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Reproductive and developmental biology of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitoid of *Homalodisca coagulata* (Hemiptera: Cicadellidae)

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Abstract

The reproductive and developmental biology of *Gonatocerus ashmeadi* Girault, a parasitoid of the glassy-winged sharpshooter *Homalodisca coagulata* (Say), was determined at five constant temperatures in the laboratory: 15; 20; 25; 30; 33 °C. At 30 °C, *G. ashmeadi* maintained the highest successful parasitism rates with 46.1% of parasitoid larvae surviving to adulthood. Lifetime fecundity was greatest at 25 °C and fell sharply as temperature either increased or decreased around 25 °C. Temperature had no effect on sex ratio of parasitoid offspring. Mean adult longevity was inversely related to temperature with a maximum of 20 days at 15 °C to a minimum of eight days at 33 °C. Developmental rates increased nonlinearly with increasing temperatures. Developmental rate data were fitted with the modified Logan model for oviposition to adult development times across each of the five experimental temperatures to determine optimal and upper lethal temperature thresholds. The lower developmental threshold estimated by the Logan model and linear regression were 1.10 and 7.16 °C, respectively. Linear regression of developmental rate for temperatures 15–30 °C indicated that 222 degree-days were required above a minimum threshold of 7.16 °C to complete development. A temperature of 37.6 °C was determined to be the upper development threshold with optimal development occurring at 30.5 °C. Demographic parameters were calculated and pseudo-replicates for intrinsic rate of increase (r_m), net reproductive rates (R_o), generation time (T_c), population doubling time (T_d), and finite rate of increase (λ) were generated using the bootstrap method. Mean bootstrap estimates of demographic parameters were compared across temperatures using ANOVA and nonlinear regression.

Keywords: Bootstrap; Degree-days; Demographics; Life table; Intrinsic rate of increase; Net reproductive rate; Biological control; *Gonatocerus ashmeadi*; *Homalodisca coagulata*

1. Introduction

The mymarid parasitoid species *Gonatocerus ashmeadi* Girault, *Gonatocerus triguttatus* Girault, *Gonatocerus morrilli* Howard, and *Gonatocerus fasciatus* Girault (Hymenoptera: Mymaridae) are the most common natural enemies associated with *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), the glassy-winged sharpshooter, in its home range of Southeastern USA and Northeastern Mexico (Triapitsyn and Phillips, 2000). *G. ashmeadi* is a self-introduced parasitoid into California, USA, and most likely came into California from Florida (USA) in parasitized *H. coagulata* eggs on ornamental plants prior to 1978 (Vickerman et al., 2004). *G. ashmeadi* is a solitary endoparasitoid that attacks eggs of sharpshooters in the cicadellid tribe Proconiini (Triapitsyn et al., 2002). *G. ashmeadi* most likely established on the California native *H. liturata* Ball and has since established a local association with *H. coagulata* following successful establishment of this insect in California around 1990 (Sorensen and Gill, 1996). In addition to establishing in California, *H. coagulata* has also invaded French Polynesia, where it established in 1999

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(Cheou, 2002), Hawaii in 2004 (Hoover, 2004), and Easter Island in 2005 (Sandra Ide pers. comm., 2005).

Since establishing, *H. coagulata* has become a major economic pest in Southern California. Feeding exclusively on xylem fluid, *H. coagulata* avoids secondary plant compounds that act as natural plant defenses (Raven et al., 1992) and it is an efficient vector of the xylem-dwelling bacterial pathogen *Xylella fastidiosa*. This bacterium causes a variety of diseases in many urban, natural, and agricultural plants (Almeida and Purcell, 2003a; Almeida and Purcell, 2003b; Costa et al., 2000). Oleander leaf scorch alone has caused damage of more than $52 million on freeway median vegetation (Costa et al., 2000), and nearly $7 million was spent on large pesticide application projects that focused on area-wide spray regimes during a time in which Riverside and San Diego counties (both in Southern California) had estimated losses of up to $38 million due to plant ailments caused by *X. fastidiosa* (Siebert, 2001). The major agricultural concern from *H. coagulata–Xylella* in California is to wine, table, and raisin grape industries. Commercial grape varieties are very sensitive to infection with *Xylella* and can not be cured once infected. A potential future threat is the disease citrus variegated chlorosis (CVC), also caused by the pathogen *X. fastidiosa* and vectored by sharpshooters (Brlansky et al., 2002). CVC is not yet present in California, but its arrival would have a negative impact on citrus production.

Insecticide control of *H. coagulata* has featured prominently in management strategies (Akey et al., 2001; Bethke et al., 2001) though the use of IPM approaches and biological control have been experiencing increasing research attention (Jones, 2002; Morgan et al., 2000). Classical biological control of *H. coagulata* is being pursued vigorously in California. One factor that may limit the successful establishment and impact of natural enemies used in a classical biological control program is imprecision in matching the environmental conditions in the natural enemy's home range with those in the area into which natural enemies are to be released (Goolsby et al., 2005; Hoddle, 2004).

A thorough understanding of the reproductive and developmental biology of candidate natural enemies is paramount when predicting potential establishment and population growth of introduced taxa (Godfray and Waage, 1991; Rochat and Gutierrez, 2001). Characterization of biological attributes such as degree-day requirements, and intrinsic rates of increase, can aid evaluation of natural enemies for use in classical biological control and to assist interpretation of impact and spread in the field (Daane et al., 2005; Dahlsten et al., 2005). The developmental biology and degree-day requirement for *H. coagulata* egg development has been determined (Al-Wahaibi and Morse, 2003) but equivalent studies for parasitoids attacking *H. coagulata* eggs have not been previously studied.

