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RESEARCH ARTICLE

Comparative assessments of *Gonatocerus ashmeadi* and the 'new association' parasitoid *G. deleoni* (Hymenoptera: Mymaridae) as biological control agents of *Homalodisca vitripennis* (Hemiptera: Cicadellidae)

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Egg age preference, competitive ability, and behavior of *Gonatocerus deleoni* ('new association' parasitoid) and *G. ashmeadi* (the co-evolved dominant parasitoid in California) were investigated in the laboratory to determine whether one species exhibited competitive superiority. When searching concurrently for *Homalodisca vitripennis* egg masses, *G. ashmeadi* consistently outperformed *G. deleoni* by parasitizing 59–89% more eggs under three different experimental systems in the laboratory with varying host densities, egg ages, and exposure times. *G. ashmeadi* parasitism in control vials containing one parasitoid ranged from 58 to 86% and was up to 28% higher at egg ages 1 and 3 days compared with 5 days. *G. deleoni* in control vials parasitized on average 7% of *H. vitripennis* egg masses in 1 h regardless of egg age. *G. deleoni* failed to parasitize *H. vitripennis* egg masses in 15 min when caged alone or in competition with *G. ashmeadi*. In a combined species treatment, parasitism by *G. ashmeadi* was 11% higher when *H. vitripennis* eggs were exposed for 5 days compared with 24 h. Conversely parasitism by *G. deleoni* was 5% lower for this comparison. *G. ashmeadi* had a significantly female biased sex ratio for all three experimental designs, whereas, *G. deleoni* offspring sex ratio was not significantly greater than 50%. In comparison to *G. ashmeadi*, behavioral observations indicated that *G. deleoni* was absent from host egg infested leaves 53% more frequently and it oviposited 66% less frequently. No incidences of females aggressively chasing competitors off *H. vitripennis* egg masses were recorded during this study.

Keywords: aggressive behavior; competitive ability; egg age preference; exposure time; neo-classical biological control

Introduction

A co-evolved natural enemy may be more efficient in finding and attacking a target pest because it has evolved to exploit it (Messenger and van der Bosch 1971). Alternatively, it has been argued that biological control agents that have not co-evolved with a pest will be more effective natural enemies. This is because co-evolution between pests and biological control agents leads to decreased effectiveness of natural enemies and increased resistance of the pest to attacks

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because the system works towards establishing a balance between these two entities (Pimentel 1963). Consequently, it has been proposed that new association biological control agents which have no evolutionary history with the target pest should be used because the pest will be highly vulnerable to attack by this novel agent. This form of biological control with non-co-evolved natural enemies is called 'new association' biological control (Hokkanen and Pimentel 1989). Hokkanen and Pimentel (1984) concluded that there was an approximately 75% greater chance for success for pest suppression with new association biological control agents when compared with success rates for co-evolved natural enemies. However, species that are most likely to be effective new association biological control agents are pre-adapted to using new hosts, and theoretically pose high risks to non-target species because of polyphagy (Roderick 1992).

Homalodisca vitripennis (Germar) (Hemiptera: Cicadellidae: Proconiini, formally *H. coagulata*), the glassy-winged sharpshooter, is native to the southeastern USA and northeastern Mexico, and has become a significant threat to agricultural and ornamental industries in California since establishing in the late 1980s. This pest is a vector of a xylem-limited bacterium, *Xylella fastidiosa* Wells et al., which causes disease in several important plants including grapes, almond, alfalfa, peach, and oleander (Blua, Phillips, and Redak 1999; UCOP 2000; Varela, Smith, and Phillips 2001). Considerable effort has been expended in California to develop a classical biological control program for this pest with egg parasitoids.

The current parasitoid guild attacking *H. vitripennis* in California consists of eight species of egg parasitoids (CDFA 2006), and 70% of species reared from host eggs consists of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) (CDFA 2006). Hoddle (2009) reported that year round parasitism of *H. vitripennis* for all parasitoid species averages $\sim$25%. 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 which can affect levels of control (Myers, Higgens, and Kovacs 1989; Briggs 1993; Denoth, Frid, and Myers 2002). Therefore, the low parasitism rates that occur in California may be attributed to competitive exclusion amongst the eight parasitoid species which reduces their collective impact. Alternatively, the system may lack an aggressive and efficacious natural enemy that can dominate the guild to provide effective and consistent year round biological control of *H. vitripennis* populations. Pimentel (1991) reported that in more than 95% of cases of successful biological control, it only took one natural enemy to suppress pest numbers to acceptable levels. It is possible that new association natural enemies of *H. vitripennis,* could provide the highly sought after early season suppression of this pest in California where parasitism by *G. ashmeadi* is extremely low (Triapitsyn, Morgan, Hoddle, and Berezovskiy 2003).

