Entomologia Experimentalis et Applicata **103:** 11–21, 2002.

Host selection by the heteronomous hyperparasitoid *Encarsia pergandiella*: multiple-choice tests using *Bemisia argentifolii* as primary host

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Accepted: April 11, 2002

Key words: Aphelinidae, heteronomous hyperparasitoids, *Bemisia*, *Eretmocerus*, *Encarsia*, superparasitism, secondary host preference, host-stage preference

Abstract

The ovipositional patterns of the heteronomous hyperparasitoid *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) in the presence of its primary host *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae), and in the presence or absence of conspecific and heterospecific secondary hosts (*Encarsia formosa* Gahan and *Eretmocerus mundus* Mercet; Hymenoptera: Aphelinidae) were examined to assess host species preferences. Host preferences by heteronomous hyperparasitoids may affect the relative abundance of co-occurring parasitoid species and may influence host population suppression by the parasitoid community. Four combinations of hosts were tested: (1) *B. argentifolii*, *E. mundus*, and *E. formosa*, (2) *B. argentifolii*, *E. formosa*, and *E. pergandiella*, (3) *B. argentifolii, E. mundus*, and *E. pergandiella*, and, (4) *B. argentifolii*, *E. mundus*, *E. formosa*, and *E. pergandiella.* Arrays of hosts (24) were constructed in Petri dishes using leaf disks, each bearing one host. Thirty arrays of each host combination were exposed to single females for 6 h. All hosts were dissected to determine number of eggs per host. *Encarsia pergandiella* parasitized *E. formosa* hosts as frequently as *E. mundus* hosts. However, *E. pergandiella* parasitized either of these heterospecific hosts more frequently than conspecific hosts in treatments including two secondary host species. When a third parasitoid species was included in host arrays, *E. pergandiella* parasitized conspecific hosts as frequently as heterospecific hosts. Developmental stage of the hosts did not significantly influence host species selection by *E. pergandiella.* Our results indicate that host selection and oviposition by heteronomous hyperparasitoids like *E. pergandiella*, vary with the composition of hosts available for parasitization, and suggest a preference for heterospecific over conspecific secondary hosts.

Introduction

Aphelinid parasitoids (Hymenoptera: Aphelinidae) have been extensively studied because of their success in biological control of several hemipteran pests especially scales and whiteflies (Daane et al., 1991; van Lenteren et al., 1996; Bográn et al., 1998; De Barro et al., 2000; van den Berg et al., 2000). Heteronomous hyperparasitoids, known only from Aphelinidae, have a unique reproductive strategy whereby males and females develop in different species of hosts. The female wasp develops as an obligate primary parasitoid of a hemipteran host, usually a mealybug, whitefly or scale, while the male wasp develops as a secondary parasitoid on the immatures of primary parasitoid species or on developing females of their own species (Walter, 1983). The species in this unique group have also been termed autoparasitoids or adelphoparasitoids (Walter, 1983; Viggiani, 1984; Godfray, 1994; Williams & Polaszek, 1996; Hunter & Woolley, 2001). Cases of successful biological control involving heteronomous hyperparaitoids include citrus

blackly, *Aleurocanthus woglumi* Ashby, citrus whitefly, *Dialeurodes citri* (Ashmed), orange spiny whitefly, *Aleurocanthus spiniferus* (Quaintance) and spiraling whitefly, *Aleurodicus dispersus* Russell among others (Rose & DeBach, 1981; Nguyen et al., 1983; Thompson et al., 1987; Nafus & Nechols, 1995; Nechols & Nafus, 1995).

The dual role of heteronomous hyperparasitopids as both predators (attacking primary parasitoids) and competitors (for shared host resources) may influence their abilities, and the abilities of co-occurring species, to regulate their host populations (Rosenheim, 1998; Sullivan & Volkl, 1999). The impact of these interspecific interactions may be mediated through host selection by heteronomous female wasps. For example, the degree to which heteronomous females deposit male eggs in conspecific hosts relative to placing female eggs in primary hosts will influence population dynamics of the heteronomous species. Further, the species of secondary hosts that are selected for oviposition of male eggs may affect relative abundance of each species within the aphelinid parasitoid complex (Briggs, 1993; Mills & Gutierrez, 1996; Hunter & Kelly, 1998). Previous research on host selection by heteronomous hyperparasitoids has been characterized by pairwise choice tests attempting to identify mechanisms associated with host preference (Buijs et al., 1981; Hunter, 1989a; Williams, 1991; Pedata & Hunter, 1996; Hunter & Kelly, 1998). However, aphelinid parasitoid communities are usually complex and frequently there are many host species that may be utilized by heteronomous hyperparasitoids (Williams, 1996).

Encarsia pergandiella Howard is a heteronomous hyperparasitoid endemic to California, New York, Florida Texas, Puerto Rico, Central and South America (Polaszek et al., 1992; Hunter, 1993; Bográn et al., 1998). Surveys conducted as a part of biological control programs against pestiferous *Bemisia* species have revealed *Encarsia pergandiella* may be numerically dominant parasitoids of *B. tabaci* and *B. argentifolii* (Riley & Ciomperlik, 1997; Bográn et al., 1998; Schuster et al., 1998; Simmons, 1998). *Encarsia pergandiella* has also been successfully introduced into Italy from California for biological control of the greenhouse whitefly *T. vaporariorum* to complement parasitism by *E. formosa* (Viggiani & Mazzone, 1980; Onillon et al., 1994.

Encarsia formosa Gahan is a thelytokous parasitoid (females produce virtually no male offspring) presumed to be native to the Western Hemisphere (Polaszek et al., 1992) and used around the world for augmentative biological control of whitefly in greenhouse crops (Hoddle et al., 1998). Several strains of *E. formosa* have been evaluated against *B. argentifolii* infesting greenhouse grown ornamental crops with good results (Heinz & Parrella, 1994; Hoddle & Van Driesche, 1999). *Eretmocerus mundus* Mercet is also a primary parasitoid, both males and females develop on whitefly hosts (Foltyn & Gerling, 1985; Powell & Bellows, 1992). *Eretmocerus mundus* is a palearctic species recently introduced and released into the United States for biological control of *Bemisia* whiteflies (Goolsby et al., 1998; Zolnerowich & Rose, 1998). *Eretmocerus mundus* was considered one of the most effective parasitoid species in laboratory and field evaluations conducted as a part of a biological control program against *B. argentifolii*, and is one of only two species of parasitoids, among 17 species imported, to have established in Texas (Goolsby et al., 2000).