Life tables are commonly used in studies addressing how the life expectancy of organisms may change if affected by environmental changes (Stäubli Dreyer et al., 1997; Tsai and Wang, 2001). Construction of vertical life tables allows the calculation of key demographic characters such as the intrinsic rate of increase. A major shortfall with life table construction is that there is no replication as data are compiled for the production of one summary table, making it difficult to assess the level of variability in the calculated life table statistics which makes statistical testing impossible (Hulting et al., 1990; Meyer et al., 1986). Some insect species are amenable to having several independent life tables developed over several sequential generations. This allows the construction of a dataset large enough to assess variability of key demographic parameters (Aryawan et al., 1993). Re-sampling procedures such as bootstrapping or jackknifing can be used to create multiple datasets from single life tables from which replicated life table parameters can be derived. These "pseudo-replicates" enable estimates of variation for parameters of interest that can be compared statistically across treatments (Efron, 1981; Maia et al., 2000; Meyer et al., 1986; Miller, 1974).

The jackknife procedure estimates standard errors for life table parameter estimates by sequentially removing one female and her offspring from the original dataset and recalculating each life table parameter from the truncated dataset. After creating this pseudo-replicate, the removed individual and her offspring is replaced in the dataset and the next female is removed to create another pseudo-replicate until all individuals have been removed once and the life table parameters of interest have been calculated (Hoddle et al., 2000). These pseudo-replicates, a maximum of $n-1$, form a set of numbers from which means and associated variances can be calculated and compared statistically (Efron, 1981; Meyer et al., 1986; Miller, 1974).

One life table statistic of interest is net reproductive rate (R_o), the number of daughters produced/female in the population of interest (Carey, 1989). When the jackknife is used to generate pseudo-values for R_o, an adjustment of the pseudo R_o value is needed to account for the reduced sample size and is expressed as:

$$R_{o.adj} = n \times R_{o.all} - (n-1) \cdot R_{o.jackknifed} \quad \text{(Meyer et al.,1986).}$$

Subsequently, the removal of a female that produces no offspring will create an increase in $R_{o.jackknifed}$ large enough to create a zero or negative $R_{o.adj}$ value (Meyer et al., 1986). The jackknife analysis has limited analytical application for populations that have a large number of females that produce no offspring, as was the case in this study (see Section 3). In datasets, where this occurs it is possible to circumvent this problem and obtain pseudo-values by using the bootstrap method which was the protocol used in this study.

The bootstrap method has been regarded as being more reliable for estimating variance in the life table values than jackknifing (Efron, 1981) although the two methods have been increasingly considered as comparable in most aspects (Meyer et al., 1986). The bootstrap procedure involves randomly removing an individual from the life table and recal-

culating the statistical values. That individual is replaced prior to another being selected randomly and the values once again recalculated and, in essence, re-samples the experimental population (Meyer et al., 1986). The bootstrap procedure may be repeated as many times as desired, creating an almost infinite number of pseudo-replicates.

Insects regulate their body temperatures through heat exchange with their surrounding environment and have a nonlinear relationship between development rate and temperature (Lactin et al., 1995). Generally, as temperature increases the development rate for poikilothermic organisms rises from a threshold of zero development to an optimal developmental rate before falling rapidly again to a developmental rate of zero, when the upper lethal threshold is reached (Lactin et al., 1995). The modified Logan model (Lactin et al., 1995), accounts for this nonlinear relationship between developmental rate and temperature, and estimates a developmental threshold above which development occurs. In addition to this nonlinear model, linear regression analysis can be used to calculate the lower developmental threshold and degree-day accumulation necessary to complete development of pre-imaginal stages. This study used the nonlinear model to determine the upper development threshold and linear modeling to calculate the lower developmental threshold.

The purpose of work presented here was to study the developmental and reproductive biology of *G. ashmeadi* under controlled laboratory conditions. Population demographic data were generated across five experimental temperatures and subjected to bootstrap analyses to enable statistical comparison of key demographic parameters. The modified Logan model was used to determine lower threshold, optimal, and upper lethal temperatures. Linear regression was used to compare Logan model estimates of minimum threshold for development and to estimate degree-day accumulation for egg to adult development.

Improved understanding of the basic biology of *G. ashmeadi*, the key natural enemy of *H. coagulata* in California, will assist mass-rearing efforts of this parasitoid; optimize timing of inoculative field releases; facilitate better understanding of parasitoid impact on *H. coagulata* in various climatic zones in California; will assist with targeted collecting for biotypes of *G. ashmeadi* in the home range of *H. coagulata* that may exhibit unique climatic adaptations that current parasitoid populations in California lack.

