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The competitive ability of three mymarid egg parasitoids (*Gonatocerus* spp.) for glassy-winged sharpshooter (*Homalodisca coagulata*) eggs

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Abstract

Parasitoid longevity, utilization of *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) eggs of different ages, and progeny survival rates were determined in the laboratory for *Gonatocerus ashmeadi* Girault, *Gonatocerus triguttatus* Girault, and *Gonatocerus fasciatus* Girault (Hymenoptera: Mymaridae). Ovipositional behavior and aggression between females were investigated when all three species were simultaneously presented *H. coagulata* egg masses in the laboratory. Results from the longevity study demonstrated that when females were provisioned with honey–water solution, female *G. ashmeadi* survived up to 171.9% longer than *G. triguttatus* and *G. fasciatus*, whereas, survival was equivalent between *G. triguttatus* and *G. fasciatus*. Results from the egg age utilization study showed that *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* most efficiently utilized eggs 1–6, 3–6, and 1–2 days of age, respectively, and that exploited egg age ranges overlapped between species. *Gonatocerus ashmeadi* parasitized a significantly higher (up to 45.0 and 62.6%) proportion of *H. coagulata* eggs aged 1–6 days of age compared with *G. triguttatus* and *G. fasciatus*, respectively. Additionally, in competition studies, overall parasitism by *G. ashmeadi* was significantly higher (up to 76.0%) compared with *G. triguttatus* and *G. fasciatus*. Results from behavioral observations of females concurrently searching for *H. coagulata* egg masses showed that *G. ashmeadi* allocated the greatest proportion of time to resting/grooming (26.5%) and oviposition (25.8%), while *G. triguttatus* allocated significantly more (up to 61.6%) time to resting/grooming compared with all other activities. Female *G. fasciatus* spent the greatest proportion of time resting/grooming (40.0%) and off leaves with *H. coagulata* egg masses (39.6%). *G. ashmeadi* and *G. triguttatus* allocated 2.1 and 1.3% of time to aggressively interacting and defending egg masses from congenerics, whereas, this was not observed for *G. fasciatus*. Results suggest that *G. ashmeadi* may show the most potential as a biological control agent of *H. coagulata*, and that successful widespread establishment and impact by *G. fasciatus* on *H. coagulata* in California is unlikely unless this species can efficiently exploit low density populations of *H. coagulata* in early spring when congeneric competition for egg masses is low.

Keywords: Aggressive behavior; Biological control potential; Cicadellidae; Egg age utilization; *Gonatocerus ashmeadi*; *Gonatocerus triguttatus*; *Gonatocerus fasciatus*; Hemiptera; *Homalodisca coagulata*; Hymenoptera; Interspecific competition; Longevity; Mymaridae; Patch defense

1. Introduction

Over the past decade the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), an insect native to southeastern USA and northeastern Mexico, has become a serious economic threat to many agricultural and ornamental industries in California. This xylem-feeding insect is a major vector of various strains of *Xylella fastidiosa* Wells et al., a bacterial pathogen that causes disease and devastating losses in various crops including grapes (*Vitis vinifera* L.), almonds

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(*Prunus amygdalus* Batsch.), plums (*Prunus domestica* L.), alfalfa (*Medicago sativa* L.), peaches (*Prunus persica* L.), and forest trees (Adlerz, 1980; Hopkins and Adlerz, 1988; Hopkins and Purcell, 2002; Purcell and Saunders, 1999). Female *H. coagulata* oviposit on the undersides of leaves. Individual eggs (~2–15) are laid side by side to form an egg mass, which is deposited in a slit cut with the ovipositor between the epidermis and parenchyma (Irvin and Hoddle, 2005). Populations of *H. coagulata* have reached inordinate densities in California, and a classical biological control program was instigated in 2000 to reduce the density and spread of *H. coagulata* and *Xylella*-related diseases vectored by this pest. Efforts have included the importation, mass rearing, and release of three mymarid parasitoids that parasitize *H. coagulata* eggs.

The three parasitoids that are the focus of mass rearing and distribution efforts are *Gonatocerus ashmeadi* Girault, *Gonatocerus triguttatus* Girault, and *Gonatocerus fasciatus* Girault (Hymenoptera: Mymaridae). These *Gonatocerus* spp. are the dominant natural enemies attacking *H. coagulata* in its home range (Triapitsyn and Phillips, 2000; Triapitsyn et al., 1998). *G. ashmeadi*, is a solitary endoparasitoid (i.e., one adult parasitoid emerges from an individual host egg) that has been resident in California since 1978 (Huber, 1988). Genetic, population interbreeding, and morphological studies indicate *G. ashmeadi* is native to the southeast USA and probably accompanied *H. coagulata* to California from its home range (Vickerman et al., 2004). *G. triguttatus*, is a solitary endoparasitoid that was imported from eastern Texas and liberated in California in 2001. *G. fasciatus*, is a gregarious endoparasitoid (i.e., more than one adult parasitoid emerges from an individual host egg [Triapitsyn et al., 2003]), known from Louisiana, Florida, Georgia, Illinois, Missouri, Tennessee, Texas, and Virginia (Triapitsyn, pers. comm.). This parasitoid was imported from Louisiana and has been liberated in California since 2002 (CDFA, 2003).

Understanding the dynamics of competitive interactions between parasitoid species can assist in the selection of effective agents for classical biological control (Mackauer, 1990), and can provide insight into predicting and interpreting field outcomes following natural enemy establishment in new locales (Murdoch and Briggs, 1996). The introduction of more than a single natural enemy to control a target pest can result in competitive interactions (Briggs, 1993; Denoth et al., 2002; Myers et al., 1989; Pemberton and Willard, 1918; Zwolfer, 1971) that may reduce agent efficacy thereby ultimately limiting their collective impact on the target. Recoveries of *G. triguttatus* and *G. fasciatus* have been made at release sites and establishment is expected (CDFA, 2003). Should *G. triguttatus* and *G. fasciatus* populations increase, it is unknown how they will compete with each other and the omnipresent *G. ashmeadi*.

For *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, the following research investigated parasitoid longevity, utilization of *H. coagulata* eggs of different ages, reproductive capacity, aggressive behavior between females concurrently searching for *H. coagulata* egg masses, and progeny survival rates when females of all three species were simultaneously presented *H. coagulata* egg masses in the laboratory. When taken together, these data could provide insight into the biological control potential of these mymarids and possible outcomes of competitive field interactions between these three species.

