THE INFLUENCE OF TEMPERATURE ON DEVELOPMENT AND REPRODUCTION OF THE EGG PARASITOID *GONATOCERUS ASHMEADI*

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ABSTRACT
The effect of temperature on the development and reproduction of *Gonatocerus ashmeadi* (*G. ashmeadi*)Girault was studied in environmentally-controlled chambers set at 12˚C, 16˚C, 20˚C, 24˚C, 28˚C and 32˚C. Our results showed that the parasitoid developed the fastest at 28˚C. The parasitoid took 27.1 days at 16˚C and 9.5 days at 28˚C to complete the development from egg to adult. The embryonic stage was 6.3 days at 12˚C, about 2 days at 16-20˚C, and 1 day at 24-32˚C. At 16-32˚C, the length of the first instar larval stage was about 1 day, but 6 days at 12˚C. The development of the second and third instars also varied with temperature. At 16˚C, the second and third instars were approximately 2 and 3 days in length, respectively, and 1 and 1.5 days at 28˚C. Continued exposure to 32˚C arrested the development of the third instar larvae. Prepupae developed faster as temperature increased, but slowed down when held at 32˚C. Pupae also developed faster as the temperature increased, but without slowing at 32˚C. Linear regression analysis showed that the threshold temperature for development was 5.5˚C, 3.4˚C, 8.3˚C, 5.2˚C, and 5.4˚C for embryos, first, second, third instar larvae, prepupae and pupae, respectively. The lower temperature threshold was 8.2˚C for egg to adult development. A total of 219.2 degree days above the minimum temperature threshold were needed to complete the development from egg to adult. Temperature also affected the emergence pattern of the *G. ashmeadi* adults. At 16˚C and 20˚C, adult emergence lasted 10 days and 5 days at 28˚C and 32˚C. The maximum emergence occurred on the first day of emergence at 20-32˚C while the emergence peaked on the second day at 16°C. At 28˚C and 32°C, about 92 and 88% parasitoids emerged within the first two days. At 20˚C and 24°C, nearly 84 and 85% parasitoids emerged within the first three days. Temperature did not influence the sex ratio of the emerging *G ashmeadi*, but significantly affected the longevity of both sexes. At 16˚C, the life spans of female and male adults were 27 and 19 days, respectively, while at 28-32˚C, their life spans ranged from 6 to 8 days. The maximum lifetime fecundity of the female parasitoid occurred at 24˚C, with an average total of 105 eggs deposited. High temperature shortened parasitoid longevity and reduced lifetime fecundity. At 24˚C and 32˚C, *G. ashmeadi* deposited >10 eggs/day. At 16˚C and 20˚C, parasitoid oviposition was 3 and 7 eggs/ day, respectively.

INTRODUCTION
Over the past decade, the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (*H. coagulata*) (Say), has become a serious economic threat to many agricultural and ornamental crops in California by serving as a key vector of the xylem-inhabiting bacterium, *Xylella fastidiosa*(*Xf*) Wells (Sorensen and Gill, 1996). The egg parasitoid, *G. ashmeadi* Griault, is one of the most common natural enemies against the GWSS. *G. ashmeadi* has established its population since it was discovered in 1978 and was most likely dispersed to California with the GWSS from the southeastern USA (Irvin and Hoddle 2005; Vickerman, *et al.* 2004). The parasitoid may have a considerable potential as an effective biological control agent of the GWSS because it accounts for 80-95% of the observed parasitism on the sharpshooter eggs in California (Phillips 2000). Previous studies on *G. ashmeadi* have focused on host age preference and parasitism (Irvin and Hoddle 2005), gene-related geographical population (Vickerman *et al.* 2004), mymarid taxonomy (Triapitsyn 2003), overwintering biology (López *et al.* 2004) and functional responses and superparasitism (Chen *et al.* submitted). To date, no studies have been conducted on establishing the relationship between temperature and the development and reproductive biology of *G. ashmeadi*.