Material thought to be *G. tuberculifemur* (Ogloblin) (Hymenoptera; Mymaridae), a common and widespread parasitoid that attacks Proconiini sharpshooters in Argentina and Chile in South America, was imported from Argentina into quarantine in Texas and California during 2001–2006 and reared on egg masses of the factitious host *H. vitripennis* (Triapitsyn, Logarzo, De León, and Virla 2008). It was later discovered that some of this material imported from the San Rafael area of Mendoza Province of Argentina in 2006 was in fact a new species, *Gonatocerus deleoni* Triapitsyn, Logarzo & Virla sp. n. (initially called 'Clade 2' of *G. tuberculifemur* [Ogloblin] as described in Triapitsyn et al. 2008, referred to hereafter

as *G. deleoni*) (Hymenoptera; Mymaridae). The parasitoids from Argentina, *G. tuberculifemur* and *G. deleoni*, have no evolutionary history with *H. vitripennis.* If released and established in California, this would make these two parasitoid species new association biological control agents of *H. vitripennis.*

To help determine whether *G. tuberculifemur* should be introduced into California, research was conducted to determine whether it could outperform *G. ashmeadi,* the dominant parasitoid of *H. vitripennis.* Studies demonstrated that *G. tuberculifemur* failed to outperform *G. ashmeadi* in variable experimental arenas, and across different host densities, egg ages and exposure times (Irvin, Hoddle, and Suarez-Espinoza 2009; Irvin and Hoddle 2010). *G. tuberculifemur* may be an inferior biological control agent of *H. vitripennis* eggs and a poor competitor compared to *G. ashmeadi*. Consequently, there may be little advantage to releasing *G. tuberculifemur* from quarantine, unless it can be demonstrated to fulfill a field niche where competition with *G. ashmeadi* is reduced. *G. deleoni* may potentially be a more suitable candidate for new association biological control of *H. vitripennis* when compared with *G. tuberculifemur* because the host and geographic range of *G. deleoni* is narrower than *G. tuberculifemur* (Triapitsyn et al. 2008). In Mendoza Province, Argentina, *G. deleoni* is limited to desert oases which have high climatic similarity to California, but not to the south-eastern USA where *H. vitripennis* is native (Jones 2003). Theoretically, *G. deleoni* would not be able flourish in the south-eastern USA which could minimize establishment risks and reduce threats of attacks to non-target native leafhoppers in this area (De León, Logarzo, and Triapitsyn 2008). Furthermore, risks to California fauna may be reduced since *G. deleoni* appears to be highly host specific as it has only been recorded attacking *Tapajosa rubromarginata* (Signoret) (Cicadellidae: Proconiini) in Argentina and not eggs of Cicadellini (Triapitsyn et al. 2008).

Research into the competitively ability of *G. deleoni* and whether it can outperform *G. ashmeadi* was required to determine whether *G. deleoni* would benefit *H. vitripennis* biological control efforts in California. These studies, which are reported here, investigated host egg age preferences, the competitive ability of *G. deleoni* when foraging simultaneously with *G. ashmeadi* on *H. vitripennis* egg masses, and aggressive interactions between these two parasitoid species when resources were contested. The results from these experiments could be used to help guide the decision to release *G. deleoni* from quarantine for liberation and establishment in California for new association biological control of *H. vitripennis.*

Materials and methods

Insect colonies and parasitoid preparation for experiments

Laboratory colonies of *H. vitripennis* and *G. ashmeadi* were maintained at the University of California, at Riverside (UCR). Colonies of *G. ashmeadi* were held at $26 \pm 2^{\circ}$C and 30–40% relative humidity (RH) under a 14 h L:10 h D photoperiod and reared on *H. vitripennis* eggs laid on 'Eureka' lemon leaves (*Citrus limon* L.), a preferred lemon variety for *H. vitripennis* oviposition and parasitoid foraging (Irvin and Hoddle 2004). *Citrus limon* cv. 'Eureka' trees, approximately 2 years old 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 Inc., Marysville, OH). Female *G. deleoni* were sourced from colonies maintained in the Insect and Quarantine building at UCR, California. These colonies had completed approximately 63 generations since arriving at UCR in January 2006. *G. deleoni* colonies were maintained in ventilated plastic cages ($9 \times 9 \times 16$ cm) and held at $24 \pm 2^{\circ}$C, 40–50% RH under a 14 h L:10 h D photoperiod. Females were held with 50% honey–water for 2–3 days before exposure to *H. vitripennis* eggs laid on 'Eureka' lemon leaves. Petioles of leaves with *H. vitripennis* egg masses were inserted into 1-cm slits cut into a piece of 0.5-cm thick polystyrene foam so that leaves had a vertical aspect. The foam was cut to fit tightly into the bottom of the *G. deleoni* colony cage. The bottom of the parasitoid colony cage was perforated with holes, and placed in a metal tray ($20 \times 20 \times 45$ cm) containing 2 cm of tap water which watered the foam pad holding leaves. *Gonatocerus ashmeadi* and *G. deleoni* colonies were provisioned with honey–water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland, CA) and checked daily for parasitoid emergence.

To prepare parasitoids for experiments, newly emerged (<12 h) female and male *G. ashmeadi* were aspirated into 130-mL plastic vials (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) and 50% honey–water (Natural uncooked honey, Wild Mountain Brand) was supplied in droplets on the lid. This was repeated for *G. deleoni.* Parasitoids were held in the laboratory for 24 h at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod prior to use in experiments. On days when parasitoids ($\sim$24–36 h of age) and host eggs were available, all experiments were set up between 10am and 1pm in the laboratory at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod with fluorescent lighting. Parasitoids were discarded if no host eggs were available that day. Irvin and Hoddle (unpublished data) found that female-applied brochosomes cover 64% of *H. vitripennis* egg masses and that the density of brochosome deposition is highly variable. Brochosomes have been demonstrated to interfere with parasitism (Velema, Hemerik, Hoddle, and Luck 2005). Therefore, to standardize the surface of egg masses, leaves were rinsed under cold water and brochosomes were gently wiped from eggs with a soft paper towel prior to presentation to parasitoids. Consequently, all *H. vitripennis* egg masses used in experiments were free of brochosomes.

