

Host-Parasite Interactions Between Whiteflies and Their Parasitoids

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There is relatively little information available concerning the physiological and biochemical interactions between whiteflies and their parasitoids. In this report, we describe interactions between aphelinid parasitoids and their aleyrodid hosts that we have observed in four host-parasite systems: *Bemisia tabaci*/*Encarsia formosa*, *Trialeurodes vaporariorum*/*E. formosa*, *B. tabaci*/*Eretmocerus mundus*, and *T. lauri*/*Encarsia scapeata*. In the absence of reported polydnavirus and teratocytes, these parasitoids probably inject and/or produce compounds that interfere with the host immune response and also manipulate host development to suit their own needs. In addition, parasitoids must coordinate their own development with that of their host. Although eggs are deposited under all four instars of *B. tabaci*, *Eretmocerus* larvae only penetrate 4th instar *B. tabaci* nymphs. A pre-penetrating *E. mundus* first instar was capable of inducing permanent developmental arrest in its host, and upon penetration stimulated its host to produce a capsule (epidermal in origin) in which the parasitoid larva developed. *T. vaporariorum* and *B. tabaci* parasitized by *E. formosa* initiated adult development, and, on occasion, produced abnormal adult wings and eyes. In these systems, the site of parasitoid oviposition depended on the host species, occurring within or pressing into the ventral ganglion in *T. vaporariorum* and at various locations in *B. tabaci*. *E. formosa*'s final larval molt is cued by the initiation of adult development in its host. In the *T. lauri*-*E. scapeata* system, both the host whitefly and the female parasitoid diapause during most of the year, i.e., from June until the middle of February (*T. lauri*) or from May until the end of December (*E. scapeata*). It appears that the growth and development of the insects are directed by the appearance of new, young foliage on *Arbutus andrachne*, the host tree. When adult female parasitoids emerged in the spring, they laid unfertilized male-producing eggs in whiteflies containing a female parasitoid [autoparasitism (development of male larvae utilizing female parasitoid immatures for nutrition)]. Upon hatching, these male larvae did not diapause, but initiated development, and the adult males that emerged several weeks later mated with available females to produce the next generation of parasitoid females. Thus, the interactions that exist between whiteflies and their parasitoids are complex and can be quite diverse in the various host-parasitoid systems. Arch. Insect Biochem. Physiol. 60:209–222, 2005. Published 2005 Wiley-Liss, Inc.[†]

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INTRODUCTION

Whiteflies are often severe pests of food, fiber, and ornamental plants. These homopterans damage plants by feeding on phloem, transmitting pathogenic viruses and producing honeydew, a

sweet sticky substance that supports the growth of sooty mold. The sweet potato whitefly (SPWF), *Bemisia tabaci*, Biotype B (also known as the silverleaf whitefly or *B. argentifolii*) (Bellows et al., Perring, 1994) is polyphagous, attacking more than 600 different species of plants in both field and

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greenhouse settings (Oliveira et al., 2001). Another polyphagous species, the greenhouse whitefly (GHWF) *Trialeurodes vaporariorum* is a pest of plants grown both in greenhouses and, more recently, out of doors, particularly in South America (Buitrago et al., 1994; Cardona et al., 1993; De Vis et al., 1999). World-wide, these pests cause billions of dollars of damage in crop losses each year (Perkins and Bassett, 1988; Gill, 1992; Zalom et al., 1995; Heinz, 1995; Henneberry et al., 1997, 1998; Chu and Henneberry, 1998). For the last several decades, chemical pesticides have been the preferred method for controlling pest species of whiteflies (Horowitz and Ishaaya, 1995; Horowitz et al., 1994). However, biological control agents including parasitoids, predators, and fungi have also received attention (Heinz, 1995; Lacey et al., 1995; Nordlund and Legaspi, 1995; Naranjo and Ellsworth 2001). The use of *Encarsia formosa* to control *T. vaporariorum* is probably the best known and most successful example of the use of a biological agent for whitefly control (Hussey and Scopes, 1985; van Lenteren and Martin, 1999). Various species of *Eretmocerus*, another genus of whitefly parasitoid, as well as other species of *Encarsia*, have also been mass reared and augmentatively released in fields in the southern United States (especially in California and Texas) and in European greenhouses, to assist in the control of *B. tabaci* (Hoelmer, 1995; Roltsch et al., 2001; Simmons et al. 2002; Gould, 2003, Urbaneja and Stansly 2004). Several exotic *Eretmocerus* species have become established in the Imperial Valley in California, and across the Rio Grande flood plain in Texas (Roltsch et al., 2001; Hoelmer and Goolsby, 2003).

While parasitoids can be useful as biocontrol agents, they also could contribute to the development of new biopesticides, if only the complex mechanisms by which they manipulate their hosts' physiology and biochemistry were well understood. To date, most research on host-parasite interactions has focused on those that occur between lepidopterans and their parasitoids, and only very little has been reported for similar interactions that occur between whiteflies and their parasitoids. In this report, we describe interactions that we have ob-

served in four host-parasite systems: *B. tabaci*/*E. formosa*, *T. vaporariorum*/*E. formosa*, *B. tabaci*/*E. mundus*, and *T. lauri*/*E. scapeata*. The parasitoids belong to the family Aphelinidae and the whiteflies to the family Aleyrodidae.

