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REALIZED LIFETIME PARASITISM OF GLASSY-WINGED SHARPSHOOTER EGG MASSES BY *GONATOCERUS ASHMEADI* (HYMENOPTERA: MYMARIDAE)

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1. Objectives

The main objective was to measure real lifetime contributions of individual female *Gonatocerus ashmeadi* to parasitism of glassy-winged sharpshooter (GWSS) egg masses in citrus orchards. However it is unknown whether *G. ashmeadi* is pro- or syn-ovigenic. *Gonatocerus* parasitoids are generally classified as strictly pro-ovigenic (Jervis and Copland, 1996). Jervis et al. (2001) calculated ovigeny index (number of mature eggs at emergence divided by potential lifetime fecundity) of 638 species of parasitoids from 28 families and concluded that ovigeny exists on a continuum from strictly pro-ovigenic species (ovigeny index = 1) to extremely synovigenic species (ovigeny index = 0). In order to investigate realized lifetime fecundity of individual *G. ashmeadi* in the field it is essential to determine whether this parasitoid is pro- or syn- ovigenic and whether egg maturation or oosorption exist.

Consequently, four laboratory experiments were conducted to determine:

1. The relationship between female *G. ashmeadi* size and egg load at time of emergence from GWSS egg masses.
2. The extent to which oosorption occurs in the absence of hosts, and the length of time without ovipositing that is required to initiate this physiological response.
3. If female parasitoids mature eggs in excess of those they emerge with
4. The relationship between wing wear and *G. ashmeadi* age. Wing wear has been used to estimate age of Hymenopteran parasitoids (Heimpel et al 1996, Lee et al. 2006, see review by Hayes and Wall 1999).

Dead *G. ashmeadi* were collected from the field and a composite index describing the correlative relationship of the above four factors was created to estimate reproductive success of individual *G. ashmeadi.*

2. Materials and Methods

All laboratory experiments were carried out at $26^{o} \pm 2^{o}$C and 30-40% RH under a L14:10D photoperiod.

2.1 Determining relationship between HTL and <24 h egg load

HTL as a measure of parasitoid size has been positively correlated with parasitoid longevity and fecundity (Bernal et al., 1999; Jervis et al., 2003). The relationship between

HTL and <24 h egg load for *G. ashmeadi* was determined in the following way. *Homalodisca vitripennis* and smoke-tree sharpshooter (*H. lacerta* Fowler; STSS) egg masses were collected from citrus and jojoba at the UCR Ag. Ops Facility during April-August, 2006, and placed into Petri dishes lined with moist filter paper. Two mated female *G. ashmeadi* (<48 h old) were placed into each Petri dish for 24 h for oviposition. Two sharpshooter hosts were used to vary the size of emerging *G. ashmeadi*. Petri dishes were checked daily for *G. ashmeadi* emergence and females were killed by freezing for 12-36 h. To select large, medium and small females for dissection, *G. ashmeadi* were sprinkled over white paper and removed with fine forceps. Female *G. ashmeadi* were dissected within 2 d of emergence. Females were placed on a microscope slide and the right metathoracic tibia was removed, mounted in Canada balsam and covered with a glass cover slip. The length of each tibia was measured to within 0.003 mm using an optical scale lens positioned in the eye piece of a compound microscope at 160 x magnification. Tibiae were measured from the point of attachment to the femur to the top of the first tarsal segment at the base of the spur. One drop of water was applied to the remaining female body, the abdomen was removed using fine forceps, and the ovaries were teased from the abdomen. A glass cover slip was placed over the dissected material and gently pressed with fine forceps to burst the ovaries and release the eggs. The number of mature eggs was counted under a dissecting microscope and the number of immature eggs was counted using a compound microscope at 160 x magnification. A total of 376 female *G. ashmeadi* (<12 h old) were dissected during April 14 –August 31, 2006. The relationship between HTL and <24 h egg load was determined for spring (April-June) and summer (July-August) using linear regression. A two-way ANOVA was performed in SAS to determine the effect of season, sharpshooter species, and season*species interaction on HTL (Box Cox 8 transformed [Cox 1972]) and egg load data (raw data).

2.2 Oosorption

Newly emerged mated female *G. ashmeadi* (<12 h old) were held individually in inverted 130 ml plastic ventilated vials without hosts. A drop of 50% honey-water was placed on the lid and renewed daily. Water was provided via a moist cotton ball placed on the top netting of each vial. After 1, 3, 5, 7, 9, 11 and 13 days, between 12-20 vial replicates were dismantled and female *G. ashmeadi* were killed and dissected. *G. ashmeadi* < 2 h of age were dissected to obtain data for day 0. The right and left forewings were removed (used in Section 2.4 below), the right metathoracic tibia was measured and the number of mature and immature eggs were counted as previously described. The number of 'reabsorbed eggs' (those eggs which had partially broken down) was also counted. Potential fecundity (mature + immature eggs) was calculated for each female. HTL was used to predict <24 h egg load of each female by using the linear relationship between HTL and <24 egg load described in Section 2.1. Parasitoid age was converted to physiological age using day-degree estimates (Pilkington and Hoddle 2006b).

