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Authors: Hu, Jing S., Gelman, Dale B., and Blackburn, Michael B.

Source: Journal of Insect Science, 3(28) : 1-10

Published By: Entomological Society of America

URL: https://doi.org/10.1673/031.003.2801

Hu JS, Gelman DB, Blackburn MB. 2003. Age-specific interaction between the parasitoid, *Encarsia formosa* and its host, the silverleaf whitefly, *Bemisia tabaci* (Strain B). 10pp. *Journal of Insect Science*, 3:28, Available online: insectscience.org/3.28

Journal of Insect Science

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Age-specific interaction between the parasitoid, *Encarsia formosa* and its host, the silverleaf whitefly, *Bemisia tabaci* (Strain B)

Jing S. Hu, Dale B. Gelman and Michael B. Blackburn

Insect Biocontrol Laboratory, USDA, ARS, PSI, Beltsville, MD 20705, USA
gelmand@ba.ars.usda.gov

Received 7 January 2003, Accepted 26 July 2003, Published 29 August 2003

Abstract

The effect of hostage, the instar of *Bemisia tabaci* (Gennadius) parasitized, on the growth and development of *Encarsia formosa* (Gahan) was studied. *E. formosa* was able to parasitize and complete its life cycle no matter which instar of *B. tabaci* (Strain B), [also identified as *B. argentifolii* (Bellows and Perring)], was provided for oviposition, but parasitoid development was significantly slower when 1st or 2nd instar *B. tabaci* rather than 3rd or 4th instars were parasitized. Host age influenced the day on which *E. formosa* nymphs hatching from eggs was first observed. Mean embryonic development was significantly longer when 1st (5.4 days) rather than 2nd, 3rd or 4th instars (4.1, 3.4 and 3.5 days, respectively) were parasitized. The duration of the 1st instar parasitoid and the pupa, but not the 2nd or 3rd instar parasitoid, were also significantly greater when 1st instars were parasitized than when older host instars were parasitized. Interestingly, no matter which instar was parasitized, the parasitoid did not molt to the 3rd instar until the 4th instar host had reached a depth of about 0.23 mm (Stage 4-5) and had initiated the nymphal-adult molt and adult development. Histological studies revealed that whitefly eye and wing structures had either disintegrated or were adult in nature whenever a 3rd instar parasitoid was present. It appears, then, that the molt of the parasitoid to its last instar is associated with the host whitefly's nymphal-adult molt. However, the initiation of the host's final molt, while a prerequisite for the parasitoid's 2nd-3rd instar molt, did not necessarily trigger this molt. In contrast to its significant effect on various aspects of parasitoid development, host instar did not significantly influence the mean size of the parasitoid larva, pupa, or adult. Larval and pupal length and adult head width were similar for all parasitoids, regardless of which host instar was parasitized as was adult longevity. Adult parasitoid emergence was more synchronous when 2nd, 3rd and 4th instars were parasitized than when 1st instars were parasitized. Results are compared with those reported when the greenhouse whitefly, *Trialeurodes vaporariorum,* was parasitized by *E. formosa,* and provide possible explanations for why *T. vaporariorum* is a more suitable host than *B. tabaci* for *E. formosa*.

Keywords: endoparasitoid, parasitoid development, host-parasite interactions, nymphal-adult molt

Introduction

Since it was first discovered in Florida in 1986 (Price et al. 1987), *Bemisia tabaci* (Strain B), also identified as *Bemisia argentifolii* (Bellows *et al.* 1994; Naranjo and Ellsworth 2001), has become one of the most important pests of broadleaf field crops worldwide, costing farmers and greenhouse growers millions of dollars each year (Perkins and Bassett 1988; Gill 1992; Zalom *et al.* 1995; Liu and Stansly 1996; Chu and Henneberry 1998). The explosive increase in the *B. tabaci* population has been attributed, in part, to the heavy application of pesticides to control this pest, which, in turn, has resulted in the development of pesticide resistance (Prabhaker et al. 1985; Cahill et al. 1996; Henneberry et al. 1998) and in the decline of the whitefly's natural enemies (Bellows et al. 1992; van Lenteren 2000). Therefore, it became important to increase the use of effective biological control agents that can efficiently control the pest, are safe for the environment and are acceptable to farmers and greenhouse growers (Heinz 1995).

Encarsia formosa, a uniparental, thelytokous hymenopteran parasitoid (Agekyan 1982), was first discovered and utilized to control the greenhouse whitefly, *Trialeurodes vaporariorum,* in greenhouses in England by Speyer (1927). Since then, this parasitoid has been used worldwide for controlling *T. vaporariorum* in greenhouses (Vet *et al.* 1980; Noldus and van Lenteren 1990), most recently by augmentative inundative release (Hoddle *et al.* 1997a, b). Although able to successfully parasitize *B. tabaci*, *E. formosa* is not as effective in controlling this pest species of whitefly as in controlling *T. vaporariorum* (Bosclair *et al.* 1990; Parrella et al. 1991; Henter and van Lenteren 1996; Hoddel et al. 1998). In order to maximize *E. formosa* success in biological control, it is important to elucidate the interactions between the parasitoid and its whitefly host, including the effects of the host on parasitoid development

(Hoddle et al. 1998). Host-parasite interactions related to the developmental regulation of *E. formosa* by *T. vaporariorum* have recently been reported (Hu *et al.* 2002). Liu and Stansly (1996) have described the effects of *B. tabaci* age on oviposition, development, and survivorship of *Encarsia pergandiella,* while Donnell and Hunter (2002) have studied the effects of differences in egg yolk content on developmental rates of *E. pergandiella* and *E. formosa* in *B. tabaci* hosts that were parasitized as 1st or 4th instars.