PARASITOID-ASSOCIATED PRODUCTS THAT REGULATE HOST DEVELOPMENT

Lepidoptera

Parasitoids that attack lepidopteran hosts have an arsenal of weapons to redirect host development to suit their own needs. These include the injection of polydnavirus, venom, and calyx fluid at the time of oviposition, the release of teratocytes that originate from the serosal membrane that surrounds the parasitoid embryo during development, and products produced by the parasitoid itself, once parasitization has occurred (most recently reviewed in Edwards and Weaver, 2001; Beckage and Gelman, 2004). Some important effects of the parasitoid and parasitoid-associated products are summarized in Table 1. It is noteworthy that parasitoids use a variety of different mechanisms to achieve a desired effect. Thus, immunity can be compromised through the action of polydnavirus and teratocytes. The activity of polydnavirus, venom, and teratocytes can contribute to the induction of developmental arrest in the host, and molting hormone levels can be manipulated by venom, teratocytes, and the parasitoid itself. It is also important to note that in different host-para-

TABLE 1. Parasitoid-Associated Products That Regulate Lepidopteran Host Development

Product	Effect
Polydnavirus	Immune response Developmental arrest Castration
+ Venom and parasitoid	Precocious metamorphosis
Venom	Developmental arrest Changes in ecdysteroid metabolism Paralysis
Calyx fluid	Refractory prothoracic glands
Teratocytes	Developmental arrest Immune response Changes in ecdysteroid metabolism
Parasitoid	Changes in ecdysteroid and JH levels

site systems, the parasitoid has often evolved unique mechanisms with specific targets to modify host development, e.g., the induction of precocious metamorphosis in *Spodoptera littoralis* parasitized by *Chelonus inanitus* is due to an inhibition in juvenile hormone (JH) induced by a precocious increase in JH esterase, which, in turn, is induced through the combined action of wasp polydnavirus, venom, and the developing parasitoid (Jones, 1996; Lanzrein et al., 2001). In other host-parasite systems, parasitization promotes a decrease in JH esterase activity, which, in turn, causes elevated JH titers in last instars (Beckage and Gelman, 2004, Table 1). The resulting high JH titers contribute to an inhibition of ecdysteroid production and a consequential inhibition of molting in the host insect.

Homoptera/Heteroptera

In homopterans, relatively little is known about the mechanisms used by parasitoids to manipulate and/or terminate their hosts' development. The aphid *Acyrtosiphon pisum* parasitized by the braconid *Aphidius ervi* exhibits ovarian atrophy within 24 hr of parasitization (Diglio et al., 2000). When injected with ovarian fluid and venom collected from *A. ervi*, *A. pisum* undergoes developmental arrest in the last (4th) instar (Diglio et al., 1998). Since the effect on the ovary is observed prior to egg hatch, and since activity is destroyed by treatment with heat and/or pronase, it has been suggested that a venom protein is responsible for the observed female castration (Diglio et al., 2000). Teratocytes have been reported to be present in parasitized *A. pisum* and experimental results support the view that these parasitoid-derived cells secrete proteins that have a nutritional function (Falabella et al., 2000). To date, polydnaviruses have not been found in parasitoids of aphids. Since the immune system of the host aphid must have been neutralized to allow the parasitoid to survive, it is likely that substances injected by the parasitoid or possibly released by teratocytes are responsible for the apparent lack of an immune response.

Teratocytes have been observed in a few species of *Encarsia* but not in *E. formosa* (summarized

in Donnell and Hunter, 2002; Pedata et al., 2003), and there are no reports to our knowledge concerning the presence of polydnavirus in *Encarsia* species or teratocytes or polydnavirus in *Eretmocerus* species. Therefore, fluid/venom injected by the parasitoid must be responsible for the immunosuppression that occurs in host whiteflies following parasitization, and the developmental arrest that occurs in *B. tabaci* parasitized by *E. mundus*.

Host-Parasite Interactions in the *T. vaporariorum*/*E. formosa* and *B. tabaci* (Biotype B)/*E. formosa* Systems

In previous studies designed to track whitefly and parasitoid development and to detect host cues that direct parasitoid development and vice versa, when young 1st, 2nd, 3rd, and 4th instar *T. vaporariorum* or *B. tabaci* whitefly nymphs were exposed to *E. formosa*, and whiteflies were dissected daily post-parasitization, parasitoid developmental rates differed significantly depending upon the host instar parasitized (Nechols and Tauber, 1977; Hu et al., 2002, 2003). Time to adult emergence was longer when 1st or 2nd rather than 3rd or 4th instar nymphs were presented for oviposition. In addition, parasitoid emergence was more synchronous and adult longevity was significantly greater when the older (3rd and 4th) nymphal instars were parasitized (Hu et al., 2002, 2003). It is logical that a parasitoid's growth and development would be enhanced when older and, thus, larger and nutritionally richer instars serve as hosts. In many host-parasite systems, although parasitization occurs in a young host, the parasitoid remains relatively dormant until the host molts to its last instar (Beckage and Gelman, 2001). It is significant that based on dissection and histological studies, no matter which whitefly instar was parasitized, *E. formosa* did not molt to its 3rd (last) instar until the host had reached its maximum depth and had initiated adult development (Hu et al., 2002, 2003; Blackburn et al., 2002). At this time, ecdysteroid titers are at their peak (Fig. 1) (Gelman et al., 2002a,b). Thus, *E. formosa*'s final lar-

