Development and Reproduction of the Egg Parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), as a Function of Temperature

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ABSTRACT The development, fecundity, and life table parameters of *Gonatocerus ashmeadi* Girault, an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), were studied in the laboratory at six constant temperatures between 12 and 32°C. At 12°C, the parasitoid failed to develop beyond the third instar, and durations of the egg stage and the first and second instars were prolonged. Development from the egg stage to adult emergence varied from 27.1 d at 16°C to 9.5 d at 28°C. Temperature thresholds for development ranged from 3.8°C for first-instar larvae to 12.8°C for the pupal stage. The thermal constants were lowest for the second-instar larvae (26.7 DD) and highest for the pupae (75.4 DD). Nearly 207 DD were required above the lower temperature threshold of 8.5°C to complete development from egg to adult. The optimum temperature for egg to adult development was 29.2°C. Survival from egg to adult was 67.4% at 16°C and ranged from 83.4 to 86.7% between 20 and 32°C. At 16–32°C, the population had a type I survivorship pattern. At 16°C, longevity of adult females and males averaged 27.1 and 19.0 d, respectively, but declined to 6.4 and 6.9 d at 32°C. At 20–32°C, peak adult emergence occurred on the first day of emergence, but at 16°C, it was greatest on the second day. When exposed to temperatures ranging from 16 to 32°C, the female:male sex ratio was similar, ranging from 3.4 to 5.6. Lifetime fecundity was greatest at 24°C and lowest at 32°C, with the maximum net reproduction also occurring at 24°C. Greatest intrinsic and finite rates of increase, shortest population doubling time, and mean generation time occurred when *G. ashmeadi* was held at 28°C. The parameters defined in this study can influence geographical distribution and are important for the mass-rearing this wasp as biological control agent for the glassy-winged sharpshooter.

KEY WORDS parasitoid, glassy-winged sharpshooter, development, fecundity, temperature

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), has become a serious economic threat to California agriculture since its introduction from the southwestern United States in the late 1980s (Blua et al. 1999, de León et al. 2004, Irvin and Hoddle 2005a). This polyphagous pest is a key vector of the xylem-inhabiting bacterium, *Xylella fastidiosa* Wells (Sorensen and Gill 1996), which causes a variety of scorch-like diseases in a range of high value agricultural crops and ornamental plants (Purcell et al. 1999, Almeida and Purcell 2003, Hoddle 2004). Over the past decade, the dispersal of *H. coagulata* throughout California has caused outbreaks of grape Pierce's disease, oleander leaf scorch (Purcell et al. 1999), and to a minor degree, almond leaf scorch (Almeida and Purcell 2003). From 1998 to 1999, grape growers in Riverside and San Diego counties lost $37.9 million because of Pierce's disease (Siebert 2001), and the California Department of Food and Agriculture (2003) has estimated that insect vectors carrying various strains of *X. fastidiosa* have put at risk $3.2 billion of grapes, $905 million of stone fruits, and $897 million of other miscellaneous crops. Additionally, oleander leaf scorch has caused damage in excess of an estimated $53 million along 2,000 miles of freeway median plantings (Costa et al. 2000).

Classical biological control is being studied for *H. coagulata.* Studies encompass species identification, introduction and safety assessment, and the exploitation and field augmentation of mymarid parasitoids (Triapitsyn and Phillips 2000, Triapitsyn 2003, Pilkington et al. 2005, Virla et al. 2005). The majority of the reported egg parasitoids of *H. coagulata* are the members of the genus *Gonatocerus*, including *G. ashmeadi* Girault, *G. triguttatus* Girault, *G. morrilli* Howard, and *G. fasciatus* Girault (Triapitsyn and Phillips 2000, Triapitsyn 2003). Three parasitoids, including the solitary *G. ashmeadi* and *G. triguttattus* species and the gregarious *G. fasciatus*, have been released across nine

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California counties, with their impact on glassy-winged sharpshooter populations currently being evaluated (California Department of Food and Agriculture 2003).

Unlike *G. morrilli,* a native species, *G. ashmeadi* was most likely introduced into California, along with host eggs, from the southern United States (Vickerman et al. 2004). It has been established in California since 1978 (Huber 1988), and in the late 1990s accounted for 80–95% of the observed parasitism of sharpshooter eggs in California (Triapitsyn et al. 1998). A recent study showed that *G. ashmeadi* outcompetes *G. triguttatus* and *G. fasciatus* in the field (Irvin and Hoddle 2005b). Studies on *G. ashmeadi* have focused on host age preference and parasitism (Irvin and Hoddle 2005a), interspecific competition (Irvin and Hoddle 2005b), population dynamics (Vickerman et al. 2004), overwintering biology (López et al. 2004), and functional responses and superparasitism (Chen et al. 2006).

We studied the effects of temperature on the development and reproductive biology of *G. ashmeadi.* Understanding such temperature effects for a particular biological control agent is a central feature for predicting its geographical distribution. This information is also necessary for assessing the potential effectiveness of *G. ashmeadi* as a biological control agent and for devising mass-rearing protocols for this parasitoid for use in augmentative releases. The objectives of our study were (1) to determine the duration of each developmental stage of the immatures; (2) to estimate the temperature threshold and thermal constant for development of each immature stage; (3) to determine the mortality of immature stages and emergence patterns at various temperatures; and (4) to evaluate the effect of temperature on fecundity, longevity, sex ratio, and life table parameters of this parasitoid.

