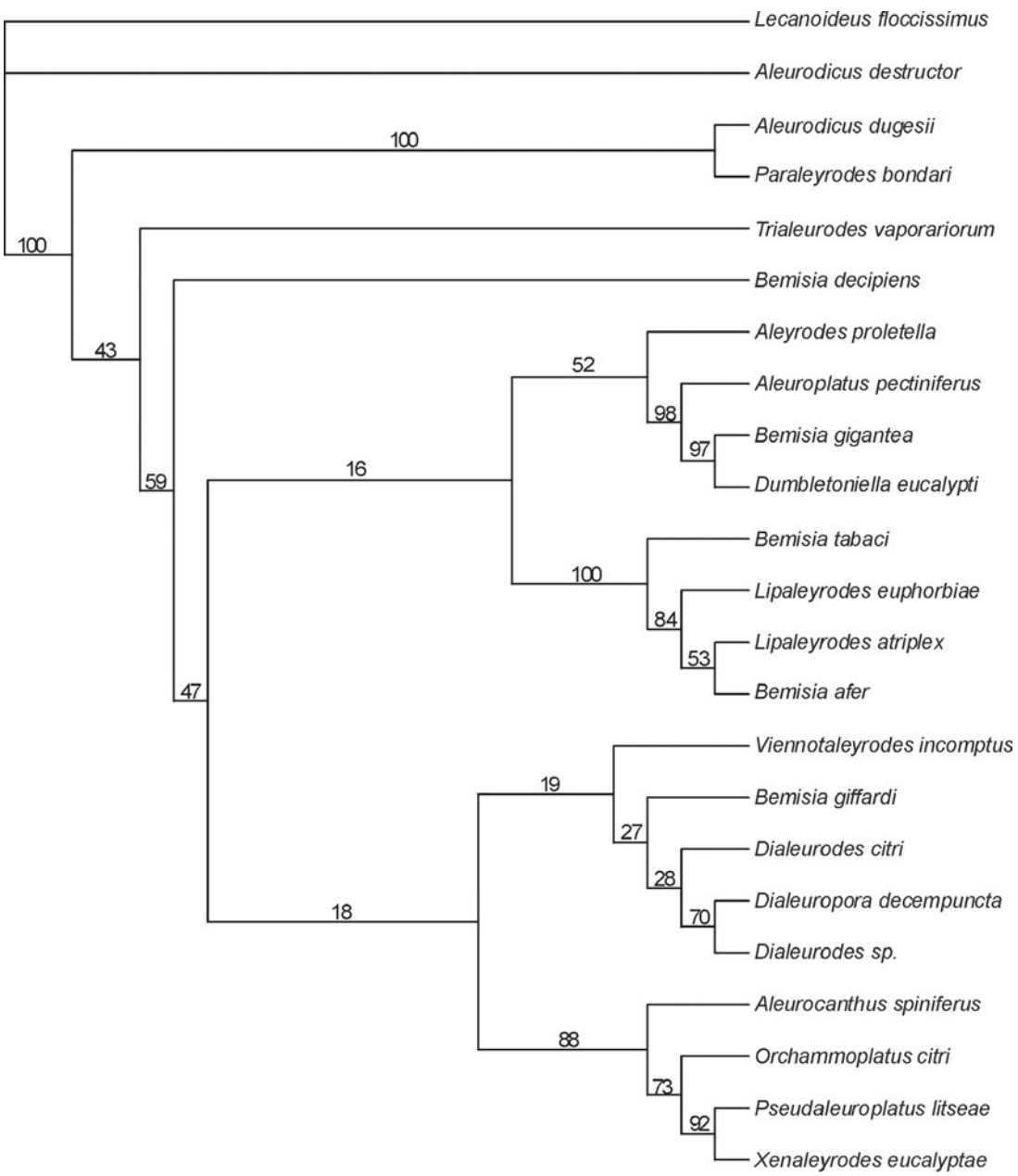


Fig. 2. Neighbour-joining phylogram inferred from a 762 to 782 bp portion of the ribosomal 18s gene. The subfamily Aleurodiciane species *Aleurodicus destructor*, *Lecanoideus floccissimus*, *A. dugesii* and *Paraleyrodes bondari* are outgroups. The remaining species belong to the subfamily Aleurodinae 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