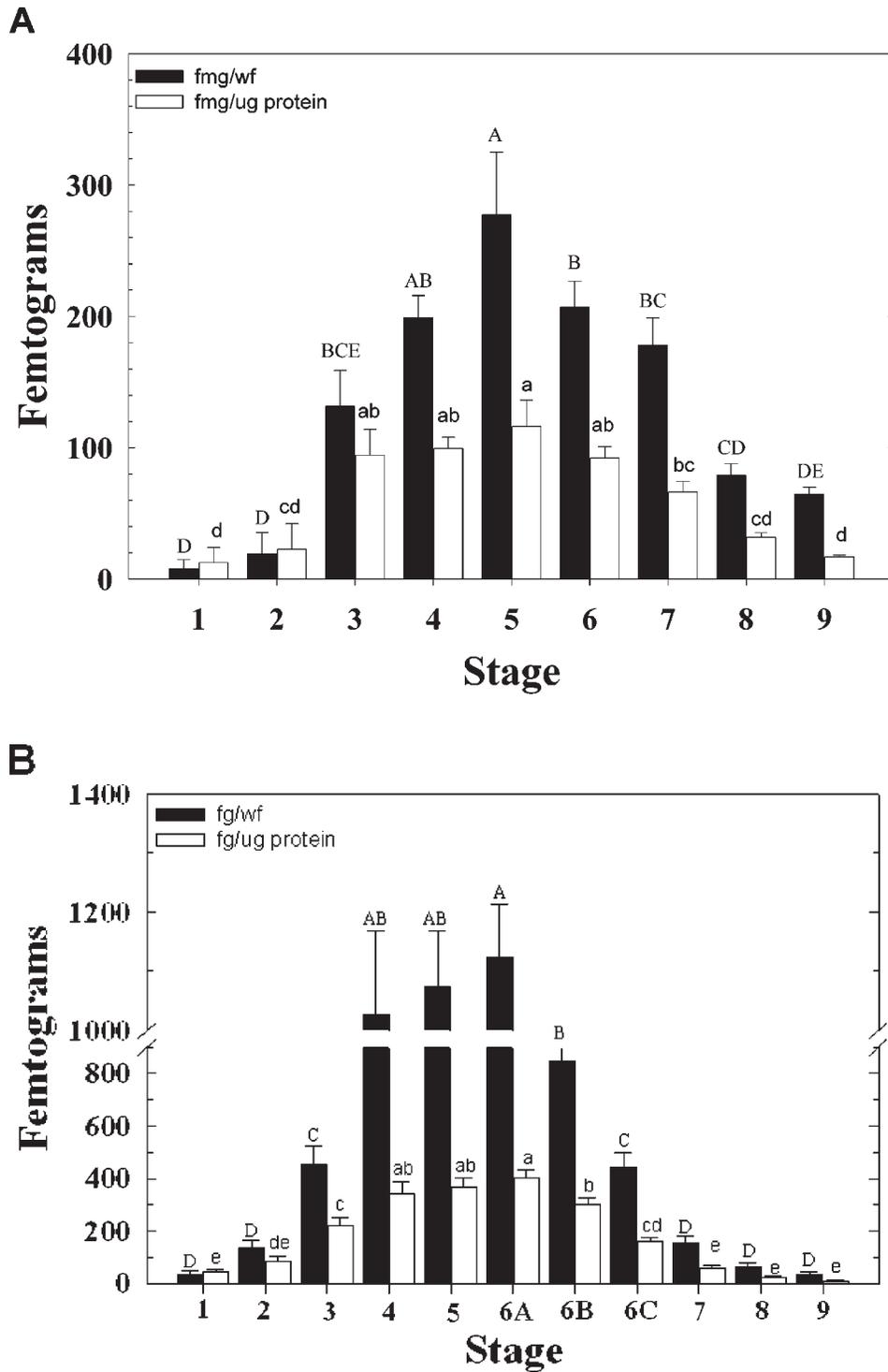


Fig. 1. Whole body ecdysteroid fluctuations in 4th instars and pharate adults of *T. vaporariorum* (A) and *B. tabaci* (B) (adapted from Gelman et al., 2002a,b) (Reprinted from Journal of Insect Physiology 48(1):63–73, 2002 with permission from Elsevier). Assignment to Stages 1–5 and 6–9 were based on increasing body depth and the color and appearance of the developing adult eye, respectively (see Fig. 5 legend). Adult development is typically initiated in Stage 4 or 5 (*T. vaporariorum*) or Stage 6 (*Bemisia tabaci*). For each stage (1–9), appropriate numbers of whiteflies were extracted in aqueous methanol and ecdysteroid titers were determined using an enzyme immunoassay. Titters are expressed as fg 20-hydroxyecdysone equivalents per whitefly and per μg protein. Each bar represents the mean \pm S. E. of at least 5 separate determinations. Means having the same letter designation were not significantly different.

val molt is cued, at least in part, by the initiation of adult development in its whitefly host.

The use of histological techniques to track whitefly and parasitoid development also yielded information of interest concerning the site of wasp

oviposition. Blackburn et al. (2002) found that the Beltsville strain of *E. formosa* almost always oviposited its egg within the ventral ganglion of *T. vaporariorum* (Fig. 2A). When the site of oviposition of a commercial strain of *E. formosa* (obtained