Host age and size affect the parasitoid's perception of host suitability (Smilowitz and Iwantsch 1973; Beckage and Riddiford 1978; Harvey et al. 1999; Hu and Vinson 2000; Hu et al. 2002). In general, a parasitoid's growth and development are enhanced when older hosts that are larger and nutritionally richer are parasitized than when younger hosts are selected for parasitization, but the effect of host age will vary with the parasitoid-host system under investigation (Beckage and Riddiford 1978; Pennacchio et al. 1993; Hu and Vinson 2000; Hu et al. 2002). Here we describe in detail the effect of *B. tabaci* host instar parasitized (1st through 4th) on the duration of parasitoid development, parasitoid size, emergence pattern and parasitoid longevity. We also provide evidence for a host cue that appears to trigger an important developmental event in the parasitoid. Results are compared with those reported for the parasitization of *T. vaporariorum* by *E. formosa* and may explain why *T. vaporariorum* is a more suitable host for *E. formosa* than is *B. tabaci.*

Materials and Methods

Insect culture

B. tabaci (Gennadius) (Homoptera: Aleyrodidae) was maintained on a variety of plants [green bean, *Phaseolus vulgaris*, cultivar Roma II (Burpee, Warminster, PA, USA), cotton, *Gossypium hirsutum*, cultivar Stoneville ST 474 (Stoneville Pedigreed Seed Co., Maricopa, AZ, USA), tomato, *Lycopersicon esculentum*, cultivar Bush Big Boy (Burpee,Warminster, PA, USA), poinsettia, *Euphorbia pulcherrima*, cultivar Freedom Red (Paul Ecke Ranch, Encinitas, CA, USA), and eggplant, *Solanum melongena*, cultivar Millionaire Hybrid (Burpee, Warminster, PA, USA)] in a walk-in growth chamber at a constant temperature of 26 ± 2° C, relative humidity 60-80%, and a photoperiodic regimen of L:D 16:8 (Hu et al. 2002). Twenty-eight fluorescent cool white 30-W bulbs (G30-TB, GE) were installed to maintain a light intensity of 17,000 lux. Plants were maintained and *E. formosa* (Gahan) (Hymenoptera: Aphelinidae) (Beltsville strain) was reared as described by Hu *et al.* (2002).

Parasitization

In all experiments, rooted green bean cuttings served as the host plant. In order to determine the effect of host instar parasitized on the *in vivo* growth and development of *E. formosa*, rooted green bean leaf cuttings were infested with *B. tabaci* (Hu et al. 2002). When the whiteflies reached the appropriate stage [sessile 1st instar (0.5-1 d post-oviposition), early 2nd, 3rd, and 4th instars], they were parasitized as described by Hu *et al.* (2002) by exposing them to the Beltsville strain of *E. formosa* (Heinz and Parella 1994; Hoddle et al. 1997b). *B. tabaci* nymphal instars were identified by measuring the body length of the whitefly, and young instars were selected based on their relatively flat appearance (Gelman et al. 2002b). Parasitized whiteflies were maintained in incubators at 26 ± 2°C, with a relative humidity of about 55%, under a photoperiodic regimen of L:D 16:8 and a light intensity of 600 lux. For histological studies of parasitized *B. tabaci*, the standard commercial strain of *E. formosa* was provided by Rincon-Vitova (Ventura, CA, USA).

Determination of parasitoid size and rate of development

For each instar parasitized, at least 10 parasitized whitefly nymphs were dissected each day post-parasitization until parasitoid adults emerged. The instar and stage within the instar (early or late) of each developing parasitoid were recorded (Hu et al. 2002). Length of the parasitoid larva and pupa and head width of the adult (measured immediately after emergence) served as measures of size. Host developmental stages were recorded at the time of dissection in order to determine chronological relationships between *B. tabaci* and *E. formosa*. Host instar was identified based on body length, and the stage of the 4th instar was determined by measuring body depth (Gelman et al. 2002a, b). Briefly, Stages 1, 2 and 3 were characterized by body depths of 0.1, 0.15 and 0.2 ± 0.02 mm, respectively; Stage 4 had a body depth of 0.23 – 0.26 mm and Stage 5 had a body depth of ≥ 0.27 mm.

Longevity was compared for adults that emerged from host whiteflies parasitized as instar 1, 2, 3 or 4. Unfed adults were maintained in petri dishes until death. The number of dead whiteflies was recorded daily.