To investigate whether female *G. ashmeadi* continued egg maturation in the absence of hosts, logistic regression was conducted in SAS (1990) to determine whether the linear relationship between mean potential fecundity and parasitoid age was significantly different from the linear relationship between predicted <25 h egg load and parasitoid age. To investigate the effect of parasitoid age on the different egg stage categories, one-way analysis of variances (ANOVA's) were conducted on mean number of mature eggs, mean number of immature eggs, predicted <24 h egg load data and mean potential fecundity in SAS (SAS 1990) (see Table 2 for transformations used). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. Data for mean number of reabsorbed eggs contained a high proportion of zeros for the earlier

age categories. Therefore, ANOVA was used to compare data (transformed using Box Cox 0.8) between 9, 11 and 13 days. For the earlier age categories, data was converted into binomial data, where "no reabsorbed eggs = 0" and "some reabsorbed eggs = 1", and Fisher's Exact test (Agresti 2002) was used to determine the effect of age on reabsorbed eggs. Finally, two parabolic models were fitted to the relationship between mature eggs and parasitoid age, and the relationship between immature eggs and parasitoid age. A Wald test (Fitzmaurice et al. 2004) was used at the 0.05 significance level to investigate the hypothesis that mature eggs data was the inverse relationship of immature eggs data.

2.3 Egg maturation

Newly emerged mated female *G. ashmeadi* (<12 h old) were held individually in inverted 130 ml plastic vials with ventilation and supplied water as previously described. A drop of honey-water was placed on the side of the inverted vial and renewed daily. Approximately 35 GWSS eggs laid on 'Eureka' *C. limon* leaves were supplied to females by placing stems through holes drilled through the lid of a 130 ml plastic vial filled with deionized water. Eggs were <72 h of age and preferred by *G. ashmeadi* for oviposition (Irvin and Hoddle, 2005a). Daily egg number was selected based on previous fecundity studies for *G. ashmeadi* (Pilkington and Hoddle 2006a, Irvin and Hoddle 2007). The inverted vial containing female *G. ashmeadi* was attached to the lid of the vial containing hosts. GWSS egg masses were renewed daily and exposed leaves bearing host egg masses were placed into Petri dishes lined with moist filter paper and left at 26^{o}C for three weeks to allow parasitoids to emerge. Realized fecundity was calculated as the total number of emerged and unemerged parasitoid progeny. After 1, 3, 4, 5, 7, 9, 11 and 13 days, between 9-20 vial replicates were dismantled and female *G. ashmeadi* were killed and dissected. *G. ashmeadi* < 2 h of age were dissected to obtain data for day 0. The right and left forewings were removed, the right metathoracic tibia was measured, and the number of mature and immature eggs were counted as previously described. Reabsorbed eggs were not present. Potential fecundity (mature + immature eggs) and predicted <24 h egg load was calculated for each female as previously described. Parasitoid age was converted to physiological age using day-degree estimates (Pilkington and Hoddle 2006b).

One-way ANOVA's were used to investigate the effect of parasitoid age on predicted fecundity, potential fecundity, realized fecundity, the number of mature eggs and the number of immature eggs in SAS (1990) (see Table 3 for transformations). To determine whether female *G. ashmeadi* continued maturing eggs after emergence in the presence of hosts, paired t-tests were used on untransformed data to compare predicted <24 h egg load and potential fecundity at each age category. To determine the number of eggs female *G. ashmeadi* mature in excess of those they emerge with, the difference between predicted fecundity (as predicted by HTL) and potential fecundity was calculated at each parasitoid age. Logistic regression was used to determine the relationship between parasitoid age and the number of eggs matured after emergence.

Ovigeny index has been defined as the number mature eggs at female emergence divided by potential lifetime fecundity (Jervis et al. 2001). Therefore, ovigeny index of *G. ashmeadi* was calculated as the mean number of mature eggs on day 0, divided by the average potential fecundity for degree days 137, 176, 215 and 254 (where potential fecundity had stabilized and there was no significant difference between days).

2.4 Estimating parasitoid age

The right and left forewings were removed from 12-20 female *G. ashmeadi* aged 0, 1, 3, 5, 7, 9, 11 and 13 days in the laboratory (see Section 2.2 above). Forewings were mounted in Canada balsam and covered with a glass cover slip. Photographs of each wing

were taken using Automontage software at 100 x magnification and imported into Microsoft Powerpoint using one slide per photograph. The number of broken setae around the entire wing fringe were counted and the mean number of setae hairs per female was calculated. Parasitoid age was converted to physiological age using day-degree estimates (Pilkington and Hoddle 2006b). Logistic regression was performed in SAS (1990) to determine the relationship between *G. ashmeadi* age and the number of broken setae per female.