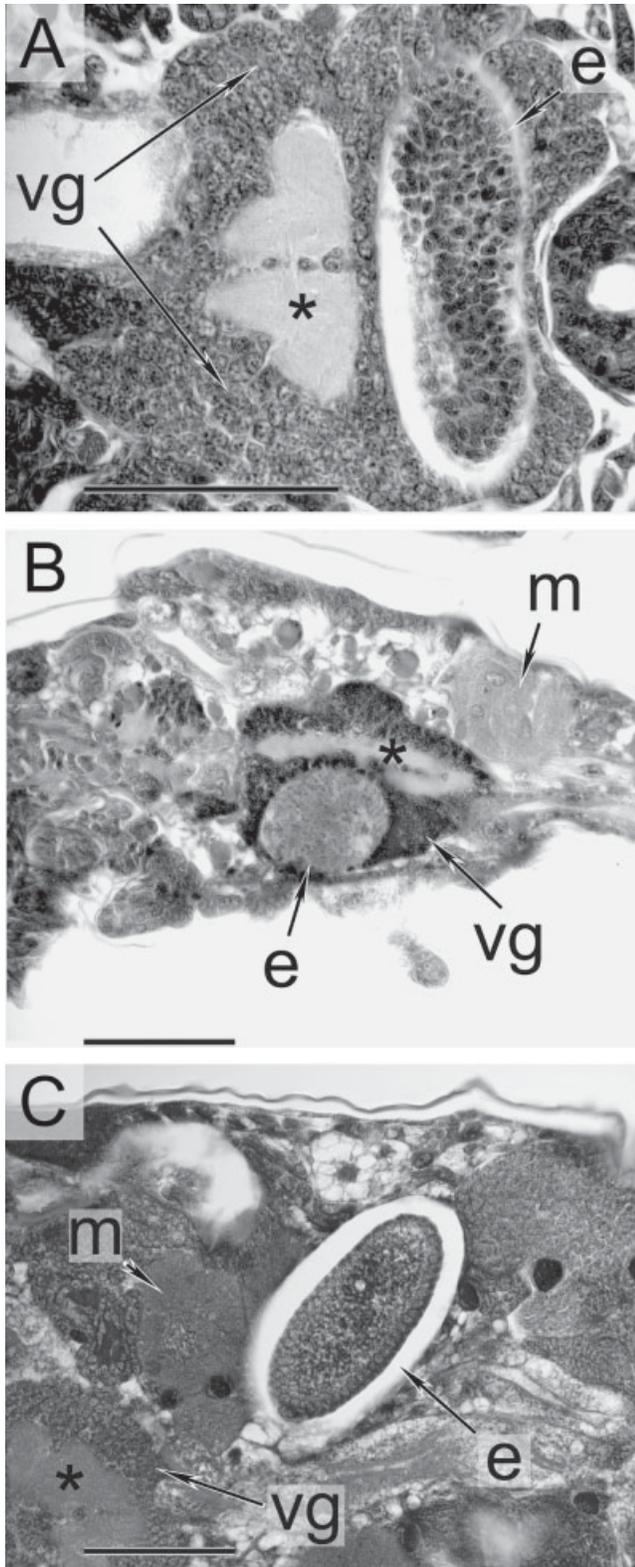


Fig. 2. Oviposition site of *E. formosa* (Beltsville strain) in *T. vaporariorum* (A), *E. formosa* (commercial strain) in *T. vaporariorum* (B), and *B. tabaci* (C). Sections were prepared as described in Blackburn et al. (2002). A and C are horizontal sections; B is a sagittal section. e, embryo; vg, ventral ganglion, *, neuropil of vg; m, mycetome. Scale bars = 50 μ m.

from Rincon-Vitova, Ventura, CA, when the Beltsville strain was no longer available) was examined using *B. tabaci* as the host, the parasitoid egg was found at many different locations within the host, including within or pressing into the ventral ganglion (Fig. 2B). To determine whether the difference in the whitefly species or the difference in the strain of *E. formosa* was responsible for the discrepancy in the sites of oviposition in the two species of whitefly, the same parasitization and histological procedures (Blackburn et al., 2002) were used to examine the site of oviposition of the commercial strain of *E. formosa* using *T. vaporariorum* as the host. We found that the egg oviposited by the commercial strain of *E. formosa* was located within or pressing into the ventral ganglion of the greenhouse whitefly more than 90% of the time (Fig. 2C). Thus, it appears that the site of oviposition is influenced by the species of whitefly (Table 2). The variability of the oviposition site of *E. formosa* when *B. tabaci* is the host whitefly may contribute to the reduced ability of the parasitoid to control *B. tabaci* as compared to *T. vaporariorum* (Bosclair et al., 1990; Henter et al., 1993). Netting and Hunter (2000) have hypothesized that oviposition within an organ offers protection to the parasitoid egg, e.g., from another parasitoid female determined to commit ovidicide

TABLE 2. Site of Oviposition of *E. formosa* in *T. vaporariorum* and *B. tabaci**

Whitefly species	Percent oviposition within or pressing into ventral ganglion	
	Strain of <i>E. formosa</i>	
	Beltsville	Commercial
<i>T. vaporariorum</i>	93	93
<i>B. tabaci</i>	—	40

*Percentages were based on at least 10 separate successful parasitizations for each host-parasite system.