2. Materials and methods

2.1. Source of plants and insects, and experimental conditions in the laboratory

Citrus limon (lemon) cv. 'Eureka' trees, approximately 2 years of age and grafted to *Marcophylla* sp. Rootstock, were obtained from C&M Nurseries, Nipomo, CA. Trees were pruned to 60 cm in height, potted into 4-L containers, and fertilized every 2 weeks with Miracle-Gro (20 ml/3.5 L of water, Scotts Miracle-Gro Products, Marysville, OH). 'Eureka' was chosen because it has been previously demonstrated that *H. coagulata* prefers this lemon variety for oviposition and parasitoid foraging is not adversely affected in comparison to other lemon cultivars (Irvin and Hoddle, 2005). Parasitoid colonies were maintained at the University of California, Riverside at 26 ± 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' leaves. Colonies were provisioned with honey–water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA) and checked daily for parasitoid emergence to assure uniform age for experiments. All experiments described here were conducted in the laboratory at 26 ± 2 °C and 30–40% RH under light intensity of 1.2 ± 0.2 log lumens/m^2 and L14:10D photoperiod. During statistical analysis, some of the data could not be transformed to fit the assumptions of ANOVA, therefore non-parametric methods were used.

2.2. Experiment 1: utilization of *H. coagulata* eggs of different ages by *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*

Stems of leaves containing 15 *H. coagulata* eggs of known age laid on 'Eureka' lemon leaves were placed through holes drilled in the lid of a 130 ml plastic vial (40 dram plastic vial, Thornton Plastics, Salt Lake City, UT) filled with deionized water and 3 ml of antiseptic (Listerine Antiseptic Mouthwash, Pfizer, New York, NY) to prevent bacterial rot. Leaf numbers were standardized to three per vial by including lemon leaves without *H. coagulata* egg masses. 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 test material and left for 2 h. This procedure was replicated approximately 30 times for *H. coagulata* eggs 1–2, 3–4, 5–6, 7–8, and 9–10 days of age that were presented to *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*.

Vials containing leaves exposed to parasitoids were held for 3 weeks to allow parasitoids and *H. coagulata* nymphs to emerge. Drying of leaves sometimes occurred which often prevented successful insect emergence, therefore unemerged eggs were dissected and the numbers of unemerged nymphs, parasitoids, and unemerged unknowns (those that could not be identified) were recorded. Percentage offspring [(the total number of emerged and unemerged parasitoids/the number of *H. coagulata* eggs) × 100], percentage parasitism [(number of *H. coagulata* eggs parasitized/total number of eggs) × 100], and percentage nymphs [(the total number of emerged and unemerged *H. coagulata* nymphs/the number of eggs) × 100] were calculated for each egg age category and parasitoid species. Progeny production and parasitism are not equivalent for *G. fasciatus* as this species is gregarious, whereas, *G. ashmeadi* and *G. triguttatus* are solitary parasitoids. The sex of parasitoid progeny was recorded and percentage of female progeny was calculated for each egg age category and parasitoid species.

To determine parasitoid utilization, percentage parasitism was compared between egg age categories for each parasitoid species using χ^2 in SAS (1990). Kruskal–Wallis χ^2 tests were used to compare percentage parasitism, percentage offspring, and percentage nymphs between species for each egg age category. Percentage female data was square root arc sine transformed and ANOVA was used to compare data between parasitoid species for egg age categories 1–2 and 3–4. Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. *T* tests were used to compare percentage of female offspring between *G. ashmeadi* and *G. triguttatus* for egg age categories 5–6 and 7–8. Percentage female pair-wise comparisons had to be conducted at these egg ages because *G. fasciatus* parasitism rates of eggs aged 5–10 days were too low to allow adequate sample sizes for analysis with ANOVA. This also occurred for egg age category 9–10 days for *G. ashmeadi* and *G. triguttatus*. Means presented here are back transformed.

2.3. Experiment 2: comparing parasitoid longevity between species

Fifteen replicates of honey–water and water treatments were set up in a randomized design. Honey–water treatments consisted of three droplets of honey–water solution (see previous description) placed with an eyedropper on the lid of a 130 ml plastic vial. A 130 ml vial with ventilation, and containing one newly emerged mated naïve male and female parasitoid (~24 h old) was inverted and placed on the lid containing the honey–water. Honey–water was applied once to the lid at the beginning of the experiment and replaced every 3 days. Water was supplied to each treatment via a moist cotton ball placed on the mesh top of the inverted 130 ml plastic vial. The water only treatment consisted of this design but with out honey–water solution. This was replicated 12–15 times for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*. Parasitoid longevity was recorded daily until death for each sex and males were not replaced once dead. All longevity data were square root transformed prior to analysis. ANOVA was used to compare longevity between parasitoid species for each treatment and those comparisons with significant *F* values were further analyzed using Tukey's multiple comparison of means test at the 0.05 level of significance. Means presented here have been back-transformed.

2.4. Experiment 3: simultaneous exposure of parasitoids to H. coagulata eggs in each of three different age categories

'Eureka' lemon leaves containing approximately 30 *H. coagulata* eggs of known age were placed in double inverted vials as described above with one newly emerged mated naïve female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* (~24 h of age) which were left to forage. This procedure was replicated 15–20 times for *H. coagulata* egg ages 1, 3, and 5 days of age. Twenty-four hours after exposure, parasitoids were removed and vials containing leaves with egg masses exposed to parasitoids were held for 3 weeks to allow parasitoids to emerge. The proportion of offspring produced and *H. coagulata* eggs parasitized was calculated for each parasitoid species and compared between species at each egg age using Friedman's χ^2. Offspring production and parasitism data were pooled over all egg ages and compared between species using the same test. *G. ashmeadi* and *G. triguttatus* offspring production and parasitism data were square root arc sine transformed and compared across egg ages using ANOVA. For *G. fasciatus* these parameters were compared between species using Kruskal–Wallis χ^2 on non-transformed data. All means presented here are back transformed.

2.5. Experiment 4: simultaneous exposure of parasitoids to H. coagulata eggs of three different ages

Three 'Eureka' lemon leaves containing approximately 15 *H. coagulata* eggs each of 1, 3, and 5 days of age were placed in double inverted vials (see above) with one mated naïve female of *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* (~24 h of age). This procedure was replicated 17 times and the percentage of offspring produced and *H. coagulata* eggs parasitized per vial was calculated as above for each parasitoid species. Offspring production and parasitism data were compared between parasitoid species using Friedman's χ^2.