Data analysis

At least three replications, each consisting of 10 sample parasitoids, were performed for each host instar parasitized. Data were analyzed using the program STATISTIX (Analytical Software, Inc., P. O. Box 12185, Tallahassee, FL, USA). One-way ANOVA ($\alpha = 0.05$) was used to compare the differences in growth and development of *E. formosa* based on host age. When F-tests were significant, mean comparisons were performed using the Tukey's HSD comparison of means test ($\alpha = 0.05$).

Histological methods

Parasitized whiteflies were collected on days 5, 6, 7, and 8 following oviposition and fixed in Carnoy's #2 [60% ethanol: 30% chloroform: 10% glacial acetic acid (Davenport 1960)] for 2-3 h. The fixed nymphs were dehydrated with three changes of absolute ethanol, then transferred through four changes of xylene and placed in paraffin (Paraplast Xtra) at 60°C overnight. After transfer to fresh paraffin in embedding molds, the whiteflies were oriented and chilled rapidly in ice water. The embedded nymphs were sectioned at 5 μm on a rotary microtome. Sections were relaxed on water at 40° C, mounted on albumin-coated slides, dried and placed horizontally in a drying oven at 40° C overnight.

Mounted sections were deparaffinized in three changes of xylene, transferred through three changes of absolute ethanol and rehydrated through a series of aqueous ethanol solutions (95%, 90%, 70% and 50%). Sections were stained with Carazzi's hematoxylin (Carazzi 1911) followed by Casson's trichrome (Kiernan 1990) and examined with a Nikon Eclipse 600 compound microscope equipped with Differential Interference Contrast optics. Photomicrographs

were taken with a Nikon DMX 1200 CCD camera. Parasitoid instars were identified based on the development of the pharyngeal musculature as described previously; the pharyngeal dilator muscles of 3rd instar *E. formosa* are significantly more developed than those of 2nd instars (Blackburn et al. 2002).

Results

Development of Encarsia formosa

The developmental rate of *E. formosa* varied with host age (instar parasitized) (Table 1). The duration of parasitoid development from oviposition to adult emergence was significantly (F = 312.73; df = 3, 998; $P \leq 0.001$) longer when 1st instars were parasitized than when 2nd, 3rd or 4th instars were parasitized. Development was also significantly slower when a 2nd instar was parasitized than when 3rd or 4th instars were parasitized. There was no significant difference in developmental times when 3rd or 4th instars were parasitized. Durations of individual parasitoid stages were also influenced by host age (Table 1). Parasitoid embryonic (from egg oviposition to hatch) (F = 30.33; df = 3, 108; $P \leq 0.001$), 1st instar larval (F = 5.97; df = 3, 101; $P \leq 0.001$) and pupal (F = 31.78; df = 3, 536; $P \leq 0.001$) developmental times were significantly different depending upon host age. Developmental times for these three stages were always significantly longer when 1st instar whiteflies were provided for oviposition (Table 1). Pupae of *E. formosa* developed significantly faster when the 4th instar was parasitized than when 2nd or 3rd instars were parasitized (Table 1). However, 2nd and 3rd instar parasitoids had similar developmental rates no matter which nymphal instar was parasitized (Table 1). Longevity of *E. formosa* adults was not significantly influenced by host age (Table 2).

Table 2. Effect of *Bemisia argentifolii* instar parasitized on the longevity of *Encarsia formosa* adults.

Host instar	Longevity (Days ± S. E)
1st	1.82 ± 0.35[a]
2nd	1.89 ± 0.38[a]
3rd	1.94 ± 0.43[a]
4th	1.89 ± 0.40[a]

Days of parasitoid adult survival served as a measure of adult longevity. Each value represents the mean ± S. E. of at least 150 separate determinations. A one-way ANOVA followed by the Tukey HSD post hoc test ($\alpha \leq 0.05$) was used to determine if there were significant differences in longevity of *E. formosa* based on the host instar parasitized. Means followed by the same letter are not significantly different.

Size of Encarsia formosa

Mean larval or pupal body length and adult head width served as measures of size (Table 3). Regardless of which instar was parasitized, there were no significant differences in larval/pupal length or in adult head width (Table 3).

Emergence pattern of Encarsia formosa

Host age affected the day on which emergence peaked (number of adults that emerged on a given day/total number of adults

Table 1. Developmental duration of *Encarsia formosa** as a function of host instar parasitized.