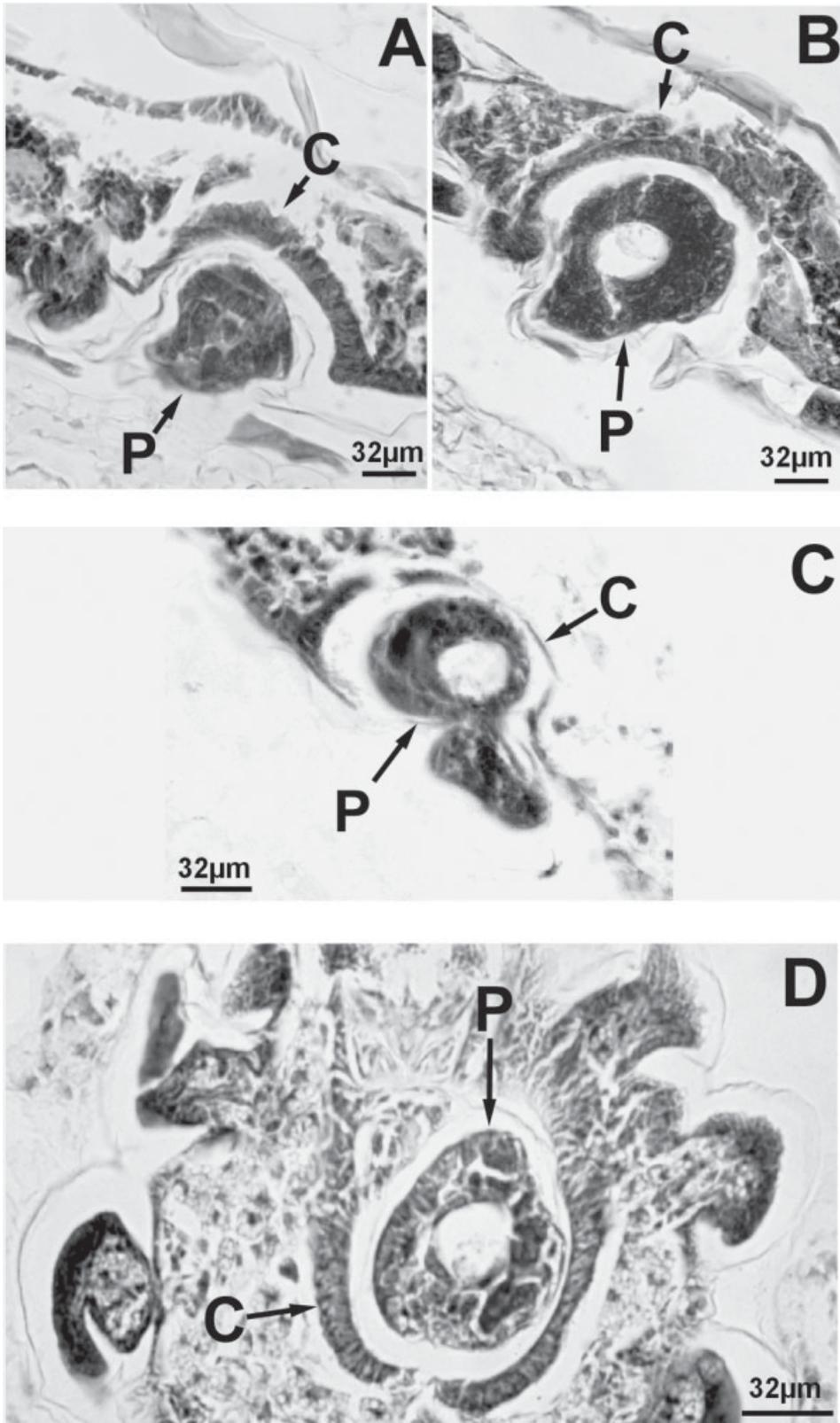


Fig. 3. Capsule formation upon penetration of an *E. mundus* 1st instar larva into a *B. tabaci* 4th instar nymph. A: Host epidermis begins to invaginate as 1st instar parasitoid begins to penetrate. B: 1st instar *E. mundus* in a later stage of penetration. C: 1st instar *E. mundus* completing penetration. D: 2nd instar *E. mundus* completely surrounded by the capsule. P, parasitoid; C, capsule. Scale bars = 32 μm.

prior to oviposition of her own egg. Whether deposition within the ventral ganglion offers this or other forms of protection is not known.

Host-Parasite Interactions in the *B. tabaci* (Biotype B)/*E. mundus* System

Unlike *Encarsia* species that oviposit within the whitefly host, *Eretmocerus* species deposit an egg between the ventral surface of the whitefly and the leaf substrate, typically near the front legs or mouthparts of the host (Clausen and Berry, 1932; Gerling et al., 1990). At $27 \pm 2^\circ\text{C}$, *E. mundus* eggs hatch 3 days post-oviposition, and remain protected under the whitefly until the latter has molted to its 4th instar (Foltyn and Gerling, 1985; Gerling et al., 1991; unpublished results). Upon receiving this cue, the wasp larva begins to penetrate its host (Fig. 3A–C) as the epidermal cells of the host, apparently cued by the parasitoid larva, undergo mitosis and engulf the parasitoid, eventually forming a capsule around it (Fig. 3D) (Gerling et al., 1990). It is probable that this unique capsule protects the parasitoid from host defensive mechanisms even though materials can pass back and forth between the parasitoid and the host through the capsule wall (Gerling et al., 1991). While it is likely that the parasitoid 1st instar larva induces capsule formation by injecting materials into its host, the mechanism of action is unknown. Unless there is a small, localized ecdysteroid peak in the area of parasitoid penetration, it appears that an increase in ecdysteroid titer is not involved since just before and during early penetration and capsule formation host ecdysteroid titers are significantly lower in hosts than in unparasitized controls (unpublished results). Following the encapsulation process, the wasp molts to its 2nd instar. When the parasitoid is late into the 2nd instar, whitefly tissues have already begun to disintegrate; the capsule begins to show large gaps when the parasitoid reaches the 3rd instar, a time when the host immune system is probably no longer functional. *E. mundus* completes its development in the body cavity of its host (Gerling et al., 1990, 1991).

The possible ability of the *E. mundus* egg and

pre-penetrating larva to interfere with *B. tabaci* development is also of interest. Thus, the effect of these parasitoid stages on adult whitefly emergence was examined. Sweet potato leaves having late 3rd instar whiteflies were placed in cages containing young *E. mundus* adults. Two and 3 days post-oviposition, when the whitefly nymphs had reached the 4th instar, they were inverted to determine if parasitization had occurred. Those having a parasitoid egg or larva were placed on moist filter paper in Petri dishes, while unparasitized whiteflies from the same cohort (controls) were similarly placed in a separate set of Petri dishes. Neither the parasitoid egg nor its larva was transferred with its whitefly host. Filter papers were kept moist throughout the observation period and Petri dishes were maintained in incubators at L:D 16:8 and a temperature of $27 \pm 2^\circ\text{C}$. Percent emergence was recorded. When Stage-6 4th instar/pharate adult whiteflies (eye pigment had begun to diffuse, adult formation had been initiated) were removed prior to parasitoid egg hatch, percent adult emergence was similar for experimental and control insects; however, when whiteflies were removed after the parasitoid egg had hatched, only one whitefly adult emerged (Table 3). Thus, as a pre-penetrating larva, but not as an egg, *E. mundus* almost always induces permanent developmental arrest and eventual mortality in its host whitefly.

Host-Parasite Interactions in the *T. lauri*/*E. scapeata* System

The whitefly *T. lauri* (Fig. 4B) only infests hardwood evergreen trees. In Israel, it is found almost

TABLE 3. Effect of *E. mundus* Egg and Pre-Penetrating Larva on *B. tabaci* Development

Stage of <i>E. mundus</i> ^a	Percent emergence of host WF	
	Parasitized WF	Unparasitized WF
Egg	>60	>60
1st instar larva	<7 ^b	>60

^aWhitefly 4th instar/pharate adults that had attained Stage 6 (adult formation initiated) and had either an egg or a pre-penetrating parasitoid larva beneath them were removed to Petri dishes containing moist filter paper. Adult emergence was monitored for the next ten days.

^bRepresents only one individual.

exclusively on *Arbutus andracne* (Fig. 4A), a tree that is a common inhabitant of the hills of several Mediterranean countries. *A. andracne* has a relatively short-growing season in Israel, with buds bursting during February and March, the first leaves appearing in March or April and young leaf growth ending in mid to late May. The former year's leaves are shed during May and June, shortly after the appearance of the new leaves (Fig. 5A). During the rest of the year, the tree is dormant. Therefore, it is not surprising that the whitefly *T. lauri*, probably cued, at least in part, by its host *A. andrachne*, enters diapause in late spring and remains in diapause until the following March (break was first observed in mid-February) (Fig. 5B). In nature, *T.*

lauri is univoltine, i.e., it exhibits only one generation per year with adults appearing in April and May, ovipositing and dying shortly thereafter.