2.5 Estimating realized lifetime fecundity of G. ashmeadi

To estimate realized lifetime fecundity of female *G. ashmeadi* in the field, funnel traps were used to capture dead female *G. ashmeadi* falling from trees. Funnel traps were deployed five days a week from May 8th, 2006 to September 1st, 2006, excluding weeks beginning June 5th, 12th and 26th due to low occurring field populations. Funnel traps consisted of a 473 ml plastic cup placed inside a white ThermoSafe Insulated polystyrene container (28 x 22.5 x 18 cm, wall thickness 4 cm) previously spray-painted black. Black traps were used to reduce the likelihood of traps attracting live parasitoids. Blocks of dry ice were shattered with a hammer and packed around the plastic cup. A 2.2 cm hole was cut in the lid of the plastic cup and polystyrene container. A red 20 cm diameter plastic funnel previously spray-painted black, was positioned through the holes in the polystyrene container and plastic cup so that dead insects falling from trees were captured in the funnel and preserved in the plastic cup surrounded in dry ice. On each sampling date, 40 funnel traps were placed underneath the canopies of 40 randomly selected orange trees in the Agricultural Operations Area, University of California, Riverside, California from approximately 9am until 5pm. At the end of each day, traps were collected and returned to the laboratory where *G. ashmeadi* were removed with fine forceps by the antennae (to prevent wing damage). Polystyrene containers were touched up with black paint at the end of each sampling week. Female *G. ashmeadi* were dissected, the number of mature, immature and dissolved eggs at time of death was counted, HTL measured, forewings mounted, and the number of broken setae counted. HTL was used to estimate the number of eggs at emergence for each female using the relationship previously determined in Section 2.1 [y = (252.77 * HTL) - 77.38). The relationship between female age and wing wear [Section 2.4; y = (6.52 * number of broken hairs per female) + 53.21] was used to estimate the age of each female at time of death. Female age was used to estimate the number of eggs matured since female emergence using the relationship previously determined in Section 2.3 [y = 0.39 * female age) – 9.68]. Collectively, this data was used to estimate the mean number of GWSS eggs parasitized by each female *G. ashmeadi* captured in funnels traps during their lifetime (i.e., realized lifetime fecundity) by the following equation:

predicted egg complement at emergence	+	additional eggs matured since emergence	-	egg load at time of death	=	no. eggs oviposited in the field

G. ashmeadi were only captured during July and August possibly due to low populations occurring during the remaining months funnel traps were deployed. Therefore, mean predicted egg load, predicted age, realized lifetime parasitism and ovigeny index was calculated for July and August, and these parameters were statistically compared between months using a t-tests on untransformed data. ‘Predicted egg load minus the number of

eggs in ovaries at death' data was square root transformed prior to performing t-test to normalize distribution. Oosorption was not included in the realized fecundity equation since results from Section 2.2 showed that oosorption was initiated after 137 degree days (or 5 days at 26^{o}C) without hosts. GWSS eggs are abundance during July and August so it is unlikely that individual *G. ashmeadi* would not encounter hosts for 137 degree days.

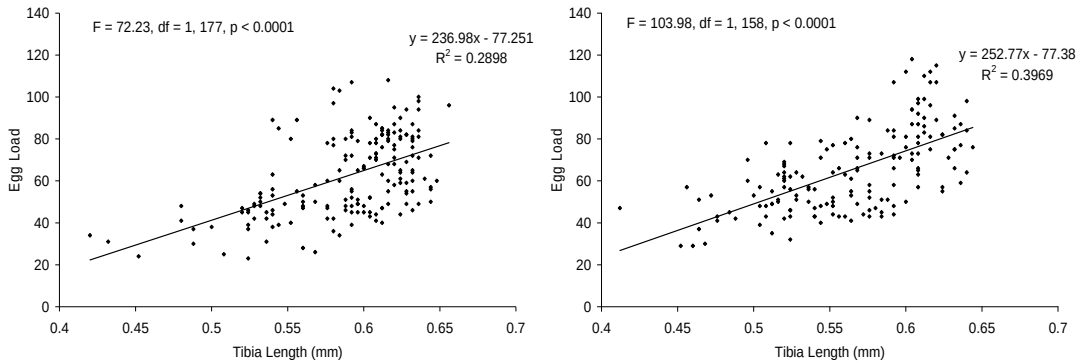
To aid interpretation of differences in age and realized fecundity between July and August, the number of adult GWSS, *G. ashmeadi* and GWSS eggs were monitored in lemon and orange orchards at Agricultural Operations, University of California using two methods. Firstly, *G. ashmeadi* and GWSS were captured daily from citrus located at Agricultural Operations, University of California, Riverside from June 6th, 2006 to August 30th, 2006. Sampling was conducted during existing collection activities conducted by the laboratory to support GWSS colonies. Collection was carried out during 6.30am-9.00am, five days per week, on orange and lemon trees. A citrus branch was placed inside a collecting net and forcefully tapped to dislodge insects. Contents were placed into field cages (34 x 32 x 38 cm) and returned to the laboratory for aspiration. *G. ashmeadi* were aspirated into a 130 ml plastic vial, labelled with date and killed by freezing for 1 day before counting. GWSS were aspirated into 130 ml plastic vials (25 per vial) and counted before transporting to the GWSS colony. The number of *G. ashmeadi* and adult GWSS were recorded each day along with the number of staff and the amount of time staff collected insects. The mean number of *G. ashmeadi* and GWSS collected per hour per person per day was calculated.