2.6. Experiment 5: simultaneous exposure of parasitoids to one H. coagulata egg mass

One *H. coagulata* egg mass (3–6 eggs and 24–48 h of age) was placed into a Petri dish (3.5 × 1 cm, Becton–Dickinson Labware, Becton–Dickinson, Franklin Lakes, NJ) lined with moist filter paper (4.25 cm, Whatman International, Maidstone, England), and exposed simultaneously to one newly emerged, mated naïve female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* (~24 h of age) for 1 h visual observations were made for each of the three foraging females every 5 min for resting/grooming, searching leaf, searching off the leaf, inspection of eggs with antennae, oviposition (insertion of ovipositor), and aggressive behavior towards congenerics. One hour following exposure, parasitoids were removed and Petri dishes containing leaves with egg masses exposed to parasitoids were held for 3 weeks to allow parasitoids and *H. coagulata* nymphs to emerge. This procedure was repeated eight times on five separate days and experiments were conducted between 9:30 am and 12:00 pm. The proportion of offspring produced and *H. coagulata* eggs parasitized was calculated as above for each parasitoid species and compared between species using Friedman's χ^2. The percentage of time spent in each behavioral event was calculated for each parasitoid species and compared between species, and within species, using Friedman's χ^2 and Kruskal–Wallis χ^2, respectively, at the 0.05 level of significance. Behavioral ethograms were constructed for each species by calculating the percentage occurrence of each behavioral path.

3. Results

3.1. Experiment 1: egg age utilization by G. ashmeadi, G. triguttatus, and G. fasciatus

There was a highly significant effect of egg age on percentage parasitism by *G. ashmeadi* ($\chi^2 = 759.59$, $df = 4$, $p < 0.005$), *G. triguttatus* ($\chi^2 = 327.13$, $df = 4$, $p < 0.005$) and *G. fasciatus* ($\chi^2 = 150.22$, $df = 4$, $p < 0.005$) (Fig. 1). Eggs 3–4 days of age resulted in up to 67.0 and 45.1% higher percentage parasitism by *G. ashmeadi* and *G. triguttatus*, respectively, compared with all remaining egg ages (Fig. 1). Percentage parasitism by *G. fasciatus* was up to 10.4% higher when females were exposed to eggs 1–2 days of age compared with all remaining egg age categories. Exposing *G. fasciatus* to *H. coagulata* eggs aged 5 days and older resulted in only 0.7–2.4% parasitism, compared with 17.4% parasitism for eggs 1–2 days of age (Fig. 1).

Comparing percentage parasitism between species showed significant results for all egg age categories (1–2: $\chi^2 = 248.72$, $df = 2$, $p < 0.005$; 3–4: $\chi^2 = 455.16$, $df = 2$, $p < 0.005$; 5–6: $\chi^2 = 245.17$, $df = 2$, $p < 0.005$; 7–8: $\chi^2 = 59.25$, $df = 2$, $p < 0.005$; 9–10: $\chi^2 = 16.88$, $df = 2$,

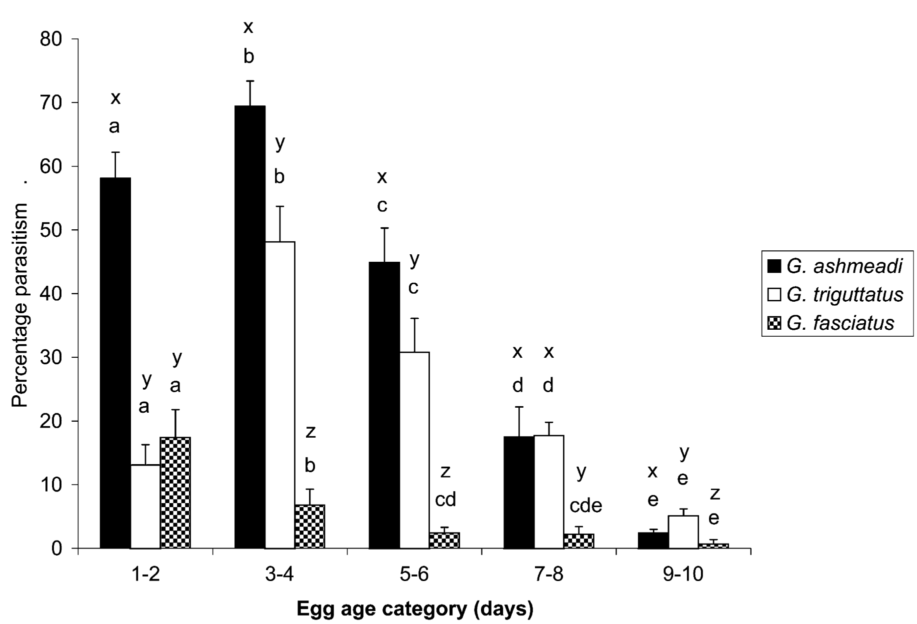

Fig. 1. Effect of *H. coagulata* egg age category and parasitoid species on percentage of *H. coagulata* eggs parasitized by *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* [different letters (a, b) indicate significant ($p < 0.05$) differences between egg age for each species; different letters (x, y) indicate significant ($p < 0.05$) differences between species for each egg age; error bars indicate ±SEM].

$p < 0.005$) (Fig. 1). Percentage parasitism by *G. ashmeadi* was up to 62.6% higher compared with *G. triguttatus* and *G. fasciatus* when females were exposed to eggs 1–6 days of age (Fig. 1). In contrast, percentage parasitism was equivalent between *G. ashmeadi* and *G. triguttatus* when females were exposed to eggs 7–8 days of age, and both species parasitized up to 15.5% more 7- to 8-day-old eggs compared with *G. fasciatus*. Percentage parasitism by *G. triguttatus* was up to 41.3% higher compared with *G. fasciatus* for egg age categories 3–4, 5–6, 7–8, and 9–10. When females were exposed to eggs 1–2 days of age, percentage parasitism by *G. fasciatus* was 4.3% higher compared with *G. triguttatus* (Fig. 1).

3.1.1. Offspring production

Offspring production on each egg age category varied significantly (1–2: $\chi^2 = 26.32$, $df = 2$, $p < 0.005$; 3–4: $\chi^2 = 40.33$, $df = 2$, $p < 0.005$; 5–6: $\chi^2 = 30.29$, $df = 2$, $p < 0.005$; 7–8: $\chi^2 = 9.67$, $df = 2$, $p < 0.01$) between parasitoid species, except for eggs aged 9–10 days of age ($\chi^2 = 5.56$, $df = 2$, $p = 0.06$) (Fig. 2A). For egg age category 1–2 days, *G. ashmeadi* offspring production was up to 45% higher compared with *G. triguttatus* and *G. fasciatus*, while *G. fasciatus* produced a higher (19.2%) proportion of offspring compared with *G. triguttatus* (Fig. 2A). When females were exposed to eggs 3–4 days of age, *G. ashmeadi* offspring production was up to 53.6% higher compared with *G. triguttatus* and *G. fasciatus*, and *G. triguttatus* produced a higher (32.3%) proportion of offspring compared with *G. fasciatus*. For egg age categories 5–6 and 7–8, offspring production by *G. ashmeadi* and *G. triguttatus* was equivalent, and both species produced up to 41.2% more offspring compared with *G. fasciatus* (Fig. 2A).