Materials and Methods

Insect Rearing. The *H. coagulata* colony used in this study was acquired from a laboratory colony maintained at the USDA-USDA/APHIS Plant Protection Laboratory, Edinburg, TX, which was originally collected near Bakersfield, CA. Our *G. ashmeadi* colony was started from the colony maintained at the Mt. Rubidoux Field Station of the California Department of Food and Agriculture, Riverside, CA. Both the host and the parasitoid colonies were maintained in the laboratory using the procedures described by Chen et al. (2006).

Development, Survival, and Sex Ratio. Host egg masses (<24 h old) were collected from the *H. coagulata* colonies maintained within cages (Bug Dorm-2; BioQuip Products, Dixon, KY) using a mixed host system consisting of sunflower (*Helianthus annuus*), an evergreen shrub (*Euonymus japonica* Thunb.), and chrysanthemum (*Chrysanthemum morifolium* L. variety White Diamond) plants in a greenhouse, augmented with high intensity sodium lighting under a photoperiod of 16 L:8 D. Only those egg masses which were deposited beneath the epidermis on the underside of euonymus leaves were used. To prevent egg desiccation, the petioles of the excised euonymus leaves bearing egg masses were placed into slits cut into a water-soaked sponge held in a petri-dish (3.5 cm diameter by 1.0 cm high). After determination of the number of eggs/egg mass under a stereoscope, a transparent container (26 cm diameter by 9 cm high; Tri-State Plastic, Rancho Dominguez, CA) was used to hold the petri dish before introduction of the parasitoids. The test container, having a lid with an insert of fine nylon mesh, was maintained in the laboratory at 22 ± 1°C under fluorescent lighting. Mated females were selected from pooled *G. ashmeadi* individuals (sex ratio of newly emerged females and males is 1:1) that had been caged together for 4–6 h and randomly mated. During this period water was provided by a wet sponge.

To reduce the frequency of superparasitism (Chen et al. 2006), mated *G. ashmeadi* females were introduced into the plastic container at a parasitoid/host ratio of 1:25 and permitted to oviposit randomly among the host egg masses for 4 h. After removal of the parasitoids, egg masses were evenly divided into six groups and were transferred into six walk-in chambers set at 12, 16, 20, 24, 28, or 32°C under a 16 L:8 D photoperiod. The number of host eggs used at each temperature was based on developmental time of *G. ashmeadi* from egg to adult. At 28 and 32°C, 250 host eggs were used, 500 eggs at 20 and 24°C, and >750 eggs for 12 and 16°C. Wet sponges provided the parasitoids access to water in the test containers.

A dissection method followed by a simple staining procedure was used to determine the effect of temperature on the development and survival of *G. ashmeadi.* Dissections were performed in a solution of 0.2% toluidine blue in saline under a stereomicroscope. The leaf tissue covering the egg masses was carefully removed with a pair of fine forceps. Host eggs were grasped by the anterior end with a pair of fine forceps and the posterior end was removed with a scalpel. Egg contents were gently squeezed into the dissecting/staining solution. Live parasitoid eggs and larvae remained unstained. Dead immature larvae and sharpshooter eggs and embryos stained blue within 2–3 min. At least 10 parasitized host eggs were dissected daily. Superparasitzed eggs were not used. Because the parasitoid egg and larval stages developed faster at 24, 28, and 32°C, 10 host eggs were dissected and stained every 8 h instead of daily to keep abreast of the changing developmental processes. Identification of *G. ashmeadi* immature stages was as described by Chen et al. (2006). Parasitoids developing to the pupal stage (as evidenced by darkening of the egg chorion of the host) were held at each respective temperature regimen until the adults emerged. Three days after adult wasp emergence had ceased, host eggs were dissected under a stereoscope and numbers of adults plus all stages of unemerged parasitoids were recorded. This process was repeated four times for each temperature.

To determine the effect of temperature on the sex ratio of *G. ashmeadi,* 80 1-d-old *H. coagulata* eggs on euonymus leaves were exposed for 24 h to a single mated female parasitoid and held at 16, 20, 24, 28, or 32°C until emergence of offspring. The sex ratio and emergence time of each progeny were recorded. This experiment was repeated at least 12 times for each temperature.

Longevity and Fecundity. Mated females were selected from *G. ashmeadi* individuals (newly emerged females and males = 1:1) pooled at each temperature from previous sex ratio experiments. Each female wasp was given 80 host eggs (<24 h old) oviposited on six euonymus leaves at its rearing temperature (six temperatures). Water was provided by a water-soaked sponge in a petri dish. Each day until the parasitoid death, host eggs were replaced by new host eggs. Female longevity was recorded and the host eggs were dissected daily to record the number of parasitoid eggs deposited/ female, including those in superparasitzed hosts.

At least 10 male and mated female parasitoids (≈4 h old) were held with water and/or honey to determine the effect of honey on the longevity of the parasitoid. Water was provided daily through water-soaked sponges. Three drops of honey (0.4 ml) were placed on a yellow paper (2.0 cm by 1.5 cm) in the plastic container and was replenished with fresh honey every 3 d. This experiment was conducted in chambers set at 16, 20, 24, 28, and 32°C but not at 12°C because the parasitoid failed to develop to adulthood at that temperature.

Statistical Analysis. Effect of temperature on mean developmental duration, sex ratio, percentage survival and emergence, longevity, and daily and lifetime fecundity were compared using analysis of variance (ANOVA) and Tukey's Studentized range test ($P = 0.05$) through the PROC GLM program (SAS Institute 1996). Before analysis, data on percentage survival and emergence were arcsine-transformed to meet the assumption of normality. A two-way ANOVA was used to determine the effect of temperature, sex, or adult diet on longevity.