Bemisia argentifolii Bellows & Perring and the closely related *B. tabaci* (Gennadius) are serious pests of crops in most tropical and subtropical regions of the world (Mound & Halsey, 1978; Gerling, 1990; Caballero, 1993). Starting in 1986, population outbreaks of *B. argentifolii* in the sourthern United States prompted field releases of exotic parasitoids to improve the natural control provided by indigenous parasitoids including the heteronomous *E. pergandiella* (Goolsby et al., 1998). It is important therefore, to understand secondary host preferences of native heteronomous hyperparasitoids like *E. pergandiella* as they may influence the abilities of introduced aphelinid parasitoids to establish and suppress populations of *B. argentifolii* in agroecosystems.

Based on the above, we undertook a laboratory study to examine the ovipositional patterns of the heteronomous hyperparasitoid *E. pergandiella* in the presence of its primary host *B. argentifolii*, and in the presence or absence of conspecific hosts and heterospecific *E. formosa* and *E. mundus* hosts. Our objectives were to (1) assess the influence of host species composition on host selection and oviposition by *E. pergandiella* using *B. argentifolii* as the primary host, and (2) evaluate the influence of host developmental stage on host species selection and oviposition by the heteronomous *E. pergandiella.*

Materials and methods

Host and parasitoid cultures. All parasitoids used in the experiment were laboratory reared on *B. argentifolii*, which were reared on sweet potato (*Ipomoea batatas* L.). Individual leaves were maintained in 10 ml floral aquapic filled with a 5-11-26 (N-P-K) hydroponic solution (Aqua-Ponics International, Los Angeles, California) in the laboratory. Leaves were exposed to one hundred adult *B. argentifolii* (approximately 1:1 male to female ratio) within two 4.5 cm (diameter) × 4.0 cm (height) clip cages (50 adults per clip cage) for a 24-h oviposition period. Infested leaves were individually placed into 12 cm (diameter) × 2.5 cm (height) polystyrene tissue culture dishes (Corning Inc., Corning NY), the top of which was replaced by mesh polyester organdy (32 × 32 openings per cm^2). Second and third instars *B. argentifolii* were used to rear *E. mundus*; third and fourth instars were used to rear *E. formosa* and *E. pergandiella* (Powell & Bellows, 1992; Enkegaard, 1993; Schuster & Price, 1996).

Immature parasitoids used as secondary hosts in the experiments were obtained by exposing *B. argentifolii* nymphs of the appropriate stage to ten female parasitoids per infested leaf (inside culture dishes) for a period of 48–72 h. *Encarsia pergandiella* used in the experiments were reared from material originally collected around Weslaco, TX in late 1999 and kept in culture (as described above) at laboratories of United States Department of Agriculture-Agricultural Research Service, Beneficial Insects Research Unit (USDA-ARS-BIRU), Subtropical Agricultural Research Center, Weslaco, TX. *Encarsia formosa* and *E. mundus* strains used in the experiments were reared from material originally collected in Egypt and Spain, respectively in early 1992 and maintained under culture at USDA-APHIS-PPQ Mission Plant Protection Center (MPPC), Mission, TX (*E. formosa* and *E. mundus* MPPC designation M92030 and M92014, respectively; see Goolsby et al., 1998).

Experimental set up. Laboratory experiments were carried out at the USDA-ARS-BIRU, Weslaco, TX in February and April 2000. Arrays of hosts were constructed in Petri dishes (60 mm diameter × 15 mm depth; Falcon®, Becton Dickinson and Co., Lincoln Park, NJ) lined with 4.25 cm (diameter), tap water-saturated filter paper (413 grade, VWR Scientific Products, West Chester, PA), containing 24 circular 10 mm^2 (area) sweet potato leaf disks each bearing one host. Since host developmental stage may influence host suitability for heteronomous hyperparasitoids (Pedata & Hunter, 1996), each host species was exposed at two developmental stages, early and late. For *B. argentifolii* the early stage was the third instar (N3) while the late stage was the fourth instar (N4). These stages have been found to be the most suitable for female *E. pergandiella* development (Liu & Stansly, 1996; Schuster & Price, 1996; Jones & Greenberg, 1999). For the three parasitoid species, the early stage was the late-larva (LL), while the late stage was the early pupa (EP). For *E. mundus* LL was the stage at which the immature had consumed its host but retained the amorphous shape of *Eretmocerus* larva (approximately ten days after oviposition), while EP was the stage immediately after pupation but preceding pigmentation of the eyes (approximately 12 days after oviposition). The LL stage in both *E. formosa* and *E. pergandiella* was the stage immediately preceding pupation, when the wasp larva had consumed its host but retained its crescent shape (approximately eight days after oviposition). The EP stage for both *Encarsia* species was the stage immediately after pupation but preceding pigmentation of metasoma and eyes (approximately ten days after oviposition).

Encarsia pergandiella females used in the experiment were obtained from the laboratory colony by removing individual parasitoid pupae from infested leaves and placing them into 3 ml (volume) glass vials one day before expected adult emergence. Vials were streaked (1cm length) once with pure honey using the tip of a small paint brush (No. 000) to provide newly emerged wasps with a source of nutrients. Two to eight hours after female emergence, one male *E. pergandiella* (24–48 h old) was introduced into each vial until mating was observed. Females that did not mate within the first 5 min of observation were discarded. All females used in the experiment were mated, less than 12 h old and had no prior contact with live hosts.

To assess the influence of host species composition and developmental stage on host selection and oviposition by *E. pergandiella* we exposed single female wasps to arrays of hosts in one of four species combinations: (1) *B. argentifolii*, (*Ba*) *E. mundus* (*Em*), and *E. formosa* (*Ef*), (*Ba+Em+Ef)*, (2) *B. argentifolii*, *E. formosa*, and *E. pergandiella* (*Ep*), (*Ba+Ef+Ep)*, (3) *B. argentifolii*, *E. mundus*, and *E. pergandiella* (*Ba+Em+Ep)*, and (4) *B. argentifolii*, *E. mundus*, *E. formosa*, and *E. pergandiella* (*Ba+Em+Ef+Ep*). In treatments 1–3, eight hosts of each species were presented, four at 'early' and four at 'late' develop-

mental stage (four individuals × three species × two stages = 24 hosts per array). In treatment 4, six hosts of each species were presented, three at 'early' and three at 'late' developmental stage (three individuals × four species × two stages = 24 hosts per array). Thus, the total number of hosts available for attack was kept constant across treatments while the host species composition was experimentally manipulated. The position of each host type within the arrays was randomly assigned and host arrays were constructed so that the 10 mm^2 leaf disks were adjacent to each other in a 6 × 4 rectangular grid.