2. Materials and methods

2.1. Homalodisca coagulata egg production

One hundred potted, insect-free 'Eureka' lemon trees, *Citrus limon* (L.) Burm., a cultivar preferred by *H. coagulata* for egg laying (Irvin and Hoddle, 2004) were planted in 1 L pots, watered twice weekly with an initial application of fertilizer (Dynamite plant food; Enviro-Safe Laboratories, Inc., Miami, FL, USA) followed with a second application at 6 months. Trees were placed in two large ($2.4 \times 1.8 \times 1.8$ m), mesh screened walk-in cages located within a temperature-controlled glasshouse ($6.5 \times 3.8 \times 3.8$ m) maintained at 15–28 °C at Ag-Ops Field Station, University of California, Riverside, California. Daily field collection of *H. coagulata* adults was sorted from other insects in the laboratory and approximately 500–1000 adults were added to these caged plants. Caged plants were inspected daily, and all *H. coagulata* egg masses were removed every 24 h to ensure the age of eggs being presented to parasitoids was consistently within the range of 24–48 h of age, an age range readily utilized by *G. ashmeadi* for parasitization (Irvin and Hoddle, 2005).

2.2. Source of parasitoids and set up of experimental arenas

Gonatocerus ashmeadi females were sourced from the CDFA mass-rearing facility at Mt. Rubidoux, Riverside, California. *H. coagulata* egg masses on 'Eureka' lemon leaves were placed through holes drilled in the lid of a 130 ml plastic vial filled with deionized water. A second 130 ml plastic vial with ventilation [three 2 cm holes (one on the bottom, and one on each of two sides) covered with mesh netting (80 μm Jelliff Corporation, Southport, CT)] was inverted and attached to the lid of the vial holding the water and lemon leaves (Irvin and Hoddle, 2005). A piece of parafilm "M" (Pechiney Plastic Packaging, Menasha, WI, USA) was stretched and placed between the bottom vial and lid. Petioles were pushed through the parafilm to reduce incidence of the parasitoid drowning and minimize condensation accumulation in the upper part of the cage.

2.3. Experimental temperatures and number of females used for experiments

Five individual growth cabinets (Percival Scientific Inc., Perry, IA) were set at 15 °C (±0.2 °C) (28 females set up), 20 °C (±0.6 °C) (18 females), 25 °C (±0.5 °C) (22 females), 30 °C (±0.5 °C) (22 females), and 33 °C (±0.3 °C) (17 females) with long days (14:10 L:D) and relative humidity (RH) set at 50%. Temperatures and RH in cabinets were measured every 30 min with HOBO data loggers (Onset Computer Co., Bourne, MA, USA).

2.4. Monitoring parasitoid progeny production

One-day-old mated female *G. ashmeadi* were placed individually in cages containing approximately 30–40-, 24–48-h-old *H. coagulata* eggs, and a 5% honey–water solution. After 24 h, females were moved to a new cage containing fresh, unparasitized *H. coagulata* eggs of the same age range. This procedure was repeated every 24 h until parasitoids died. The cause of death was recorded as natural or other (e.g., drowning in condensation or escape).

When parasitoid or *H. coagulata* emergence was first detected from experimental egg masses, leaves were

removed from cages, and placed in sealed, 9 mm Petri dishes (Becton–Dickinson Labware, Franklin Lakes, NJ, USA) lined with moistened filter paper, maintained at the same experimental temperature, and checked daily for continued emergence. The length of time between oviposition and progeny emergence, sex of offspring, and percentage parasitism were recorded for every egg mass at each experimental temperature. At temperatures where high instances of no emergence was observed (i.e., 33 °C), *H. coagulata* eggs were dissected and the number of developed but dead parasitoid larvae and pupae was recorded and used as estimates of parasitism. At each experimental temperature, 60 *H. coagulata* eggs were placed in separate cages not containing parasitoids, and these eggs were used as a control to determine if parasitism of eggs in the *H. coagulata* colony prior to presentation to females used in experiments had occurred. Preoviposition, oviposition to adult development times, and oviposition rates were calculated using all female parasitoids regardless of their cause of death. Total progeny, mean daily progeny, and sex ratio were calculated using data from parasitoids that died of natural causes.

2.5. Calculation of demographic growth parameters

Time from oviposition to emergence, adult parasitoid survivorship rates, daily fecundity, and sex ratio of offspring at each experimental temperature were used to construct $l_\chi m_\chi$ life tables from which demographic growth parameters were calculated. Only data from adult parasitoids that died naturally were used for calculation of demographic growth parameters (15 °C, $n = 10$; 20 °C, $n = 10$; 25 °C, $n = 10$; 30 °C, $n = 9$; 33 °C, $n = 10$). The following demographic parameters were calculated from $l_\chi m_\chi$ life tables:

1. Net reproductive rates ($R_o = \sum l_\chi m_\chi$, where $l_\chi m_\chi$ is the net female maternity, l_χ is the fraction of females alive at age χ and m_χ is the number of daughters born to surviving females at age χ), express the per generation growth rate of the population as the number of daughters produced by females ($R_o > 1.0$ the population is increasing, $R_o = 1.0$ population is at equilibrium, and $R_o < 1.0$ population growth is declining (Deevey, 1947)).
2. Mean generation time ($T_c = \sum \chi l_\chi m_\chi / R_o$) is the average interval separating births of one generation from the next (Deevey, 1947). Direct estimates of generation time were calculated from individual parasitoid offspring development time from oviposition to adult emergence.
3. The intrinsic rate of natural increase, r_m, is the maximum exponential rate of increase by a population growing within defined physical conditions (Birch, 1948). The value of r_m is iterated until the equation $1 = \sum l_\chi m_\chi \exp(-r_m)$ is solved.
4. Finite rate of increase, $\lambda = \exp(r_m)$, is the factor by which a population multiplies between each time step (Birch, 1948).
5. Population doubling time, $T_d = \ln(2)/r_m$, is the time required by a population, growing exponentially without limit, to double in size when increasing at a given r_m (Carey, 1989).