Parasitoid egg age preferences and competitive abilities

One mated female *G. ashmeadi* and *G. deleoni* ($\sim$24–36 h old) were presented simultaneously to one *H. vitripennis* egg masses (4–8 eggs) laid on 'Eureka' lemon leaves and camouflaged amongst three other similar sized lemon leaves in a double ventilated vial system as described in Irvin et al. (2009). This experiment was replicated 15 times for *H. vitripennis* eggs aged 1, 3, and 5 days of age. Female parasitoids were left to forage for 1 h and then leaves containing egg masses were placed into Petri dishes (3.5×1 cm, Becton-Dickinson Labware, Becton-Dickinson and Co., Franklin Lakes, NJ) lined with moist filter paper (4.25 cm, Whatman Ltd International, Maidstone, England) and labeled with replicate number and egg age. Petri dishes were held at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod for 3 weeks to allow insects to emerge. The number of *H. vitripennis* nymphs and emerged male and female adults of each parasitoid species was recorded. Premature drying of leaves sometimes occurred which occasionally

prevented successful insect emergence. Therefore, unemerged eggs were dissected and the numbers of easily identifiable nymphs and unemerged pupae, and adult males and females were also recorded for each parasitoid species. Unemerged *G. deleoni* could be identified because 4–7 days after oviposition the host egg turns orange/red (Jones et al. 2005; Virla, Logarzo, Jones, and Triapitsyn 2005a), whereas, *G. ashmeadi* turns grey/black. Presence or death of parasitoid eggs and larvae were not determined and host egg mortality was attributed to unknown causes. Fifteen control vials containing one female parasitoid were set up for each species to investigate whether two parasitoid species foraging concurrently affected overall parasitism of *H. vitripennis* eggs. Control vials also provided information on egg age preference by allowing comparison of parasitism between egg ages for each parasitoid species.

Statistical analysis

All statistical analyses were conducted in SAS Institute (1990). A one factor logistic regression model was used to determine the effect of treatment (*G. ashmeadi* control, *G. deleoni* control, and combined species treatment) on overall *H. vitripennis* nymph emergence and total parasitism. Pair-wise contrast tests at the 0.05 level of significance were used to separate means. A two factor logistic regression model with an interaction term was used to determine the effect of treatment and egg age on parasitism by *G. ashmeadi* and parasitism by *G. deleoni.*

To compare the competitive ability of *G. ashmeadi* and *G. deleoni* within the combined species treatment, a one factor logistic regression model was used to compare parasitism levels between *G. ashmeadi* and *G. deleoni.* In this model, the Intercept represented the species effect, and an age class variable was used to test for species effects across the three host egg age categories (Agresti 2002). All percentage parasitism and nymph means presented in the results section are calculated as percentages of total number of *H. vitripennis* eggs.

Finally, a two factor logistic regression model with an interaction term was used to determine the effect of treatment (i.e., individual controls or mixed species treatment) and egg age on percentage of female offspring (referred to hereafter as 'sex ratio') for *G. ashmeadi*. This analysis could not be conducted for *G. deleoni* due to low parasitism rates and insufficient data. Sex ratio data were pooled over treatments and egg ages and a chi-squared analysis at the 0.05 level was used to determine whether sex ratio varied significantly between the two parasitoid species. A χ^2-test was also used to determine whether overall sex ratio was equivalent to 50% for each parasitoid species.

Parasitoid behaviors and competitive abilities

One mated female *G. ashmeadi* and *G. deleoni* ($\sim$24–36 h) were presented simultaneously to one *H. vitripennis* egg masses (4–8 eggs, 1–3 days of age an age category preferred by both *G. ashmeadi* and *G. deleoni*, see results in section 3.1) for 15 min in a double ventilated vial as outlined in section 2.2. In contrast to the 'egg age preferences' experiment (section 2.2), egg masses were not camouflaged amongst three other similar sized leaves. Therefore, one leaf was present per vial. This experiment was replicated 31 times. For 15 min, visual observations were made for

each female every 60 s (total of 15 observations for each vial) for activity that was characterized as either off leaf (walking on vial), searching leaf (antennating leaf surface), searching egg mass (inspecting egg mass with antennae), oviposition (insertion of ovipositor into an egg), resting (standing still), grooming, aggressively chasing competitor (female on egg mass chasing the other female), antennating competitor, searching egg mass from top side of leaf (antennating leaf surface directly opposite the egg mass), ovipositing from top side of leaf (inserting ovipositor into an egg from the opposite side of the leaf) or drinking (mouthing moist filter paper). After the 15-min exposure time, leaves containing egg masses were placed into labeled Petri dishes lined with moist filter paper and held at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod for 3 weeks. The numbers of emerged and unemerged nymphs and males and females of each parasitoid species for each treatment were recorded. Thirty replicates of two types of control vials were also set up for each species. The first control treatment consisted of one female per vial to determine whether the frequency of non-aggressive behaviors (e.g., stationary or grooming behaviors) were the result of having a competitor present. The second control consisted of two females of the same species and was used to determine whether 'aggressive behavior' was due to having congenerics competing for an egg mass, or was the result of having another female present irrespective of species. The control vials containing two females of the same species were used to determine whether competition between two species reduced or increased parasitism of *H. vitripennis* eggs and if competition between females affected offspring sex ratio (Irvin and Hoddle 2006). Means presented in the results section are calculated as percentages of the total number of *H. vitripennis* eggs.