On the day of the experiments, individual females were released at mid-day (1100–1200 h) into Petri dishes containing host arrays (see below) and kept in the previously described incubator for 6 h. Petri dishes were sealed with parafilm (Parafilm®, American National Can™, Neenah, WI) to prevent parasitoid escape. The 6-h exposure time was chosen to avoid host limitation and subsequent high levels of parasitism that would diminish the detectability of significant differences among host types. The time of the day in which experiments were run was chosen to coincide with peak activity periods of aphelinid parasitoids (Walter, 1988). Thirty female wasps were individually exposed to each of the four treatments. Female wasps were removed from the Petri dishes after the 6-h exposure period and the dishes were kept in the incubation chamber for 48–72 h prior to host dissections. Dissections were performed to assess the number of eggs deposited in each host type and were conducted in a drop of glycerol under a stereo microscope (Olympus® SZ60, Olympus America Inc. Melville, NY) with a transmitted light illumination base, and at approximately 126× power. The use of glycerol, compared to water, facilitated the dissection process because it evaporates more slowly than water and its oil-like texture eases the handling of hosts.

Data analyses. The number of eggs deposited by *E. pergandiella* in each host type was tabulated from counts made during dissections. To examine the influence of species composition on host selection and oviposition by *E. pergandiella*, the frequency of parasitized hosts was compared among all species within each of the host species combinations (treatments). Analyses were performed using replicated G-tests of goodness of fit (Sokal & Rohlf, 1995). Since each host species was given the same opportunity to be attacked by the female wasp, the null expectation was that of no preference and each host species would be attacked with equal frequency. Whenever significant deviations from the null expectation were found for comparisons among all host species, parasitism within each treatment was compared between individual host species using single classification G-tests of goodness of fit, and test statistics were adjusted for type I error using William's correction (Sokal & Rohlf, 1995).

To assess the influence of host developmental stage on host selection and oviposition by *E. pergandiella*, we compared parasitism among host types (of each species and developmental stage) within each treatment. Comparisons were made using log linear model analyses for multi-way contingency tables (replicate, host species, and developmental stage as variables) in SAS (PROC CATMOD, SAS Institute. Inc. Cary, NC). The log linear model analysis is analogous to the analysis of variance and it is used for the analysis of response functions (including frequencies) and the partitioning of variation among those functions into various sources (Sokal & Rohlf, 1995). A significant interaction between host species and host developmental stage would indicate that host species preferences by *E. pergandiella* are influenced by the developmental stage of the hosts.

Differences in the frequency of superparasitism (frequency of hosts with more than one egg) between host species may reflect host species preferences by heteronomous hyperparasitoids (Pedata & Hunter, 1996). Therefore in addition to parasitism, the frequency of superparasitism (defined here as self-superparasitism *sensu* Waage, 1986) was compared among species and among host types within each of the host-species combinations tested. The observed levels of superparasitism ($< 5\%$ of all hosts) resulted in low cell frequencies that prevented the use of replicated G-tests; therefore data on superparasitism were pooled across replicates before analyses. Comparisons on the frequency of superparasitism among host species were made using single classification G-tests and the test statistics were adjusted for type I error using William's correction (Sokal & Rohlf, 1995). The null hypothesis tested was that the observed frequency of superparasitism was independent of host species. Since each host species was given the same opportunity to be attacked, significant deviations from this expectation would suggest host species preferences by *E. pergandiella* as reflected by superparasitism (Pedata & Hunter, 1996). Comparisons among host types on the frequency of superparasitism (pooled across replicates) within each treatment were made using G-tests of independence for two-way contingency tables

(host species and developmental stage as classification variables) (Sokal & Rohlf, 1995). Test statistics were adjusted for type I error using William's correction (Sokal & Rohlf, 1995). Significant effects would indicate an association between host species and host developmental stage on the frequency of superparasitism by *E. pergandiella.*

Results

The average number of parasitized hosts by *E. pergandiella* during a single 6-h period ranged between 1.9 ± 0.26 and 3.0 ± 0.43 (mean ± 1 SEM, $n = 30$ female wasps), and was always much less than the 6–8 hosts of each species available for parasitization in the different species combinations. In addition, the highest mean number of parasitized hosts of any single species in a host array was 1.5 ± 0.3 ($n = 30$ females). Thus, choices made by female *E. pergandiella* were unlikely influenced by host availability.

Host species composition and E. pergandiella *parasitism.* The frequencies of parasitized hosts of each species within each of the species combinations tested were analyzed using replicated G-tests of goodness of fit (Table 1). In all cases, G values for heterogeneity tests were not significant ($P > 0.05$) indicating the direction of deviations, from the expectation of no preference, were uniform across replicates (Table 1). Significant deviations were found in all host-species combinations, as G values for pooled tests were always significant (Table 1).

Encarsia pergandiella oviposited more often in *E. mundus* (adjusted G value [G_{adj}] = 4.9, df = 1, $P < 0.05$) or *E. formosa* ($G_{adj} = 9.4$, df = 1, $P < 0.005$) than in the primary host *B. argentifolii*, but similarly ($G_{adj} = 0.8$, df = 1, $P > 0.5$) into both secondary hosts in treatment *Ba+Em+Ef* (Figure 1A). In treatment *Ba+Ef+Ep*, the number of hosts parasitized by *E. pergandiella* was higher for *E. formosa* hosts than for *E. pergandiella* ($G_{adj} = 5.1$, df = 1, $P < 0.05$) or *B. argentifolii* ($G_{adj} = 14.2$, df = 1, $P < 0.01$) hosts, but the number of parasitized *B. argentifolii* hosts was not different ($G_{adj} = 2.4$, df = 1, $P > 0.05$) than the number of parasitized *E. pergandiella* hosts (Figure 1B). The number of hosts parasitized by *E. pergandiella* was slightly but significantly higher ($G_{adj} = 3.9$, df = 1, $P < 0.05$) for *E. mundus* hosts than for *E. pergandiella* hosts in treatment *Ba+Em+Ep* (Figure 1C). In this treatment, *E. pergandiella* parasitized *E. mundus* hosts more often than *B. argentifolii* hosts ($G_{adj} = 9.5$, df = 1, $P < 0.005$) but similarly parasitized *B. argentifolii* and conspecific hosts ($G_{adj} = 0.3$, df = 1, $P > 0.1$) (Figure 1C). No significant differences in the number of parasitized hosts were found in treatment *Ba+Em+Ef+Ep*, between *E. mundus, E. formosa* and *E. pergandiella* hosts ($G_{adj} = 1.4$, df = 1, $P > 0.5$). However, in this treatment the number of parasitized hosts was higher for the secondary hosts *E. mundus* ($G_{adj} = 8.0$, df = 1, $P < 0.005$), *E. formosa* ($G_{adj} = 11.1$, df = 1, $P < 0.005$) and *E. pergandiella* hosts ($G_{adj} = 4.5$, df = 1, $P < 0.05$), than for the primary host *B. argentifolii* (Figure 1D).

