Biological Control

Determination of *Homalodisca coagulata* (Hemiptera: Cicadellidae) egg ages suitable for oviposition by *Gonatocerus ashmeadi*, *Gonatocerus triguttatus*, and *Gonatocerus fasciatus* (Hymenoptera: Mymaridae)

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Abstract

Homalodisca coagulata (Say) (Hemiptera: Cicadellidae) eggs 1–10 days of age were exposed to *Gonatocerus ashmeadi* Girault, *Gonatocerus triguttatus* Girault, and *Gonatocerus fasciatus* Girault (all Hymenoptera: Mymaridae) in no choice laboratory trials to investigate egg age utilization and to determine which egg ages are vulnerable to attack by these three parasitoids. The *H. coagulata* egg ages that were most suitable for oviposition by *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* were eggs 3, 4, and 2 days of age, respectively. Egg ages least suitable for parasitoid development were 6–10 days for *G. ashmeadi* (resulting in <50% parasitism), 1–2 and 7–10 days for *G. triguttatus* (resulting in <25% parasitism), and 3–10 days for *G. fasciatus* (resulting in <11% parasitism). Pooling parasitism data across all egg ages showed that parasitism by *G. ashmeadi* was 12.9 and 28.5% higher compared with *G. triguttatus* and *G. fasciatus*, respectively, and *G. triguttatus* resulted in 15.6% higher percentage parasitism compared with *G. fasciatus*. Egg age had a significant effect on the percentage of female *G. ashmeadi* offspring produced, but this was not significant for *G. triguttatus*, and low *G. fasciatus* parasitism prevented statistical analyses for comparisons. Results from tests where females were offered a choice for oviposition between eggs 1, 3, and 5 days of age demonstrated that *G. ashmeadi* and *G. triguttatus* showed no significant oviposition preference, while percentage parasitism by *G. fasciatus* was 29.4 and 7.4% higher when females were presented eggs 1 and 3 days of age, respectively, compared with eggs 5 days of age. Choice tests indicated that an overlap in egg age suitability for oviposition exists between *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, and that interspecific competition for eggs 1, 2, and 3 days of age may occur in the field environment.
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1. Introduction

Over the past decade, the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has become a serious economic threat to many agricultural and ornamental crops in California,

USA. This xylem-feeding insect is native to the southeastern USA and northeastern Mexico (Triapitsyn and Phillips, 2000), and was first discovered in California in 1989 (Sorensen and Gill, 1996). It is the key vector of various strains of *Xylella fastidiosa* Wells et al., a bacterial pathogen that causes scorch-like diseases in numerous crops, including; grapes (*Vitis vinifera* L.), almonds (*Prunus amygdalus* Batsch.), plums (*Prunus domestica* L.), alfalfa (*Medicago sativa* L.), peaches (*Prunus persica* L.), oleander (*Nerium oleander* L.), *Liquidambar* spp.,

and forest trees (Adlerz, 1980; Hopkins and Adlerz, 1988; Hopkins and Purcell, 2002; Purcell, 2002; Purcell and Saunders, 1999). *Homalodisca coagulata* lays its eggs beneath the epidermis on the undersides of leaves and multiple eggs are deposited side by side forming an easily identifiable egg mass. In California, there are two generations (spring and summer) of *H. coagulata* per year, and the adult stage overwinters (Blua et al., 1999; Nielson, 1968; Turner and Pollard, 1959). Recently, *H. coagulata* has become the focus of a major classical biological control program in California. In 2002, more than 290,000 egg parasitoids (all Hymenoptera: Mymaridae), comprising almost exclusively of the following three *Gonatocerus* species, were released across nine Californian counties (CDFA, 2003). *Gonatocerus ashmeadi* Girault has been resident in California since 1978 (Huber, 1988) and genetic studies indicate it is most likely invaded California with *H. coagulata* from the southeastern USA (Vickerman et al., 2004). *Gonatocerus triguttatus* Girault is native to Texas and central Florida, and has been imported from Texas and released in California since 2001 (CDFA, 2003). Finally, *Gonatocerus fasciatus* Girault is known from Louisiana, Florida, Georgia, Illinois, Missouri, Tennessee, Texas, and Virginia (Triapitsyn, pers. comm.) and this parasitoid collected from Louisiana has been released in California since 2002 (CDFA, 2003). *G. ashmeadi* and *G. triguttatus* are solitary egg endoparasitoids and *G. fasciatus* is a gregarious endoparasitoid of sharpshooter eggs (Triapitsyn et al., 2003). *Gonatocerus* parasitoids may show considerable potential as biological control agents of *H. coagulata* since *G. ashmeadi* parasitism rates of up to 80% have been reported from July collections in Riverside, California (Triapitsyn et al., 1998).

Understanding the host preferences of natural enemies is vital to the success of classical biological control programs as it assists with mass-rearing efforts and can facilitate explanation of observed outcomes in the field. *Gonatocerus ashmeadi*, *G. triguttatus*, and *G. fasciatus* all attack *H. coagulata* eggs, however it is not known which egg ages are most preferred for oviposition by females of each species, and what range of egg ages are suitable for progeny production following oviposition. This information is essential for mass-rearing parasitoids and for understanding egg age phenology in the field and associated parasitism rates. Consequently, we sought to determine the age range of *H. coagulata* eggs that are vulnerable to attack by *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* under no choice, and choice conditions.

2. Materials and methods

2.1. Insect colonies

Laboratory colonies of *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* were maintained at the University of California, at Riverside. Parasitoid colonies were held at $26 \pm 2\,°C$ and 30–40% RH under a L14:10D photoperiod in cages ($50 \times 40 \times 40$ cm) on *H. coagulata* eggs laid on 'Eureka' lemon leaves. Parasitoids were provisioned with honey–water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA). Host cultures were maintained in greenhouses by collecting *H. coagulata* adults from citrus orchards in Riverside and placing them into $30 \times 60 \times 35$ cm cages with two potted 'Eureka' lemon trees [fertilized every 2 weeks with Miracle-Gro (1 tablespoon/3.5 L water, Scotts Miracle-Gro Products, Marysville, OH)] for feeding and oviposition. Lemon trees with *H. coagulata* egg masses were changed daily, labelled, and held at $26 \pm 2\,°C$ and 30–40% RH under a L14:10D photoperiod until use in egg age experiments. To maintain parasitoid cultures, a proportion of 24–48 h old *H. coagulata* egg masses were harvested and exposed to parasitoids by placing the petioles of harvested leaves through the lid of a 130 ml plastic vial (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) filled with tap water. The vial with vertically positioned lemon leaves bearing *H. coagulata* egg masses were placed in cages with parasitoids. After 24 h exposure to parasitoids, leaves were removed and parasitized egg masses were placed in Petri-dishes (10 diameter × 2 cm deep Optiluz Petri-dish, Becton–Dickinson Labware, Frankin Lakes, NJ) lined with damp filter paper (10 cm diameter Midwest Scientific Brand, Valley Park, MO). Petri-dishes were checked daily for parasitoid emergence to assure uniform age for experiments.