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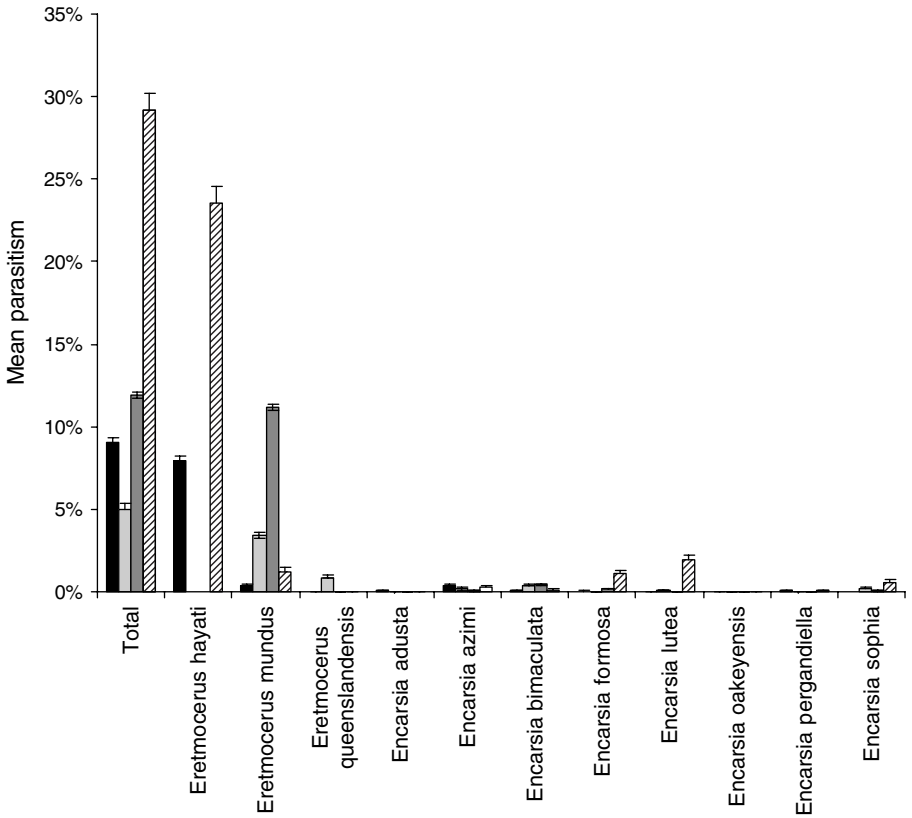


Fig. 3. Percent parasitism for each of the collections made during the pre-release (1995–1999) and post-release (2004–2008) surveys ranked from highest to lowest % parasitism. In the pre-release surveys and post-release, there were 937 and 173 collections, respectively, in which no parasitism was observed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

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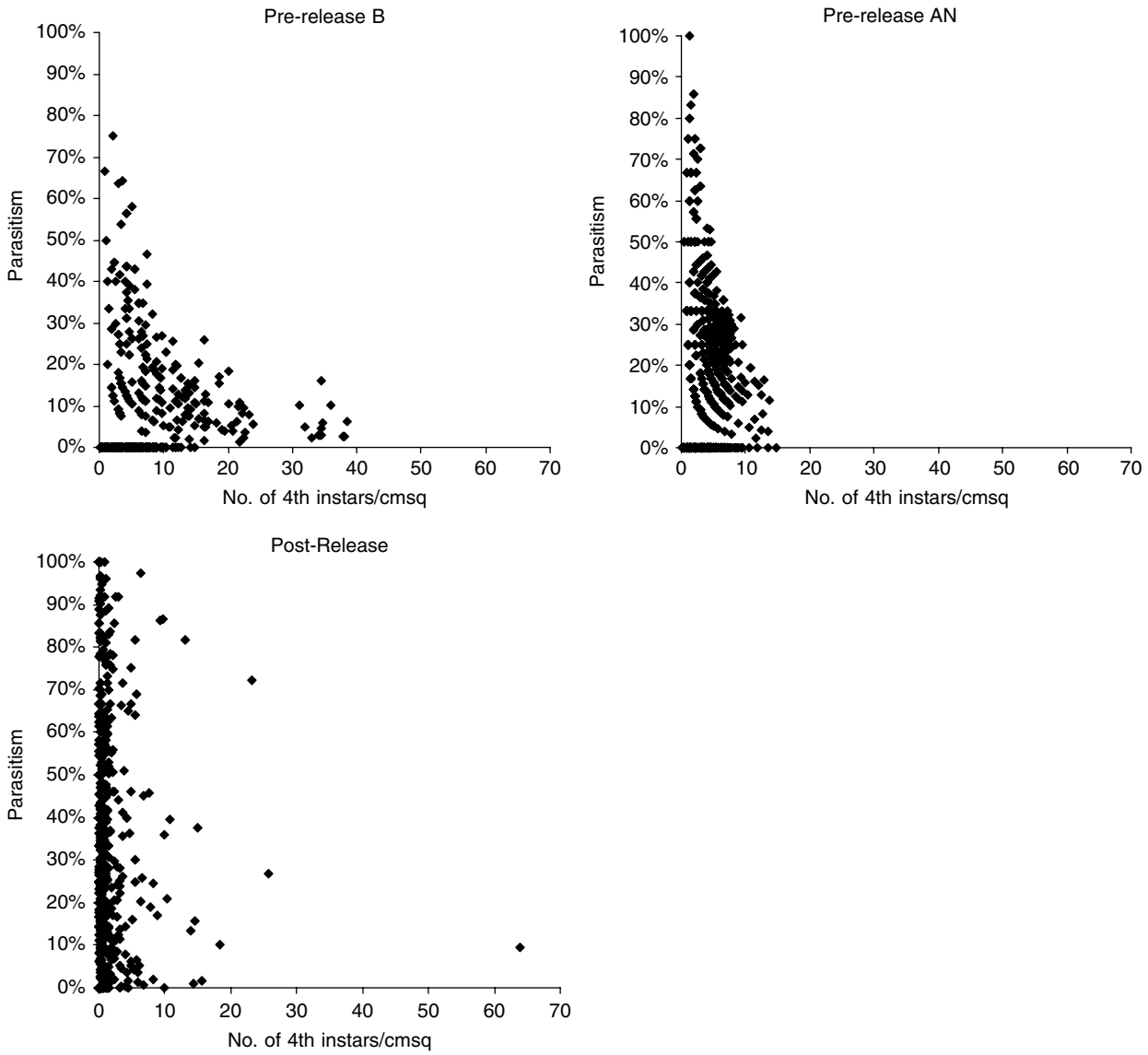