Host instar parasitized	Developmental Duration of the Parasitoid (Day ± S.E.)					
	Embryonic	First instar	Second instar	Third instar	Pupa	Oviposition to adult emergence
1st	5.44 ± 1.48[a]	3.06 ± 1.94[a]	2.12 ± 1.44[a]	3.27 ± 1.98[a]	4.82 ± 3.07[a]	18.14 ± 2.77[a]
2nd	4.12 ± 0.65[b]	2.26 ± 1.05[ab]	2.28 ± 1.04[a]	3.11 ± 1.10[a]	3.79 ± 1.20[b]	14.90 ± 1.31[b]
3rd	3.39 ± 0.50[b]	1.65 ± 0.86[b]	2.21 ± 1.18[a]	2.85 ± 1.32[a]	3.83 ± 0.96[b]	13.93 ± 0.96[c]
4th	3.50 ± 0.51[b]	1.86 ± 0.95[b]	2.42 ± 1.10[a]	2.97 ± 1.08[a]	3.25 ± 1.18[c]	14.03 ± 1.20[c]

* Duration for each parasitoid instar and for the pupa was determined by subtracting the mean day of a given stage from the mean day of the following stage. Each value represnts the mean ± S. E of the means of at least 3 separate experiments. For each experiment, between 180 and 200 host whiteflies were dissected. A one-way ANOVA followed by the Tukey's HSD post hoc test ($\alpha \leq 0.05$) was used to determine if there were significant differences in developmental duration. Means in the same column followed by a different letter are significantly different.

Table 3. Effect of *Bemisia argentifolii* instar parasitized on the size of *Encarsia formosa*.

Host instar parasitized	Body size of the parasitoid (mm ± S.E.)				
	1st instar	2nd instar	3rd instar	Pupa	Adult
1st	0.20 ± 0.03[a]	0.41 ± 0.03[a]	0.64 ± 0.07[a]	0.64 ± 0.04[a]	0.25 ± 0.01[a]
2nd	0.22 ± 0.03[a]	0.45 ± 0.05[a]	0.66 ± 0.04[a]	0.65 ± 0.04[a]	0.26 ± 0.02[a]
3rd	0.30 ± 0.05[a]	0.49 ± 0.05[a]	0.69 ± 0.05[a]	0.68 ± 0.05[a]	0.28 ± 0.04[a]
4th	0.28 ± 0.03[a]	0.48 ± 0.06[a]	0.67 ± 0.06[a]	0.66 ± 0.03[a]	0.27 ± 0.03[a]

Mean length of larvae and mean adult head width were used to compare parasitoid size. Each value represents the mean ± S. E. of three replications. For each replication, n ≥ 10. A one-way ANOVA followed by the Tukey's HSD post hoc test ($\alpha \leq 0.05$) was used to determine if there were significant differences in mean parasitoid larval and pupal body length and for adults, mean head width, based on the *B. tabaci* instar parasitized. Means in the same column followed by the same letter are not significantly different.

that emerged) (Fig. 1). Emergence occurred on the day of (day 1) and the day after (day 2) emergence was initiated when 3rd and 4th instars were parasitized, but on day 2 when younger instars were parasitized (Fig. 1). Thus, the pattern of emergence varied with host age. When 3rd or 4th instars were parasitized, 77% of adults emerged by day 2. When 2nd instar whiteflies were parasitized, most of the parasitoids emerged on days 2 (41%) and 3 (30%); only 13% emerged on day 1. When 1st instars served as hosts, there was no significant difference in the percent parasitoids emerged on days 1, 2, 3 and 4 ($F = 1.35$; df = 3, 108; $P = 0.306$). Thus, emergence of *E. formosa* was much less synchronous when 1st instar as compared to 2nd through 4th instars were parasitized.

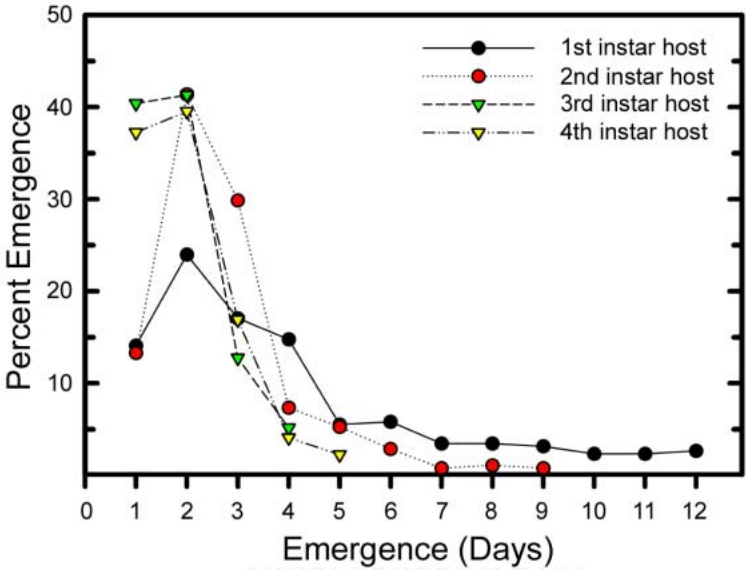

Figure 1. Emergence pattern of *Encarsia formosa* as a function of host instar parasitized. Parasitized whiteflies were examined daily and the time of parasitoid emergence was recorded. The day on which adult parasitoid emergence was first observed was designated day 1 ("onset"). Percent adult emergence for day 1 and for each succeeding day = number of adults that emerged on a given day/total number of adults that emerged. Each point represents the mean of at least three separate determinations. For each determination, n ≥ 100. To avoid confusion, standard errors have not been indicated. However, for any given point, the value of the standard error was ≤ 10% of the point's value.