2.2. Parasitoid egg age utilization under no choice conditions

Leaves containing 15 *H. coagulata* eggs of known age laid on 'Eureka' lemon leaves were placed through the lid of a 130 ml plastic vial filled with deionized water and 3 ml of antiseptic (Listerine Antiseptic Mouthwash, Pfizer, New York, NY) to prevent bacterial rot. When necessary, leaf number was standardized to three per vial by including lemon leaves without *H. coagulata* egg masses, however egg mass number per vial varied from 1 to 8 to ensure that a total of 15 eggs were presented. 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, Southport, CT)] was inverted and attached to the lid of the vial holding the water and lemon leaves. One newly emerged, mated, naïve female parasitoid (~24 h old) was placed inside the inverted vial that covered the leaves bearing *H. coagulata* eggs and left to forage for 2 h. Since no previous research on fecundity of these *Gonatocerus* spp. had been published, the exposure period used in this study was based on small preliminary studies conducted at the beginning of these experiments. This procedure was replicated 15 times for eggs 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days

of age for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*. Females who were not mated (producing male only progeny) were excluded from the analysis. Fifteen replicates of control vials for each egg age were set up to determine naturally occurring *H. coagulata* mortality for each egg age in the absence of parasitoids.

After 2 h exposure, parasitoids were removed and vials containing the leaves were held at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod for 3 weeks to allow parasitoids and *H. coagulata* nymphs to emerge. Vials were topped up with water every second day. Three weeks post-parasitoid exposure, the number of emerged nymphs and parasitoids was recorded. Unemerged eggs were dissected and the numbers of unemerged nymphs, identifiable parasitoids, and 'unidentifiable' unknowns were recorded. Percentage parasitism (number of *H. coagulata* eggs parasitized/total number of eggs), percentage nymphs (total emerged and unemerged nymphs/total number of eggs), and percentage unknowns (total unknowns/total number of eggs) were calculated for each egg age and parasitoid species. Sex of parasitoid progeny was also recorded and percentage female progeny was calculated for each egg age and species. *G. ashmeadi* and *G. triguttatus* are solitary species producing one offspring per *H. coagulata* egg, whereas, *G. fasciatus* is gregarious in nature. Therefore, the average clutch size for *G. fasciatus* was also calculated for each egg age.

2.3. Parasitoid egg age utilization under choice conditions

Oviposition utilization tests for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* were conducted by simultaneously presenting to individual mated females (∼24 h of age) eggs 1, 3, and 5 days of age. One 'Eureka' lemon leaf containing approximately 8–12 *H. coagulata* eggs of each of the three egg age categories were used. This procedure was replicated 17–38 times for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* to obtain a minimum of 10 replicates for each species where parasitism occurred. After 1-h exposure, parasitoids were removed and vials containing the leaves were held at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod. Although a 2 h exposure period was used for the no choice experiment (containing 15 eggs/vial), 1 h was used for the choice study to ensure that only one egg age (containing 10 eggs/vial) was parasitized per vial. After 3 weeks, leaves were placed under a binocular microscope and the number of emerged and unemerged, nymphs, and parasitoids, was recorded. Percentage parasitism, percentage nymphs, and percentage unknowns were calculated for each egg age and parasitoid species.

2.4. Statistical analyses

Percentage parasitism and percentage nymphs were compared between egg ages for each parasitoid species using Pearson Chi-square in SAS (1990). Percentage unknowns were compared between egg ages, and between vials containing parasitoids and controls at each egg age using non-parametric Kruskal Wallis tests for each species. For *G. fasciatus*, average clutch size was compared between egg ages using Kruskal Wallis. Additionally, percentage parasitism, percentage nymphs, percentage unknowns, and average clutch size was pooled over all egg ages and compared between species using Kruskal Wallis tests. Individual Wilcoxon two-sampled tests were used to separate means. For *G. ashmeadi* and *G. triguttatus*, percentage of female offspring was square root and arcsine transformed prior to analysis and compared between egg ages 1–7 days of age, using analysis of variance (ANOVA). Tukey's test at the 0.05 level of significance was used to separate means. For egg ages 8–10 days of age, *G. ashmeadi* and *G. triguttatus* parasitism rates were too low to allow adequate sample sizes for analysis on percentage female offspring data. This also occurred for *G. triguttatus* when females were exposed to eggs 2 days of age. Similarly, for *G. fasciatus* this percentage female offspring analysis could not be conducted for any egg age category as progeny production was too low to be amenable to statistical analysis. Logistic regression was used to determine whether percentage parasitism (for all three *Gonatocerus* species) and percentage of female offspring (for *G. ashmeadi* and *G. triguttatus* only) were significantly correlated with the number of *H. coagulata* masses per vial.

For choice tests, female parasitoids that failed to oviposit within the 1 h exposure period were considered 'incompetent' and removed from analyses. The proportion of replicates parasitized was compared between the three egg age categories for each species, and between species using Pearson χ^2 tests. Percentage parasitism, percentage nymphs, and percentage of unknowns were compared between egg ages for each species using Friedman's χ^2 test on non-transformed data. Additionally, these parameters were compared between species at each egg age using Kruskal Wallis tests. Individual Wilcoxon two-sampled tests were used to separate means.