Mean demographic parameter estimates with standard errors were generated using bootstrap pseudo-replication of $l_\chi m_\chi$ life table data. At each temperature, life tables and associated demographic parameters were constructed for each possible outcome for the random selection, removal, and replacement of individual female parasitoids from the cohort of 9–10 females that died from old age for each specific experimental temperature. Female parasitoids dying prematurely from other causes were discarded for these analyses. The results for each value of R_o, r_m, T_c, T_d, and λ calculated for each life table generated by random female removal were placed in a table in rows, with each row being allocated a sequential number to identify it. A second table was created that contained 1000 pseudo-replicates for each demographic parameter. This was achieved by adding a column that contained a random number generated between 1 and 9 or 10, inclusive (i.e., the number of females dying from old age in the experimental cohort that was used to generate the $l_\chi m_\chi$ life table).

Each row in the new table used the random value to auto-populate the adjacent cells by using the MS Excel "vlookup table" protocol to return values that matched the identifying number with the generated random number. This simple procedure emulated the recalculation of the life table parameters with a random individual extracted and then replaced after calculation as many times as needed. Spreadsheet templates for the manual calculation of life table parameters and bootstrapping procedure may be downloaded from http://www.biocontrol.ucr.edu/devbiology.

The new dataset calculated by bootstrapping contains many pseudo-replicates and no adjustment for the removed individual is necessary, and bias in the generated mean R_o value ($R_{o.adj}$) is reduced with the equation:

$$R_{o.adj} = 2 \times R_{o.all} - R_{o.bootstrapped}$$ (Meyer et al.,1986).

From this bootstrap analysis, mean and variance estimates for each demographic parameter of interest (i.e., equations 1–5 above) were calculated for *G. ashmeadi* at each experimental temperature.

2.6. Developmental rates, temperature thresholds, and degree-day calculations

Developmental rates for each experimental temperature were calculated as the reciprocal time in days from oviposition to the emergence of adult parasitoids. The modified Logan model was fitted to the reciprocal of mean developmental time in days for each temperature (Lactin et al., 1995). The nonlinear fitted line from the modified Logan model intercepted the x-axis and identified the lower developmental threshold. The optimum temperature for insect

development was identified as the peak in the fitted line and the upper lethal threshold was identified as the point where the line crossed the *x*-axis at a higher temperature. The highest temperature, 33 °C, was omitted from the fitting of the linear regression model to the same data to obtain a more accurate indication of the linear portion of the data. Degree-day requirements were calculated using the inverse slope of this fitted linear regression line (Campbell et al., 1974).

2.7. Statistical analysis

Mean adult longevity, successful oviposition rates (oviposition resulting in viable offspring), parasitism rates (oviposition events that include dissected eggs containing *G. ashmeadi* larvae that did not emerge), daily and lifetime fecundity, and preoviposition means were compared with ANOVA and Tukey's Studentized Range Test at the 0.05 level of significance using SAS 9.1 (SAS Institute, 2002). The number of offspring emerging from parasitized eggs were transformed ($\sqrt{(\chi + 0.001)}$) before analysis.

The demographic parameter means were subjected to nonlinear regression to estimate the affect of different temperatures on life table values outside the experimental temperature range. The pseudo-values for R_o, r_m, T_c, T_d, and λ were analyzed with ANOVA and Tukey's Studentized Range Test ($P = 0.05$) to determine if there were statistical differences between the temperatures and quadratic regression lines fitted to the data using SAS 9.1 (SAS Institute, 2002).

3. Results

3.1. Partial life tables and developmental and reproductive estimates

Life tables were constructed for the number of *G. ashmeadi* adults entering each age class and their realized and actual mortality rates (Table 1). Analysis of variance showed that mean adult longevity, the length of time from adult emergence to natural death, was significantly different between temperatures ($F = 6.155$, $df = 4,44$, $P < 0.001$) and was greatest with a value of 20.0 days at 15 °C, declining to a low of 7.9 days at 33 °C (Table 2).

Oviposition that led to emergence of offspring was highest at 30 °C (Fig. 1) and was significantly different between temperatures ($F = 57.204$, $df = 4,102$, $P < 0.001$). At 30 °C, 46.1% of *H. coagulata* eggs presented to parasitoids produced viable progeny. Parasitism rates decreased to 3.7 and 10.0% at 15 and 33 °C, respectively, and emerged progeny at 15 and 33 °C were significantly lower than 20, 25, and 30 °C ($F = 38.441$, $df = 4,44$, $P < 0.001$). Longevity of adult *G. ashmeadi* was greatest at lower temperatures and progeny production increased with higher temperatures (Fig. 2).