Developmental chronology of Encarsia formosa

As expected, the presence of a given parasitoid instar in a particular whitefly instar varied according to the age of the host at the time of parasitization. When 1st instar whitefly nymphs were parasitized, 1st instar parasitoids were observed in 2nd, 3rd and 4th instar whiteflies, and 2nd instar parasitoids were observed in 3rd and 4th instars (Fig. 2A). When 2nd instars were parasitized, 1st instar parasitoids were observed in 3rd and 4th instar whiteflies, and 2nd instar parasitoids were observed only in 4th instar whiteflies (Fig. 2B). When 3rd instar whiteflies were parasitized, 1st, 2nd and 3rd instar parasitoids were observed only in 4th instar hosts (Fig. 2C). Importantly, regardless of which instar was parasitized, 3rd instar parasitoids were never found until the host had reached a depth equivalent to that of a Stage-4/5 4th instar.

Horizontal sections of parasitized B. tabaci

Sections from parasitized whiteflies fixed on days 5, 6, 7 and 8 post-oviposition revealed primarily 2nd and 3rd instar parasitoids. The appearance of 3rd instar parasitoids was always preceded by disintegration of the host wingbuds and eye structures, or in one case, adult development of these structures (Table 4, Fig. 3). In no case did we find a 3rd instar parasitoid in a host that had intact, immature wings or eyes.

Discussion

When placed in a no-choice arena, *E. formosa* parasitized all nymphal instars of *B. tabaci* and was able to complete development through the adult stage. These results are similar to those reported for *B. argentifolii* parasitized by *E. pergandiella* (Liu

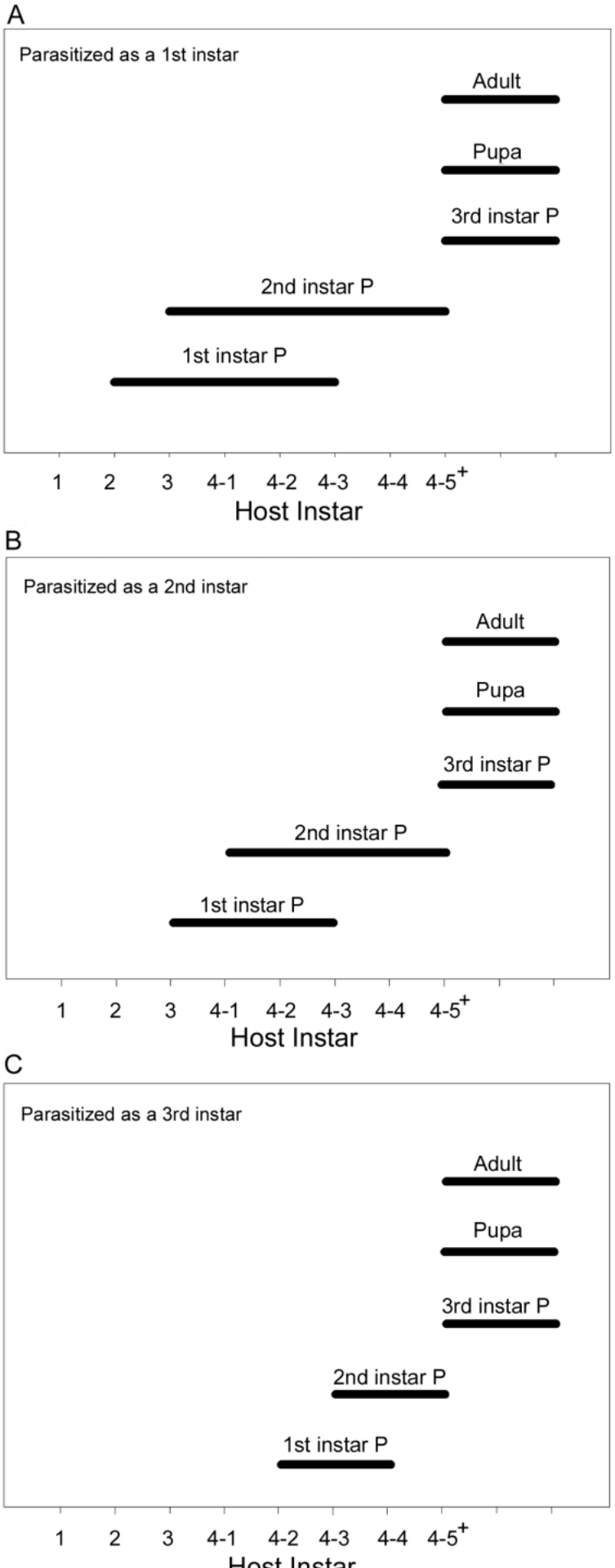

Figure 2. (Left) Developmental chronology of *Encarsia formosa* as a function of host instar parasitized. A, B and C, host parasitized as 1st, 2nd and 3rd instar nymphs, respectively. At least 10 parasitized whitefly nymphs were dissected each day post-parasitization and the stage of parasitoid development was ascertained. The whitefly instar, and for the 4th instar, stage (x-axis) in which each parasitoid instar, pupa, or adult was present is represented by a horizontal line. For example, when a first instar whitefly was parasitized, 1st instar parasitoids were detected in 2nd, 3rd and 4th instar hosts, and 2nd instar parasitoids were detected in 3rd and 4th (stages 1-5) instar hosts. P = parasitoid larva; Pupa = parasitoid pupa; Adult = adult parasitoid.