The second sampling protocol was carried out each week during 6.30am-9.00am in Rough Lemon plots located at Agricultural Operations. Sixty healthy trees were divided into groups of 15 and tagged with one of four colored ribbons. Each sampling week, a different colored set of trees were sampled so that the same trees were only sampled every 4 weeks. Trees were divided into quadrants and one randomly selected branch in each quadrant was enclosed with a collecting net and forcefully tapped to dislodge GWSS. Nets were emptied after each branch (four times per tree) into collection boxes as previously described. In the laboratory, GWSS were aspirated, counted and the total number of adult GWSS per tree was recorded. Additionally, leaves located in a 1-2 meter band around each tree were searched for GWSS egg masses and removed. For each egg mass, the number of eggs was counted under a binocular microscope at 10x magnification. The total number of eggs per sampling date was recorded. Daily average air temperature, relatively humidity and precipitation occurring at station UC Riverside (Station 44) was downloaded from CIMIS weather database (http://www.cimis.water.ca.gov/cimis/dataInfoType.jsp). The mean number of adult GWSS recorded for each sampling method, *G. ashmeadi* and weather data were calculated for July and August compared between months using t-tests for each variable. Finally, to determine the contribution of individual *G. ashmeadi* to biological control of GWSS in the field, overall mean estimated age, estimated realized lifetime parasitism and ovigeny index was calculated.

3. Results and Discussion

3.1 Determining relationship between HTL and <24 h egg load

There was a significant positive correlation between HTL and <24 h egg load for both spring and summer *G. ashmeadi* generations (Fig. 1).



a) b)

Figure 1: Relationship between <24 h egg load and female size as measured by hind tibia length for different sized *G. ashmeadi* reared from GWSS and STSS eggs in (a) spring and (b) summer.

There was a significant effect of season ($F = 17.50$, df = 1, 332, $p < 0.0001$) and species ($F = 164.62$, df = 1, 332, $p < 0.0001$) on HTL, whereas, the interaction between season and species was not significant ($F = 2.53$, df = 1, 332, $p = 0.11$). Similarly, there was a significant effect of season ($F = 6.91$, df = 1, 335, $p < 0.01$) and species ($F = 84.23$, df = 1, 335, $p < 0.0001$) on <24 h egg load, and no significant interaction effect ($F = 2.06$, df = 1, 335, $p = 0.15$). Female *G. ashmeadi* emerging from the summer collected egg masses were 0.025 mm smaller, but contained on average 3 eggs more than those emerging from spring egg masses (Table 1). *G. ashmeadi* emerging from GWSS egg masses were 0.057 mm larger and contained on average 19 eggs more than those females emerging from STSS egg masses. This can be attributable to the smaller size of STSS eggs in comparison to GWSS eggs.

Table 1: Hind tibia length (HTL) and <25 h egg load of dissected *G. ashmeadi* emerging from GWSS and STSS egg masses in spring and summer [means ± SEM's; different letters (a, b) indicate significant differences between seasons; different Arabic numerals (i, ii) indicate significant differences between sharpshooter species].

	Spring	Summer	GWSS	STSS
HTL (mm)	0.590 ± 0.003 a	0.565 ± 0.003 b	0.594 ± 0.002 i	0.537 ± 0.003 ii
Egg load	62.19 ± 1.44 a	65.11 ± 1.55 b	68.84 ± 1.25 i	49.84 ± 1.09 ii

3.2 Oosorption

Gonatocerus parasitoids are generally classified as strictly pro-ovigenic (Jervis and Copland, 1996) where females emerge with a full load of mature eggs and do not mature more eggs as they age (Quicke, 1997). Oosorption results showed that the number of mature eggs significantly increased 24 eggs from 0 to 59 degree days suggesting that *G. ashmeadi* may be partially syn-ovigenic. Interestingly, the parabolic relationship between mature egg production and parasitoid age ($y = 32.3034 + 9.3742x - 0.701x^2$; $F = 69.93$, df = 2, 119, $p < 0.0001$) was not significantly ($\chi^2 = 5.99$, df = 2, $p > 0.05$) different from the inverse parabolic relationship between immature egg production and parasitoid age (i.e. they are inversely equivalent) ($y = 30.7375 - 7.048x + 0.485x^2$; $F = 105.82$, df = 2, 119, $p < 0.0001$).

The number of reabsorbed eggs counted 0-176 degree days after *G. ashmeadi* emergence remained statistically equivalent, then increased 9 eggs from 176 to 215 degree days (Fig. 2, Table 2). It is unknown whether these eggs were reabsorbed allowing additional energy reserves for survival, or if mature eggs merely expired and degraded. Irvin et al. (2007) showed that female *G. ashmeadi* given access to honey-water survived 269% longer than those provided with honey-water and hosts for oviposition indicating that it is likely that oosorption did occur. *G. ashmeadi* oosorption was initiated after 137 degree days, or 5 days at 26^{o}C. In this species, oosorption is obligatory because egg maturing continues in the absence of hosts (Quicke, 1997). Females may reabsorb mature eggs theoretically enabling them to redirect energy into host seeking and survival, a characteristic of syn-ovigenic species (Jervis et al., 1996).