Among other factors, temperature has dominant influence on developmental rate, survival and fecundity of animals. Some temperature-related analyses, such as temperature threshold, optimal and upper temperature, and thermal constant for development have been extensively used as the index for studies of behavior, abundance and geographical distribution of arthropods (Messenger 1959). On the other hand, determination of relationship between development and reproduction in response to temperature is vital to an understanding of the life history and population dynamics of the insects. Our research on *G. ashmeadi* will assist us to assess parasitoid laboratory production and colony management. Meanwhile, knowledge of temperature threshold and thermal constant will be helpful to design the protocol for cold storage of parasitized eggs used in later augmentative release in the biological control program.

OBJECTIVES
1. Determine the effect of temperature on the rate of development from the egg to the adult stage.
2. Determine the temperature threshold and thermal constant of immature stages of *G. ashmeadi*.
3. Determine the emergence pattern, lifetime and daily fecundity and longevity of *G. ashmeadi* at a range of temperatures.

RESULTS AND CONCLUSIONS

Developmental rate determination

To determine the length of time required for development of *G. ashmeadi* at various temperatures, 250-700 *H. coagulata* eggs (< 24 h old) were exposed to the mated female parasitoids (< 24 h old) at a parasitoid/host ratio of 1: 25 in order to reduce the effect of super-parasitism (22 ±1˚C and 10 L: 8 D). After 4 h, the parasitized eggs were transferred into six environmentally-controlled chambers set at a constant 12ºC, 16ºC, 20ºC, 24ºC, 28ºC, and 32ºC while operating on a 16 L: 8 D photoperiod and 60% RH**.** At least 10 parasitized eggs were dissected daily for each temperature to determine the development rates of the parasitoids. Dissections were performed in an alcoholic solution of 0.02% eosin under a stereomicroscope. Our results showed that the developmental rate of *G. ashmeadi* varied with temperature (Table 1). The duration of parasitoid development at 16˚C from egg to adult emergence was significantly longer than that at 20˚C, 24˚C, 28˚C and 32˚C ($F = 1687.06.40$, $df = 4,195$, $P < 0.0001$). There was no significant difference between developmental times at 28˚C and 32˚C or between 20˚C and 24˚C from oviposition to adult emergence. The duration of the individual stages was also significantly affected by temperature as evidenced by the following values: embryonic ($F = 293.75$, $df = 5,234$, $P < 0.0001$), first instar larval ($F = 1456.57$, $df = 5,230$, $P < 0.0001$), second instar larval ($F = 27.96$, $df = 4,193$, $P < 0.0001$), third instar larval ($F = 52.47$, $df = 4,195$, $P < 0.0001$), prepupal ($F = 58.03$, $df = 1,194$, $P < 0.0001$) and pupal ($F = 952.93$, $df = 4,195$, $P < 0.0001$) (Table 1). The time to complete embryonic development at 12˚C was highly significantly longer than those of the other temperatures, but there was no difference in embryonic development time between 16˚C and 20˚C or among the range of temperatures from 24˚C to 32˚C. The developmental rate for first instar larvae was similar at 16-32˚C, but they were significantly faster than those held at 12˚C. Second instar larvae developed significantly slower at 16˚C than that at other temperatures. At 28˚C and 32˚C, second instar larvae developed faster than they did at 20˚C and 24˚C. Third instar larvae at 16˚C took 3 days to complete their development, and 2 days at 20˚C, 24˚C and 32 ˚C. When held at 28˚C, third instar larvae developed faster than the other temperatures. The prepupae developed at the same rate as third instar larvae. Pupae developed faster as the temperature increased. At 28˚C and 32˚C, the pupal stage was nearly 3 days, but at 24˚C, 20˚C and 16˚C, the pupal stage is 6.9 and 16 days, respectively (Table 1).