Preoviposition was longest at 15 °C, total progeny and mean daily offspring was highest at 25 °C (Table 2). Direct

Table 1
Partial life table for *Gonatocerus ashmeadi* reared at five constant temperatures

Age class	l_χ[a]					d_χ[b]					q_χ[c]					M_r[d]				
	15 °C	20 °C	25 °C	30 °C	33 °C	15 °C	20 °C	25 °C	30 °C	33 °C	15 °C	20 °C	25 °C	30 °C	33 °C	15 °C	20 °C	25 °C	30 °C	33 °C
0–3	10	10	10	9	10	0	0	0	1	2	0.00	0.00	0.00	0.11	0.20	0.00	0.00	0.00	0.11	0.20
4–7	10	10	10	8	8	0	2	3	1	4	0.00	0.20	0.30	0.13	0.50	0.00	0.20	0.30	0.11	0.40
8–11	10	8	7	7	4	2	1	2	2	4	0.20	0.13	0.29	0.29	1.00	0.20	0.10	0.20	0.22	0.40
12–15	8	7	5	5	0	3	3	4	5	0	0.38	0.43	0.80	1.00	0.00	0.30	0.30	0.40	0.55	0.00
16–19	5	4	1	0	0	0	2	1	0	0	0.00	0.50	1.00	0.00		0.00	0.20	0.10	0.00	
20–23	5	2	0	0	0	2	1	0	0	0	0.40	0.50	0.00			0.20	0.10	0.00		
24–27	3	1	0	0	0	1	1	0	0	0	0.33	1.00				0.10	0.10			
28–31	2	0	0	0	0	2	0	0	0	0	1.00	0.00				0.20	0.00			

Life stage shown is adult and age class is representative of this stage and disregards earlier life stages.
[a] l_χ = number entering stage.
[b] d_χ = number dying in stage.
[c] q_χ = apparent mortality, the proportion dying within the age class.
[d] M_r = real mortality, the proportion dying within the age class reflected as a function of the number entering the first age class.

Table 2
Mean adult longevity (±SE), mean preoviposition period (±SE), mean daily fecundity, lifetime fecundity (±SE), direct generation time (±SE) and female sex ratio of progeny of mated female *Gonatocerus ashmeadi* at each experimental temperature

	Temperature (°C)				
	15	20	25	30	33
Adult longevity (days)	20.00 ± 2.69a	15.90 ± 2.02ab	12.00 ± 1.23bc	10.56 ± 1.36bc	7.90 ± 0.98c
Preoviposition period (days)	4.53 ± 0.486a	0.61 ± 0.293b	0.182 ± 0.125b	0.227 ± 0.113b	0.235 ± 0.106b
Total progeny	7.07 ± 1.767a	46.35 ± 8.387b	63.75 ± 8.362b	59.88 ± 6.931b	10.37 ± 1.562a
Mean daily progeny	0.24 ± 0.098a	1.66 ± 0.353ab	2.24 ± 0.685b	2.10 ± 0.685b	0.37 ± 0.152a
Mean developmental time (days)	36.16 ± 1.859a	18.86 ± 0.172b	12.59 ± 0.106c	9.87 ± 0.075d	10.91 ± 0.226d
Sex ratio (% female offspring)	65.11 ± 7.348a	65.03 ± 6.807a	71.54 ± 6.020a	64.54 ± 5.690a	65.08 ± 6.461a

Values with different letters indicate significant differences at 0.05 level of confidence.

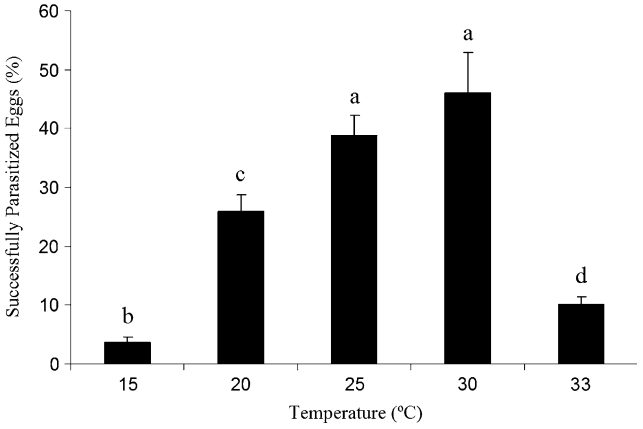

Fig. 1. Parasitized *H. coagulata* eggs (excluding dissection of parasitoids) from which adult *G. ashmeadi* emerged shown as a percentage over the entire number of eggs made available to mated female parasitoids at each experimental temperature. Values with different letters indicate significant differences at 0.05 level of confidence.

estimates of development time (i.e., mean number of days [±SE) from oviposition to emergence of adult parasitoids) derived from temperature development studies were significantly different for all temperatures except 30 and 33 °C (Table 2). There were no statistically significant differences in sex ratio among the temperatures (Table 2).

3.2. Demographic growth parameters

Mean net reproductive rate (R_o) ($F = 73857.9$, $df = 4,4995$, $P < 0.001$), intrinsic rate of increase (r_m) ($F = 732354$, $df = 4,4995$, $P < 0.001$), and finite rate of increase (λ) ($F = 683820$, $df = 4,4995$, $P < 0.001$) were all significantly different between temperatures and was highest for *G. ashmeadi* reared at a constant 25, 30, and 30 °C, respectively. Population doubling times, T_d, showed a statistically significant difference between temperatures ($F = 178{,}515$, $df = 4$, 4995, $P < 0.001$) and were lowest when parasitoids were reared at 30 °C. Mean generation time, T_c, was significantly different between temperature and was lowest at 33 °C ($F = 1821157$, $df = 4,4995$, $P < 0.001$). Quadratic lines were fitted to the means for each life table parameter and accounted for 79.6 to 99.7% of the observed variance (Fig. 3).