A modification of the Logan nonlinear model (Logan et al. 1976) by Lactin et al. (1995) was used to estimate the lower and upper temperature thresholds, and optimal temperatures for development of immature stages, because the original Logan model cannot be used to estimate the low temperature developmental threshold (Lactin et al. 1995). The model formula used in this situation was as follows:

$$R(T) = \exp^{\rho T} - \exp\left(^{\rho Tm - (Tm - T)/\Delta}\right) + \lambda \quad (1)$$

where $R(T)$ is mean developmental rate, T is temperature (°C), T_m is the upper temperature, and ρ, Δ and λ are fitted coefficients. The nonlinear regression model was fitted using PROC NLIN program (SAS Institute 1996).

A linear model also was used to determine the relationship between temperature (T) and developmental rates (R) and to estimate the low temperature threshold only when nonlinear model failed to estimate valid parameters. The linear model is as follows

$$R(T) = a + bT \quad (2)$$

where a and b are the regression parameters. The lower temperature threshold ($t_b = -a/b$) was estimated by extrapolating the linear portion of the temperature-developmental curve (first- through third-instar stages and combined immature stage) or a straight line (egg and pupal stages). The model was fitted using the PROC GLM program (SAS Institute 1996). The degree-day requirements for development of each life stage were calculated using the model described by Campbell et al. (1974): $K = (T - t_b) \times$ developmental time, where K is the thermal constant (degree-days) and developmental time is the mean number of days to complete development at a constant temperature (T).

Life Table Analysis. Observations on life history, including adult fecundity and longevity, were used to construct a time-specific life table for *G. ashmeadi* when maintained under laboratory conditions. Life table parameters for females held at different temperatures were estimated using methods described by Carey (1993). Estimates were generated for intrinsic rate of increase, $r_m = (\ln R_0)/T$; net reproduction rate, $R_0 = \Sigma l_x m_x$; mean generation time, $T_c = \Sigma(l_x m_x x)/\Sigma(l_x m_x)$; finite rate of increase, $\lambda = \exp(r_m)$; and doubling time, $T_d = \ln(2)/r_m$ (where l_x is the proportion of individuals alive at age x, and m_x is the number of female offspring produced per female during age interval x) (Carey 1993).

The jackknife technique was used to estimate mean demographic parameters of $l_x m_x$ of the life table and their SE. This method was first applied to life table analysis as proposed by Meyer et al. (1986) and has been widely used to estimate population growth rates of animals in the past two decades. The jackknife analysis method removes one observation at a time from the original data set and recalculates the statistic of interest from the truncated data set. The method can estimate R_0, T_c, r_m, λ, and T_d with their respective jackknife variances and confidence intervals. A Student *t*-test was used to perform pairwise comparisons (two-tailed) (Maia et al. 2002) to determine if temperature had significant effects on the *G. ashmeadi* population growth statistics.

The Weibull frequency distribution was used to describe the age-specific survival curve for all females at the different temperatures (Pinder et al. 1978): $S(t) = \exp[-(t/b)^c]$, where $S(t)$ is survival rate to a given age (t) and b and c are, respectively, the scale and shape parameters of the Weibull frequency distribution. The shape parameters c >1, =1 and <1, respectively, correspond to the type I, II, and III survivorship curves of Deevey (1947), which, respectively, reflect that mortality rate increases, remains constant, and decreases with increasing age.

Table 1. Developmental time of *G. ashmeadi* as a function of temperature[a]

Temperature (°C)	Duration of life stages (d)					
	Egg	First instar	Second instar	Third instar	Pupa	Egg to adult
12	6.33 ± 0.37a	10.83 ± 0.38a	18.60 ± 0.18a	Failed to pupate	—	—
16	1.94 ± 0.02b	1.29 ± 0.12b	2.15 ± 0.21b	3.13 ± 0.37a	18.60 ± 0.95a	27.10 ± 0.87a
20	1.75 ± 0.20b	1.26 ± 0.24b	1.90 ± 0.09b	2.13 ± 0.09ab	11.51 ± 0.23b	18.55 ± 0.52b
24	1.15 ± 0.05c	1.28 ± 0.06b	1.52 ± 0.04c	1.99 ± 0.05b	7.40 ± 0.36c	13.35 ± 0.45c
28	1.06 ± 0.01c	0.99 ± 0.04b	1.03 ± 0.08d	1.53 ± 0.18b	4.84 ± 0.13d	9.45 ± 0.47d
32	1.05 ± 0.01c	1.15 ± 0.10b	1.25 ± 0.03cd	2.08 ± 0.14b	4.07 ± 0.38d	9.60 ± 0.27d
F	293.75	410.68	3079.13	3.56	155.27	1687.06
Df	4,195	5,18	5,18	4,15	4,15	4,195
P	<0.0001	<0.0001	<0.0001	0.0311	<0.0001	<0.0001

[a] Developmental time for each parasitoid instar and the pupal stage was determined by subtracting the mean day of a given stage from the mean day of the following stage. At least 10 parasitized eggs were dissected daily for each stage and repeated four times. Each value represents mean ± SE of four separate determinations for the larval and pupal stages and 40 separate determinations for the egg stage and whole immature stage (egg stage up to adulthood). Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$ (one-way ANOVA).