Table 1. Developmental duration of *G. ashmeadi* as a function of temperature

(°C)	Duration of Parasitoid Stages (Day ± S.E.)*						
	Embryonic	**1st instar**	**2nd instar**	**3rd instar**	**prepupal**	**pupal**	**Egg to female adult emergence**
12	6.3 ± 0.4a	10.8 ± 0.4a	In progress	In progress	In progress	In progress	In progress
16	1.9 ± 0.1b	1.3 ± 0.1b	2.2 ± 0.2a	3.1 ± 0.4a	2.7 ± 0.1a	16.1 ± 0.7a	27.1 ± 0.9a
20	1.7 ± 0.2b	1.3 ± 0.2b	1.9 ± 0.1b	2.1 ± 0.1b	1.9 ± 0.2b	9.3 ± 0.5b	18.6 ± 0.5b
24	1.2 ± 0.1c	1.3 ± 0.1b	1.5 ± 0.1c	1.9 ± 0.1b	1.5 ± 0.1c	5.9 ± 0.5c	13.4 ± 0. 5c
28	1.1 ± 0.1c	1.0 ± 0.1b	1.1 ± 0.1d	1.5 ± 0.2c	1.2 ± 0.1d	3.3 ± 0.2d	9.5 ± 0.5d
32	1.1 ± 0.1c	1.2 ± 0.1b	1.3 ± 0.1d	2.1 ± 0.1b	1.3 ± 0.1cd	2.8 ± 0.4e	9.6 ± 0.3d

*Duration for each parasitoid instar, prepupa and pupa was determined by subtracting the mean day of a given stage from the mean day of the following stage. For each experiment, between 280 to 350 *H. coagulata* eggs (at 12°C, > 700 eggs) were dissected. A one-way ANOVA followed by the LSD test ($P < 0.05$) was used to determine if there were significant differences in developmental time. Means within columns followed by a different letter are significantly different.

Table 2. Threshold temperatures and thermal constants for *G. ashmeadi* calculated by linear regression analysis.

Stage	Temperature threshold (°C)	Fitting regression equation	R^2	Thermal constant (K) (degree-day)[a]
Egg[b]	5.48	$R_T = 0.0241*T - 0.1325$	0.26	23.75
First instar[b]	3.44	$R_T = 0.0308*T - 0.1058$	0.47	21.61
Second instar	8.30	$R_T = 0.0322*T - 0.2667$	0.28	20.74
Third instar	5.20	$R_T = 0.0313*T - 0.1626$	0.41	34.37
Prepupal	5.44	$R_T = 0.0391*T - 0.2129$	0.52	27.33
Pupal	13.16	$R_T = 0.0177*T - 0.2328$	0.82	55.57
Egg to adult	7.49	$R_T = 0.0046*T - 0.0348$	0.88	219.21

[a]Thermal constants were calculated by using the mean temperature method proposed by Soto et al. (1999).
[b]Data collected at 12°C were used in calculating the linear regression.

Temperature threshold and thermal constant

The minimum temperature thresholds for the various stages were determined by using the linear regression model of Campbell *et al.* (1974), and the thermal constants were calculated using the mean temperature method of Soto *et al.* (1999). Our results show that the lower temperature threshold for development was 7.49˚C for egg to adult development and that a total of 219.21 degree days above the minimum temperature threshold were needed to complete the development from egg to adult (Table 2). For first instar larvae, the minimum temperature threshold (3.44˚C) was lower than other stages, suggesting that this stage may be more cold tolerant than other stages. Embryos required 23.75 degree days above the minimum temperature threshold (5.48˚C) to complete development. The temperature thresholds for the development of second and third instar larvae were 8.3˚C and 5.2˚C, respectively. The temperature thresholds for completion of prepupal and pupal development were 5.44˚C and 13.16˚C, respectively. Pupae needed 57.57 degree days above temperature threshold to complete development. Our previous research on cold storage of parasitized eggs also showed that no *G. ashmeadi* survive storage temperatures at 2˚C, about 7% survive 10 days at 4˚C and nearly 35% survive for 20 days at 4.5˚C (Leopold *et al*. 2004).