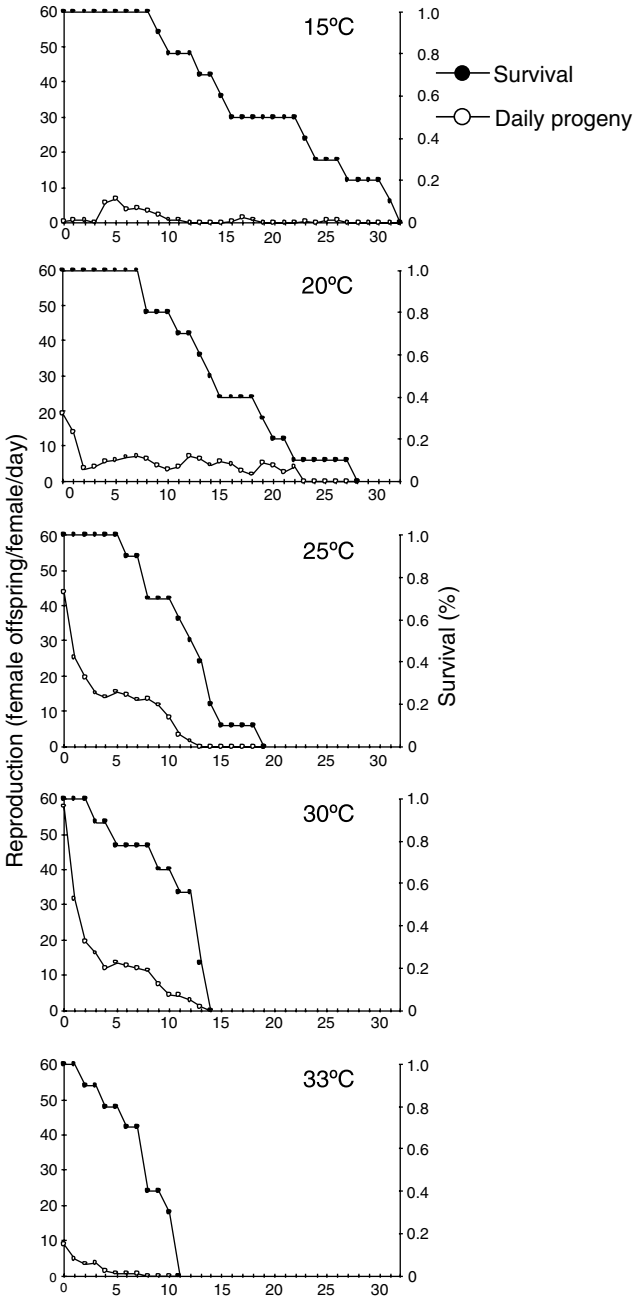

Fig. 2. Mean (±SE) observed age-specific female progeny from individual parasitoids and survivorship data for *G. ashmeadi* at five temperatures.