Statistical analyses

All statistical analyses were conducted in SAS Institute (1990). A one factor logistic regression was used to determine the effect of treatment (combined species treatment – AD; vials containing one female *G. ashmeadi* only – A-control; vials containing one *G. deleoni* only – D-control; vials containing two *G. ashmeadi* – AA-control; and vials containing two *G. deleoni* – DD-control) on parasitism by *G. ashmeadi* and *H. vitripennis* nymph emergence. Pair-wise contrast tests at the 0.05 level of significance were used to separate means. Due to poor parasitism rates by *G. deleoni* (no *G. deleoni* offspring were recorded in the combined species treatment or *G. deleoni*-control, see results), a two by two table Fisher exact test was used to determine the effect of treatment (D-control, DD-control, and the combined species treatment) on parasitism by *G. deleoni*. A one factor logistic regression, excluding treatment D-control, was used to determine the effect of treatment on total parasitism. Pair-wise contrast tests at the 0.05 level of significance were used to separate means. The results from using a Fisher exact test to determine the effect on treatment on parasitism by *G. deleoni* outlined above can be directly applied to compare total parasitism between treatments D-control and DD-control because total parasitism was identical to parasitism by *G. deleoni* in these treatments.

To compare the competitive ability of *G. ashmeadi* and *G. deleoni* when *G. ashmeadi* and *G. deleoni* were concurrently foraging on the same *H. vitripennis* egg mass, an Exact two-sided binomial test (for $\rho = 0.5$) was used to compare the number of *H. vitripennis* eggs parasitized between *G. ashmeadi* and *G. deleoni* for the

combined species treatment. A one factor logistic regression model was used to determine the effect of treatment on sex ratio for *G. ashmeadi*. This analysis could not be conducted for *G. deleoni* due to low parasitism rates and insufficient data. Sex ratio data were pooled over treatments and a Fishers Exact test at the 0.05 level was used to determine whether sex ratio significantly varied between the two parasitoid species. A χ^2-test and Fishers Exact test were used to determine whether overall sex ratio was equivalent to 50% for *G. ashmeadi* and *G. deleoni*, respectively.

Behavior data were used to calculate the percentage of time spent in each behavioral event for each treatment. For the combined species treatment (AD), the behavior of each female was identified as being either *G. ashmeadi* (AD-A) or *G. deleoni* (AD-D) (i.e., there were 6 treatments: A, AA, D, DD, AD-A, and AD-D). When comparing between the percentage of observations allocated to each behavior within each treatment, data are considered dependent because the frequency of one behavior may affect the frequency of subsequent behaviors. Consequently, the multivariate modeling technique MANOVA (Agresti 2002) was conducted to determine whether the mean percentage of observations was equal between all 10 behaviors for each treatment. For this test, a significant difference between behaviors existed when $P < 0.05$ (Agresti 2002). Pair-wise contrast tests at the 0.05 level of significance were used to separate means. To determine the effect of treatment on the percentage of observations allocated to each behavior (data considered independent when comparing between treatments), data were analyzed in two stages: (1) for a specific behavior, ANOVA was used to determine whether the three percentages associated with the *G. ashmeadi* treatments were equivalent. This was repeated for *G. deleoni* and (2) if the three percentages associated with the *G. ashmeadi* treatments were not statistically equal, three pair-wise contrast tests at the 0.05 level of significance were used to separate means. This was repeated for *G. deleoni*. To compare the behavior of *G. ashmeadi* and *G. deleoni* when *G ashmeadi* and *G. deleoni* were concurrently foraging on the same *H. vitripennis* egg mass, contrast tests at the 0.05 level of significance were used to compare the percentage of observations between *G. ashmeadi* and *G. deleoni* for each behavior.

Assessing the effect of longer exposure times and higher H. vitripennis densities on parasitism rates

The previous experiments involved simultaneously exposing one *H. vitripennis* egg mass to *G. ashmeadi* and *G. deleoni* for 15–60 min which may have been more favorable for one species of parasitoid. To reduce possible bias resulting from the length of exposure time because some parasitoids show patch defense behaviors (Field 1998; Field, Calbert, and Keller 1998), additional experiments were conducted that increased experimental exposure times and numbers of host eggs available for attack. Approximately 50 *H. vitripennis* eggs (1–2 days of age; 6–8 egg masses) were placed in a double ventilated vial cage, and exposed simultaneously to one mated female *G. ashmeadi* and *G. deleoni* ($\sim$24–48 h old) for either 24 h or 5 days. Vials were held at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod under fluorescent lighting. After the exposure period was complete, leaves containing egg masses were placed into Petri dishes lined with moist filter paper and held at $26 \pm 2^{\circ}$C and 30–40% RH under a 14 h L:10 h D photoperiod for 3 weeks. Twenty replicates were set up for each exposure time. The number of male and female *G. ashmeadi* and

G. deleoni offspring were recorded for each vial. Fifteen replicates of *G. ashmeadi*-control vials and 15 replicates of *G. deleoni*-controls were set up for exposure time 24 h to verify *G. deleoni* used in this experiment successfully parasitized *H. vitripennis* eggs (since parasitism of *G. deleoni* in this study was so low, see results).