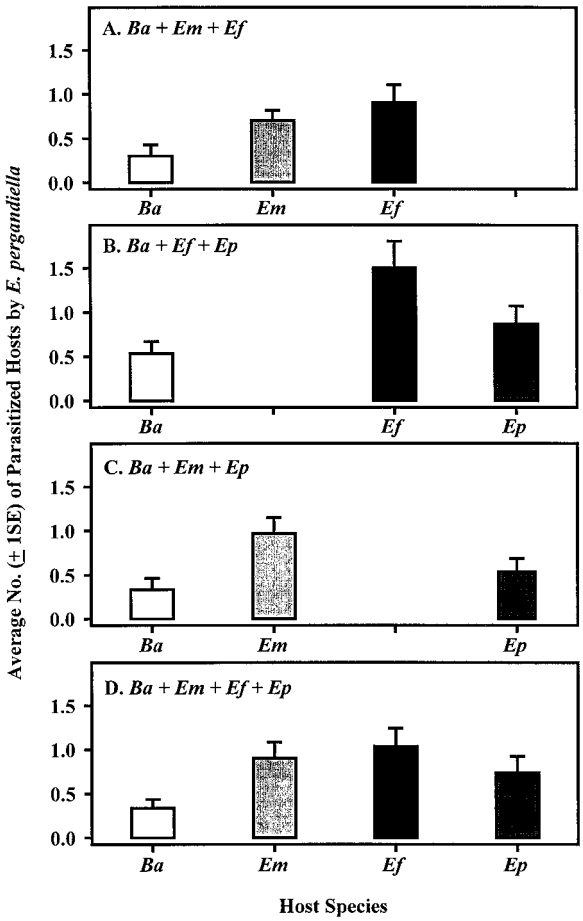

Figure 1. Average number of parasitized hosts by *E. pergandiella* during a 6-h exposure to one of four host species combinations: (A) *B. argentifolii*, *E. mundus* and *E. formosa*; (B) *B. argentifolii*, *E. formosa* and *E. pergandiella*; (C) *B. argentifolii*, *E. mundus* and *E. pergandiella*; (D) *B. argentifolii*, *E. mundus*, *E. formosa* and *E. pergandiella* (in all cases $n = 30$ female wasps, see text for details on statistical analyses).

Table 1. Results from replicated G-tests comparing the frequency of parasitized hosts of each species within each of the species combinations tested

Host species composition	Test	df[a]	G[a]	P[a]	
Ba + *Em*+ *Ef*	Pooled	2	9.84	<0.01	
	Heterogeneity	58	66.28	>0.05	n.s.
	Total	60	76.13	>0.05	n.s.
Ba + *Ef* + *Ep*	Pooled	2	15.01	<0.001	
	Heterogeneity	58	64.58	>0.05	n.s.
	Total	60	79.59	<0.05	
Ba + *Em* + *Ep*	Pooled	2	10.23	<0.01	
	Heterogeneity	58	50.11	>0.05	n.s.
	Total	60	60.34	>0.05	n.s.
Ba + *Em* + *Ef*+ *Ep*	Pooled	3	12.51	<0.01	
	Heterogeneity	87	84.78	>0.05	n.s.
	Total	90	97.29	>0.05	n.s.

[a]G = G test statistic, df = degrees of freedom, P = probability, n.s. = not significant at the 0.05 level.

Host developmental stage and E. pergandiella *parasitism.* The influence of host developmental stage on host selection and oviposition by *E. pergandiella* was tested using log linear model analyses (Table 2). In all cases, likelihood ratio tests of three way interactions (replicate × host species × developmental stage) were not significant ($P > 0.05$) indicating effects were consistent across replicates; therefore tests on main effects and their interactions were appropriate. Consistent with results from replicated *G*-tests (see previous section Host species composition and *E. pergandiella* parasitism), a significant ($P < 0.05$) host species effect was found for all host species combinations tested (Table 2). In treatment *Ba*+*Em*+*Ef* no significant differences were found between host stages for either *B. argentifolii* or *E. formosa* and this caused a lack of an overall host-stage effect (Table 2). In the same treatment however, *E. mundus* late larvae were parasitized more frequently than *E. mundus* early pupae ($G_{adj} = 8.5$, df = 1, $P < 0.005$) (Figure 2A). No significant differences in parasitism were found between host developmental stages in treatments *Ba*+*Ef*+*Ep* and *Ba*+*Em*+*Ep* (Figure 2B-C). In treatment *Ba*+*Em*+*Ef*+*Ep*, all parasitized *B. argentifolii* hosts were 3^{rd} instars (no 4^{th} instars were parasitized), and slight (but not significant) differences favoring parasitoid late larvae over early pupae (in all species) caused an overall significant host-stage effect (Table 2 and Figure 2D). In this treatment, the earlier stages of the hosts were consistently utilized more frequently than the later stages of the hosts (Figure 2D). No significant host-species × developmental stage interaction terms were detected for any of the host species combinations tested, as in all cases the later stages of the hosts experienced lower levels of parasitism (Table 2).

Superparasitism by E. pergandiella. Twenty three percent of all hosts containing eggs (66/289) were superparasitized. Most superparasitism occurred in secondary hosts (64/66). Two *B. argentifolii* 3^{rd} instars in treatment *Ba*+*Em*+*Ep* contained more than one egg per host. Superparasitism ranged between 14% and 38% of the parasitized hosts within each species combination. Superparasitism by *Encarsia pergandiella* was more frequent in *E. formosa* hosts ($G_{adj} = 6.8$, df = 1, $P < 0.05$) than in conspecific hosts in treatment *Ba*+*Ef*+*Ep* (Figure 3B). No significant differences in the number of superparasitized hosts were detected between host species for any other host species combination (Figure 3). The frequency of superparasitism on secondary hosts was independent of the developmental stage of the hosts in all host-species combinations tested except for treatment *Ba*+*Ef*+*Ep* in which a significant association ($G_{adj} = 4.7$, df = 1, $P < 0.05$) was detected (Figure 3B). In this treatment, larval *E. formosa* were superparasitized more frequently than pupae ($G_{adj} = 4.9$, df = 1, $P < 0.05$) but no difference was found in superparasitism between larval and pupal *E. pergandiella* (Figure 3B).