3. Results

3.1. Parasitoid egg age utilization under no choice conditions

3.1.1. Gonatocerus ashmeadi

There was a significant ($\chi^2 = 825.84$, $df = 9$, $P < 0.005$) effect of egg age on percentage parasitism by *G. ashmeadi*, with eggs 3 days of age resulting in highest levels of parasitism (Fig. 1A). The 4 *H. coagulata* egg ages that produced maximum percentage parasitism for *G. ashmeadi* were 1, 2, 3, and 4 days of age (means = 59.2% ± 7.3, 56.6% ± 11.5, 79.8% ± 4.0, and 58.1% ± 6.7, respectively),
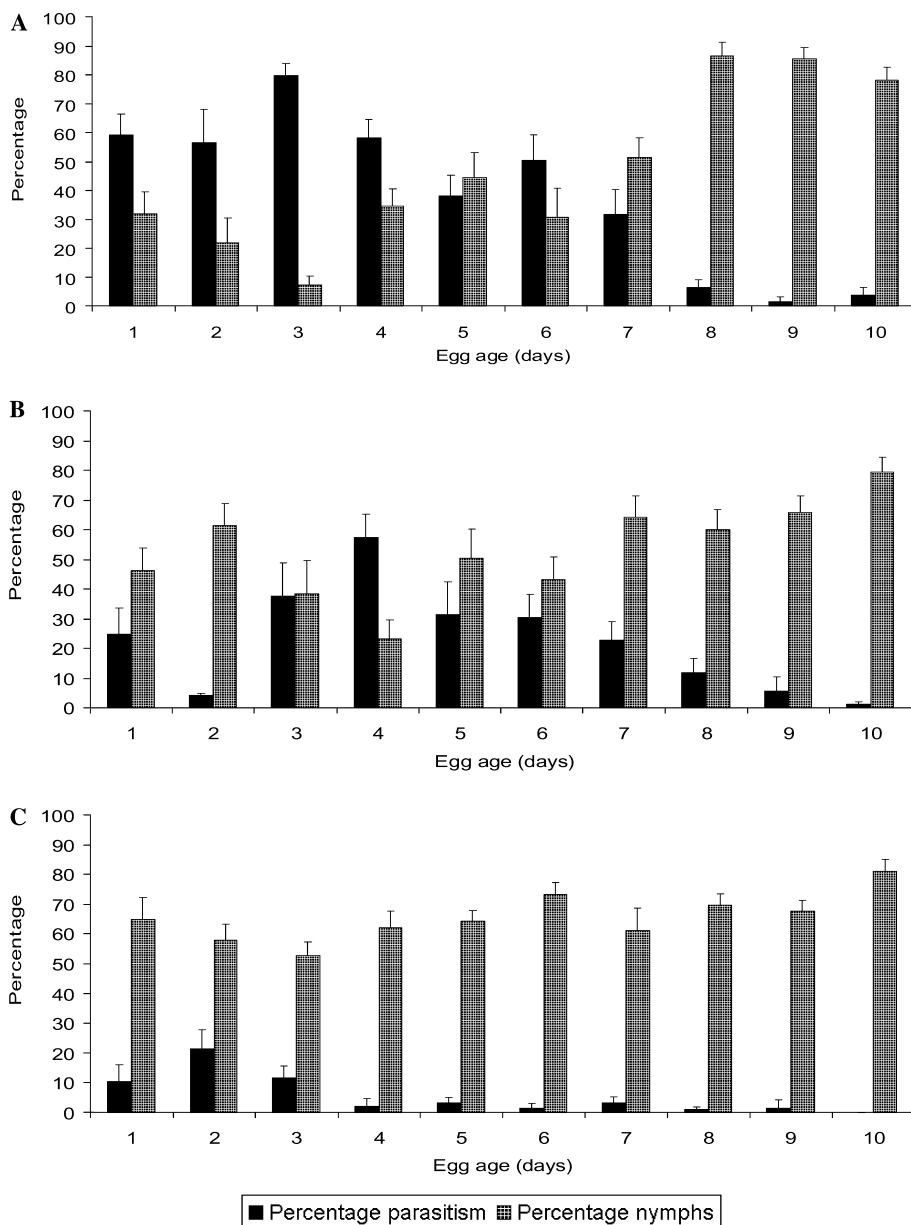
Fig. 1. Percentage parasitism and percentage *H. coagulata* nymph emergence when *H. coagulata* egg ages 1–10 days of age were exposed to female (A) *G. ashmeadi*, (B) *G. triguttatus*, and (C) *G. fasciatus* in the laboratory (error bars indicate ± SEM).

and these four egg age categories produced up to 41.5% higher percentage parasitism compared with eggs 5 days of age (Fig. 1A). There was a highly significant ($\chi^2 = 759.17$, $df = 9$, $P < 0.005$) effect of egg age on nymph survival, with eggs 3 days of age resulting in up to 79.1% lower percentage emergence of nymphs compared with all other egg age categories. Eggs 8, 9, and 10 days of age resulted in maximum mean *H. coagulata* survival rates (8 days = 86.5% ± 4.6; 9 = 85.5% ± 3.9; and 78.2% ± 4.4) (Fig. 1A).

There was no significant ($\chi^2 = 10.35$, $df = 9$, $P = 0.32$) difference in percentage unknown mortality between egg ages for *G. ashmeadi* (Table 1). Comparing percentage unknowns between vials containing *G. ashmeadi* and

controls that lacked *G. ashmeadi* at each egg age, showed that undetermined egg mortality was significantly higher (up to 30.9%) in control vials compared with *G. ashmeadi* for eggs 1, 3, 4, 7, and 8 days of age (1: $\chi^2 = 15.58$, $df = 1$, $P < 0.005$; 3: $\chi^2 = 7.74$, $df = 1$, $P < 0.01$; 4: $\chi^2 = 10.68$, $df = 1$, $P < 0.01$; 7: $\chi^2 = 6.09$, $df = 1$, $P < 0.05$; and 8: $\chi^2 = 4.16$, $df = 1$, $P < 0.05$) (Table 1). There was no significant ($P > 0.05$) difference in percentage of unknowns between *G. ashmeadi* and controls for all remaining egg ages (Table 1).