Conversely, the linear relationship between mean potential fecundity and parasitoid age ($y = 1.0069x + 63.691$; $R^2 = 0.4834$) was not significantly ($z = -0.04$, $p>0.05$) different from the linear relationship between predicted <25 h egg load and parasitoid age ($y = 0.0763x + 65.703$; $R^2 = 0.0119$). This demonstrates that in the absence of hosts, female *G. ashmeadi* do not mature more eggs than what they emerge with, a characteristic of pro-ovigenic species. There was no significant effect of age on predicted <24 h egg load and mean potential fecundity (Fig. 2, Table 2).

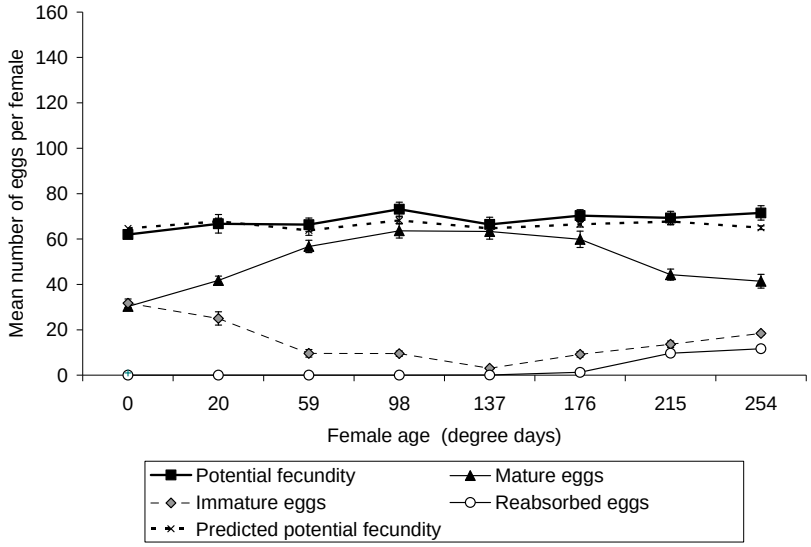


Figure 2: The effect of age on the mean number of mature eggs, immature eggs, reabsorbed eggs, potential fecundity and predicted potential fecundity (as predicted from hind tibia length) in ovaries of female *G. ashmeadi* presented with no hosts for 254 degree days (13 days at 26^{o}C).

Table 2: Statistical significance and test statistics for oosorption data presented in Fig. 2 above (different letters indicate significant differences between age categories).

Parasitoid Age (day degrees)	Significance (different letters indicate significant differences between ages)				
	Mature eggs	Immature eggs	Reabsorbed eggs	Predicted <24 h egg load	Potential fecundity

0	c	a	a	a	a
20	b	ab	a	a	a
59	a	de	a	a	a
98	a	d	a	a	a
137	a	e	a	a	a
176	a	de	a	a	a
215	b	cd	b	a	a
254	b	bc	b	a	a
F	25.31	41.29	29.50	1.47	1.48
df	7, 113	7, 113	2, 40	7, 113	7, 113
p	< 0.0001	<0.0001	<0.0001	0.19	0.18
Transformation performed to normalize data	square root	square root	Box Cox 0.8	Box Cox 3	raw data

3.3 Egg maturation

Parasitoid age had a significant effect on all egg count parameters (Fig 3, Table 3). Figure 3 shows that at 254 degree days, potential fecundity was 77 eggs higher than the predicted <24 hr potential fecundity as estimated from HTL. This suggests that female *G. ashmeadi* matured more eggs as they parasitized hosts during their lifetime and indicates that this species is partially syn-ovigenic. It is possible that potential fecundity was underestimated in this study because some eggs oviposited by females may be unaccounted for due to superparasitism or early larval death. The potential fecundity of females given hosts 20 degree days after emergence was 26 eggs lower than the predicted <24 hr potential fecundity indicating that 26 eggs were lost due to superparasitism or early larval death. For the remaining age categories potential fecundity was statistically higher (up to 77 eggs higher) than predicted <24 h egg load demonstrating the syn-ovigenic characteristic of *G. ashmeadi*.

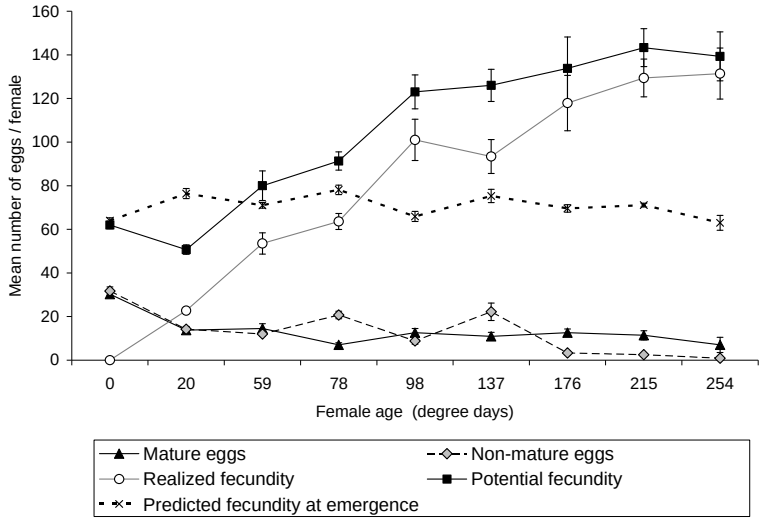


Figure 3: The number of mature and non-mature eggs present in ovaries, realized fecundity (successful parasitism), potential fecundity (realized fecundity + eggs present in ovaries) and predicted fecundity (estimated from hind tibia length) of female *G. ashmeadi* offered hosts daily for 0-254 degree days after female emergence.