Statistical analyses

All statistical analyses were conducted in SAS Institute (1990). A one factor logistic regression model was used to determine the effect of exposure time on percentage parasitism by *G. ashmeadi* and *G. deleoni* in the combined species treatment. Similarly, this model was also used to determine whether parasitism varied significantly between *G. ashmeadi* and *G. deleoni* in the combined species treatment. For exposure time 24 h, a one factor logistic regression model was used to determine the effect of treatment on total parasitism, parasitism by *G. ashmeadi* and parasitism by *G. deleoni*. The effect of exposure time on sex ratio for each species was determined using a one factor logistic regression model. Sex ratio data were pooled over exposure times and a χ^2-test at the 0.05 level of significance was used to determine whether sex ratio significantly varied between the two parasitoid species. A χ^2-test was also used to determine whether overall sex ratio was equivalent to 50% for each parasitoid species.

Results

Parasitoid egg age preferences and competitive abilities

There was a significant effect of treatment on total percentage parasitism ($\chi^2 = 216.28$, df = 2, $P < 0.001$) and the overall percentage of *H. vitripennis* nymphs emerging from exposed egg masses ($\chi^2 = 155.84$, df = 2, $P < 0.001$) (Figure 1). Vials containing one female *G. deleoni* resulted in significantly less parasitism (i.e., 68–70% lower) and up to 66% higher percentage of *H. vitripennis* nymph emergence when compared with the *G. ashmeadi* control vials and mixed vials containing one female of each species (Figure 1). Total percentage parasitism and overall percentage nymph emergence were statistically equivalent between *G. ashmeadi* control vials and the combined species treatment (Figure 1).

Results from vials containing one *H. vitripennis* egg mass camouflaged among leaves without egg masses and exposed simultaneously to one *G. ashmeadi* and *G. deleoni* for 1 h showed that overall parasitism by *G. ashmeadi* was 75% higher compared to *G. deleoni* ($\chi^2 = 60.17$, df = 1, $P < 0.001$) (Figure 1). The effect of egg age on the difference in parasitism between species was not significant ($\chi^2 = 0.38$, df = 2, $P = 0.83$) thereby indicating that parasitism by *G. ashmeadi* was consistently and significantly higher (i.e., by up to 89%) than *G. deleoni* for all three egg ages (Figure 2).

Egg age had a significant effect on percentage parasitism by *G. ashmeadi* ($\chi^2 = 49.20$, df = 2, $P < 0.001$) (Figure 2). The treatment ($\chi^2 = 0.02$, df = 1, $P = 0.88$) and interaction terms were not significant ($\chi^2 = 3.25$, df = 1, $P = 0.20$) (Figures 1 and 2). Results from the *G. ashmeadi* control vials indicated that *G. ashmeadi* parasitism ranged from 58 to 86% across all egg ages (Figure 2). Percentage parasitism was significantly higher (i.e., 26–28%) when *G. ashmeadi* was presented

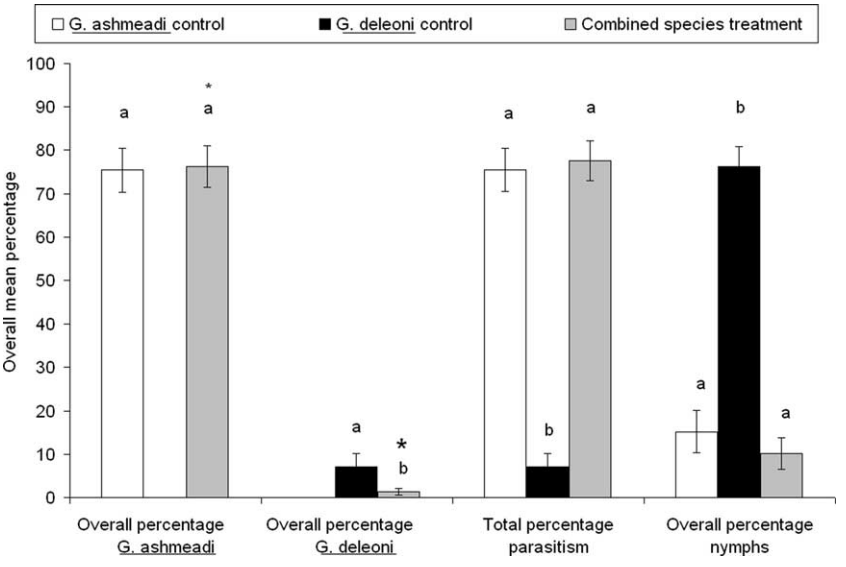

Figure 1. Overall percentage parasitism by *G. ashmeadi,* percentage parasitism by *G. deleoni*, total percentage parasitism, and percentage nymphs emerging when *H. vitripennis* egg masses were exposed to three parasitoid treatments (vial containing one female *G. ashmeadi* only – *G. ashmeadi* control; vial containing one *G. deleoni* only – *G. deleoni* control; vial containing both *G. ashmeadi* and *G. deleoni* – combined species treatment) for 1 h at 26°C (error bars indicate $\pm$ standard error of the means, SEMs; different letters indicate significant, $P < 0.05$, differences between treatments for each percentage category; asterisks indicate a significant difference, $P < 0.05$, in parasitism between *G. ashmeadi* and *G. deleoni* for the combined species treatment).