Fig. 4. The relationship between percentage parasitism by *Er. mundus* and B and AN *B. tabaci* densities and the relationship between percentage parasitism by *Er. hayati* and the B biotype.

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 arrenokous 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 $\leqslant 3\%$ (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 $cm^{-2}$, it is not possible to predict the response to the higher densities that were often encountered in our surveys. Further research is,

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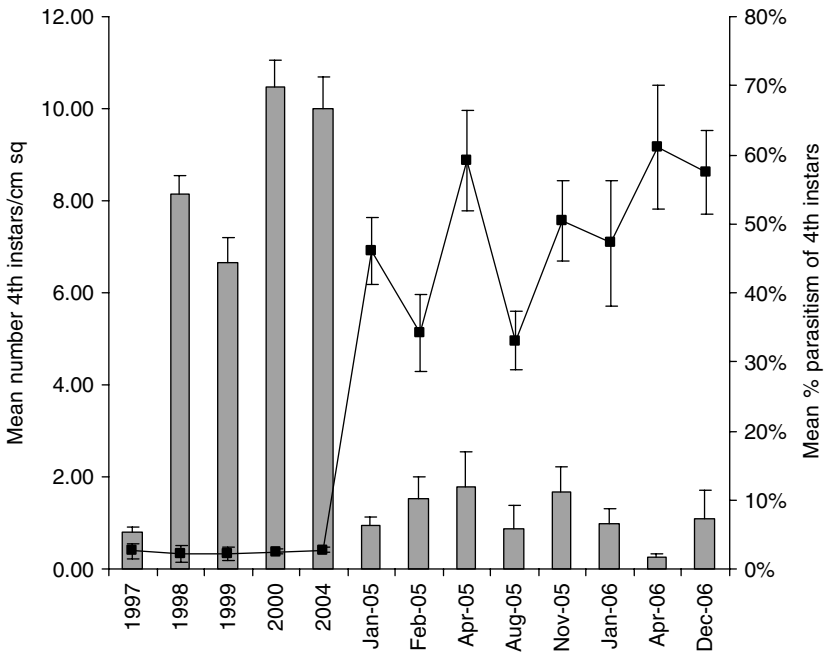


Fig. 5. The mean number of 4th instar *B. tabaci* between 1997 and 2006 together with the mean percentage parasitism over the same period. A total of 57,000 *Er. hayati* were released in Bundaberg between 5 November and 14 December 2004 (▒, 4th instars/cmsq; -■-, % parasitism).

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 supports 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 difference 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 (Bukovinszky *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

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

Data associated with host plants common to both surveys denoted by [1].

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 lead 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.

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