Table 3: Significance and test statistics for egg maturation data presented in Fig. 3 above [different letters indicate significant differences between age categories, asterisks indicate a significant (* = p<0.05, ** = p<0.01, *** = p<0.001) difference between potential fecundity and predicted <24 h egg load for each age category].

Parasitoid Age (day degrees)	Significance				
	Mature eggs	Immature eggs	Realized fecundity	Predicted <24 h egg load	Potential fecundity
0	a	a	c	c	c
20	b	bc	b	a	c ***
59	b	c	b	abc	c
78	b	b	b	a	bc **
98	b	c	a	bc	ab ***
137	b	ab	ab	ab	ab ***
176	b	d	a	abc	a ***
215	b	d	a	abc	a ***
254	b	d	a	c	a ***
F	16.19	61.53	74.52	6.70	23.41
df	8,108	8, 104	8,104	8,104	8,104
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Transformation performed to normalize data	raw data	square root	Box Cox -0.5	raw data	raw data

There was a significant positive correlation between female age and the number of eggs matured by female *G. ashmeadi* after emergence ($F = 36.09$ df = 1, 7, $p < 0.0001$) (Fig. 4). This demonstrates that female *G. ashmeadi* given access to honey-water and hosts continue to mature eggs throughout their lifetime. Furthermore, ovigeny index equalled 0.22. Jervis et al. (2001) defined strictly pro-ovigenic species as having an ovigeny index of 1, whereas, the index is less than one for synovigenic species. In the current study, Figure 4 and the ovigeny index calculated based on Jervis et al. (2001) conclude that *G. ashmeadi* is a partially synovigenic species when provided honey-water and hosts in the laboratory.

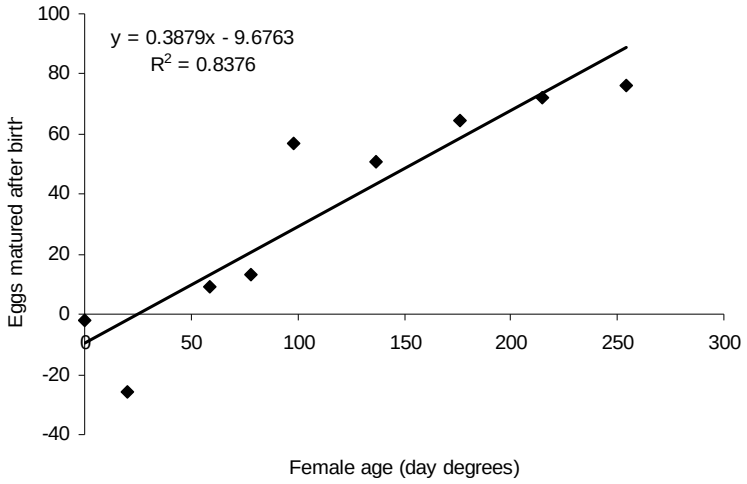


Figure 4: The relationship between *G. ashmeadi* age and the number of eggs matured by female *G. ashmeadi* after emergence.

3.4 Relationship between wing wear and parasitoid age

There was a significant positive correlation between parasitoid age and the number of broken setae on the forewings of female *G. ashmeadi* ($F = 39.79$ df = 1, 100, $p < 0.0001$) (Fig. 5). This wing wear index shows potential as a tool for estimating age of *G. ashmeadi*.

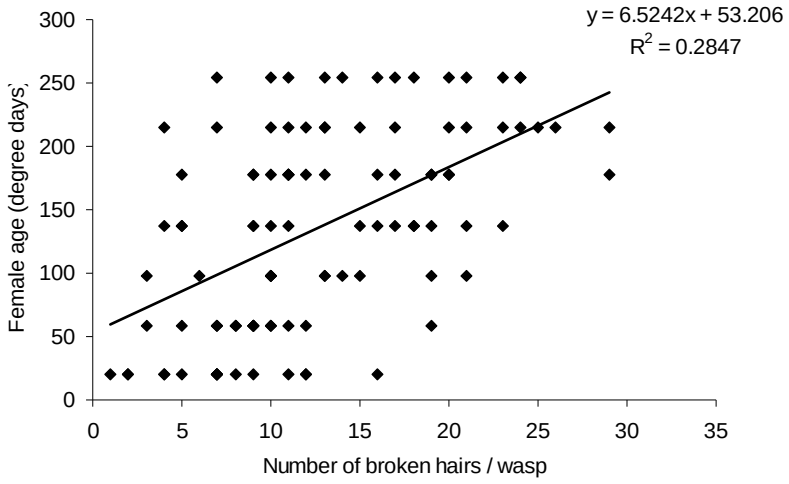


Figure 5: The relationship between parasitoid age and number of broken setae (hairs) counted on the forewings of female *G. ashmeadi* reared in the laboratory at 26°C.