with *H. vitripennis* eggs aged 1 and 3 days of age when compared with eggs 5 days of age (Figure 2). For *G. deleoni*, treatment had a significant effect on parasitism ($\chi^2 = 6.91$, df = 1, $P < 0.01$) (Figure 1 and 2), where the presence of *G. ashmeadi* in the combined species treatment (AD treatment) reduced overall parasitism by *G. deleoni* by up to 6%, compared with the *G. deleoni*-control vials (Figure 1 and 2). In the *G. deleoni*-control vials, *G. deleoni* parasitized on average 7% of *H. vitripennis* egg masses regardless of egg age ($\chi^2 = 0.36$, df = 2, $P = 0.83$) (Figure 2). The interaction term was not significant ($\chi^2 = 0.24$, df = 2, $P = 0.89$).

Parasitoid behaviors and competitive abilities

There was a significant effect of treatment on total percentage parasitism ($\chi^2 = 43.95$, df = 3, $P < 0.001$) and percentage of *H. vitripennis* nymphs emerging from exposed egg masses ($\chi^2 = 79.83$, df = 4, $P < 0.001$). Vials containing one or two female *G. deleoni* resulted in just 0–7% total parasitism which was significantly lower (by 48–62%) when compared with the *G. ashmeadi* controls and the combined species treatment (Figure 3). Similarly, both of the *G. deleoni* controls resulted in up to 52% more *H. vitripennis* nymphs emerging when compared to vials containing one or two *G. ashmeadi* controls and the combined species treatment (Figure 3). Total parasitism and nymph emergence was statistically equivalent between the *G. ashmeadi* controls

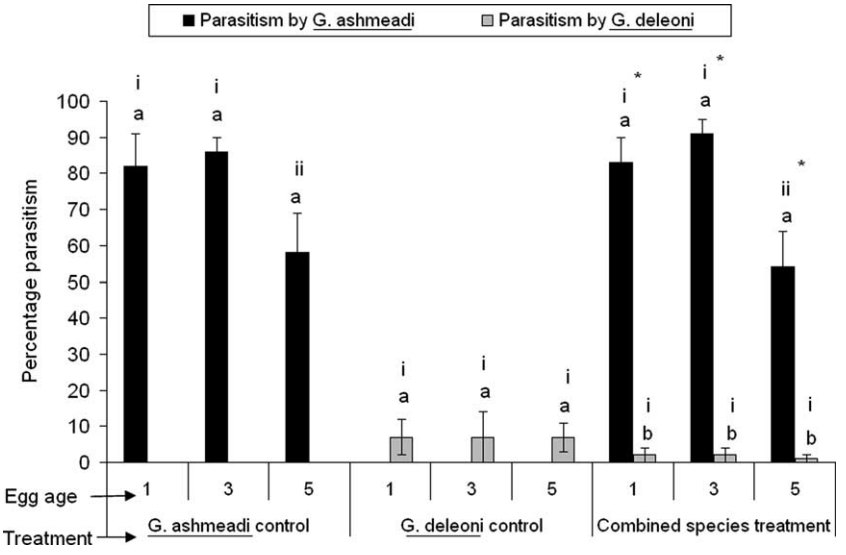

Figure 2. Percentage parasitism by *G. ashmeadi* and *G. deleoni* resulting when *H. vitripennis* egg masses aged 1, 3, and 5 days of age were exposed to three parasitoid treatments (vial containing one female *G. ashmeadi* only – *G. ashmeadi* control; vial containing one *G. deleoni* only – *G. deleoni* control; vial containing both *G. ashmeadi* and *G. deleoni* – combined species treatment) for 1 h at 26°C (error bars indicate SEMs'; different roman numerals – i, ii, iii, indicate significant, $P < 0.05$, differences between egg ages within each control; different letters – a, b, c, indicate significant, $P < 0.05$, differences in parasitism of *G. ashmeadi* or *G. deleoni* between controls and the combined species treatment for each egg age; an asterisk indicates a significant difference, $P < 0.05$, in parasitism between *G. ashmeadi* and *G. deleoni* at each egg age for the combined species treatment).

and the combined species treatment demonstrating the dominant effect of *G. ashmeadi* (Figure 3).

One *H. vitripennis* egg mass exposed simultaneously to one *G. ashmeadi* and *G. deleoni* for 15 min resulted in zero parasitism by *G. deleoni* and 59% parasitism by *G. ashmeadi* (two-sided Binomial test, $P < 0.001$) (Figure 3). There was no significant effect of treatment on parasitism by *G. ashmeadi* ($\chi^2 = 4.37$, df $= 2$, $P = 0.11$) (Figure 3). *G. deleoni* failed to parasitize *H. vitripennis* egg masses in 15 min when caged alone or in competition with *G. ashmeadi*. When two *G. deleoni* females were present, parasitism by *G. deleoni* was 7% which was significantly higher than the D-control (Fishers Exact test, $P < 0.05$) and combined species treatment (two-sided Fishers Exact test, $P < 0.05$). Within each treatment, the percentage of observations allocated to each behavior differed significantly among behavior events for all treatments (Table 1). Female *G. ashmeadi* in the A-control vials and the combined species treatment allocated up to 42% more observations to oviposition compared with all remaining behavioral events (Table 1). In contrast, female *G. deleoni* in the D-control, DD-control vials and the combined species treatment spent up to 43% of observations off the leaf compared with all remaining behavioral events. Female *G. deleoni* spent 0.5% of observations searching the egg mass from the opposite side of the leaf in the DD-controls, but no observations of ovipositing through the leaf were recorded for *G. deleoni* in any treatment (D-controls, DD-controls, or combined