Results

Development and Survivorship of Immature Stages. Development from the egg stage to adulthood occurred at five of our six test temperatures (Table 1). At 12°C, the third instars did not pupate within the host eggs and eventually died. For each immature stage and for the combined immature stages (egg to adult emergence), duration of the stages varied significantly with temperature (Table 1). For egg and pupal stages, developmental rate increased with temperature (Fig. 1A and 1E). For larvae and the combined immature stages, the developmental rate increased with temperature up to 28°C but decreased at 32°C (Figs. 1B, 1C, 1D, and 1F). Therefore, a linear model was used to fit the developmental data for egg and pupal stages and the modified Logan nonlinear model was used for fitting data for the larval and combined immature stages.

The lowest temperature tested (12°C) significantly delayed egg development (Table 1). The time between oviposition to hatching at 12°C was approximately six times longer than that at 24–32°C, four times at 20°C, and three times at 16°C. Similar trends were observed for developmental rate of first and second instars. The pupal stage, including the prepupal stage, was the longest developmental stage of all immature stages assayed at each temperature. The proportion of pupal stage relative to the whole immature stage (oviposited egg up to adult emergence) was 66, 62, 56, 51, and 42% at 16, 20, 24, 28, and 32°C, respectively.

Lower and upper temperature thresholds, optimum temperatures for development, and thermal constants for all immature stages and combined immature stages are shown in Table 2. The egg stage and first instars had low temperature thresholds around 4°C, whereas the pupal stage was nearly 13°C. The thermal constants correlate well to the durations of each developmental stage. The pupal stage required the longest time to complete development (Table 1) and likewise commanded the greatest number of degree-days, more than two-fold greater than that of the other stages.

Temperature had no significant effect on survival of the egg stage and individual larval stages within the range of 16–32°C (Table 3). Survival of the pupal stage and combined immature stages varied significantly with temperature. The lowest survival of these two groups occurred at 16°C, whereas the highest occurred at 28°C.

Emergence Pattern. Temperature significantly influenced the incidence of adult emergence as well as the amount of time over which emergence occurred. Adult emergence lasted 10 d at 16 and 20°C, 7 d at 24°C, and 5 d at 28 and 32°C (Fig. 2). Peak emergence occurred on the first day of emergence at 20–32°C and on the second day at 16°C. At 28 and 32°C, ≈92 and 88% of the parasitoids emerged within the first 2 d, whereas nearly 84 and 85% of the parasitoids emerged within the first 3 d at 20 and 24°C, respectively.

Percentage emergence at 28°C on the first day was significantly higher than that at 16–24°C ($F_{4,69} = 4.93$, $P = 0.0015$). On the second day of emergence, there was no difference in the percentage emergence among the five temperatures assayed ($F_{4,69} = 0.96$, $P = 0.44$). On the third day of emergence, there were still 16 and 15% of parasitoids emerging at 20 and 24°C, and 11 and 9% on the fourth day, respectively. On the fifth day of emergence, $< 4\%$ of the parasitoids emerged at the temperatures between 20 and 32°C, and 8% at 16°C (Fig. 2).

Sex Ratio, Longevity, and Reproduction. Unmated females ($n = 4$) could oviposit on the first day of emergence but produced only male progeny (data not shown). Mated females produced both sexes, but the sex ratio (female: male) was strongly female-biased, ranging from 3.4 to 5.6. Exposure to the various test temperatures during development did not significantly influence the gender allocation of *G. ashmeadi* colonies (Table 4).

Ingestion of honey did not significantly affect the longevity of females ($F_{1,195} = 0.73$, $P = 0.3947$; Table 5). Longevity of females varied with temperature ($F_{4,195} = 120.35$, $P < 0.0001$). There was no interaction between temperature and diet ($F_{4,195} = 1.93$, $P = 0.1062$).

The longevity of female and male adults reared with water and/or honey varied significantly with temper-