3.1.2. Gonatocerus triguttatus

Egg age had a highly significant ($\chi^2 = 391.74$, $df = 9$, $P < 0.005$) effect on percentage parasitism by *G. trigutt-*

Table 1
Comparing undetermined egg mortality between control vials and vials containing female *Gonatocerus ashmeadi*, *G. triguttatus* or *G. fasciatus*, at each *H. coagulata* egg age (asterisk indicates significantly difference from control where *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Egg age	Control	G. ashmeadi	G. triguttatus	G. fasciatus
1	39.3 ± 6.8	8.4 ± 2.7 ***	29.4 ± 4.8	24.6 ± 3.1
2	34.8 ± 5.3	21.1 ± 7.8	22.8 ± 4.9	20.7 ± 3.5 *
3	32.1 ± 5.3	12.9 ± 3.2 **	24.6 ± 5.4	35.5 ± 5.1
4	29.3 ± 4.9	7.6 ± 2.8 **	19.9 ± 5.3	28.8 ± 4.2
5	31.8 ± 5.0	19.1 ± 5.7	19.3 ± 5.7	32.6 ± 3.4
6	21.4 ± 3.0	19.2 ± 9.1	27.2 ± 4.4	25.7 ± 4.1
7	31.3 ± 4.0	16.7 ± 3.7 *	13.8 ± 3.2 **	35.8 ± 6.8
8	15.5 ± 3.4	7.7 ± 2.7 *	28.7 ± 5.4	29.2 ± 4.0 *
9	12.5 ± 2.6	14.2 ± 3.2	25.5 ± 3.9 *	31.0 ± 3.1 ***
10	23.4 ± 3.4	17.3 ± 3.8	19.5 ± 5.0	18.6 ± 4.0

atus, with eggs 4 days of age resulting in highest levels of parasitism (Fig. 1B). The four *H. coagulata* egg ages that produced maximum percentage parasitism for *G. triguttatus* were 3, 4, 5, and 6 days of age (means = 37.8% ± 11.0, 57.5% ± 7.9, 31.5% ± 11.0, and 30.4% ± 7.8, respectively). There was a highly significant ($\chi^2 = 236.01$, $df = 9$, $P < 0.005$) effect of egg age on nymph survival, with eggs 4 days of age resulting in up to 56.4% lower percentage nymph emergence compared with all other egg age categories (Fig. 1B). Eggs 10 days of age resulted in maximum *H. coagulata* survival (mean = 79.6% ± 5.0).

There was no significant ($\chi^2 = 15.31$, $df = 9$, $P = 0.08$) difference in percentage unknowns between egg ages for *G. triguttatus* (Table 1). Comparing percentage of unknowns between vials containing *G. triguttatus* and controls showed significant differences for eggs 7 and 9 days of age (Table 1). For eggs 7 days of age, percentage unknowns was 17.5% higher in control vials compared with *G. triguttatus* ($\chi^2 = 9.55$, $df = 1$, $P < 0.01$), whereas for eggs 9 days of age, percentage unknowns was 13.0% higher in vials containing *G. triguttatus* compared with controls ($\chi^2 = 6.06$, $df = 1$, $P < 0.05$). There was no significant difference ($P > 0.05$) in percentage of unknowns between *G. triguttatus* vials and controls for all remaining egg ages (Table 1).

3.1.3. Gonatocerus fasciatus

Egg age had a highly significant ($\chi^2 = 198.55$, $df = 9$, $P < 0.005$) effect on percentage parasitism by *G. fasciatus*, with eggs 2 days of age resulting in highest levels of parasitism (Fig. 1C). The three *H. coagulata* egg ages that produced maximum percentage parasitism for *G. fasciatus* were 1, 2, and 3 days of age (means = 10.4% ± 5.4, 21.5% ± 6.2, and 11.6% ± 4.1, respectively). Beyond this egg age, percentage parasitism of *H. coagulata* eggs reached a maximum of 3.3% (Fig. 1C).

Egg age had a significant ($\chi^2 = 58.43$, $df = 9$, $P < 0.005$) effect on *H. coagulata* nymph survival for *G. fasciatus*, with eggs 3 days of age resulting in lowest

nymph survival (52.7% ± 4.7). Eggs 10 days of age resulted in maximum mean *H. coagulata* survival (81.1% ± 4.0) (Fig. 1C).

There was no significant ($\chi^2 = 16.58$, $df = 9$, $P = 0.06$) difference in percentage unknowns across all egg ages for *G. fasciatus* (Table 1). Comparing undetermined egg mortality between vials containing *G. fasciatus* and controls showed significant results for eggs 2, 8, and 9 days of age (Table 1). For eggs 2 days of age, percentage unknowns was 14.1% higher in control vials compared with vials holding *G. fasciatus* ($\chi^2 = 4.27$, $df = 1$, $P < 0.05$). For eggs 8 and 9 days of age, percentage of unknowns was significantly higher (up to 18.5%) in vials containing *G. fasciatus* compared with controls (8 days: $\chi^2 = 5.63$, $df = 1$, $P < 0.05$; 9 days: $\chi^2 = 11.29$, $df = 1$, $P < 0.005$). There was no significant ($P > 0.05$) difference in undetermined egg mortality between *G. fasciatus* and controls for all remaining egg ages (Table 1).

There was no significant ($\chi^2 = 3.91$, $df = 8$, $P < 0.97$) effect of egg age on average clutch size for *G. fasciatus* (Fig. 2). The average clutch size remained consistently above one *G. fasciatus* offspring per *H. coagulata* egg for all host ages, and ranged between 1.5 and 2 (Fig. 2).