3.1.2. Nymphal H. coagulata survival rates

Homalodisca coagulata nymph emergence rates varied significantly within each egg age category (1–2: $\chi^2 = 96.85$, $df = 2$, $p < 0.005$; 3–4: $\chi^2 = 173.28$, $df = 2$, $p < 0.005$; 5–6: $\chi^2 = 86.51$, $df = 2$, $p < 0.005$; 7–8: $\chi^2 = 8.85$, $df = 2$, $p < 0.05$; 9–10: $\chi^2 = 20.39$, $df = 2$, $p < 0.005$) (Fig. 2B). When parasitoids were exposed to eggs 1–6 days of age, percentage nymph emergence was up to 37.1% lower for *G. ashmeadi* compared with *G. triguttatus* and *G. fasciatus*. For egg age category 1–2 days, nymph survival was equivalent between *G. triguttatus* and *G. fasciatus*, whereas, for females exposed to egg age categories 3–4 and 5–6, nymph survival was up to 28.5% higher for *G. fasciatus* compared with *G. triguttatus* (Fig. 2B).

3.1.3. Female offspring production

Percentage female offspring production between species showed significant ($F = 4.24$, $df = 2$, 57, $p < 0.05$) results for egg age category 3–4 days, where *G. fasciatus* produced 17.1% more female offspring compared with

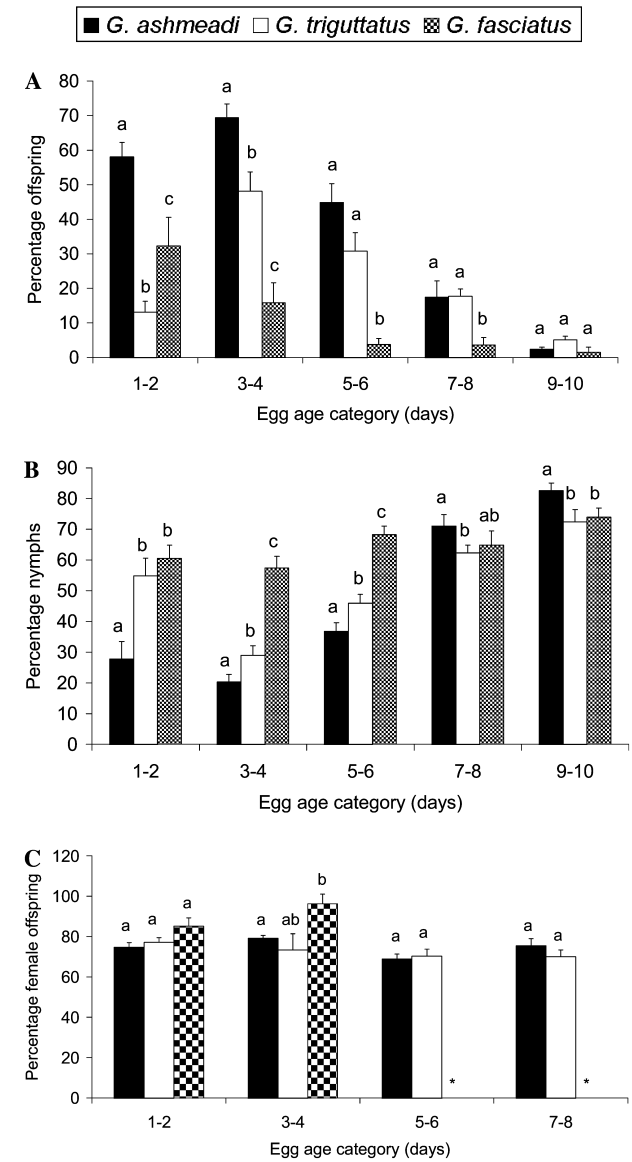

Fig. 2. The effect of parasitoid species on (A) percentage of offspring produced per vial, (B) percentage *H. coagulata* nymphs, and (C) percentage female offspring when *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* were exposed individually to *H. coagulata* eggs of varying age [different letters (a, b) indicate significant ($p < 0.05$) differences between species for each egg age; error bars indicate ±SEM; * = no data available due to low parasitism rates].

G. ashmeadi, while all remaining egg ages were not significant (1–2: $F = 1.90$, $df = 2$, 36, $p = 0.16$; 5–6: $t = 0.40$, $df = 1$, $p = 0.69$; 7–8: $t = 1.67$, $df = 1$, $p = 0.11$) (Fig. 2C).

3.2. Experiment 2: comparing parasitoid longevity between species

Species had a significant effect on longevity of female and male parasitoids provisioned honey–water (female: $F = 11.12$, $df = 2$, 28, $p < 0.005$; male: $F = 11.10$, $df = 2$, 28, $p < 0.005$) and water (female: $F = 10.68$, $df = 2$, 29,

$p < 0.005$; male: $F = 3.52$, $df = 2$, 29, $p < 0.05$). Of these treatments, survival of female *G. ashmeadi* (honey–water: mean = 46.5 days; water: 2.5 days) was up to 171.9 and 87.5% higher compared with *G. triguttatus* (honey–water: 17.1 days; water: 1.7 days) and *G. fasciatus* (honey–water: 24.8 days; water: 1.7 days), respectively, whereas, survival was equivalent between female *G. triguttatus* and *G. fasciatus*. Survival of male *G. ashmeadi* (mean = 13.5 days) and *G. triguttatus* (17.0 days) on honey–water was up to 325.0% higher compared with *G. fasciatus* (4.0 days), whereas, survival was equivalent between *G. ashmeadi* and *G. triguttatus*. When males were provisioned water only, survival of *G. ashmeadi* was 60.6% higher compared with *G. fasciatus*.