and Stansly, 1996), and for *T. vaporariorum* parasitized by *E. formosa* (Nechols and Tauber 1977; Hu et al. 2002). As reported for *T. vaporariorum* (Hu et al. 2002), 3rd and 4th instar *B. tabaci* were more suitable hosts for parasitization than 1st or 2nd instars. Parasitoids developed more rapidly and adult emergence was more synchronous when 3rd and 4th instars were parasitized than when younger instars were parasitized. Donnell and Hunter (2002) also reported significantly faster developmental times and more synchronous adult emergence rates for *E. formosa* when 4th as compared to 1st instar whiteflies were parasitized. However, the time required for 50% of *E. formosa* eggs to hatch (a measure of the duration of embryonic development) was 63.2 h (2.6 days) and 56.31 h (2.3 days) when these investigators parasitized 1st and 4th instars, respectively, while we report mean embryonic development times of 5.44 days and 3.5 days, respectively, when 1st and 4th instars were parasitized. Since in the two studies, total development times (ovipositon to adult emergence) were quite similar, the differences in embryonic developmental times are surprising. Incubation temperatures in the two studies were similar; 27 ± 1° C (Donnell and Hunter 2002) and 26 ± 2° C (this study). However, a slightly higher mean temperature, differences in relative humidity and/or host plant identity (cotton in the Donnell and Hunter study) could be responsible for the reduced times required for embryonic development reported by Donnell and Hunter.

Parasitoid developmental rates in *B. tabaci* differed from those reported for *T. vaporariorum* (Hu et al. 2002). When 1st or 2nd instar whiteflies were parasitized, *E. formosa* tended to develop faster in *B. tabaci* than in *T. vaporariorum*. Under the same rearing conditions, the developmental duration (time from oviposition through adult emergence) was 18.1 days and 14.9 days when 1st and 2nd instar *B. tabaci*, respectively, were parasitized. In contrast, the parasitoid took 21.2 and 18.4 days, respectively, to complete development when 1st and 2nd instar *T. vaporariorum* were parasitized. This difference in the parasitoid's developmental rate in the two hosts could be due to differences in host size, in host developmental rate and/or in the environmental milieu in which the parasitoid grows and develops. First and 2nd instar *B. tabaci* are smaller than their *T. vaporariorum* counterparts (Gelman et al. 2002a, b). Although it is well known that typically, the larger the host, the greater is its suitability for its parasitoid (Vinson 1990; Pennacchio et al. 1993; Hu and Vinson 2000; Hu et al. 2002), the larger *T. vaporariorum* effects a slower developmental time than the smaller *B. tabaci*, especially when 1st and 2nd instars are parasitized. Interestingly, however, soluble protein content for the various stages of 3rd and 4th instar *T. vaporariorum* is lower than