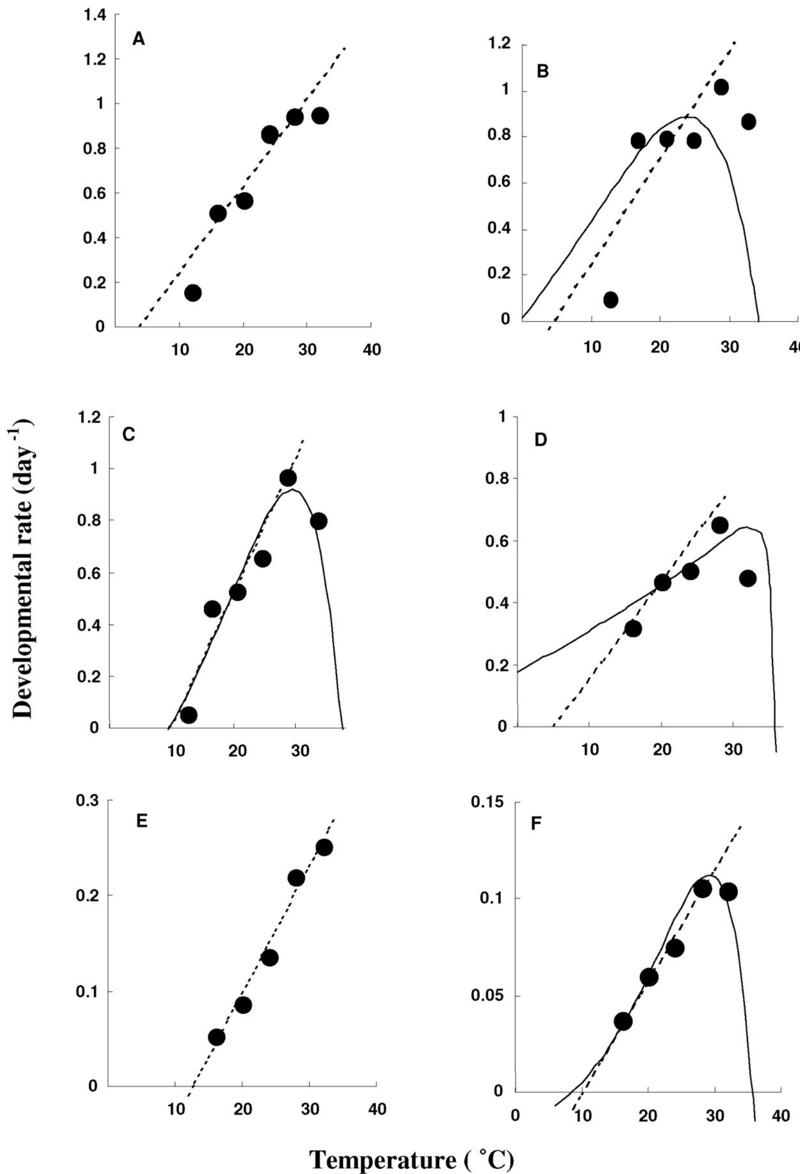

Fig. 1. Developmental rate (1/d) of egg stage (A), first instar (B), second instar (C), third instar (D), pupal stage (E), and combined immature stage (egg stage up to adult; F) of *G. ashmeadi* as a function of temperature. Observed (solid circles) and predicted values by linear regression model (straight line) for the temperature range of 12–28°C (12–32°C for egg and pupa), and the modified Logan model after Lactin et al. (1995) (curved line) for the range of 12–32°C. The parameter estimates derived from these figures are given in Tables 2 and 3.

ature (Table 5), with longevity increasing as temperature decreased. Female and male adults survive over three and two times longer, respectively, at 16°C than at 32°C, respectively. Longevity at 20°C was significantly shorter than at 16°C, but longer than at other temperatures. Adult longevity varied significantly with sex ($F_{1,178} = 8.26$, $P = 0.0045$) and temperature ($F_{4,178} = 94.24$, $P < 0.0001$). There was a significant interaction between sex and temperature ($F_{9,178} = 45.20$, $P < 0.0001$).

Observed and simulated daily survival (l_x) and age-specific oviposition at different temperatures are shown in Fig. 3. The data set of age-specific survivorship fitted by a Weibull frequency distribution analysis showed that the *G. ashmeadi* population maintained by water in the temperature range of 16–32°C had a type I survivorship curve ($c > 1.0$).

Mated females oviposited into host eggs on the first day of adult emergence (Fig. 3), and their fecundity was significantly influenced by temperature (Table 4).

Table 2. Calculated lower and upper temperature thresholds, optimum temperature of development, and thermal constant for *G. ashmeadi* Girault

Life stage	Lower threshold (°C)	Upper threshold (°C)[b]	Optimum temp (°C)[b]	n[c]	r^2	Thermal constant (degree-day)
Egg	4.02[ad]	—	—	240	0.68[a]	29.92[a]
First instar	3.79[ad]	37.18	23.87	240	0.90[b]	34.99[a]
Second instar	9.10[bd]	38.41	29.92	240	0.86[b]	26.70[b]
Third instar	6.15[a]	32.44	31.22	200	0.48[b]	32.32[a]
Pupa	12.76[a]	—	—	200	0.91[a]	75.44[a]
Egg-adult	8.50[b]	36.32	29.16	200	0.96[b]	206.68[b]

[a] Determined by linear regression.
[b] Determined with Logan model modified by Lactin et al. (1995).
[c] No. of observations.
[d] The temperature ranged from 12 to 32°C for the egg and pupal stages. For each experiment within the range of 16 to 32°C, 280–350 parasitized *H. coagulata* eggs (at 12°C, > 1450 eggs) were dissected. The unparasitized and superparasitized eggs were discarded and not calculated.

The highest number of eggs oviposited/female was recorded at 24°C, and this was 90% higher than those oviposited at 32°C. The reproductive rate (number of eggs/female/d) also varied significantly with temperature (Table 4). The highest reproductive rate occurred at 28°C, and the lowest at 16°C. Peak reproduction occurred on the first day of oviposition at all temperatures assayed (16–32°C). The adult females died within 1–2 d after cessation of oviposition when exposed to 20–32°C, but survived ~7 more d when exposed to 16°C (Fig. 3).

The intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T_c), population doubling time (T_d), and infinite rate of increase (λ) were estimated by a jackknife analysis of $l_x m_x$ life table data at the different temperatures (Table 6). These demographic growth parameters were significantly affected by temperature. The rearing temperature of 28°C had the largest r_m and λ and the shortest T_d and T_c. At 32°C, a decline in r_m and λ resulted in an increase of T_d and T_c compared with 28°C. Although this group had the highest net reproductive rate (R_0) at 24°C, the longer generation time (T_c) resulted in a decrease in r_m and λ values.