3.1.4. Comparing overall means between species

Pooling data across all egg ages showed that there was a significant effect of species on mean percentage parasitism ($\chi^2 = 60.79$, $df = 2$, $P < 0.005$), nymph survival ($\chi^2 = 10.89$, $df = 2$, $P < 0.01$), undetermined egg mortality ($\chi^2 = 60.15$, $df = 2$, $P < 0.005$) and average clutch size ($\chi^2 = 151.76$, $df = 2$, $P < 0.005$) (Fig. 3). Total parasitism by *G. ashmeadi* was 12.9 and 28.5% higher compared with *G. triguttatus* ($\chi^2 = 10.86$, $df = 2$, $P < 0.005$) and *G. fasciatus* ($\chi^2 = 59.71$, $df = 2$, $P < 0.005$), respectively. *Gonatocerus triguttatus* showed significantly ($\chi^2 = 23.1$, $df = 2$, $P < 0.005$) higher parasitism (15.6% greater) when compared with *G. fasciatus* (Fig. 3).
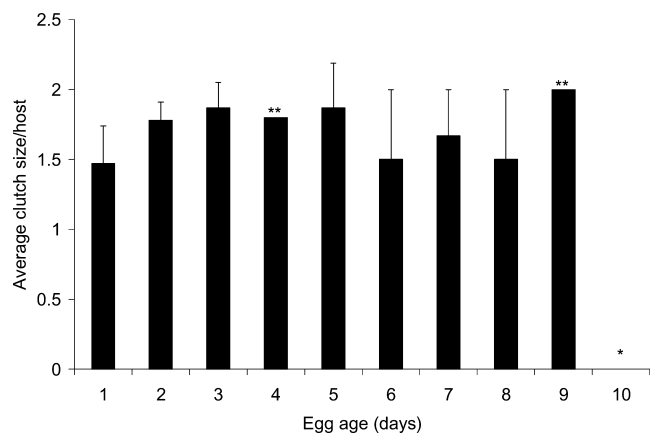

Fig. 2. Average clutch size when *H. coagulata* egg ages 1–10 days of age were exposed to female *Gonatocerus fasciatus* (error bars indicate ± SEM; *indicates data unavailable due to zero parasitism; **indicates error bar unavailable since $n = 1$).
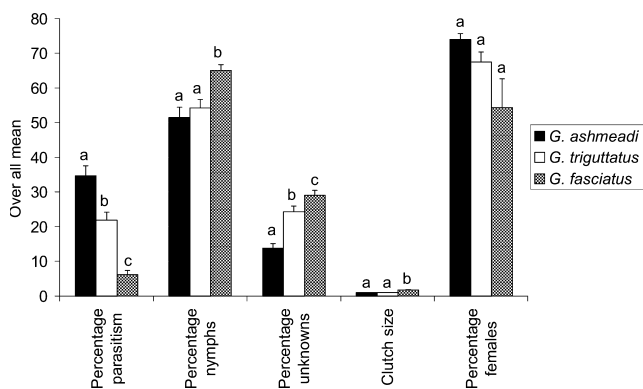
Fig. 3. Over all mean percentage parasitism, percentage *H. coagulata* nymph emergence, percentage of unknown egg mortality, percentage female offspring, and clutch size for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* in the laboratory (different letters indicate significant ($P < 0.05$) differences between species; error bars indicate $\pm$ SEM).
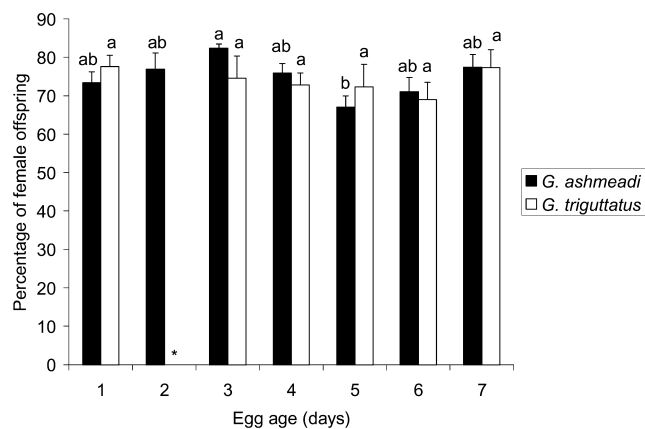

Fig. 4. Mean percentage female offspring produced when *H. coagulata* egg ages 1–10 days of age were exposed to female *G. ashmeadi* and *G. triguttatus* (different letters indicate significant ($P < 0.05$) differences between egg ages within each species; Asterisk indicate no data available for *G. triguttatus*; error bars indicate $\pm$ SEM).

Over all *H. coagulata* nymph survival was significantly higher at 13.5 and 10.8% for eggs exposed to *G. fasciatus* when compared with *G. ashmeadi* ($\chi^2 = 9.04$, $df = 2$, $P < 0.01$) and *G. triguttatus* ($\chi^2 = 7.17$, $df = 2$, $P < 0.01$). There was no significant ($\chi^2 = 0.24$, $df = 2$, $P = 0.63$) difference in percentage nymphs between *G. ashmeadi* and *G. triguttatus* (Fig. 3). Undetermined egg mortality was significantly higher (15.2 and 4.7% higher, respectively), when *H. coagulata* eggs were exposed to *G. fasciatus* when compared with *G. ashmeadi* ($\chi^2 = 56.91$, $df = 2$, $P < 0.005$) and *G. triguttatus* ($\chi^2 = 6.39$, $df = 2$, $P < 0.05$). *G. ashmeadi* resulted in significantly ($\chi^2 = 27.43$, $df = 2$, $P < 0.005$) lower (10.5%) percentage unknowns compared with *G. triguttatus* (Fig. 3).

Over all average clutch size was significantly higher (70.0%) for *G. fasciatus* compared with *G. ashmeadi* ($\chi^2 = 94.00$, $df = 1$, $P < 0.005$) and *G. triguttatus* ($\chi^2 = 74.52$, $df = 1$, $P < 0.005$).

3.1.5. The effect of the number of H. coagulata egg masses in vials on percentage female progeny and percentage parasitism

Mean percentage female offspring for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* was $74.0\% \pm 1.6$, $67.5\% \pm 2.9$, and $54.3\% \pm 8.3$, respectively. However, differences between species were not significant ($\chi^2 = 3.01$, $df = 2$, $P = 0.22$) (Fig. 3). Logistic regression demonstrated that there was no significant correlation between percentage of female *G. ashmeadi* and *G. triguttatus* offspring and the number of *H. coagulata* egg masses present per vial replicate (*G. ashmeadi*: $\chi^2 = 2.29$, $df = 1$, $P = 0.13$; *G. triguttatus*: $\chi^2 = 0.51$, $df = 1$, $P = 0.47$). This analysis could not be conducted for *G. fasciatus* as too few progeny were produced.

Comparing percentage of female offspring across egg ages one through seven showed that there was a significant effect of egg age on percentage of female *G. ashmeadi* offspring ($F = 3.07$, $df = 6$, $P < 0.005$), whereas there

was no significant difference between egg ages for *G. triguttatus* female offspring production ($F = 0.59$, $df = 6$, $P = 0.71$) (Fig. 4). For *G. ashmeadi*, percentage of female offspring was significantly 15.4% higher for eggs 3 days of age compared with eggs 5 days of age ($P < 0.05$) but similar across remaining egg ages (Fig. 4).