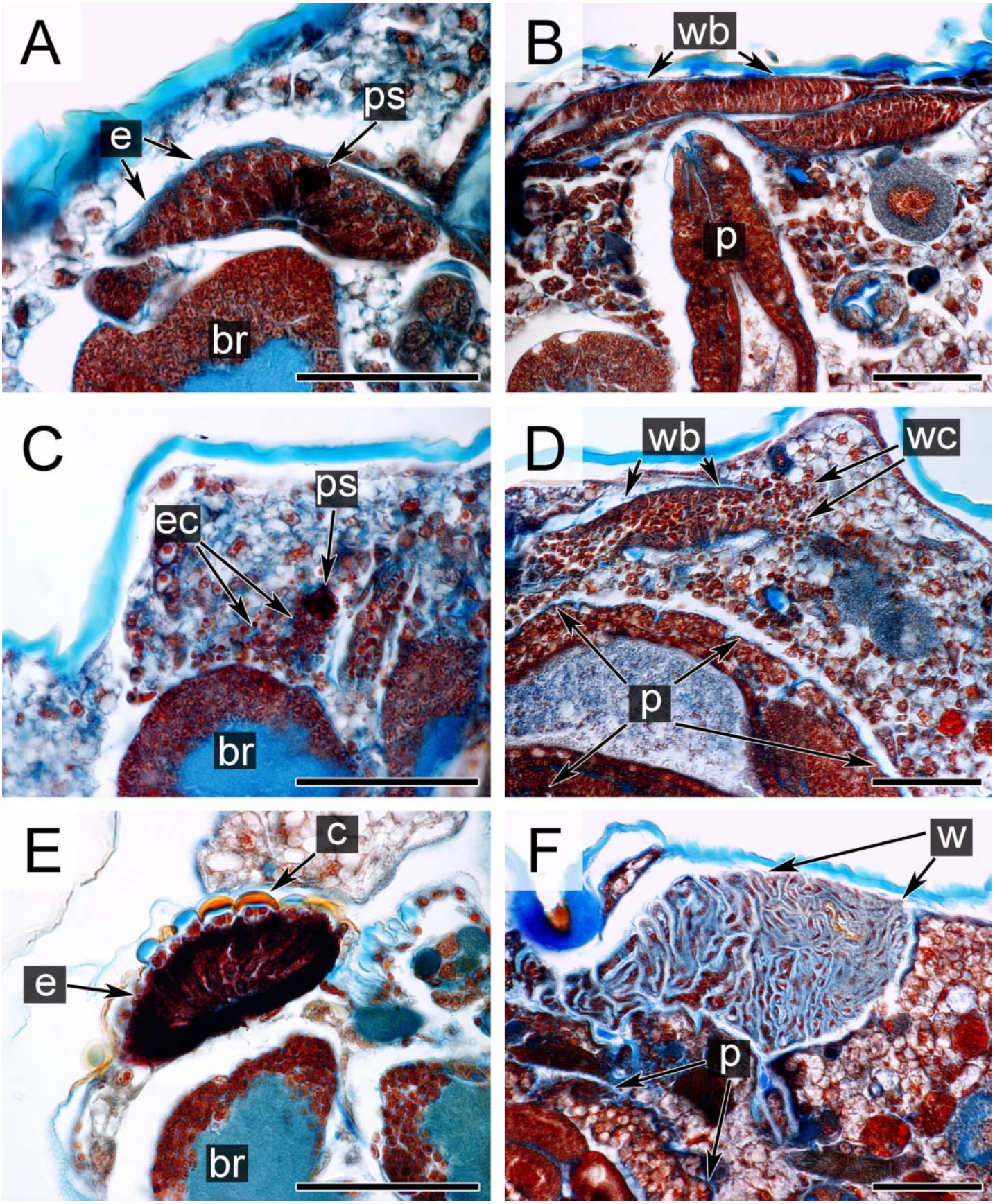

Figure 3. *Bemisia tabaci* eye and wing structure as a function of parasitoid instar present within the host whitefly. Prior to the onset of adult development, *B. tabaci* containing 2nd instar *E. formosa* have typical immature eye (A) and wing (B) structures. Upon adult development, host eye and wing structures either disintegrate (C and D, respectively) or successfully develop into adult eyes and wings (E and F). **br**, host brain; **e**, host eye; **ec**, detached host eye cells; **c**, host adult cornea; **ps**, host eye pigment spot; **wb**, host wingbud; **wc**, detached wing epidermal cells; **w**, adult host wing; **p**, parasitoid larva. Scale bars = 50 µm.

Table 4. Condition of *Bemisia argentifolii* eye and wing as a function of the parasitoid instar

		Condition of *Bemisia argentifolii* Wingbud and Eye					
Parasitoid instar	No.	Wingbuds immature	Wingbuds disintegrating	Wings adult	Eyes immature	Eyes disintegrating	Eyes adult
1st	2	2	0	0	2	0	0
2nd	24*	11	10	3	10	7	4
3rd	18*	0	17	1	0	15	1

Horizontal sections of parasitized *Bemisia argentifolii* were prepared. Results describe the condition of the host *B. argentifolii* eyes and wings five-eight days post-parasitization.

*Since the state or disintegration of *B. argentifolii* eye structures could not always be determined, there is a difference between the sample number (column 2) and the sum of reports (columns 6, 7 and 8) for 2nd and 3rd instar parasitoids.

for comparative stages of *B. tabaci* (Gelman et al. 2002a, b). Thus, increased protein content may explain the faster developmental rates of *E. formosa* in *B. tabaci*. Another explanation that is not mutually exclusive is that the time for *B. tabaci* to complete its life cycle (T = 26 ± 2° C) on green bean is less (approximately 21 days) than for *T. vaporariorum* to complete its life cycle (approximately 26 days) (unpublished results). Thus *E. formosa* may be adjusting its developmental timing to that of its host.

B. tabaci has been reported to be inferior to *T. vaporariorum* as a host (Bosclair et al. 1990; Enkegaard 1993; Szabo et al. 1993; Henter and van Lenteren 1996; Hoddle et al. 1998). The number of eggs laid, the percent of immature survival and the quality of adults is lower when B. tabaci is the host insect. In addition, although rearing *E. formosa* on *B. tabaci* for 17 generations has been reported to improve the parasitoid's performance (Bethke et al. 1991), the parasitoid performed significantly better when conditioned on *T. vaporiariorum* as compared to *B. tabaci* prior to being released on an experimental *B. tabaci* population (Henter and van Lenteren 1996). Ecdysteroid titers are approximately three times higher during the 4th instar and between two and three times lower during the 3rd instar in *B. tabaci* as compared to *T. vaporariorum* (Gelman et al. 2002a, b). The importance of host-parasite hormonal interactions is well-documented (Beckage 1985; Lawrence 1986; 1990; Beckage and Gelman 2001), and these differences in ecdysteroid titer may be contributing to the reduced suitability of *B. tabaci* as compared to *T. vaporariorum*. In addition, the nymphal cuticle of *B. tabaci* appears to be thicker and more leather-like than that of *T. vaporariorum* (unpublished results). The more flexible cuticle of the *T. vaporariorum* nymph may be less restrictive to the developing parasitoid, and may also contribute to the greater parasitization rate observed for *T. vaporariorum* as compared to *B. tabaci* (Bosclair et al. 1990).