Discussion

This study documents the influence of temperature on the development, survival, reproduction, and life table parameters of *G. ashmeadi,* which are of importance in understanding the activity of this parasitoid in relation to controlling the glassy-winged sharpshooter. As expected, developmental durations of the egg, larval, pupal, and combined immature stages decreased as temperature increased from 16 to 28°C (Table 1). At 12°C, the duration of the egg stage, first and second instars were significantly longer, and development from the third instar to the pupal stage ceased. At 32°C, developmental durations of the larval stages as well as combined immature stages (egg, larval, and pupal) was slightly longer that that at 28°C. The total duration for development of combined immature stages of *G. ashmeadi* at 24°C was consistent with previous studies conducted by Triapitsyn et al. (2003) and Chen et al. (2006). Developmental times similar to that of *G. ashmeadi* have been reported for other solitary and gregarious species of *Gonatocerus* parasitoids. Solitary *G. tuberculifemur* (Ogloblin), took 12.6 d to complete development from egg to adult within *Tapajosa rubromarginata* (Signoret) eggs at 22.5–27.5°C (Virla et al. 2005), and *G. cicadellae* Nikolskaya, an egg parasitoid of the green leafhopper (*Tettigella viridis* Linné), took 10.1 d at 30°C (Miura and Yano 1988). A gregarious parasitoid, *G. fasciatus,* took 14–15 d to complete its life cycle within *H. coagulata* eggs at 20.5–25.5°C (Triapitsyn 2003).

Linear and nonlinear models are often used to describe the relationship between developmental rates of insects with temperature (Campbell et al. 1974, Logan et al. 1976, Lactin et al. 1995). In this study, the

Table 3. Survivorship (percentage) of immature stages of *G. ashmeadi* as a function of constant temperature and results of one-way ANOVA

Temperature (°C)	Egg	First instar	Second instar	Third instar	Pupa	From egg to adult
16	95.0 ± 2.9	92.5 ± 2.5	95.0 ± 2.9	90.0 ± 4.1	89.3 ± 1.4c	67.4 ± 6.0b
20	97.5 ± 2.5	95.0 ± 2.9	97.5 ± 2.5	100.0 ± 0.0	94.9 ± 1.0ab	85.7 ± 3.7a
24	100.0 ± 0.0	92.5 ± 2.5	100.0 ± 0.0	97.5 ± 2.5	95.5 ± 0.7ab	86.0 ± 1.3a
28	100.0 ± 0.0	97.5 ± 2.5	97.5 ± 2.5	95.0 ± 5.0	95.8 ± 1.1a	86.7 ± 6.3a
32	100.0 ± 0.0	97.5 ± 2.5	97.5 ± 2.5	95.0 ± 5.0	92.1 ± 1.4cb	83.4 ± 6.1ab
F	1.71	0.94	0.58	1.19	4.64	2.43
df	4,15	4,15	4,15	4,15	4,72	4,15
P	0.1991	0.4689	0.6838	0.3560	0.0022	0.0421

Each value represents the mean ± SE of four separate determinations for egg and larval stages. At least 10 parasitized eggs were dissected daily for the egg and larval stages and repeated four times for each temperature. For the pupal stage, at least 15 parasitized egg masses were dissected 3 d after adult emergence ceased. Data were arcsine-transformed to meet the assumption of normality before applying one-way ANOVA. Means within a column followed by the same letters are not significantly different at $\alpha = 0.05$.

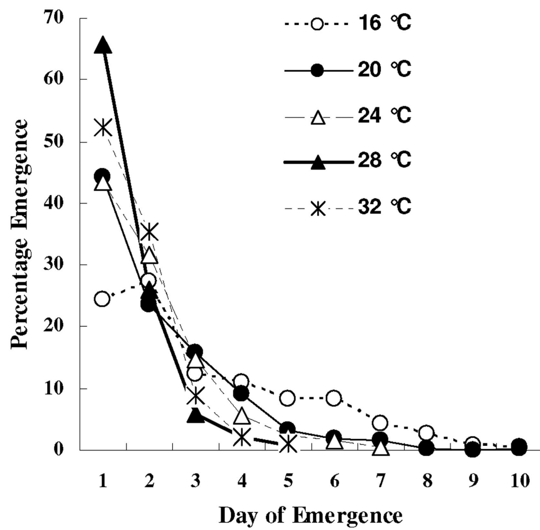

Fig. 2. Adult emergence pattern of *G. ashmeadi* as a function of temperature. Each point equals the means of at least 12 separate replicates. To avoid confusion, SEs are not included. For any given point, the value of the SE was <8% of the point's value.

Logan nonlinear model as modified by Lactin et al. (1995) was used to estimate optimum temperatures and upper temperature thresholds for development of first through third instars and combined immature stages. However, the model failed to correctly estimate lower temperature thresholds for development of the first and third instar stages because it produced negative values, and these values were incongruous with our experimental observations. Thus, the linear-degree day model was used to estimate the thermal constants and lower temperature thresholds for development of the embryo, pupa, and first and third instars. Hoddle (2002) found that the developmental rate of *Scirtoshrips perseae* also fit both linear and nonlinear degree-day models. Development of this thrip's egg stage was best fit to a linear regression, whereas development of the other stages was described by a curvilinear rate.

López et al. (2004) and Pilkington et al. (2005) reported that honey and honeydew from the citrus whitefly significantly increased the longevity of adult egg parasitoids, whereas our results showed that longevity of mated female *G. ashmeadi* provided honey and water was similar to those given only water (Table 4). However, those *G. ashmeadi* feeding on citrus flowers and *H. coagulata* excretion lived no longer than those fed on only water (Pilkington et al. 2005). Water is essential for maintaining these parasitoids because they die within 2–3 d if it is not available (data not shown). Like *G. cicadellae* (Miura and Yano 1988), honey may be needed by *G. ashmeadi* for extended reproduction.