Diapause in insects is not an immediate response to environmental conditions, but rather is induced by cues that the environment will become unfavorable (Saunders et al., 2002; Chapman, 1998). Thus, in temperate zones, insects prepare for diapause in late summer and fall, typically cueing on shorter day lengths. Falling temperatures can also play a role in the onset of diapause, a state in which insects have increased tolerance to environmental extremes (Saunders et al., 2002; Denlinger, 1985). In insects, diapause termination may or may not require special conditions, but

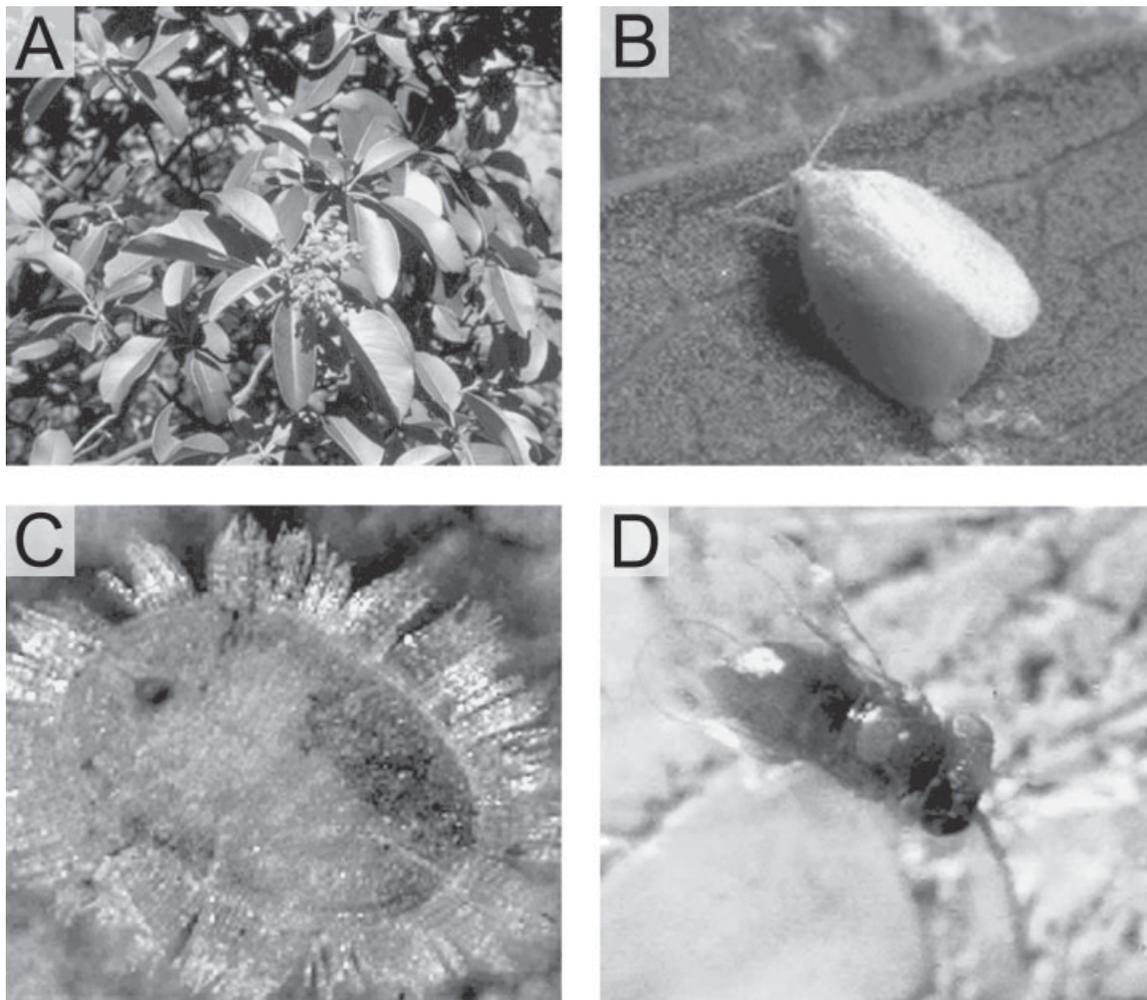


Fig. 4. The host plant, *A. andracne* (A), the host whitefly, *T. lauri*, adult and nymph (B and C, respectively),

and the parasitoid, *E. scapeata* (D).