As has been reported for *T. vaporariorum* parasitized by *E. formosa*, the parasitoid did not molt to its 3rd instar until *B. tabaci* initiated the nymphal-adult molt and adult development (Hu et al. 2002). At the time of the molt, it is likely that differentiating tissues are quite fragile. Thus, parasitoid activity during the period when eye and wing structures are differentiating into adult structures probably contributed to the observed disintegration of wing and eye tissues. As was the case for *T. vaporariorum*, 3rd instar parasitoids were found in the dorsum of *B. tabaci*, with the internal organs of the whitefly compressed ventrally. However, in *B. tabaci*, 3rd instar parasitoids often appeared to have less room for their development than in *T. vaporariorum*. This may be due to the geometry of *B. tabaci* which has a more dome-like dorsum than *T. vaporariorum*, the latter being typically described as pill box (having vertical sides) in shape.

Whereas adult longevity of *E. formosa* was influenced by *T. vaporariorum* host age (Hu et al. 2002), when *B. tabaci* served as the host, the instar parasitized had no significant effect on adult longevity. Unfed parasitoid adults survived approximately 1.8-1.9 days, regardless of which *B. tabaci* instar was parasitized. Parasitoid adults survived approximately 2.0 days and 2.5 days, respectively, when 1st and 2nd instars versus 3rd and 4th instar *T. vaporariorum* were parasitized. Thus, unfed adult survival was longer when *T. vaporariorum* served as the host insect, suggesting that parasitoids infesting *T. vaporariorum* are more robust than those infesting *B. tabaci*.

Mean sizes of *E. formosa* (larvae, pupae and adults) were not significantly different regardless of which *B. tabaci* instar was parasitized, although parasitoids that developed in hosts that were parasitized as 3rd and 4th instars were somewhat larger than those that began their development in younger instars. Similar results were reported for *E. formosa* that developed in *T. vaporariorum* (Hu et al. 2002). Donnell and Hunter (2002), however, reported that mean tibia size of parasitoids developing in *B. tabaci* parasitized as 4th instars (0.205 mm) was significantly greater than tibia size of parasitoids developing in whiteflies parasitized as 1st instars (0.195 mm), n = 20. The lack of influence of host age on parasitoid larval and pupal body length and adult head capsule width when *E. formosa* parasitizes either *T. vaporariorum* or *B. tabaci* (Strain B) indicates that parasitoid size, at least for these parameters, is not sensitive to the age of the nymph upon parasitization.

The emergence pattern of *E. formosa* was affected by host age when either *B. tabaci* or *T. vaporariorum* served as hosts (this study; Hu et al. 2002). The emergence curve was flatter, i.e., emergence was less synchronous when the parasitoid began its development in first instars as opposed to older nymphs. Donnell

and Hunter (2002) reported similar results when they compared the emergence patterns of *E. formosa* reared in *B. tabaci* parasitized as 1st and 4th instars. Nevertheless, the pattern of parasitoid emergence was different in the two whitefly hosts. When *B. tabaci* was parasitized by *E. formosa*, the emergence peak occurred on days 1 and 2 when 3rd and 4th instars were parasitized and on day 2 when younger instars were parasitized, the peak being much lower and broader when 1st instars were parasitized. In contrast, adult emergence peaked on day 2 when 3rd or 4th instar *T. vaporariorum* served as the host and exhibited two or more small peaks when younger instars were parasitized (Hu et al. 2002).

In summary, whether *T. vaporariorum* or *B. tabaci* served as the host, *E. formosa* developed more synchronously when 3rd or 4th instar whiteflies were parasitized than when younger instars were parasitized. Total developmental time was reduced, emergence was more synchronous, and in the case of *T. vaporariorum*, longevity was significantly greater, when 3rd or 4th instars served as the host. In addition, when either whitefly was parasitized, the parasitoid's molt to the 3rd instar appeared to be linked to the nymphal-adult molt and the initiation of adult development in the host. Yet *T. vaporariorum* is the preferred host for *E. formosa*. This may be due, in part, to differences in host ecdysteroid titers and/or differences in body size and body shape in the two species of whiteflies.

Acknowledgements

We wish to thank M. Chvatal for technical assistance, Paul Ecke Ranch (Encinatas CA) for providing the poinsettia plants that were used to maintain our *B. tabaci* colony and Drs. Dan Gerling, TX Liu and Donald Weber for their critical readings of the manuscript.

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