Logistic regression analysis indicated that for *G. ashmeadi* and *G. triguttatus*, percentage parasitism was significantly positively correlated with the number of *H. coagulata* egg masses per vial (*G. ashmeadi*: $\text{Log}[P_i/(1 - P_i)] = -0.84 + 0.07\text{MASS}$, $\chi^2 = 4.14$, $df = 1$, $P < 0.05$; *G. triguttatus*: $\text{Log}[P_i/(1 - P_i)] = -1.93 + 0.22\text{MASS}$, $\chi^2 = 10.74$, $df = 1$, $P < 0.01$). This was not observed for *G. fasciatus* ($\text{Log}[P_i/(1 - P_i)] = -2.35 + -0.13\text{MASS}$, $\chi^2 = 1.38$, $df = 1$, $P = 0.24$). Comparing logistic regression slopes between species demonstrated that *G. ashmeadi* parasitism was more positively correlated to the number of masses per vial when compared with *G. triguttatus* ($\chi^2 = 9.10$, $df = 1$, $P < 0.01$) and *G. fasciatus* ($\chi^2 = 33.66$, $df = 1$, $P < 0.005$).

3.2. Parasitoid egg age utilization under choice conditions

For *G. ashmeadi* and *G. triguttatus*, there were no significant ($P > 0.05$) differences in percentage parasitism, percentage nymphs, and percentage unknowns between eggs 1, 3, and 5 days of age (Figs. 5A and B). In contrast, there was a significant effect of egg age on percentage parasitism ($\chi^2 = 10.18$, $df = 2$, $P < 0.01$) and percentage nymphs ($\chi^2 = 9.26$, $df = 2$, $P < 0.01$) when *H. coagulata* eggs were exposed to female *G. fasciatus* (Fig. 5C). Exposing *G. fasciatus* to eggs 1 ($\chi^2 = 6.98$, $df = 2$, $P < 0.01$) and 3 ($\chi^2 = 3.95$, $df = 2$, $P < 0.05$) days of age resulted in 29.4 and 7.4% higher percentage parasitism, respectively, compared with eggs 5 days of age (Fig. 5C). There was no significant ($\chi^2 = 3.51$, $df = 2$, $P = 0.06$)
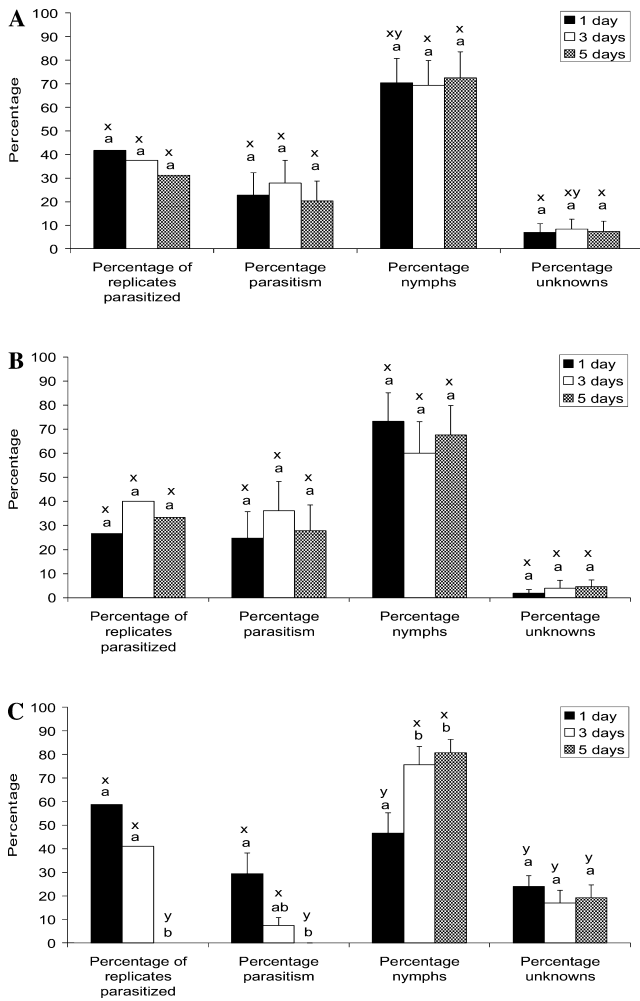
Fig. 5. Percentage of replicates parasitized, percentage parasitism, percentage *H. coagulata* nymph emergence, and percentage of unknown egg mortality when female (A) *G. ashmeadi*, (B) *G. triguttatus*, and (C) *G. fasciatus* were given an oviposition choice between *H. coagulata* eggs 1, 3, and 5 days of age (different letters (a, b, c…) indicate significant ($P < 0.05$) differences within species; different letters (x, y, z) indicate significant ($P < 0.05$) differences between species; error bars indicate ± SEM).

difference in percentage parasitism between eggs 1 and 3 days of age.

For eggs 5 days of age, *G. ashmeadi* ($\chi^2 = 6.26$, $df = 2$, $P < 0.05$) and *G. triguttatus* ($\chi^2 = 6.72$, $df = 2$, $P < 0.01$) parasitized a significantly higher proportion of replicates compared with *G. fasciatus*, while the proportion of replicates parasitized was equivalent between *G. ashmeadi* and *G. triguttatus* ($\chi^2 = 0.02$, $df = 2$, $P = 0.90$) (Fig. 5). Additionally, *G. fasciatus* (38) required 111.1 and 65.2% additional vial replicates, compared with *G. ashmeadi* (18) and *G. triguttatus* (23), respectively, to obtain sufficient replication for analysis, where parasitism occurred.