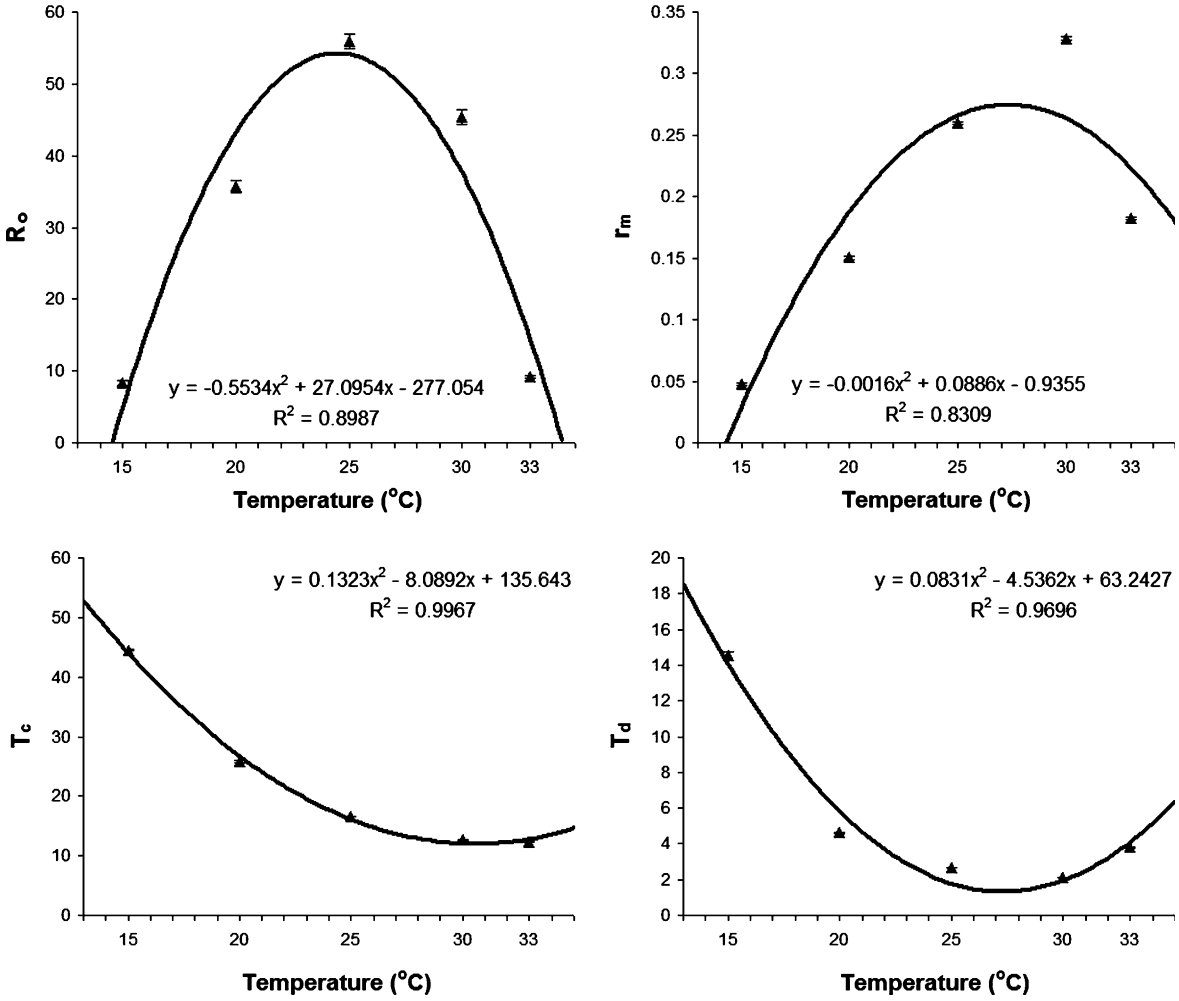

Fig. 3. Fitted quadratic lines for life table statistics R_o, r_m, T_c, and T_d for *G. ashmeadi* at each experimental temperature.

3.3. Developmental rates, temperature thresholds, and degree-days

The developmental rate for *G. ashmeadi* was nonlinear and the fitted Modified Logan model was highly significant ($F = 1292.27$, $df = 4,495$, $P < 0.005$) (Fig. 4). The fitted model converged on a lower developmental threshold for *G. ashmeadi* of 1.1 °C. The modified Logan model estimated upper maximum temperature for development at 37.6 and 30.5 °C was the estimated as the optimal temperature for development. Linear regression indicated immature *G. ashmeadi* required a total of 222 degree-days to complete development from oviposition to adult emergence and the lower temperature threshold for development was estimated at 7.16 °C from this analysis. The modified Logan model was used to ascertain the upper lethal limit and regression on the linear portion of the development data was used to estimate the lower development threshold.

4. Discussion

Gonatocerus ashmeadi is the key mymarid parasitoid species contributing to biological suppression *H. coagulata* in its native area of Southeastern USA and Northeastern Mexico (Triapitsyn and Phillips, 2000). The impact of *G. ashmeadi* as a regulating factor of populations of *H. coagulata* appears to be lower in California than in its

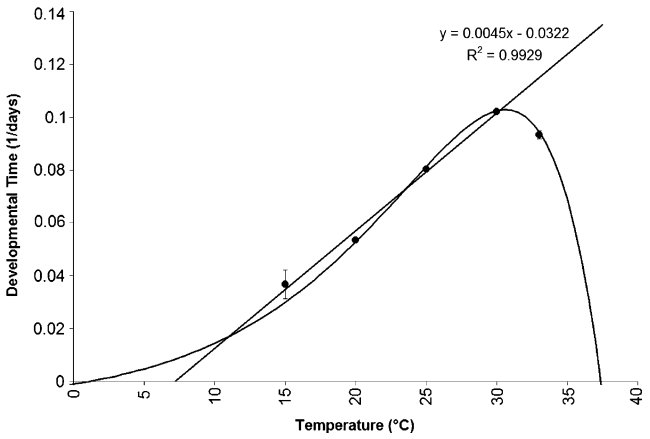

Fig. 4. The developmental rate of *G. ashmeadi* from time of oviposition to adult emergence expressed as the relationship of developmental rates and temperature fitted to the modified Logan model as described by Lactin et al. (1995) and using linear regression (Campbell et al., 1974).

home range. This reduced regulation of *H. coagulata* by the biological control agent *G. ashmeadi* may be influenced by climatic conditions in the invaded areas. Low temperatures over winter appear reduce or prevent oviposition by *H. coagulata* for extended periods which results in a shortage of hosts for *G. ashmeadi* (Pilkington et al., 2005).

The survival rates and longevity for adult *G. ashmeadi* were highest at 15 °C although progeny production was negligible at this temperature. The number of offspring produced by individual parasitoids was greatest at 20–30 °C with each female producing two to three times more offspring than females maintained at 15 or 33 °C. The net reproductive rate, R_o, was highest at 25 °C, being 83.6–85.2% higher than the highest (33 °C) and lowest (15 °C) temperatures evaluated, respectively. Population doubling time, T_d, was lowest at 30 °C, reflecting lower numbers and survivorship rates of offspring produced. Population doubling times were 6.8 times and 1.8 times greater at 15 and 33 °C, respectively, than 20 °C indicating that much longer periods of time is needed at these temperatures for populations to increase. Population doubling time increased by 20% when temperature was reduced from 30 to 25 °C.