Emergence patterns

After *H. coagulata* eggs were exposed to *G. ashmeadi* (parasitoid-to-egg ratio, 1: 80) for 24 hrs, they were placed at chambers set at 16˚C, 20˚C, 24˚C, 28˚C and 32˚C. The parasitized eggs were examined daily and the date of emergence, the number of adults emerging, and the sex of the emerging adults were recorded. Our results (Figure 1) show that temperature not only influenced when emergence occurred, but also length of time it took for the majority of the adults to emerge from their hosts. Adult emergence spanned 10 days at 16˚C and 20˚C, 7days at 24˚C and 5 days at 28˚C and 32˚C (Figure 1). Temperature also affected the day on which emergence peaked. At 20˚C, 24˚C, 28˚C and 32˚C, the maximum emergence occurred on the first day of emergence, with approximately 44%, 43%, 66%, and 52% parasitoids emerged, respectively. The percentage emergence on the 1st day of emergence at 20-32°C was significantly higher than that on other days of emergence by comparing the emergence within the first four days (20°C, $F = 13.76$, $df = 4,56$, $P < 0.0001$) or first three days (24˚C, $F = 9.20$, $df = 2,42$, $P = 0.0005$; 28˚C, $F = 31.66$, $df = 2,48$, $P < 0.0001$; 32°C, $F = 20.54$, $df = 2,45$, $P < 0.0001$). At 28˚C and 32°C, about 92 and 88% parasitoids emerged within the first two days. At 20˚C and 24°C, nearly 84 and 85% parasitoids emerged within the first three days. At 16°C, emergence peaked on the second day though there was no difference in percentage emergence between the first and second day. The percentage emergence on the third to sixth day of emergence was significantly less than that on the first (24%) or second (27%) day ($F = 4.24$, $df = 5,60$, $P = 0.0023$). From the seventh day on, only < 8% parasitoids emerged at 16°C.

On the first day of emergence, the percentage emergence at 28˚C was significantly higher than that at 16-24˚C ($F = 4.93$, $df = 4, 69$, $P = 0.0015$). On the second day of emergence, there was no difference in the percentage emergence among five temperatures ($F = 0.96$, $df = 4,69$, $P = 0.44$). On the third day of emergence, there were still 16 and 15% parasitoids emerging at 20˚C and 24˚C, respectively, significantly greater than the 6% of emergence at 28˚C ($F = 2.45$, $df = 4,69$, $P = 0.0474$). On the fourth day, 11 and 9 % of the parasitoids emerged from *H. coagulata* eggs, significantly higher than at 28˚C and 32°C ($F = 4.14$, $df = 4,69$, $P = 0.0046$). On the fifth day of emergence, < 4% parasitoids emerged from hosts held at 20-32°C, significantly lower than 8% of those held at 16°C ($F = 4.38$, $df = 4,69$, $P = 0.0032$).

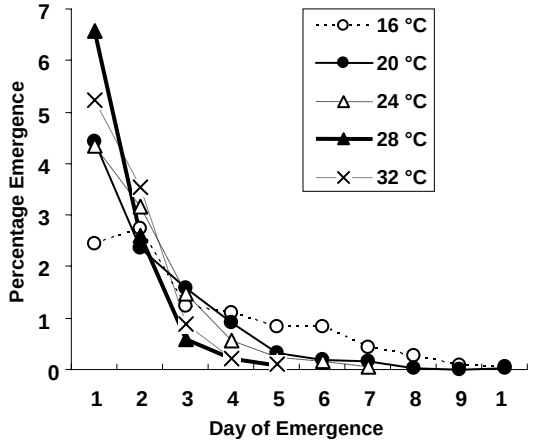

Figure 1. Emergence patterns of *G. ashmeadi* as a function of the holding temperature**.** Each point equals the means of at least 12 separate replicates. To avoid confusion, standard errors are not displayed. However, for any given point, the value of the standard error was < 8% of the point's value. The difference in percentage emergence among temperatures was analyzed using one-way ANOVA followed by LSD test (PROC GLM, SAS). The percentage emergences occurring on the day of emergence at same temperature were also compared using one-way ANOVA followed by LSD.