Table 2. Log linear analyses for the frequency of parasitized hosts relative to host species, developmental stage of the host and their interaction, for each of the species combinations in the study

Species composition	Source of variation	df	X^2	P	
Ba + Em + Ef	Host species	2	8.5	0.01	
	Stage	1	2.3	0.13	n.s.
	Species × stage	2	4.6	0.10	n.s.
	Likelihood ratio*	174	137.2	0.98	n.s.
Ba + Ef + Ep	Host species	2	14.6	<0.01	
	Stage	1	1.76	0.18	n.s.
	Species × stage	2	4.1	0.13	n.s.
	Likelihood ratio*	174	187.4	0.23	n.s.
Ba + Em + Ep	Host species	2	8.5	0.01	
	Stage	1	0.3	0.47	n.s.
	Species × stage	2	3.2	0.60	n.s.
	Likelihood Ratio*	174	146.5	0.93	n.s.
Ba + Em + Ef + Ep	Host species	3	8.3	0.04	
	Stage	1	7.1	<0.01	
	Species × stage	3	4.3	0.23	n.s.
	Likelihood ratio*	232	197.9	0.94	n.s.

*In all cases the model used to test the likelihood ratio (three way interaction) was: response = replicate × host species × host developmental stage; a non significant likelihood ratio ($P > 0.05$) indicates appropriateness of chi-square tests on main effects and their interaction (see Sokal & Rohlf, 1995).

Discussion

Encarsia pergandiella did not show a secondary host preference when two heterospecific hosts, *E. mundus* and *E. formosa*, were included as choices for female wasps (treatments *Ba+Em+Ef* and *Ba+Em+Ef+Ep*). However, *E. pergandiella* showed a preference for heterospecific secondary hosts over conspecific hosts in treatments were only one heterospecific secondary host was presented as a choice for female wasps (treatments *Ba+Ef+Ep* and *Ba+Em+Ep*). Parasitism and superparasitism by *E. pergandiella* were higher in *E. formosa* than in *E. pergandiella* hosts in treatment *Ba+Ef+Ep*, and parasitism was higher in *E. mundus* than in *E. pergandiella* hosts in treatment *Ba+Em+Ep*. Host species preferences by *E. pergandiella* were not influenced by the developmental stage of the hosts; however, *E. formosa* late larvae were parasitized and superparasitized more often than early pupae.

Results of this study are similar to previous studies on host selection by heteronomous hyperparasitoids in which *Encarsia* species preferred to parasitize heterospecific hosts over conspecific hosts, or exhibited no secondary host preferences (Buijs et al., 1981; Dowell et al., 1981; Gerling et al., 1987; Avilla et al., 1991; Williams, 1991; Pedata & Hunter, 1996). The observed pattern may result from kin-selection whereby females should prefer heterospecific hosts if there is a risk of killing a related individual by parasitizing conspecific hosts (Williams, 1991; Godfray, 1994). Thus, avoidance of conspecific hosts may result in greater inclusive fitness of discriminating females. Although host preferences have been reported for other heteronomous hyperparasitoids, our study is the first one to suggest a secondary host preference by *E. pergandiella*. Both Buijs et al. (1981) and Pedata & Hunter (1996) found no preference by *E. pergandiella* between *E. formosa* hosts and conspecific hosts. This lack of secondary host preferences for heterospecific hosts over conspecific hosts by *E. pergandiella* in previous studies may have been due to the suitability of heterospecific hosts relative to that of conspecific

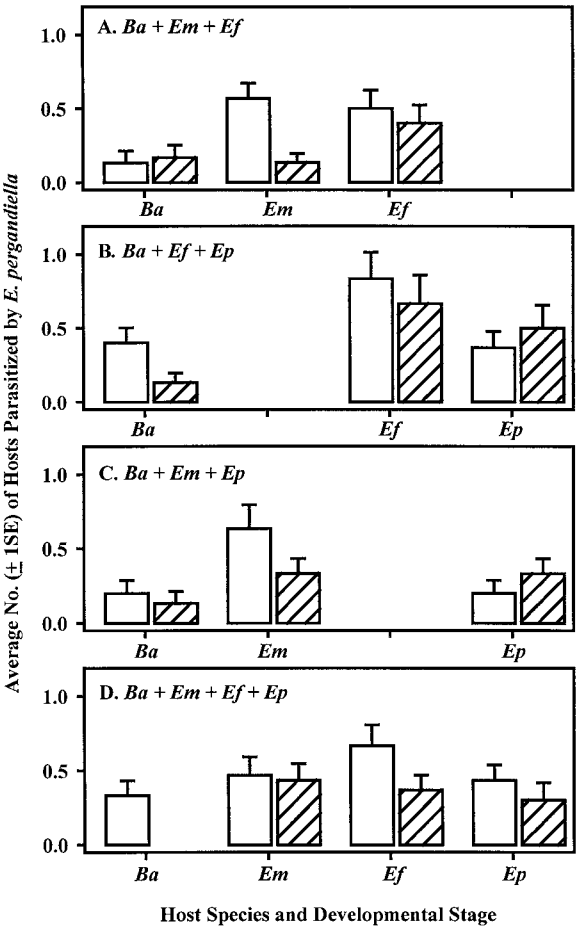

Figure 2. Average number of parasitized hosts by *E. pergandiella* of each host species and developmental stage during a 6-h exposure to one of four host species combinations: (A) *B. argentifolii*, *E. mundus* and *E. formosa*; (B) *B. argentifolii*, *E. formosa* and *E. pergandiella*; (C) *B. argentifolii*, *E. mundus* and *E. pergandiella*; (D) *B. argentifolii*, *E. mundus*, *E. formosa* and *E. pergandiella.* Clear bars represent early developmental stage of the host, hatched bars represent late developmental stage (in all cases $n = 30$ female wasps, see text for details on statistical analyses and developmental stages for each species).