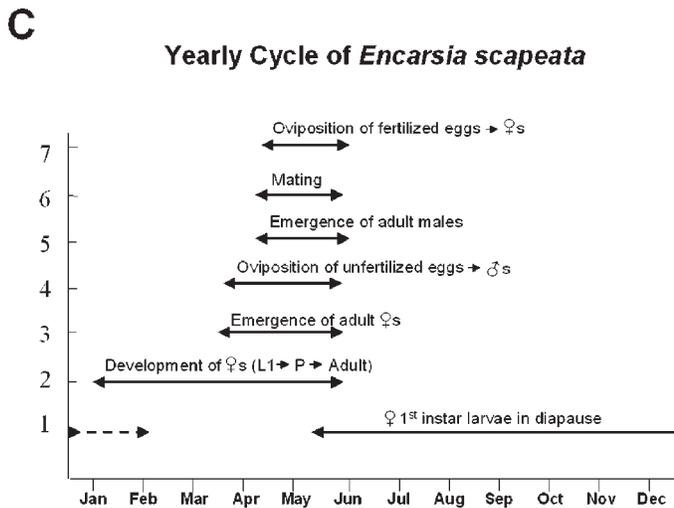
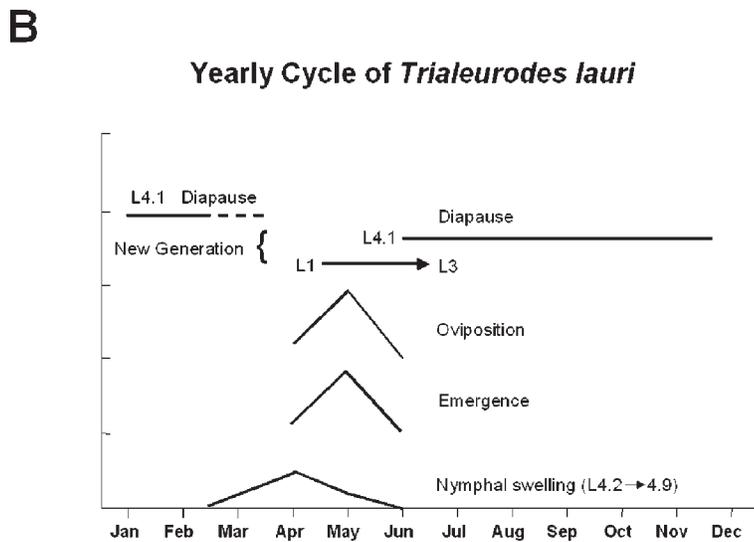
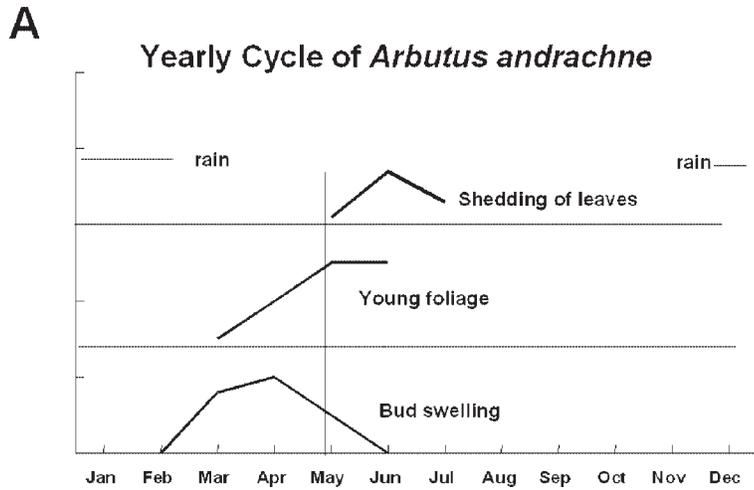


Fig. 5. Yearly cycle of *A. andrachne* (A), *T. lauri* (B), and *E. scapeata* (C) in the Mediterranean Chaparral of Israel. Results were based on field observations in which leaves heavily infested with *T. lauri* were collected every 7–10 days in April and May and monthly during the rest of the year. Whiteflies infesting these leaves were characterized as: (1) living, (2) shells with adult whitefly emergence holes, and (3) shells with parasitoid emergence holes. In addition, in the laboratory, *T. lauri* adults were placed on leaves of *A. andrachne* seedlings and whitefly development was monitored. B: L1 → L3 refers to nymphal instars 1 and 3; L4.1, 4.2, and 4.9 refers to stage of 4th instar (Gelman et al., 2002a). Briefly, Stages 1, 2, and 3 were characterized by body depths of 0.1 ± 0.02 mm, 0.15 ± 0.02 mm, and 0.2 ± 0.02 mm, respectively. Stages 6 through 9 were identified based on the appearance of the developing adult eye. Nymphs entered Stage 6 when the small intense red dot characteristic of the eye of Stages 1 through 3 began to diffuse. Stages 7, 8, and 9 were characterized by a light red, medium red bipartite, and dark red or red-black bipartite adult eye, respectively. C: L1, 1st instar wasp larva; P, pupa.

typically, for diapause break to be synchronous, specific environmental cues such as temperature changes, increased moisture, and/or longer days are utilized (Sehnal, 1985). Typically, diapause development is completed when conditions remain unfavorable, and later, when the insect receives cues that conditions will support development, diapause break is initiated (Sehnal, 1985). Some insects must experience a prolonged period of cold (e.g., the gypsy moth, *Lymantria dispar*), a period of heat (the Australian small plague grasshopper *Austroicetes cruciata*), or other unfavorable condition if they are to undergo diapause break (Masaki, 1956; Andrewartha and Birch, 1954).

Changes from spring to the relatively unfavorable conditions of summer apparently induce the cessation of development in *A. andrachne* and this in turn induces diapause in *T. lauri*. The whitefly attains Stage 1 of the 4th instar in May or early June before entering diapause, and remains in that stage (depth ≤ 0.01 mm, Gelman et al., 2002a,b) until diapause is terminated the following spring (Fig. 5B). Upon the completion of diapause development, and when conditions are favorable, *T. lauri* passes through stages 2 and 3 of the 4th instar, molts to the pharate adult (Stages 6–9) (Gelman et al., 2002a,b), completes adult development, and emerges. Adult *T. lauri* only lay eggs on new, young foliage. Hatched nymphs develop to the 4th instar, Stage 1 within approximately 3 weeks at which time they enter diapause (Fig. 5B). The signals and conditions needed for *T. lauri* to enter or break diapause have not been studied in detail, but it is likely that the levels and ratios of the growth hormones within the plant, which accompany the seasonal changes and dictate plant development, may serve as cues, either directly or via changes in the physiology of *A. andrachne*.

The aphelinid wasp *E. scapeata* (Fig. 4D) exhibits a similar period of diapause as its host whitefly (Fig. 5C), the timing of which is apparently cued by *T. lauri*. In some host-parasitoid systems, parasitoids adjust to environmental conditions indirectly by responding to changes in host endocrinology, while in other systems, the parasitoid responds directly to environmental stimuli (Sehnal, 1985).