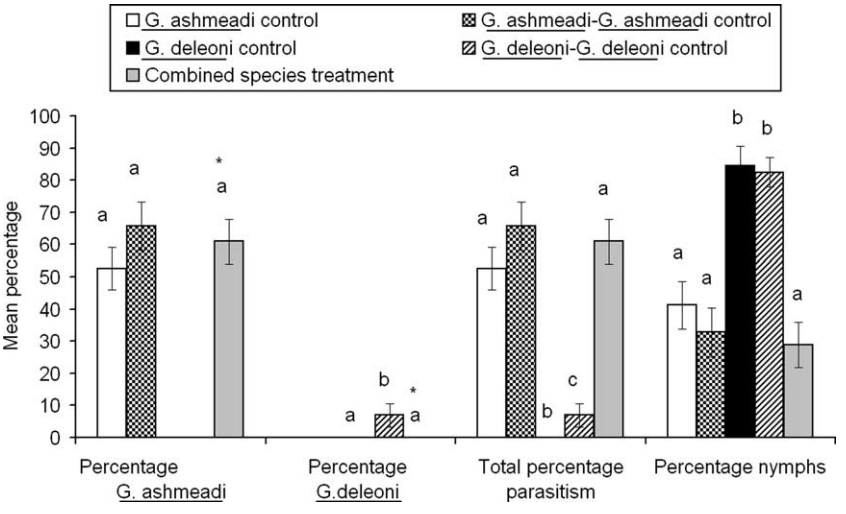

Figure 3. Mean percentage parasitism by *G. ashmeadi,* percentage parasitism *G. deleoni*, total percentage parasitism, and percentage nymphs resulting when *H. vitripennis* egg masses were exposed to parasitoids either alone or with intraspecific or interspecific competition for 15 min at 26°C (error bars indicate ± SEMs; different letters indicate significant, $P < 0.05$, differences between treatments for each percentage category; asterisks indicate a significant difference, $P < 0.05$, in parasitism between *G. ashmeadi* and *G. deleoni* for the combined species treatment).

species treatment) (Table 1). Female *G. ashmeadi* spent up to 6% of observations searching for and/or ovipositing into host eggs on the opposite side of the leaf in all treatments (A-controls, AA-controls, and combined species treatment) and these behaviors were not confined to treatments containing a competitor. *G. ashmeadi* and *G. deleoni* spent up to 2% of observations antennating competitors in vials containing two females of the same species, whereas, this behavior did not occur in the combined species treatment. No accounts of females aggressively chasing competitors off *H. vitripennis* egg masses were recorded during this study (Table 1).

Comparisons among parasitoid treatments indicated that the percentage of observations allocated to oviposition, off the leaf, grooming, antennating competitors, searching egg masses from the opposite side of the leaf and oviposition from the opposite side of leaves varied significantly among treatments (Tables 1 and 2). There was no significant effect of treatment on the frequency of observations allocated to searching the leaf, searching egg masses, resting, chasing competitors, and drinking water (Tables 1 and 2). When *G. ashmeadi* and *G. deleoni* were presented simultaneously with *H. vitripennis* egg masses for 15 min, *G. ashmeadi* allocated 16% more observations to searching the leaf, 53% more observations to ovipositing, and 66% fewer observations were recorded off the leaf when compared with *G. deleoni* (Tables 1 and 2). No accounts of oviposition by *G. deleoni* were recorded. Oviposition by *G. ashmeadi* from the opposite side of the leaves was recorded but never for female *G. deleoni* who were not observed to conduct this activity in the combined species treatment (Tables 1 and 2). The frequency at which *G. ashmeadi* was observed off the leaf was statistically equivalent between the A-controls, AA-controls, and combined species treatment suggesting that the presence of a

Table 1. The frequency (mean [±SEM] percentage) of 11 behaviors observed once every 1 min when *G. ashmeadi* and *G. deleoni* were exposed to one *H. vitripennis* egg mass for 15 min under five parasitoid treatments (A, control vial containing one female *G. ashmeadi*; AA, control vial containing two female *G. ashmeadi*; AD, one female *G. ashmeadi* and *G. deleoni*, DD, two female *G. deleoni*; D, one female *G. deleoni*).