These results indicate that modest changes in constant temperature can affect the reproductive capabilities of *G. ashmeadi* and, possibly, the parasitoid's ability to impact on populations of its host, *H. coagulata*. The development of offspring has been predicted to occur at temperatures above a developmental threshold ranging 1.1–7.16 °C (lower estimate from modified Logan model; upper estimate from linear regression) and below the upper lethal temperature of 37.6 °C. Daily weather data for 10 years over the period 1995–2004 recorded at the University of California, Riverside Agricultural Operations Facility, a major citrus production center where *H. coagulata* research is conducted, indicated that over the year, daily mean minimum and maximum temperatures were 3.8 °C (±1.31 SE) and 37.33 °C (±1.09 SE), respectively, which are within the tolerance levels of *G. ashmeadi* as indicated from the results of the modified Logan model analysis conducted in this study.

Temperature can have a significant impact on R_o estimates for *G. ashmeadi*. The fitted quadratic model for R_o, a measure of a population's growth rate, indicated that at approximately 14.6 °C the value of R_o falls below 1.0, indicating that parasitoid population growth will cease and begin to contract. During this 10 year span, the weather station at the University of California, Riverside Agricultural Operations Facility recorded the average daily temperature fall below 14.6 °C 107 times or 28% of the year. The 10 year average daily temperature in Riverside falls below 14.6 °C in a single, discrete block of 100 days typically over the period November–March. During this three month time span, temperatures fluctuate from a minimum of 3.8 °C to a maximum high temperature of 22.89 °C. Although temperatures rise above the development threshold required by the parasitoid (determined using linear regression analysis) calculations indicate that the population would accumulate enough degree-days to complete 2.4 generations in this time. Temperatures may rise enough to prompt sporadic oviposition by parasitoids if host eggs are available but the persistent low temperatures will retard parasitoid population growth. Host availability notwithstanding, this suggests populations of *G. ashmeadi* in Riverside California would contract markedly over the period November–March each year because of impaired reproductive performance at temperatures below 14.6 °C periods for prolonged periods.

The quadratic equation for R_o returns a temperature of 34.3 °C where the population will begin to contract above this temperature. There are no days where the maximum daily average temperature is above this limit in Riverside, California. The value for R_o reaches zero at approximately 34.4 °C predicting that above this temperature the population will not produce any offspring. This value is within 10% of the estimate returned via the modified Logan model. Temperature range estimations for $R_o > 1.0$ are useful because it allows prediction of population growth outside a laboratory environment. The temperature at which a population increases may provide information on the success and impact of a parasitoid population infiltrating areas that experience widely varying temperature regimens (e.g., Southern California) or more stable year round temperatures (e.g., Hawaii where *G. ashmeadi* has established independently of deliberate human assistance). Further, in new areas where releases of *G. ashmeadi* are planned (e.g., French Polynesia and Easter Island), these demographic data allow for the estimation of biofix points that can be used to time parasitoid releases in the field, assist with monitoring, and assess climatic factors affecting levels of control.

Population surveys undertaken bi-weekly since 2001 in Riverside indicate *G. ashmeadi* is present in the field for 75% of the year and populations drop below detectable levels during December, January, and February (Pilkington et al., 2005). These data reinforce predictions from R_o analyses that indicate wild populations of *G. ashmeadi* would contract for 25% of the year in Riverside, California regardless of the availability of hosts as low winter temperatures do not facilitate parasitoid reproduction.

Conversely, near Weslaco in Southeastern Texas (USA), where populations of *G. ashmeadi* impact *H. coagulata* significantly (Vickerman et al., 2004), the average daily temperature, between January 1, 1995, and December 31, 2004, was above the minimum R_o threshold of 14.6 °C for 355 days (i.e., 97%) in the year. These temperature data for Weslaco suggests that temperatures fall to a significantly low level too infrequently to adversely affect *G. ashmeadi* populations to a significant level. The warmer winter temperatures in Texas may, in part, explain why *G. ashmeadi* is thought to have a greater impact on *H. coagulata* in this region of the USA.

By understanding the effects temperature and climate have on the development and reproduction of potential biological control agents and optimizing the match to receiving environments, a greater degree of successful

establishment and efficacy may be achieved (Baker, 2002). It has been proposed that environmental conditions such as temperature, rainfall, and humidity should be monitored in a proposed biological control agent's native region to identify conditions that may prevent their successful establishment in introduced areas (Goolsby et al., 2005). Life table statistics, such as those presented here, provide a valuable tool in the evaluation of a potential biological control agent in an area that is climatically different to that of its native area and this approach has demonstrated utility (Bernal and González, 1997).

Consequently, we emphasize that all data presented in this study were produced by providing single parasitoid females unlimited host resources and by removing all adverse influences that could affect reproduction (e.g., interspecific and intraspecific competition for egg masses, predation, and fluctuating temperatures). Thus extrapolation of laboratory data derived at constant temperatures to definitively explain field observations needs to be assessed with caution.

In addition to establishing a set of protocols for the timely release of parasitoids in the field, these demographic data can also help explain why populations of *G. ashmeadi* are more successful in other areas of the USA (e.g., Texas) for suppressing *H. coagulata* when compared to California. Given an adequate time series of weather data, and developmental threshold temperatures and temperature influenced demographic estimates from this study, it should be possible to assess the probable establishment and expected level of control of *H. coagulata* by *G. ashmeadi* in any area in which this parasitoid is being considered for release. For example, data presented here suggest that in French Polynesia, *G. ashmeadi* can be expected to have a significant impact on *H. coagulata* because of favorable year round temperatures. Conversely, parasitoid performance in cooler Northern areas of California that could be infiltrated by *H. coagulata* in the future may be impaired because of low winter temperatures.

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