3.3. Experiment 3: simultaneous exposure of parasitoids to one individual host egg age

The percentage of eggs parasitized varied significantly between species for all experimental egg age categories (one: $\chi^2 = 26.61$, $df = 2$, $p < 0.005$; three: $\chi^2 = 22.23$, $df = 2$, $p < 0.005$; five: $\chi^2 = 24.57$, $df = 2$, $p < 0.005$) (Fig. 3A). For eggs 1 and 3 days of age, parasitism by *G. ashmeadi* was up to 63.7 and 81.1% higher compared with *G. triguttatus* and *G. fasciatus*, respectively, and parasitism by *G. triguttatus* was up to 18.4% higher compared with *G. fasciatus* (Fig. 3A). Parasitism of eggs 5 days of age was 62.8 and 36.3% higher for *G. ashmeadi* and *G. triguttatus*, respectively, compared with *G. fasciatus*, while there was no significant difference in percentage of eggs parasitized between *G. ashmeadi* (63.1%) and *G. triguttatus* (36.6%) (Fig. 3A).

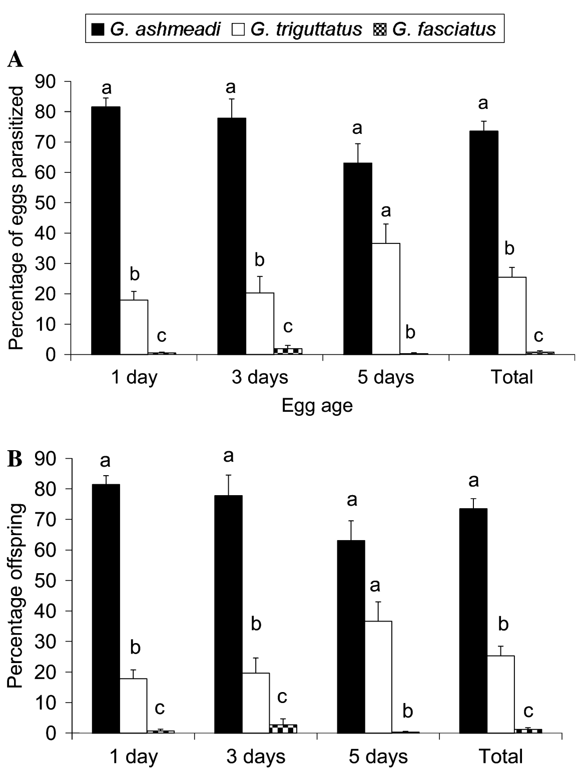

Fig. 3. (A) Percentage of eggs parasitized and (B) percentage offspring production by *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* when *H. coagulata* eggs one, three, and five days of age were exposed individually to one female of each species [different letters (a, b) indicate significant ($p < 0.05$) differences between species for each egg age; no significant differences occurred between egg ages for each species; error bars indicate ±SEM].

Comparing parasitism data across egg ages showed that the percentage of eggs parasitized by *G. triguttatus* increased from 17.8% for eggs one day of age, to 36.6% for eggs 5 days of age ($F = 3.14$, $df = 2$, 43, $p = 0.053$) (Fig. 3A). The percentage of eggs parasitized did not significantly vary between egg ages for *G. ashmeadi* ($F = 2.62$, $df = 2$, 43, $p = 0.08$) and *G. fasciatus* ($\chi^2 = 1.93$, $df = 2$, $p = 0.38$) (Fig. 3A). Pooling percentage data over all egg ages showed that the percentage of *H. coagulata* eggs parasitized varied significantly between species ($\chi^2 = 94.02$, $df = 2$, $p < 0.005$), where parasitism by *G. ashmeadi* was 48.1 and 72.8% higher compared with *G. triguttatus* and *G. fasciatus*, respectively. Overall percentage parasitism by *G. triguttatus* was 24.7% higher compared with *G. fasciatus* (Fig. 3A). Comparing percentage offspring production between and within species showed similar results to percentage parasitism results described here (Fig. 3B).

3.4. Experiment 4: simultaneous exposure of parasitoids to three host egg ages

When females were exposed simultaneously to *H. coagulata* eggs 1, 3, and 5 days of age, species had a significant effect on the proportion of offspring produced ($\chi^2 = 19.73$, $df = 2$, $p < 0.005$) and percentage of eggs parasitized by each species ($\chi^2 = 22.12$, $df = 2$, $p < 0.005$) (Fig. 4A). *G. ashmeadi* and *G. triguttatus* parasitized a higher (49.7 and 39.2%, respectively) proportion of *H. coagulata* eggs compared with *G. fasciatus*, whereas, parasitism by *G. ashmeadi* and *G. triguttatus* was equivalent (Fig. 4A). Comparing percentage offspring production between species showed similar results to percentage parasitism (Fig. 4A).

3.5. Experiment 5: simultaneous exposure of parasitoids to one H. coagulata egg mass

There was a significant effect of species on percentage offspring produced ($\chi^2 = 24.91$, $df = 2$, $p < 0.005$) and the proportion of *H. coagulata* eggs parasitized ($\chi^2 = 27.52$, $df = 2$, $p < 0.005$) by each parasitoid species (Fig. 4B). Parasitism by *G. ashmeadi* was 39.6% and 64.6% higher compared with *G. triguttatus* and *G. fasciatus*, respectively, while *G. triguttatus* parasitized 25.0% more

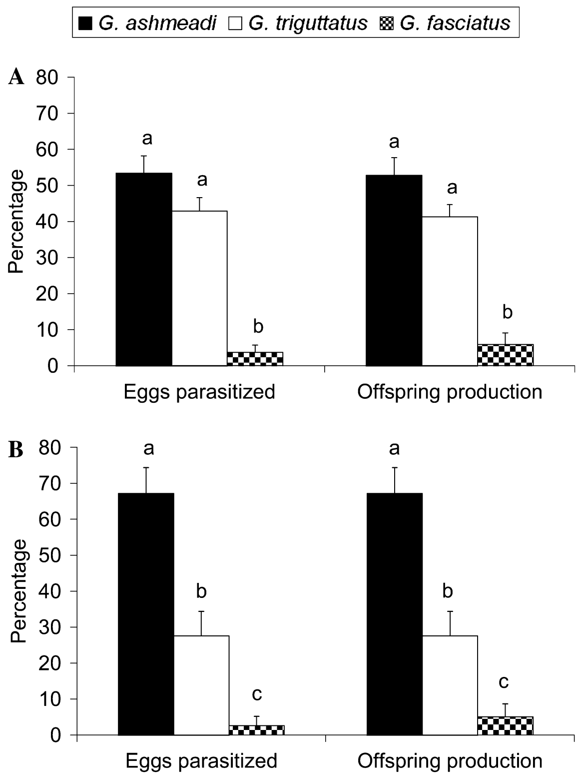

Fig. 4. Percentage of eggs parasitized and percentage offspring production by *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* when (A) *H. coagulata* eggs 1, 3, and 5 days of age were exposed simultaneously to one female of each species; and (B) one *H. coagulata* egg mass (1–2 days of age) was exposed to one female of each species [different letters (a, b) indicate significant ($p < 0.05$) differences between species; error bars indicate ±SEM].