Reproduction, sex ratio and longevity

Temperature did not significantly influence the sex ratio of *G. ashmeadi* (Table 3). The male-female sex ratios ranged from 3.4 to 5.6 when the temperature was at 16-32°C. However, longevity of female and male adults varied significantly with the temperature at which they were held. Their life spans were extended as the temperature was decreased. For both female and male adults, longevity at 16˚C was significantly longer than that at the other temperatures. Further, the longevity of insects held at 20˚C was also significantly longer than those held at 24˚C, 28˚C and 32˚C. Employing two-way ANOVA analysis,

using sex and temperature as factors, showed that the longevity not only varied significantly with sex ($F = 8.26$, $df = 1,178$, $P = 0.0045$) and temperature ($F = 94.24$, $df = 4, 178$, $P < 0.0001$), but also with interaction of sex X temperature ($F = 45.20$, $df = 9, 178$, $P < 0.0001$).

Daily fecundity also varied significantly with temperature (Table 3). At 24˚C, *G. ashmeadi* deposited about 105 eggs during its lifetime, significantly greater than 80, 81 and 55 eggs at 16˚C, 28˚C, and 32˚C, respectively. There was no difference in lifetime fecundity between females ovipositing at 20˚C and 24˚C. At 24-32˚C, the parasitoids deposited more than 10 eggs per day. There was no difference in daily fecundity among 24-32˚C. At 16˚C, the females oviposited only about 3 eggs per day, and at 20˚C about 7 eggs per day (Table 3).

Table 3. Fecundity, sex ratio and longevity of *G. ashmeadi* as a function of temperature

(˚C)	Fecundity/female[a] (means ±S. E.)			Sex ratio (female/male)	Longevity (day ±S. E.)[b]	
	n	Lifetime fecundity	Daily fecundity		female	male
16	20	79.5 ± 3.2 c	3.5 ± 0.2 c	5.6 ± 1.2 (n = 12)	27.1 ± 1.2 a (n = 16)	19.0 ± 2.2 a (n = 10)
20	20	94.3 ± 4.5 ab	6.9 ± 0.5 b	3.4 ± 0.7 (n= 15)	16.0 ± 0.1 b (n = 29)	14.0 ± 0.2 b (n= 20)
24	24	105.2 ± 6.2 a	10. 3 ± 0.8 a	3.9 ± 0.6 (n = 17)	9.4 ± 0.9 c (n = 23)	9.0 ± 0.7 c (n = 18)
28	28	81.3 ± 5.6 bc	10.7 ± 1.1 a	5.3 ± 0.6 (n = 20)	8.2 ± 0.6 cd (n = 20)	7.3 ± 1.0 c (n = 16)
32	32	55.3 ± 4.8 d	10.3 ± 1.4 a	5.4 ± 0.7 (n =16)	6.4 ± 0.7 d (n = 19)	6.9 ± 0.8 c (n = 17)
F		13.50	13.26	1.75	68.05	25.45
df		4,93	4,93	4,75	4,102	4,76
P		< 0.0001	< 0.0001	0.1480	< 0.0001	< 0.0001

[a]Females were provided water and *H. coagulata* eggs (< 23 h) on excised euonymus leaves.
[b]Female and male adults were provided with water and excised euonymus leaves.
A one-way ANOVA followed by LSD test ($p< 0.05$) was used to determine whether there were significant differences in developmental duration. Means within columns followed by a different letter are significantly different.

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