Our results showed that the temperature effects on longevity varied with the sex of the adult parasitoid. The reason(s) why female *G. ashmeadi* outlived the males at 12 and 16°C remains unknown. However, our results indicate that both sexes have similar longevities in the temperature range of 20–32°C (Table 4). Virla et al. (2005) likewise reported that the longevity of male and female *G. tuberculifemur* also was similar within the range of 22.5–27.5°C.

This study shows that *G. ashmeadi* reproduces by gamogenesis and parthenogenesis. Mating usually takes place as soon as females emerge because the first-emerging males remain around the egg mass on the underside of the leaf surface. At 16–32°C, females have no premating or preovipositional period. They oviposited on the first day of emergence even if they had not mated, indicating that *G. ashmeadi* is a pro-ovigenic species that emerges with a full complement of mature eggs. The daily fecundity per female at five temperatures showed that 50–60% of the eggs were laid at 16–28°C and ≈32% at 32°C within 2 d after emergence and that the length of the ovipositional period varied with temperature (Fig. 3). Similar characteristics have been reported for other *Gonatocerus* species (Sahad 1982), *G. cincticipitis* (Miura 1990), *G. triguttatus* (Leopold et al. 2003), and *G. tuberculifemur* (Virla et al. 2005)

Miura (1990) reported that net reproductive rate (R_0), intrinsic increase rate (r_m), and finite increase rate (λ) of *G. cincticipitis* Sahad increased with the increase of temperature from 20 to 32°C. However, the same parameters estimated in this study for *G. ashmeadi* decreased at 32°C compared with 28°C. The life cycle of *G. ashmeadi* in this study showed that the

Table 4. Fecundity and sex ratio of *G. ashmeadi* as a function of temperature[a] and results of one-way ANOVA

Temperature (°C)	Fecundity/female[b] (means ± SE)			Sex ratio	
	n	Lifetime fecundity	Daily fecundity	*N*	Female/male
16	20	79.50 ± 3.23c	3.49 ± 0.23c	12	5.60 ± 1.18
20	20	94.30 ± 4.49ab	6.91 ± 0.48b	15	3.41 ± 0.66
24	20	105.15 ± 6.21a	10.30 ± 0.78a	17	3.93 ± 0.64
28	20	81.30 ± 5.65bc	10.75 ± 1.08a	20	5.29 ± 0.61
32	18	55.28 ± 4.83d	10.25 ± 1.36a	16	5.43 ± 0.70
F		13.50	13.26		1.75
df		4,97	4,97		4,75
P		<0.0001	<0.0001		0.1480

[a] Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$ (one-way ANOVA).
[b] Data were collected during the experiment assessing reproduction, where the females were provided with water after emergence.
n, no. of individuals; *N*, no. of replicates.

Table 5. Mean longevity (±SE) of female and male *G. ashmeadi* fed either water or water and honey at different temperatures and results of one-way AVOVAs

Temperature (°C)	Water		Water + honey			
	N	Female	*n*	Female	*n*	Male
16	20	24.80 ± 1.29a	16	27.06 ± 1.24a	10	19.00 ± 2.19a
20	20	15.60 ± 0.96b	29	16.02 ± 0.99b	20	14.01 ± 0.16b
24	20	11.90 ± 0.80c	23	9.44 ± 0.90c	18	9.00 ± 0.69c
28	20	9.50 ± 0.67cd	20	8.15 ± 0.60cd	16	7.31 ± 1.00c
32	18	7.78 ± 0.75d	19	6.42 ± 0.70d	17	6.94 ± 0.85c
F		52.93		68.05		25.45
df		4,93		4,102		4,76
P		<0.0001		<0.0001		<0.0001

A one-way ANOVA followed by a LSD test was used to determine if there were significant differences in longevity at the level of $\alpha = 0.05$. Means in the same column followed by the same letter are not significantly different.
n, no. of individuals in the analysis.

optimum temperature range for development (Table 1) and reproduction (Table 5) was between 24 and 28°C. Although the fecundity, longevity and oviposition period were higher at 24 than 28°C, the highest intrinsic rate of increase (r_m), fastest development, highest survivorship of immature stages (Table 3), and shortest net generation time and population doubling time were obtained at 28°C. The highest temperature (32°C) tested caused a decline in the r_m value.

The determination of low temperature thresholds for development and oviposition is essential for estimating the potential of *G. ashmeadi* in controlling *H. coagulata* populations under cool temperature conditions and for identifying the potential geographical