H. coagulata eggs compared with *G. fasciatus* (Fig. 4B). Comparing percentage offspring production between species showed similar results to percentage parasitism (Fig. 4B).

The proportion of time spent in each behavioral event was significantly different within each *Gonatocerus* species (*G. ashmeadi*: $\chi^2 = 65.62$, $df = 5$, $p < 0.005$; *G. triguttatus*: $\chi^2 = 27.52$, $df = 2$, $p < 0.005$; *G. fasciatus*: $\chi^2 = 27.52$, $df = 2$, $p < 0.005$) (Fig. 5). *G. ashmeadi* allocated the greatest proportion of time to resting/grooming (26.5%) and oviposition (25.8%), and females allocated up to 24.4% more time to these behaviors, compared with egg inspection, searching leaf, and aggressive chasing of congenerics (Fig. 5). Female *G. ashmeadi* spent 2.1% of observations demonstrating aggressive behavior towards competitors. *Gonatocerus triguttatus* allocated the greatest proportion of time to resting/grooming (62.9%) and the time females allocated to this behavior was up to 61.6% higher compared with all remaining behaviors (Fig. 5). *Gonatocerus triguttatus* spent 1.3% of observations aggressively interacting with *G. ashmeadi* and *G. fasciatus* on egg masses. Finally, female *G. fasciatus* spent the greatest proportion of time resting/grooming (40.0%) and off the leaf (39.6%), being up to 40.0% higher compared with all remaining behaviors. Aggressive behavior was not observed for *G. fasciatus* (Fig. 5).

Parasitoid species had a significant effect on the proportion of time females spent resting ($\chi^2 = 28.68$, $df = 2$, $p < 0.005$), searching off the leaf ($\chi^2 = 27.65$, $df = 2$, $p < 0.005$), inspecting *H. coagulata* eggs with antennae ($\chi^2 = 15.57$, $df = 2$, $p < 0.005$) and oviposition ($\chi^2 = 14.48$, $df = 2$, $p < 0.005$). There was no significant difference between species searching leaves ($\chi^2 = 5.48$, $df = 2$, $p = 0.06$) or aggressively interacting with congenerics ($\chi^2 = 4.11$, $df = 2$, $p = 0.13$) (Fig. 5). Aggressive behavior by *G. ashmeadi* involved running directly at *G. triguttatus* and making contact. Although *G. ashmeadi* and *G. triguttatus* showed aggressive behavior towards other females, six accounts of simultaneous oviposition (1.3% of total observations, or 2.7% of observed oviposition events) by one or more species on the same egg mass was recorded.

A total of 274, 212, and 215 behavioral transition events were recorded for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, respectively. Female *G. ashmeadi* allocated a higher proportion of time transitioning from oviposition to resting/grooming (9.1%), resting/grooming to oviposition (8.8%), egg inspection to oviposition (8.8%), and from oviposition to egg inspection (10.6%) (Fig. 6A). Female *G. ashmeadi* aggressively chased *G. triguttatus* and *G. fasciatus* off the egg mass, after egg inspection or oviposition (Fig. 6A). When chased by *G. triguttatus*, *G. ashmeadi* showed submissive behavior by resting/grooming (1.1%), and accosted female *G. ashmeadi* did not immediately return to oviposition or egg inspection.

Gonatocerus triguttatus allocated a higher proportion of time transitioning from oviposition to resting/grooming (8.0%), resting/grooming to oviposition (8.0%), egg inspection to resting/grooming, resting/grooming to off the leaf (12.3%), and off the leaf to resting/grooming (15.6%) (Fig. 6B). Aggressive behavior by *G. triguttatus* occurred after egg inspection or oviposition, and females either returned to egg inspection, oviposition, or initiated further aggressive behavior. When chased by *G. ashmeadi*, *G. triguttatus* did not continue egg inspection, instead females searched leaves for hosts (0.9%) or rested/groomed (0.5%) (Fig. 6B).

Female *G. fasciatus* allocated a higher proportion of time transitioning from resting/grooming to off the leaf (28.0%), and from off the leaf to resting/grooming (24.0%) (Fig. 6C). No aggressive events were observed for *G. fasciatus*. Female *G. fasciatus* chased by congenerics during egg inspection (2.8%) or leaf searching (1.9%), subsequently rested/groomed (2.3%), or returned to egg inspection (2.3%).

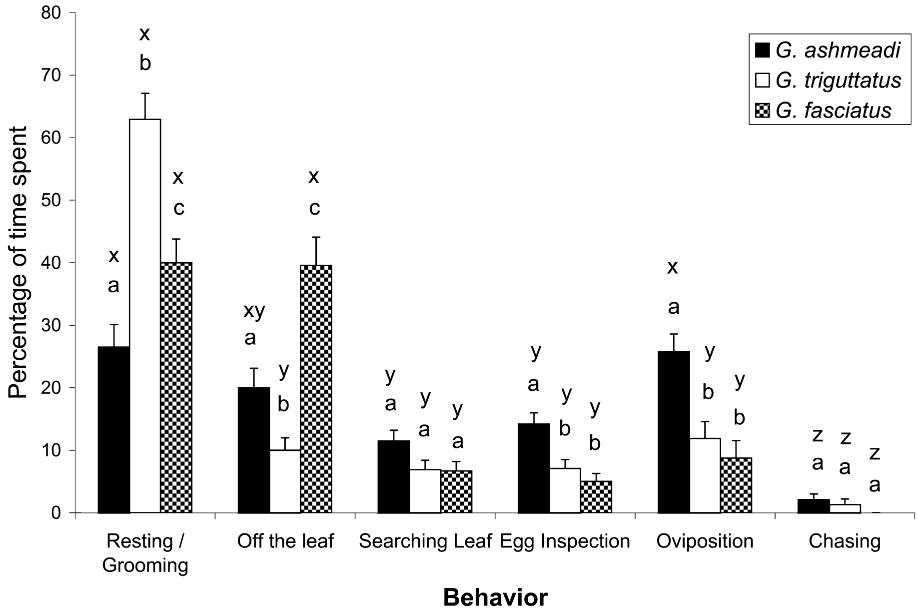

Fig. 5. The proportion of time spent in each quantified behavior for female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*, when one *H. coagulata* egg mass was exposed to one female of each species [different letters (a, b) indicate significant ($p < 0.05$) differences between species for each behavior; different letters (x, y) indicate significant ($p < 0.05$) differences between behaviors for each species; error bars indicate ±SEM].