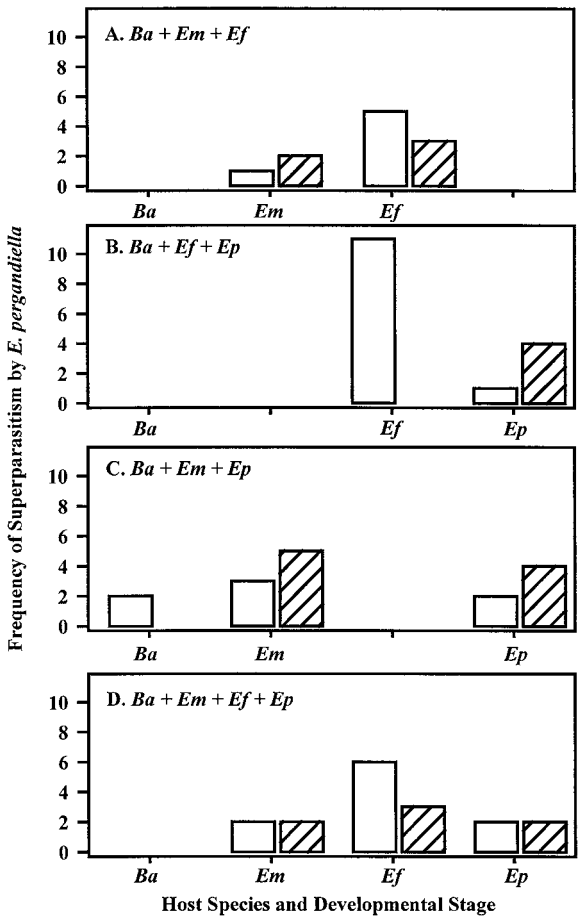

Figure 3. Frequency of superparasitized hosts (those bearing more than one egg) by *E. pergandiella* during a 6-h exposure to one of four host species combinations: (A) *B. argentifolii*, *E. mundus* and *E. formosa*; (B) *B. argentifolii*, *E. formosa* and *E. pergandiella*; (C) *B. argentifolii*, *E. mundus* and *E. pergandiella*; (D) *B. argentifolii*, *E. mundus*, *E. formosa* and *E. pergandiella.* Clear bars represent early developmental stage of the host, hatched bars represent late developmental stage (in all cases $n = 30$ female wasps, see text for details on statistical analyses and developmental stages for each species.

hosts. Previous studies have exposed *E. pergandiella* to the prepupal or pupal stage of *E. formosa* and conspecific hosts, and have used *T. vaporariorum* as the primary host for the parasitoids. *Encarsia formosa* is known to cause *T. vaporariorum* cuticle to melanize (Pedata & Hunter, 1996) and this may play a role determining its suitability as a secondary host relative to unmelanized conspecific hosts. In contrast, *E. formosa* does not cause *B. argentifolii* cuticule to melanize, and in this primary host its suitability may be higher than that of conspecific hosts when the two secondary hosts are available for parasitization. In our studies we found that *E. pergandiella* parasitized *E. formosa* more frequently than conspecific hosts in treatment *Ba+Ef+Ep*. The use of *B. argentifolii* as the primary host in our experiments may have influenced the relative suitability of *E. formosa* as a secondary host for *E. pergandiella* and may have caused the observed secondary host preference for heterospecific over conspecific hosts.

Results from log-linear analyses of the frequency of parasitism relative to host species and developmental stage indicated that host developmental stage did not significantly influence host species selection

by *E. pergandiella*. This was apparent from the lack of a significant host species × developmental stage interaction term for all host species combinations tested. These results indicate that observed differences in parasitism by *E. pergandiella* favoring heterospecific hosts over conspecific hosts (treatments *Ba+Ef+Ep* and *Ba+Em+Ep*) were not due to differences in host suitability between developmental stages of these hosts. Interstingly however, *E. mundus* LL were parasitized more often than *E. mundus* EP in two of the treatments. In pair-wise choice tests, Hunter & Kelly (1998) found no difference in host preference by the heteronomous *E. sophia* (= *E. transvena*) between conspecific prepupae and *E. eremicus* prepupae. However, *E. sophia* successfully attacked only late larvae-prepupae of conspecific females compared to all life-stages (prepupae, early pupae, and late pupae) of *E. eremicus* (Hunter & Kelly, 1998). A preference for heterospecific *E. eremicus* hosts by *E. sophia* may have been found in multiple choice tests including all susceptible life stages of both conspecific and heterospecific hosts.

The levels of superparasitism observed in our study are similar to those reported previously in tests of host selection by *E. pergandiella* (Hunter, 1989b; Pedata & Hunter, 1996). Pedata & Hunter (1996) found that superparasitism by *E. pergandiella* was higher on *E. meritoria* hosts than conspecific hosts. They suggested that higher levels of superparasitism may reflect a secondary host preference for the heterospecific *E. meritoria* over conspecific hosts but their conclusion was tentative because they could not separate host species effect from host developmental effect because *E. meritoria* hosts were presented at a different developmental stage than conspecific hosts. In our studies we simultaneously exposed *E. pergandiella* to both heterospecific and conspecific hosts at two developmental stages and found that *E. pergandiella* superparasitized *E. formosa* hosts more frequently than conspecific hosts in treatment *Ba+Ef+Ep*. In addition to higher superparasitism, a larger number of *E. formosa* hosts than conspecific hosts were parasitized by *E. pergandiella* in that treatment. The consistency of results from parasitism and superparasitism levels suggests that superparasitism may indeed be a reflection of a secondary host preference by heteronomous species like *E. pergandiella* as suggested by Pedata & Hunter (1996).

The results of the present study are important in the assessment of the role of heteronomous hyperparasitoids like *E. pergandiella* on biological pest control. In our study *E. pergandiella* preferred to oviposit on heterospecific hosts over conspecific hosts in two of the three treatments containing conspecific hosts. This suggests that *E. pergandiella* may disrupt host population suppression by effective primary parasitoids like *E. formosa* and *E. mundus*. However, the lack of secondary host preferences in treatments *Ba+Ef+Em* and *Ba+Em+Ef+Ep* suggests that under field situations where several species of secondary host may be available for male development, *E. pergandiella* may not differentially influence populations of secondary host species. Cases of successful biological control involving heteronomous species have been reported for other whitefly species including *A. woglumi* and *D. citri* in citrus (Rose & DeBach, 1981; Nguyen et al., 1983; Thompson et al., 1987). In a recent field cage study, releases of *E. pergandiella* with *E. mundus* and releases of *E. pergandiella* with *E. formosa* and *E. mundus*, were successful in reducing *B. argentifolii* populations to levels well below those in the absence of parasitoids. In addition, interactions among these parasitoids did not inhibit the ability of individual species to suppress their host populations (Bográn et al., 2002). Our results and the available field evidence indicate that other mechanisms, in addition to host species preferences, may influence the effectiveness of heteronomous hyperparasitoids like *E. pergandiella* as biological control agents. Heteronomous hyperparasitoids should not be dismissed as potential candidates for future biological pest control.