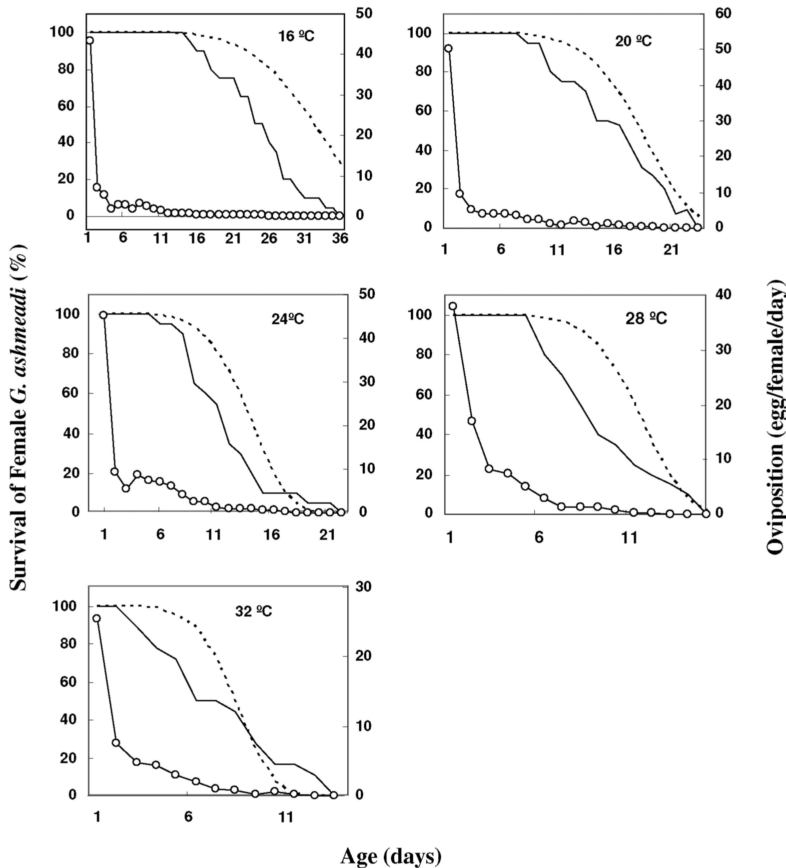

Fig. 3. Oviposition (circles), observed age-specific survivorship (solid line), and simulated age-specific survivorship by Weibull frequency distribution (dotted line) for *G. ashmeadi* as a function of temperature (°C). The shape and scale parameters of the distribution are as follows: 16°C, c = 5.34, b = 34.57, R^2 = 0.69; 20°C, c = 5.58, b = 19.08, R^2 = 0.78; 24°C, c = 5.54, b = 14.86, R^2 = 0.83; 28°C, c = 6.24, b = 11.98, R^2 = 0.76; 32°C, c = 5.92, b = 8.48, R^2 = 0.78.

Table 6. Jackknife estimates of demographic growth parameters (±SE) for *G. ashmeadi* populations at different temperatures

Temperature (°C)	*n*	R_0	T_c	r_m	λ	T_d
16	20	46.729a (±1.785)	26.711c (±0.214)	0.144a (±0.001)	1.155a (±0.002)	4.813e (±0.047)
20	20	68.415b (±3.340)	19.775b (±0.258)	0.215b (±0.002)	1.238b (±0.002)	3.243d (±0.029)
24	20	119.399d (±14.827)	18.142b (±0.799)	0.264c (±0.006)	1.303c (±0.008)	2.620c (±0.059)
28	20	72.139c (±8.665)	11.695a (±0.613)	0.366e (±0.011)	1.442e (±0.017)	1.892a (±0.056)
32	18	67.334abc (±9.837)	13.127a (±0.912)	0.321d (±0.014)	1.379d (±0.019)	2.151b (±0.083)

Means followed by the same letters within columns are not significantly different at $\alpha = 0.05$ according to a Student *t*-test used to perform pair-wise comparisons (two-tailed) of parameter estimates (Maia et al. 2000).

n, number of females in analysis; R_0, net reproductive rate; T_c, mean generation time; r_m, intrinsic rate of increase; λ, finite rate of increase. T_d, popuation doubling time in days.

range of this parasitoid. We found that *G. ashmeadi* was unable to complete its life cycle at 12°C and that the low temperature threshold for pupal development was nearly 13°C. The thermal constant for development from egg to adult was 207 DD. The average temperature during January and February in Riverside County, CA, is 11–12°C (89-yr weather records). This temperature would slow or stop development. This may account for the observation that *H. coagulata* eggs collected early in spring were not parasitized by *G. ashmeadi* and three other *Gonatocerus* species (Triapitsyn et al. 1998). It may also be the reason why other parasitoids including *G. ashmeadi* do not readily increase their numbers during the spring generation of *H. coagulata* in the colder regions of central and northern California. During the winter of 2000–2001 *G. ashmeadi* was reported to overwinter successfully in north Florida at a temperature range of 10–15°C (López et al. 2004). Under this temperature range, survival could be accomplished by an extended egg to adult developmental period as opposed to initiation of a dormancy mechanism.

Gonatocerus ashmeadi is primarily found in the southern tier of states of the United States and in northeastern Mexico (Vickerman et al. 2004). This distribution is in accord with the known geographic range of *H. coagulata* in the United States (Triapitsyn and Phillips 2000, Hoddle 2004). This suggests that the two insect species may have similar temperature requirements for development. Al-Wahaibi and Morse (2003) reported that the minimum temperature threshold of *H. coagulata* for complete embryonic development was 11.9°C, close to the threshold of 12.8°C for pupal development of *G. ashmeadi.* However, because information on other immature stages is lacking, it is uncertain if the thermal requirements for *H. coagulata* development is a factor influencing the natural distribution of the parasitoid. Hoddle (2004) suggested that cold stress in the form of an inadequate thermal summation may be a principle factor that limits the northern extent of the *H. coagulata* distribution.

In conclusion, this study clearly shows that optimum temperature for development, reproduction, and emergence of *G. ashmeadi* is near or at 28°C, although the parasitoid can complete its development within a temperature range of 16–32°C. Furthermore, the low temperature thresholds and thermal constants for development differed with each of the immature stages. This information is useful for developing an efficient protocol to mass rear these parasitoids for field release and for predicting their seasonal fluctuations and geographical distribution. Our baseline data on low temperature thresholds and day degrees for development indicate that *G. ashmeadi* would have difficulty in rapidly increasing their numbers in the cooler regions of California. Therefore, it is necessary to find cold tolerant egg parasitoids of the *H. coagulata* that have overwintering capabilities allowing effective biological control in these areas. Fortunately, this type of activity is in progress (Morse et al. 2005).

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