3.5 Estimating realized lifetime fecundity of individual G. ashmeadi *in the field*

There was no significant difference in predicted egg load at female emergence between funnel traps deployed in July and August (Fig. 6). Similarly, month had no significant effect on predicted egg load minus the number of eggs counted in ovaries at time of death (Fig. 6). Predicted age and estimated realized lifetime parasitism of female *G. ashmeadi* captured in July funnel traps was significantly 84 day degrees and 50 eggs higher, respectively, compared with those females captured in August (Fig. 6). This indicates that *G. ashmeadi* may implement higher biological control of GWSS in July.

Figure 7 shows that the number of adult GWSS and GWSS eggs in the field were higher in August compared with July, therefore any increase in estimated realized fecundity observed in July could not be attributable to a higher abundance of hosts. Mean HTL for July and August was 0.586 ± 0.008 and 0.578 ± 0.013, respectively, and there was no significant difference between months ($t = 0.54$, df = 15, $p < 0.05$). Therefore, the difference in realized fecundity between months was not attributable to differences in female size. The number of *G. ashmeadi* captured in sweep nets in July was almost twice as high compared to August (Fig. 7). Higher populations of female *G. ashmeadi* may have increased competition for hosts causing females to reabsorb eggs and allowing additional energy reserves for survival. This may explain why females captured in July lived longer than those captured in August. Alternatively, Figure 8 shows that temperature was significantly 3°C higher in July compared with August. It is possible that higher temperatures in July increased activity by *G. ashmeadi* causing higher wing wear and correlating to an increase in estimated age and realized fecundity.

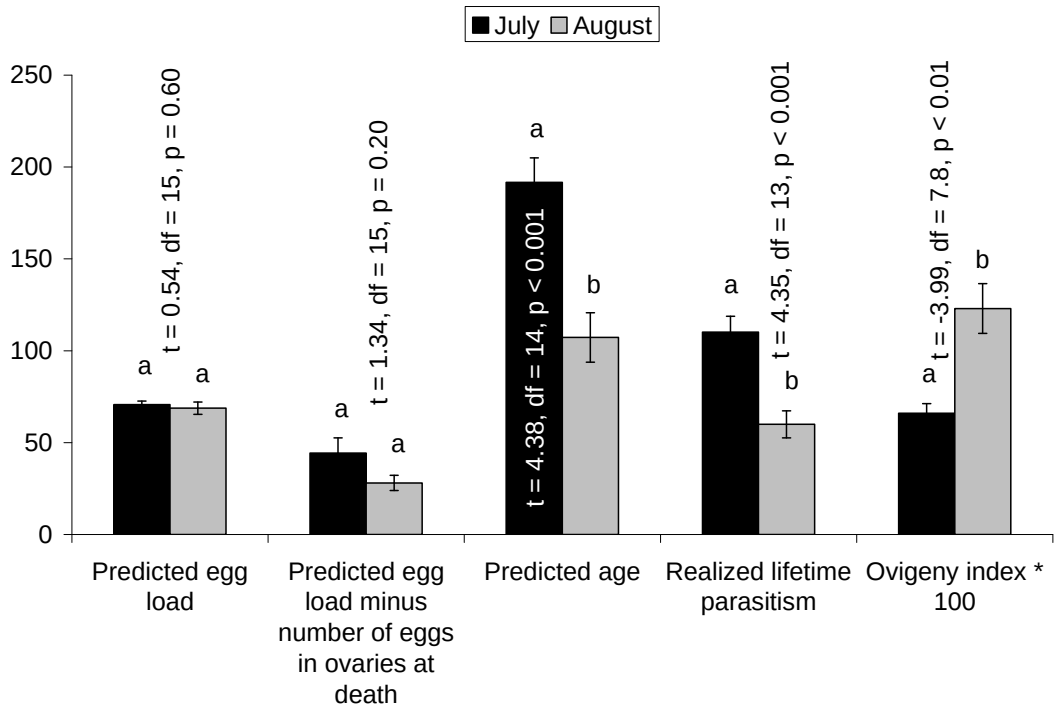


Figure 6: Egg load data, realized lifetime parasitism and estimated age for dead *G. ashmeadi* captured in funnel traps in July and August 2006 at Agricultural Operations, UCR.

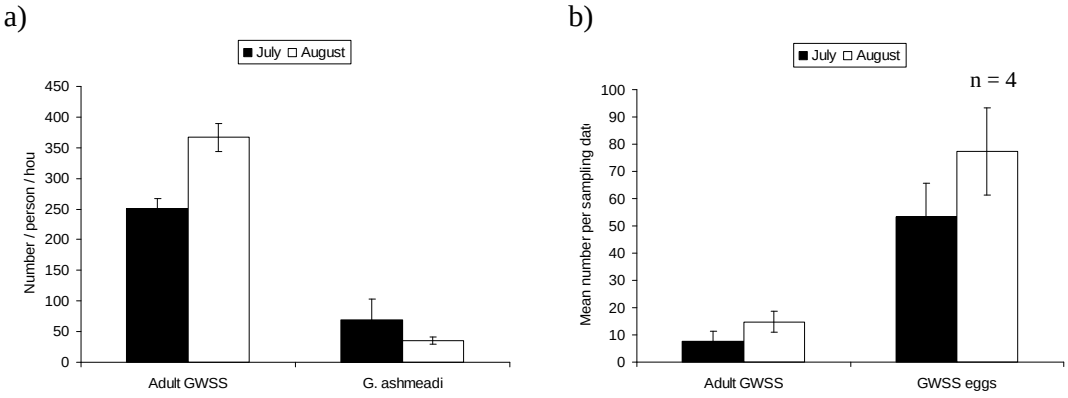


Figure 7: The mean number of adult GWSS, *G. ashmeadi* and GWSS eggs captured during a) daily net sampling in citrus orchards and b) weekly net sampling and egg searches in rough lemon plots in July and August 2006.