Maslennikova (1968) reported that many host-parasitoid systems are characterized by an intermediate condition in which stimuli from both the host and the environment regulate parasitoid diapause. In two species of aphid (*Aphis fabae*) parasitoids, *Aphidius matricariae* and *Praon volucre*, Polgár et al. (1991) reported that diapause induction in the parasitoid larva is cued by hormonal differences in the aphid morphs and is independent of the environment. However, diapause induction in *Aphidius ervi*, a parasitoid of *Acyrtosiphon pisum*, is cued directly by environmental conditions although parasitoids in oviparae enter diapause more readily than those in virginoparae so that synchrony between host and parasitoid with regard to diapause development is fostered (Christiansen-Weniger and Hardie, 1999). The relative roles of *T. lauri* and/or the environment in controlling diapause in *E. scapeata* have not yet been investigated. However, it is likely that the role of *T. lauri* is paramount. *E. scapeata* has been successfully reared on *B. tabaci*, a whitefly that does not undergo diapause with a resulting life cycle of 3 weeks versus 11 months in *T. lauri*.

Results shown in Figure 5A–C were based on field observations in which heavily infested leaves were collected every 7–10 days in April and May and monthly during the rest of the year. Whiteflies infesting these leaves were characterized as: (1) living, (2) shells with adult whitefly emergence holes, (3) whitefly nymphs containing a developing parasitoid larva or pupa, and (4) shells with parasitoid emergence holes. Our counts showed that approximately 3% of the whiteflies had been parasitized.

As previously mentioned, whiteflies break diapause and start to develop in the spring, typically in the month of March, and adults emerge in April and May (Fig. 5B). Parasitoid larvae diapause as first instars into which they hatched shortly following oviposition (Fig. 5C). Most parasitoids were observed to break diapause and begin their development during March with their molt into second and then third instars. These parasitoids emerged in April and May. However, some *E. scapeata* were found as second instars as early as the 27th of December, when all of the unparasitized hosts were still diapausing as 1st-stage 4th instars. Thus, it appears that

some *E. scapeata* begin to break diapause prior to their host (Fig. 5C), resulting in the emergence of some adult parasitoids in March and early April.

Laboratory observations showed that all parasitoids that emerged during March and early April were females, and since there were no male parasitoids, these females could only lay unfertilized or male-producing eggs. *E. scapeata*, like most other *Encarsia* species, exhibits autoparasitism; unfertilized male-producing eggs are always laid in whiteflies already containing a developing parasitoid (Hunter and Woolley, 2001). Upon hatching, the male wasp larvae would forego diapause, undergo development, and emerge within about 3 weeks during the spring. Thus, the first laid female parasitoid is sacrificed whenever a male-producing egg is oviposited in the same whitefly. In mid April-early May, when most adult male parasitoids emerge, they will mate with available females who will lay female-producing eggs in 2nd or older instar whitefly nymphs. This new *E. scapeata* female generation will diapause and emerge the following spring. Thus, virgin females that emerge in early spring oviposit male-developing eggs in whiteflies containing their sibling 3rd instar larvae and young pupae. In other species of *Encarsia*, it has been reported that parasitoid females will not oviposit in host whiteflies containing a female parasitoid that has progressed beyond the young pupal stage (Gerling and Rejouan, 2005). The resulting males emerge about 3 weeks later, mate with newly emerging or young female adults, and give rise to the next parasitoid generation.

Complex regulatory mechanisms in this example of a tritrophic interaction (plant, whitefly, parasitoid) must be deciphered. It is probable that the initiation of dormancy in *A. andrachne* contributes to the induction of diapause in *T. lauri*, which, in turn, may contribute to the induction of diapause in *E. scapeata*. During March, cued by physiological changes occurring in the plant and/or by environmental changes associated with the coming of spring, whiteflies undergo swelling, i.e., diapause break. However, it appears that some parasitoids begin to develop in late December, one or two months earlier than unparasitized white-

flies. The cue(s) that triggers early diapause break in female parasitoids (Fig. 5C) is open to speculation, as are those that determine which parasitoids will undergo early diapause break.

Future studies will address a number of questions concerning the complex interactions between *E. scapeata* and *T. lauri* that have arisen from observations reported here. For example, how is diapause induced in *T. lauri* and *E. scapeata*? What triggers *E. scapeata* to break diapause and initiate development? Is all parasitoid male production limited to eggs that are laid by virgin females that emerge between February and April? Results of studies that capitalize on the ability of *B. tabaci* to support the growth and development of *E. scapeata* should contribute to answering these questions.

CONCLUSIONS

Relatively little is known about the physiological and biochemical aspects of interactions between whiteflies and their parasitoids. Our studies have shown that, as expected, reciprocal reactions exist whereby whiteflies both affect and are affected by their parasitoids. Whitefly parasitoids manipulate their hosts' growth and development. Successful parasitoids must interfere with their hosts' immune system, and since polydnviruses and teratocytes do not appear to be present, it is likely that whitefly parasitoids must inject regulatory substances at the time of parasitization. *Eretmocerus* species have the unique ability to stimulate the host nymph to produce a capsule, which, upon the completion of parasitoid penetration, surrounds the parasitoid, perhaps to aid the parasitoid in avoiding its host's immune response. *E. mundus* pre-penetrating and penetrating larvae reduce host ecdysteroid titers and pre-penetrating larvae induce developmental arrest in their host, *B. tabaci*. *T. vaporariorum* and *B. tabaci* parasitized by *E. formosa* initiate but do not complete adult development, and adult wing and eye structures, when present, are abnormal. Whiteflies also regulate parasitoid development. The site of oviposition of *E. formosa* was influenced by the species of whitefly that was targeted for parasitization, and *E. formosa* requires a host cue to initiate its final larval molt. A 1st instar *E. mundus* larva will

not initiate host penetration until *B. tabaci* has molted to its last instar. In the *T. lauri*-*E. scapeata* system, it is likely that the induction of parasitoid diapause is cued, at least in part, by its host whitefly. However, some *E. scapeata* females break diapause one to two months earlier than *T. lauri*, probably to insure that male parasitoids are produced at the appropriate time for mating and to insure the production of the new parasitoid generation.

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