4. Discussion

4.1. Egg age utilization and interspecific competition

Results from the egg age utilization study showed that all three *Gonatocerus* species utilized most efficiently eggs of specific ages, and that egg age ranges used for oviposition overlapped between species. The *H. coagulata* egg age category that *G. ashmeadi* and *G. triguttatus* most preferred to parasitize was eggs 3–4 days of age which may suggest that interspecific competition will occur between these two species in the field environment. Given the numerical superiority of *G. ashmeadi* in California, this may prevent *G. triguttatus* from becoming abundant and widespread under prevailing conditions.

Gonatocerus ashmeadi and *G. fasciatus* parasitized 58.1 and 17.4% of eggs 1–2 days of age, respectively, and this age category was second most preferred by *G. ashmeadi*, and was the only host age *G. fasciatus* parasitized effectively. This may indicate that competition between these two species for hosts 1–2 days of age may occur in the field, and that *G. fasciatus* may have difficultly proliferating in California because 1- to 2-day-old hosts are fundamental for successful *G. fasciatus* larval development but may be unavailable due to parasitism by the more aggressive and abundant *G. ashmeadi.*

Egg age preference results showed that *G. ashmeadi* parasitized a significantly higher proportion of *H. coagulata* eggs aged 1–6 days of age compared with *G. triguttatus* and *G. fasciatus*, and vials containing *G. ashmeadi* produced significantly lower nymph survival compared with *G. triguttatus* and *G. fasciatus*. Additionally, for the competition studies (Experiments 3–5), overall parasitism by *G. ashmeadi* was up to 76.0% higher compared with *G. triguttatus* and *G. fasciatus* for all three experimental designs. These results indicate that *G. ashmeadi* may be more efficient at finding and processing hosts than *G. triguttatus* and *G. fasciatus*. Furthermore, results from the longevity study (Experiment 2) demonstrated that female *G. ashmeadi* survived up to 171.9% longer than *G. triguttatus* and *G. fasciatus* when females were provisioned honey–water. Greater longevity would enable *G. ashmeadi* females to encounter and parasitize a higher number of *H. coagulata* in the field.

Gonatocerus triguttatus and *G. fasciatus* were introduced into California in 2001 and 2002, respectively, as part of a classical biological control program against *H. coagulata*. The introduction of more than a single natural enemy to control a pest may induce interspecific competition and result in either competitive exclusion or coexistence (Briggs, 1993; Denoth et al., 2002; Myers et al., 1989; Pemberton and Willard, 1918; Zwolfer, 1971). Results presented here indicate that *G. ashmeadi* could out compete *G. triguttatus* and *G. fasciatus* in the field and may prevent their widespread proliferation in California. Furthermore, results showed that *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* demonstrated distinct utilization rates for *H. coagulata* eggs aged 1–4, 3–6, and 1–2 days, respectively. Field competition for egg masses may be less prevalent compared with laboratory no choice situations, and results from experiments three and four suggest that in the field where a range of host ages are

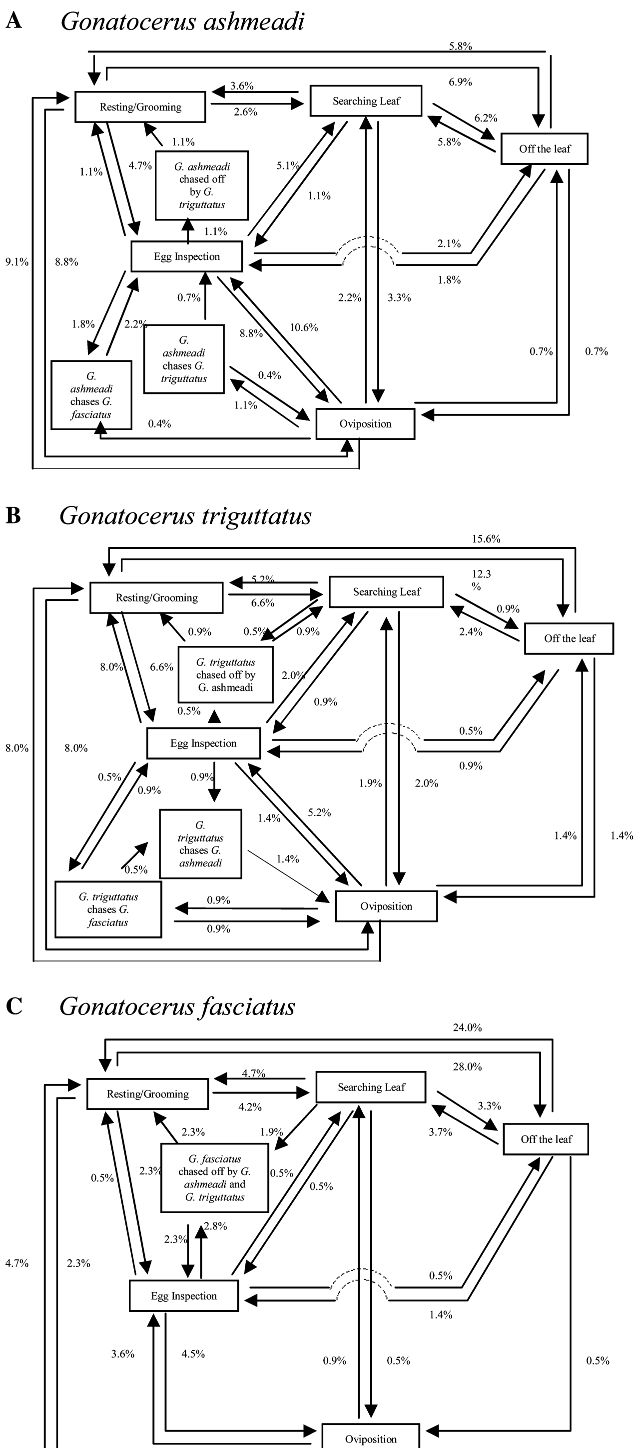

Fig. 6. Ethograms for (A) *G. ashmeadi*, (B) *G. triguttatus*, and (C) *G. fasciatus* when one female of each species was exposed simultaneously to one *H. coagulata* egg mass in the laboratory. Numbers indicate percentage of behavioral events calculated from total observed behavioral events.

present abundantly, interspecific competition may be reduced. This situation could change if host eggs become scarce.