There was a significant effect of species on percentage parasitism when parasitoids were exposed to eggs 5 days of age ($\chi^2 = 6.78$, $df = 2$, $P < 0.05$), whereas this was not significant for eggs 1 ($\chi^2 = 1.71$, $df = 2$, $P = 0.43$) and 3

days of age ($\chi^2 = 0.87$, $df = 2$, $P = 0.65$) (Fig. 5). When females were exposed to *H. coagulata* eggs 5 days of age, *G. ashmeadi* ($\chi^2 = 6.02$, $df = 2$, $P < 0.01$) and *G. triguttatus* ($\chi^2 = 6.45$, $df = 2$, $P < 0.01$) produced 20.2–27.8% higher mean percentage parasitism compared to *G. fasciatus*, and parasitism was equivalent between *G. ashmeadi* and *G. triguttatus* ($\chi^2 = 0.15$, $df = 2$, $P = 0.70$) (Fig. 5).

4. Discussion

4.1. Parasitoid egg age utilization under no choice conditions

Percentage parasitism by *G. ashmeadi* of *H. coagulata* eggs 3 days of age in no choice tests was up to 78.3% higher compared with all remaining egg age categories, indicating that 3 day old host eggs are more easily utilized by *G. ashmeadi* for oviposition. The four egg age categories that produced maximum mean percentage parasitism by *G. ashmeadi* were eggs 1, 2, 3, and 4 days old (mean parasitism = 59.2, 56.6, 79.8, and 58.1%, respectively).

Lower parasitism rates of eggs 5–7 days of age may have resulted because the *H. coagulata* embryos in these eggs had developed beyond a stage that most *G. ashmeadi* larvae were able to use after hatching. Alternatively, *H. coagulata* embryonic physiology may have inhibited *G. ashmeadi* development. In some instances, success in parasitizing host eggs in an advanced stage of development depends on whether the parasitoid egg is oviposited directly into the embryo thereby killing it (Eidmann, 1934). Therefore, a proportion (1.5–50.3%) of ovipositions by *G. ashmeadi* into *H. coagulata* eggs 5 days of age and older may have terminated development of some *H. coagulata* embryos allowing larval parasitoids to develop successfully.

Percentage parasitism by *G. ashmeadi*, of eggs 8 through 10 days of age, was only 1.5–6.4%, and significantly lower than all remaining egg age categories. Since *H. coagulata* nymphs began to emerge from egg masses on day 8 at 26 °C, parasitism rates may be an indication of the proportion of sterile host eggs that successfully supported *G. ashmeadi* development but failed to produce *H. coagulata* nymphs. Al-Wahaibi (2004) reported that 4–11% of field collected *H. coagulata* eggs did not show any signs of development, and were therefore sterile. Strand (1985) reported that *Telenomus heliothidis* (Scelionidae) and *Trichogramma pretiosum* (Trichogrammatidae) were able to successfully parasitize infertile host eggs. Our results suggest *G. ashmeadi* may be able to do likewise.

Female *G. triguttatus* parasitized a higher proportion (up to 56.3%) of *H. coagulata* eggs 4 days of age compared with all remaining egg ages, and maximum percentage parasitism was produced when females were

presented egg ages 3, 4, 5, and 6 days old. Furthermore, parasitism by *G. triguttatus* of hosts 2 days of age was up to 53.4% lower compared with eggs 1–9 days of age. These results suggest that female *G. triguttatus* may be better adapted at parasitizing older *H. coagulata* eggs. Younger hosts may have limited nutritional resources and *G. triguttatus* could require a partially developed *H. coagulata* embryo for optimum nutrition for larval development hence its better performance on older aged eggs.

Host egg ages that resulted in maximum mean percentage parasitism for *G. fasciatus* were 1, 2, and 3 days of age. Once *H. coagulata* embryos developed beyond 3 days, parasitism by *G. fasciatus* declined to 0–3.3%, indicating that this species is unable to efficiently exploit older hosts. This may occur because *G. fasciatus* larvae are less able to contend with developmental changes associated with host embryogenesis. Alternatively, adult *G. fasciatus* which are much smaller than female *G. ashmeadi* and *G. triguttatus* (Triapitsyn et al., 2003), maybe restricted to parasitising young *H. coagulata* eggs because of ovipositor length and strength. Leibee et al. (1979) observed that the mymarid parasitoid *Patasson lameerei* Debauche had difficulty with ovipositor insertion into older hosts eggs [*Sitona hispidulus* (Fabricus) (Curculionidae)]. This was possibly due to the hardening of the egg chorion, which made it difficult for female parasitoids to insert their ovipositor (Safavi, 1968). This may occur for *G. fasciatus* thereby restricting this parasitoid to attacking young host eggs. Parasitism by *G. fasciatus* of eggs 4–9 days of age ranged from 1.1–3.3%. It is possible that these parasitism rates represent levels of sterile host eggs exploited by *G. fasciatus* in which chorion hardening did not occur.

Providing parasitoids with suitable egg ages for oviposition during mass rearing is essential for efficient production of high quality offspring (Lekswasdi, 1999; Lu and Oloo, 1990; Patel and Habib, 1993; Teggellis and Biradar, 1997). The most optimum egg age for parasitism of *S. hispidulus*, by the mymarid *P. lameerei*, is 2 days of age, and it has been found that providing female *P. lameerei* with hosts of the most suitable age increased parasitism rates by up to 42% (Leibee et al., 1979). Results here showed that *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* adult emergence can be increased by up to 78.3% by providing females with their most preferred egg age. It is therefore recommended that host egg age is closely regulated in mass rearing facilitates that require production of large quantities of *Gonatocerus* parasitoids for biological control programs against *H. coagulata*.

A wide window of susceptibility in which host eggs are vulnerable to parasitoid attack could be a favourable attribute because it increases the probability that *H. coagulata* eggs will be successfully parasitized upon discovery. *H. coagulata* egg ages that produced parasitism rates of above 25% were eggs 1–7 days of age for *G. ashmeadi*, and eggs 1–6 days of age for *G. triguttatus*. In contrast, *G. fasciatus* failed to parasitize 25% of hosts of any egg age category, and only *H. coagulata* eggs 2 days of age were suitable for producing *G. fasciatus* parasitism rates above 20%. This demonstrates that *G. ashmeadi* and *G. triguttatus* have a larger attack window in which *H. coagulata* eggs are vulnerable to parasitization and may indicate that these two species show more potential as biological control agents of *H. coagulata*.