Acknowledgements

We would like to thank W. Jones (USDA ARS-BIRU) for access to laboratory facilities and insect colonies, and B. Warfield (USDA ARS-BIRU) and A. Chavarria (USDA APHIS-MPPC) for their help rearing whitefly and parasitoids. We would also like to thank USDA-CSREES National Research Initiative Grants Program (grant: 95-37302-1938) and the California Department of Food and Agriculture (CDFA; grant: RF96-00647) for grants awarded to K.M.H, and the Department of Entomology, Texas A&M University for a Graduate Research Assistantship in Biological Control awarded to C.E.B that funded this research.

References

Avilla , J., J. Anadón, M. J. Sarasúa & R. Albajes, 1991. Egg allocation of the autoparasitoid *Encarsia tricolor* at different relative densities of the primary host (*Trialeurodes vaporariorum*) and two secondary hosts (*Encarsia formosa* and *E. tricolor*). Entomologia Experimentalis et Applicata 59: 219–227.

Berg, M. A. van den, G. Hoeppner & J. Greenland, 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.

Bográn, C. E., J. Obrycki & R. Cave, 1998. Assessment of biological control of *Bemisia tabaci* in Honduras. Florida Entomologist 81: 284–295.

Bográn, C. E., K. M. Heinz & M. A. Ciomperlik, 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology 83: 653–668.

Briggs, C. J., 1993. Competition among parasitoid species on a stage-structured host and its effect on host suppression. American Naturalist 141: 372–397.

Briggs, C. J. & T. R. Collier, 2001. Autoparasitism, interference and parasitoid-pest population dynamics. Theoretical Population Biology 60: 33–57.

Buijs, M. J., I. Pirovano & J. C. van Lenteren, 1981. *Encarsia pergandiella*, a possible biological control agent for the greenhouse whitefly, *Trialeurodes vaporariorum.* A study on intra- and interspecific host selection. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit (Gent) 46: 465–475.

Caballero, R., 1993. Moscas blancas neotropicales (Homoptera: Aleyrodidae): hospederos, distribucion, enemigos naturales e importancia economica. In: L. Hilje & O. Arboleda (eds), Las Moscas Blancas (Homoptera: Aleyrodidae) en America Central y el Caribe. Centro Agronomico Tropical de Investigación y Enseñanza; Serie Técnica No. 205, Turrialba, Costa Rica, pp. 10–15.

Daane, K. M., M. S. Barzman, C. E. Kennett & L. E. Caltagirone, 1991. Parasitoids of black scale in California: establishment of *Prococcophagus probus* Annecke & Mynhardt and *Coccophagus rusti* Compere (Hymenoptera: Aphelinidae) in olive orchards. Pan-Pacific Entomologist 67: 99–106.

De Barro, P. J., P. J. Hart & R. Morton, 2000. 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.

Dowell, R. V., D. Puckett & M. Johnson, 1981. Searching and ovipositional behavior of *Amitus hesperidium* (Hym.: Platygastridae) and *Encarsia opulenta* (Hym.: Aphelinidae) parasitoids of the citrus blackfly (Hom.: Aleyrodidae). Entomophaga 26: 233–239.

Enkegaard, A., 1993. *Encarsia formosa* parasitizing the poinsettia-strain of the cotton whitefly *Bemisia tabaci* on poinsettia: bionomics in relation to temperature. Entomologia Experimentalis et Applicata 69: 251–261.

Foltyn S. & D. Gerling, 1985. The parasitoids of the aleyrodid *Bemisia tabaci* in Israel: development, host preference and discrimination of the Aphelinid wasp *Eretmocerus mundus.* Entomologia Experimentalis et Applicata 38: 255–260.

Gerling, D., 1990. Whiteflies: their Bionomics, Pest Status and Management. Intercept Ltd., Andover, Hants, UK.

Gerling, D., D. Spivak & S. B. Vinson, 1987. Life history and host discrimination of *Encarsia deserti* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Homoptera: Aleyrodidae). Annals of the Entomological Society of America 80: 224–229.

Godfray, H. C. J., 1994. Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press. Princeton, NJ.

Goolsby, J. A., M. A. Ciomperlik, B. C. Legaspi Jr., J. C. Legaspi & L. E. Wendel, 1998. Laboratory and field evaluation of exotic parasitoids of *Bemisia tabaci* (Gennadius) (Biotype 'B')(Homoptera: Aleyrodidae) in the Lower Rio Grande Valley of Texas. Biological Control 12: 127–135.

Goolsby, J. A., M. A. Ciomperlik, A. A. Kirk, W. A. Jones, B. C. Legaspi, J. C. Legaspi, R. A. Ruiz, D. C. Vacek & L. E. Wendel, 2000. Predictive and empirical evaluation for parasitoids of *Bemisia tabaci* (biotype 'B'), based on morphological and molecular systematics. In: A. D. Austin & M. Dowton (eds), Hymenoptera: Evolution, Biodiversity and Biological Control. CSIRO Publishing, Collingwood, Australia, pp. 347–358.

Heinz, K. M. & M. P. Parrella, 1994. Poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz.) cultivar-mediated differences in performance of five natural enemies of *Bemisia argentifolii* Bellows and Perring, n.sp. (Homoptera: Aleyrodidae). Biological Control 4: 305–318.

Hoddle, M. S. & R. G. Van Driesche, 1999. Evaluation of *Eretmocerus eremicus* and *Encarsia formosa* (Hymenoptera: Aphelinidae) Beltsville strain in commercial greenhouses for biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on colored poinsettia plants. Florida Entomologist 82: 556–569.

Hoddle, M. S., R. G. Van Driesche & J. P. Sanderson, 1998. Biology and use of the whitefly parasitoid *Encarsia formosa.* Annual Review of Entomology 43: 645–669.

Hunter, M. S., 1989a. Suitability of stages of female *Encarsia pergandiella* (Hymenoptera: Aphelinidae) for development of conspecific male hyperparasitoids. Entomophaga 34: 265–274.

Hunter, M. S., 1989b. Sex allocation and egg distribution of an autoparasitoid, *Encarsia pergandiella* (Hymenoptera: Aphelinidae). Ecological Entomology 14: 57–67.

Hunter, M. S., 1993. Sex allocation in a field population of an autoparasitoid. Oecologia 93: 421–428.

Hunter, M. S. & S. E. Kelly, 1998. Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of vulnerability of conspecific and native heterospecific hosts. Entomologia Experimentalis et Applicata 89: 249–259.

Hunter, M. S. & J. B. Woolley, 2001. Evolution and behavioral ecology of heteronomous aphelinid parasitoids. Annual Review of Entomology 46: 251–290.