Competition experiments three and four demonstrated that pooling results from females exposed to individual egg ages (Experiment 3) showed that *G. ashmeadi* parasitized a significantly higher proportion of *H. coagulata* eggs compared with *G. triguttatus*, whereas, when females were exposed to all egg ages simultaneously (Experiment 4), parasitism by *G. ashmeadi* and *G. triguttatus* was equivalent. This may be due to *G. ashmeadi* out competing *G. triguttatus* when females were only provided eggs 1 or 3 days of age, since these ages are more favorable for *G. ashmeadi* development, and reproductive success for *G. triguttatus* is higher in older host eggs. We speculate that when both females were exposed simultaneously to eggs 1, 3, and 5 days of age, female *G. ashmeadi* and *G. triguttatus* parasitized the egg ages most preferred by each species, thereby decreasing interspecific competition and resulting in equivalent parasitism rates between species. These results may have important implications for the field environment, where a range of host ages are present at one time, and may suggest that *G. ashmeadi* and *G. triguttatus* can coexist in California without significant interference competition when host eggs of mixed ages are abundant as currently seen.

Parasitism by *G. fasciatus* was consistently lower compared with *G. ashmeadi* and *G. triguttatus* for all three competition experimental studies. Furthermore, comparing parasitism rates and nymph survival between species for the egg age utilization study showed that *G. fasciatus* parasitized a significantly lower proportion of *H. coagulata* eggs 3–10 days of age compared with *G. ashmeadi* and *G. triguttatus*, and nymph survival from eggs 1–6 days of age was consistently higher in vials containing *G. fasciatus* compared with *G. ashmeadi* and *G. triguttatus*. The gregarious nature of *G. fasciatus* (Triapitsyn et al., 2003) may have caused poor performance in the current studies because exposure to *H. coagulata* eggs was restricted to 1, 2 or 24 h, and *G. fasciatus* may require a longer period of time for individual host handling and multiple egg deposition into hosts, compared with *G. ashmeadi* and *G. triguttatus*. In the field environment where females are only restricted by life time fecundity and longevity, *G. fasciatus* may perform more efficiently due to a higher reproductive capability per discovered egg mass.

The low parasitism rates of *G. fasciatus* shown in these studies may be attributed to poor competitive ability of *G. fasciatus* larvae. First, eggs oviposited by female *G. fasciatus* are conceivably smaller than *G. ashmeadi* and *G. triguttatus* due to comparative differences in female size between species. Large eggs may give rise to larger parasitoid larvae, which tend to out compete smaller competitors, especially if they hatch earlier (Collier and Hunter, 2001; Collier et al., 2002). Second, gregarious parasitoid larvae, such as *G. fasciatus*, may not participate in larval combat since *G. fasciatus* larvae frequently contact each other during normal development, and therefore gregarious species that have evolved to co-utilize hosts may be at a competitive disadvantage (Laing and Corrigan, 1987; Salt, 1961).

Alternatively, the low parasitism rates of *G. fasciatus* may be due to low oviposition rates and subordination. Irvin and Hoddle (2004) showed that an additional 115 and 65% female *G. fasciatus* were required to obtain sufficient replication where oviposition occurred, in 1 h egg age choice studies (egg ages 1, 3, and 5 days of age) compared with *G. ashmeadi* and *G. triguttatus*, respectively. Results from experiment five presented here showed that *G. ashmeadi* and *G. triguttatus* demonstrated aggressive behavior towards congenerics, whereas no such behavior was observed for *G. fasciatus*. Furthermore, 39.6% of time allocated by female *G. fasciatus* was spent off leaves with egg masses when congenerics were present. In the laboratory, it appears that when female *G. ashmeadi* or *G. triguttatus* are present on an *H. coagulata* egg mass, *G. fasciatus* is less assertive and attempts to search for host eggs elsewhere.

The lower parasitism rates, increased *H. coagulata* nymph survival, and poor competitive performance of *G. fasciatus* demonstrated in these studies may suggest that *G. ashmeadi* and *G. triguttatus* will out compete *G. fasciatus* in the field. Although *G. fasciatus* performed poorly in the current studies, we speculate that in the field *G. fasciatus* may be superior due to potentially higher reproduction rates, younger host age attacked and greater host finding efficiency to locate the narrow 'window' of acceptable hosts. In fact, results from Irvin and Hoddle (unpublished data) demonstrated that when 30 *H. coagulata* eggs were exposed to 2–5 ovipositing females of the same species, the number of female *G. fasciatus* produced and percentage offspring, respectively, was up to 148.1 and 126.8% higher compared with *G. ashmeadi* and *G. triguttatus*. Additionally, results from experiment one in this study showed that *G. fasciatus* produced a significantly higher proportion of female progeny compared with *G. ashmeadi* when females were presented eggs 3–4 days of age. In California, gregarious reproduction by *G. fasciatus* and female biased progeny production, if coupled with efficient host finding may give this species a competitive advantage early in the spring when *H. coagulata* egg masses are rare in the field, overwintering *G. ashmeadi* are uncommon, and parasitism levels are low (Triapitsyn et al., 2003).

4.2. Behavior between females concurrently searching for H. coagulata egg masses

Results from the behavioral data showed that both *Gonatocerus triguttatus* and *G. ashmeadi* performed a repetitive sequence consisting of transitioning from oviposition to resting/grooming, and then returning to oviposition on egg masses. This result may be indicative of patch defense by *G. ashmeadi* and *G. triguttatus*. Previous research has shown that resting or stationary behavior in the egg parasitoid *Trissolcus basalis* (Wollaston) (Scelionidae) was a component of antagonistic behavior, and combined with patrolling the egg mass, functioned as pre-emptive patch defense (Field, 1998; Field et al., 1998). *G. fasciatus* failed to initiate aggressive behavior towards congenerics. The lack of aggressive behavior in *G. fasciatus* is most probably related to the smaller size of this species in comparison to *G. ashmeadi* and *G. triguttatus*. Larger females are more likely to chase smaller females, and have a competitive advantage in contest outcomes (Lawrence, 1981; Petersen and Hardy, 1996). When *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* are present on an *H. coagulata* egg mass, *G. fasciatus* adopts a submissive role and leaves egg masses, presumably to search for suitable hosts elsewhere.

Although *G. ashmeadi* and *G. triguttatus* showed aggressive behavior towards congenerics, six accounts of simultaneous oviposition by at least two females was recorded during the experiment. This demonstrates that females can co-exploit a patch and encounter one another without initiating aggression. Initiation of aggressiveness may be related to the size of females (Lawrence, 1981; Petersen and Hardy, 1996), the number of progeny each female has invested in the patch, and the rate of encounters with unparasitized hosts (Field and Calbert, 1998). It is unknown what factors favor simultaneous co-exploitation of *H. coagulata* egg masses by *G. ashmeadi* and *G. triguttatus*. More work may be warranted in this area.

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