Gonatocerus fasciatus may have performed poorly in this study because females were restricted to a 2-h exposure period. Since *G. fasciatus* is a gregarious parasitoid, producing an average clutch size of 1.8 in the current no choice study, it may require a longer period of time to complete individual host handling compared with *G. ashmeadi* and *G. triguttatus* which are both solitary species (Triapitsyn et al., 2003). Heimpel (2000) similarly reported that lower parasitism rates maybe associated with higher clutch sizes, due to limitations on parasitoid searching efficiency and fecundity. However, maximum fecundity can be more easily achieved at low densities by those parasitoids with higher clutch sizes (Heimpel, 2000). Therefore, gregarious reproduction may enable *G. fasciatus* to be competitive with other mymarid species early in spring when *H. coagulata* egg masses are relatively rare and parasitism levels are low in California (Triapitsyn et al., 2003).

When comparing undetermined egg mortality between *G. ashmeadi* vials and controls, the percentage of *H. coagulata* eggs dying of unknown causes was consistently significantly higher (up to 30.9%) in control vials that lacked parasitoids. This may be attributed to *G. ashmeadi* parasitizing a proportion of sterile or dead *H. coagulata* eggs, therefore removing them from the 'unknown' category into 'parasitized'. Furthermore, upon dissection of unemerged *H. coagulata* eggs, undeveloped *G. ashmeadi* were more easily identifiable, compared with undeveloped *H. coagulata* nymphs. Undetermined egg mortality was significantly different between control vials and vials containing female *G. triguttatus* and *G. fasciatus* at only three egg ages. The lack of significant results for *G. triguttatus* and *G. fasciatus* is probably attributable to these species resulting in 10.5 and 15.2% higher percentage unknowns, respectively, compared with *G. ashmeadi*, thereby producing undetermined egg mortality levels more similar to controls.

The over all proportion of female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* offspring did not significantly vary between species and ranged from 54.3–74.0%. Similarly, Heimpel and Lundgren (2000) reported that 63% of 27 species of commercially reared parasitoid species tested possessed female bias sex ratios.

The percentage of female offspring was equivalent across all *H. coagulata* egg ages presented to *G. triguttatus*. Zhu et al. (1993) observed that the sex ratio of the

mymarid parasitoid, *Anagrus nilaparvatae* Pang and Wang, was not influenced by age of the host, *Sogatella furcifera* (Horvath). In contrast, results from the current study showed that egg age had a significant effect on the proportion of female *G. ashmeadi* offspring, where maximum percentage female offspring was produced from eggs 3 days of age, and was up to 15.4% higher than all other age categories. We speculate that *G. ashmeadi* sex ratio was more strongly female biased when females were presented eggs 3 days of age because these hosts were most suitable for oviposition, giving rise to higher parasitism rates and longer oviposition sequences, which increased the proportion of fertilized eggs oviposited into hosts (Waage, 1982).

There was no significant correlation between percentage female progeny and number of egg mass per vial for either *G. ashmeadi* or *G. triguttatus*. Percentage parasitism by *G. ashmeadi* and *G. triguttatus* was significantly positively correlated with the number of *H. coagulata* egg masses per vial, indicating that within a 2 h search these two parasitoid species were able to find an increasing number of egg masses and successfully parasitize eggs within discovered patches. This correlation was not observed for *G. fasciatus*. Two reasons for this may exist: (1) this parasitoid may be more adapted to low *H. coagulata* egg densities and have a tendency to reduce searching after locating a suitable egg mass due to its gregarious egg laying habit (Quicke, 1997); (2) its smaller size in comparison to *G. ashmeadi* and *G. triguttatus* may result in it having fewer eggs to deposit, especially after laying multiple eggs into each suitable *H. coagulata* egg.

4.2. Parasitoid egg age utilization under choice conditions

In the choice experiment, parasitism of at least one egg age within a vial replicate was required for analysis. Results showed that compared with *G. ashmeadi* and *G. triguttatus*, respectively, *G. fasciatus* required 111.1 and 65.2% additional replicates to obtain sufficient replication for analysis. These results suggest that *G. fasciatus* may be less efficient at finding and processing hosts compared with *G. triguttatus* and *G. ashmeadi*. Alternatively, *G. fasciatus* may require a pre-oviposition period longer than 24 h thus failed to compete effectively with female *G. ashmeadi* and *G. triguttatus* of equivalent age.

When given a choice between hosts 1, 3 or 5 days of age, *G. ashmeadi* and *G. triguttatus* showed no significant oviposition preference. Although the no choice study demonstrated that 3 and 4 day old hosts are most favourable for *G. ashmeadi* and *G. triguttatus* development, respectively, 25–59% of eggs 1 and 5 days of age were also parasitized. Therefore, the lack of significant oviposition preference by *G. ashmeadi* and *G. triguttatus* in the choice study may be attributable to these species not directly selecting between hosts of varying age when all experimental age categories suitable for oviposition

were present simultaneously. These results indicate that an overlap in egg age suitability for oviposition exists between *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, and that interspecific competition for eggs 1–3 days of age may occur in the field environment.

The significance of an overlap in egg age oviposition utilization may be of considerable importance for *G. fasciatus* given that this species possesses a narrower 'window of susceptibility', and its inability to develop in *H. coagulata* eggs greater than 4 days of age. *G. triguttatus* may have a competitive advantage over *G. ashmeadi* and *G. fasciatus* because females can parasitize a wider range of egg ages and more easily utilize unfertile *H. coagulata* eggs. However, female *G. fasciatus* are capable of producing two or more offspring per *H. coagulata* egg and gregarious reproduction may be advantageous early in the spring when parasitism levels are extremely low and host eggs are relatively scarce (Triapitsyn et al., 2003).

4.3. Concluding remarks

Under no choice conditions, *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* produced significantly more progeny from *H. coagulata* eggs 3, 4, and 2 days of age, respectively. It is recommended that eggs of these ages are used in mass rearing programs for each parasitoid species. Furthermore, each parasitoid species was able to utilize a range of egg ages around their most preferred age. These being *H. coagulata* eggs 1–4, 3–6, and 1–3 days of age for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, respectively. Consequently, interspecific competition may occur under field conditions, where all host egg ages are present at one time, and females can chose between eggs of suitable and varying age for oviposition. Further laboratory work is needed to determine the strength of egg age preferences under conditions that allow parasitoids to forage and choose amongst egg mass of varying age that are presented simultaneously for oviposition.

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