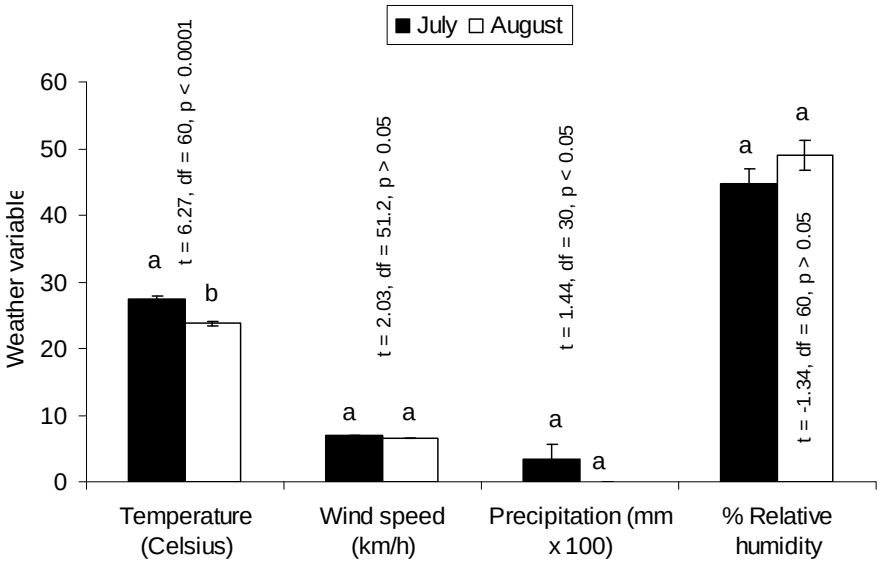


Figure 8: Weather data for July and August 2006.

Calculating the overall mean realized lifetime fecundity and estimated age for field captured *G. ashmeadi* showed that the average female *G. ashmeadi* lives 154.7 ± 14.2 degree days and parasitizes a total of 87 ± 9 eggs throughout her lifetime. Actual field realized fecundity may be lower than this estimation since wing wear may be higher in the field compared to laboratory, and this calculation fails to consider those *G. ashmeadi* that are lost through predation, superparasitism or oosorption. Overall mean field ovigeny index was 0.93 ± 0.10. This indicates that although laboratory results showed that *G. ashmeadi* is a partially syn-ovigenic species, *G. ashmeadi* performance in the field is closer to being pro-ovigenic.

4. Intellectual property and publications resulting from this work

No intellectual property was generated during this work. A publication is currently being written to submit to the leading journal Ecological Implications by the end of 2007.

5. Conclusions and contribution to solving the PD problem

Results from this study increased our understanding of the biology of *G. ashmeadi*. Oosorption in *G. ashmeadi* was initiated after 137 degree days, or 5 days at 26^{o}C in the absence of hosts. *G. ashmeadi* given access to honey-water and hosts continue to mature eggs throughout their lifetime. On average, *G. ashmeadi* matured 76 eggs in excess of those they were born with. Until now, *Gonatocerus* parasitoids were generally classified as strictly pro-ovigenic (Jervis and Copland, 1996). However, results from our study demonstrated that *G. ashmeadi* exhibits both egg maturation and oosorption, and has an ovigenic index of 0.22 in the laboratory. These are all characteristics of syn-ovigenic species. Results from this study also showed that HTL can be used as a predictor of <24 h egg load, and that wing wear demonstrates potential at estimating parasitoid age in the field.

This study aimed to measure real lifetime contributions of individual female *Gonatocerus ashmeadi* to parasitism of GWSS egg masses. Results showed that the

average female *G. ashmeadi* lives 154.7 ± 14.2 degree days and parasitizes a total of 87 ± 9 eggs throughout her lifetime. Results also suggested that *G. ashmeadi* may implement higher biological control of GWSS in July compared with August.

Additionally, results from this study demonstrated the difference in ovigencity between *G. ashmeadi* parasitizing GWSS in the laboratory and field. Although ovigeny index of laboratory reared *G. ashmeadi* was 0.22 (close to synovigenic), results showed that functional ovigeny of females caught in funnel traps was only 0.93 (close to pro-ovigenic). Sowing flowering plants in agricultural systems to supply nectar to parasitoids and increase longevity and fecundity is a form of conservation biological control. This technique could improve functional ovigeny of *G. ashmeadi* and increasing biological control of GWSS by increasing the lifespan of female *G. ashmeadi* and allowing females to fulfil their maximum potential fecundity.

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