Jones, W. A. & S. M. Greenberg, 1999. Host instar suitability of *Bemisia argentifolii* (Homopetera: Aleyrodidae) for the parasitoid *Encarsia pergandiella* (Hymenoptera: Aphelinidae). Journal of Agricultural and Urban Entomology 16: 49–57.

Lenteren, J. C. van, H. J. W. van Roermund & S. Sütterlin, 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa*: how does it work? Biological Control 6: 1–10.

Liu, T. X. & P. Stansly, 1996. Oviposition, development and survivorship of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in four instars of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Annals Entomological Society of America 89: 96–102.

Mills, N. J. & A. P. Gutierrez, 1996. Prospective modelling in biological control: an analysis of the dynamics of heteronomous hyperparasitism in a cotton-whitefly-parasitoid system. Journal of Applied Ecology 33: 1379–1394.

Mound, L. & S. Halsey, 1978. Whitefly of the World, a Systemic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data. British Museum (Natural History), John Wiley and Sons Inc, London, UK.

Nafus, D. M. & J. R. Nechols, 1995. Orange spiny whitefly. In: J. R. Nechols (ed.), Biological Control in the Western United States. University of California, Division of Agriculture and Natural Resources, Publication 3361. Oakland, California, USA.

Nechols, J. R. & D. M. Nafus, 1995. Spiralling whitefly. In: J.R. Nechols (ed.), Biological Control in the Western United States. University of California, Division of Agriculture and Natural Resources, Publication 3361. Oakland, California, USA.

Nguyen, R., J. R. Brazzel & C. Poucher, 1983. Population density of the citrus blackfly, *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae) and its parasites in urban Florida in 1979–1981. Environmental Entomology 12: 78–884.

Onillon, J. C., P. Maignet & C. Cocquepot, 1994. Premiers resultats sur l'efficacité d'*Encarsia pergandiella* (Hymenopt., Aphelinidae) dans le controle de *Bemisia tabaci* (Homopt. Aleurodidae) en serres de tomate. In: R. Albajes (ed), Integrated Control in Protected Crops; Mediterranean Climate. International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section (IOBC/WPRS) Bulletin, Vol. 17, pp. 71–80.

Pedata, P. A. & M. S. Hunter, 1996. Secondary host choice by the autoparasitoid *Encarsia pergandiella.* Entomologia Experimentalis et Applicata 81: 207–214.

Polaszek, A., G. A. Evans & F. D. Bennett, 1992. *Encarsia* parasitoids of *Bemisia tabaci* (Hymenoptera: Aphelinidae, Homoptera: Aleyrodidae): a preliminary guide to identification. Bulletin of Entomological Research 82: 375–392.

Powell, D. A. & T. S. Bellows Jr., 1992. Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). Environmental Entomology 21: 651–658.

Riley, D. G. & M. A. Ciomperlik, 1997. Regional population dynamics of whitefly (Homopetera: Aleyrodidae) and associated parasitoids (Hymenoptera: Aphelinidae). Environmental Entomology 26: 1049–1055.

Rose, M. & P. DeBach, 1981. Citrus whitefly parasites established in California. California Agriculture 36 (7&8): 21–23.

Rosenheim, J. A., 1998. Higher order predators and the regulation of insect herbivore populations. Annual Review of Entomology 43: 421–447.

Schauff, M. E., G. A. Evans & J. M. Heraty. 1996. A pictorial guide to the species of Encarsia (Hymenoptera: Aphelinidae) parasitic on whiteflies (Homoptera: Aleyrodidae) in North America. Proceedings of the Entomological Society of Washington 98: 1–35.

Schuster D. J. & J. F. Price, 1996. Parasitization of *Bemisia argentifolii* (Hom.: Aleyrodidae) by *Encarsia pergandiella* (Hym.: Aphelinidae). Entomophaga 41: 95–103.

Schuster, D. J., G. A. Evans, F. D. Bennett, P. A. Stansly, R. K. Janssonii, G. L. Leibee & S. E. Webb, 1998. A survey of parasitoids of *Bemisia* spp. whiteflies in Florida, the caribbean and Central and South America. International Journal of Pest Management 44: 255–260.

Simmons, A. M., 1998. Survey of the parasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in costal South Carolina using yellow sticky traps. Journal of Entomological Science 33: 7–14.

Sokal, R. R. & F. J. Rohlf, 1995. Biometry: the Principles and Practice of Statistics in Biological Research, 3rd ed. W. H. Freeman and Company. New York, USA.

Sullivan, D. J. & W. Völkl, 1999. Hyperparasitism, multitrophic ecoloy and behaviour. Annual Review of Entomology 42: 291–315.

Thompson, C. R., J. A. Cornell & R. I. Sailer, 1987. Interactions of parasites and a hyperparasite in biological control of citrus blackfly*, Aleurocanthus woglumi* (Homoptera: Aleyrodidae), in Florida. Environmental Entomology 15: 140–144.

Viggiani, G., 1984. Bionomics of the Aphelinidae. Annual Review of Entomology 29: 257–276.

Viggiani, G. & P. Mazzone, 1980. Sull'introduzione in Italia di *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae), parassita di *Trialeurodes vaporariorum* (Westw.) (Homoptera: Aleyrodidae). Bolletino del Laboratorio Entomologia Agraria Fillipo Silvestri 37: 39–43.

Waage, J. K., 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation. In: J. K. Waage & D. Greathead (eds), Insect Parasitoids. Academic Press, London, pp. 63–95.

Walter, G. H., 1983. 'Divergent male ontogenies' in Aphelinidae (Hymenoptera: Chalcidoidea): a simplified classification and a suggested evolutionary sequence. Biological Journal of the Linnean Society 46: 261–282.

Walter, G. H., 1988. Activity patterns and egg production in *Coccophagus barletti*, an aphelinid parasitoid of scale insects. Ecological Entomology 13: 95–105.

Williams, T., 1991. Host selection and sex ratio in a heteronomous hyperparasitoid. Ecological Entomology 16: 377–386.

Williams, T., 1996. Invasion and displacement of experimental populations of a conventional parasitoid by a heteronomous hyperparasitoid. Biocontrol Science and Technology 6: 603–618.

Williams, T. & A. Polaszek, 1996. A re-examination of host relations in the Aphelinidae (Hym.:Chalcidoidae). Biological Journal of the Linnean Society 57: 35–45.

Zolnerowich, G. & M. Rose, 1998. *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) imported and released in the United States for control of *Bemisia* (*tabaci* complex) (Homoptera: Aleyrodidae). Bulletin of the Entomological Society of Washington 100: 310–323.