	Parasitoid treatment					
			AD			
Behavior	A	AA	*G. ashmeadi*	*G. deleoni*	D	DD
Off leaf	6.7±1.8 BE	16.4±4.2 AC	6.7±2.7 BCD	72.9±7.2 A	68.9±6.6 A	48.9±6.2 A
Searching leaf	17.1±5.1 B	13.3±2.7 BC	12.4±2.2 BC	12.0±5.5 B	8.0±3.0 BC	14.9±16.3 BC
Searching egg mass	11.0±2.9 BC	12.2±2.3 BC	17.8±2.3 B	1.8±1.4 C	0±0 C	2.3±0.8 E
Oviposition	41.9±7.5 A	26.8±4.3 A	52.4±3.8 A	0±0 C	0±0 C	9.4±3.7 BCD
Resting	8.6±5.5 BCD	6.9±3.7 CD	1.8±0.8 CD	4.0±4.0 B	4.4±2.4 C	1.6±1.0 DE
Grooming	7.7±3.5 BE	20.9±4.5 AB	5.8±2.1 BC	9.3±4.1 B	18.2±4.9 C	20.2±5.2 B
Chasing Competition		0±0 G	0±0 D	0±0 C		0±0 E
Anntenaeing Competitor		1.8±0.8 DF	0±0 D	0±0 C		0.5±0.3 E
Searching egg top side	1.0±0.7 DE	0±0 G	1.8±1.2 D	0±0 C	0±0 C	0.5±0.3 E
Oviposition top side	5.7±5.7 CE	1.6±0.9 EFG	0±0 D	0±0 C	0±0 C	0±0 E
Drinking	0.5±0.5 E	1.6±0.9 EFG	1.3±1.0 D	0±0 C	0.4±0.4 C	1.6±1.0 E
Between behaviors test statistics from MANOVA[1]	$F = 66.36$, df = 9, 104, $P < 0.001$	$F = 124.14$, df = 9, 104, $P < 0.001$	$F = 66.24$, df = 9, 104, $P < 0.001$	$F = 80.99$, df = 9, 104, $P < 0.001$	$F = 79.81$, df = 9, 104, $P < 0.001$	$F = 134.48$, df = 9, 104, $P < 0.001$

[1] Different letters (A, B, C) indicate significant differences between behaviors within each parasitoid treatment.

Table 2. Hypothesis tests and resulting *F*- and *P*-values (*F*, *P*) for ANOVA tests conducted to detect significant differences between five parasitoid treatments within each of 11 behaviors (degrees of freedom for 'overall tests' = 2, 112; degrees of freedom for remaining tests = 1, 112; see Table 2; ADA = *G. ashmeadi* from the combined species treatment, ADD = *G. deleoni* from the combined species treatment; pair-wise contrast tests were conducted between percentages associated with the same parasitoid species only if the overall test was significant for that species).

	Hypothesis tests								
Behavior	Overall *G. ashmeadi* test (A = AA = ADA)	A = AA	A = ADA	AA = ADA	Comparing species within treatment AD (ADA = ADD)	Overall *G. deleoni* test (D = DD = ADD)	D = DD	D = ADD	DD = ADD
Off leaf	1.15, 0.320	–	–	–	53.67, <0.001	5.88, 0.004	6.40, 0.013	0.20, 0.659	9.23, 0.003
Searching leaf	0.38, 0.687	–	–	–	0.01, 0.939	0.96, 0.385	–	–	–
Searching egg mass	2.85, 0.062	–	–	–	26.56, <0.001	0.37, 0.693			
Oviposition	9.45, <0.001	5.83, 0.017	2.18, 0.143	17.69, <0.001	55.86, <0.001	1.77, 0.174			
Resting	0.94, 0.393	–	–	–	0.18, 0.671	0.25, 0.779	–	–	–
Grooming	3.25, 0.042	3.64, 0.059	0.05, 0.818	4.94, 0.028	0.21, 0.651	1.31, 0.275	–	–	–
Anntenaeing Competitor	4.28, 0.016	5.54, 0.020	0, 1.000	5.80, 0.017	0, 1.000	0.29, 0.752	–	–	–
Searching egg top side	3.95, 0.022	2.08, 0.152	1.18, 0.279	7.58, 0.007	5.68, 0.019	0.37, 0.689	–	–	–
Oviposition top side	3.29, 0.041	5.87, 0.017	4.46, 0.037	0, 1.000	0, 1.000	0, 1.000	–	–	–
Drinking	0.35, 0.707	–	–	–	0.82, 0.367	0.92, 0.402	–	–	–

[1]A + AA + ADA = D + DD + ADD.

competitor did not affect the frequency at which female *G. ashmeadi* were observed off the leaf (Tables 1 and 2). In contrast, female *G. deleoni* in the DD-controls were observed up to 24% more frequently off the leaf compared with the D-controls and combined species treatment.

The frequency that female *G. ashmeadi* were observed ovipositing was up to 26% higher in the control vials containing one female of the same species and the combined species treatment, compared with the control vials containing two female *G. ashmeadi* (Tables 1 and 2). Results for *G. deleoni* demonstrated that oviposition was not observed unless there were two female *G. deleoni* present (Tables 1 and 2). Frequency of female *G. ashmeadi* antennating competitors and grooming was significantly 2 and 13% higher, respectively in the AA-controls compared with the combined species treatment (Tables 1 and 2). Finally, *G. ashmeadi* were observed to oviposit into egg masses from the opposite side of leaves 6% more frequently when females were caged alone, compared with both treatments containing a competitor (Tables 1 and 2). There was no significant difference in oviposition on the opposite side of leaves by *G. deleoni* between the three treatments containing *G. deleoni* (Tables 1 and 2).

Assessing the effect of longer exposure times and higher H. vitripennis *densities on parasitism rates*

For the combined species treatment, mean parasitism by *G. ashmeadi* was significantly higher (69–84%) than *G. deleoni* for both exposure times ($\chi^2 = 768.21$, df $= 1$, $P < 0.001$) (Figure 4). Parasitism by *G. ashmeadi* was significantly higher

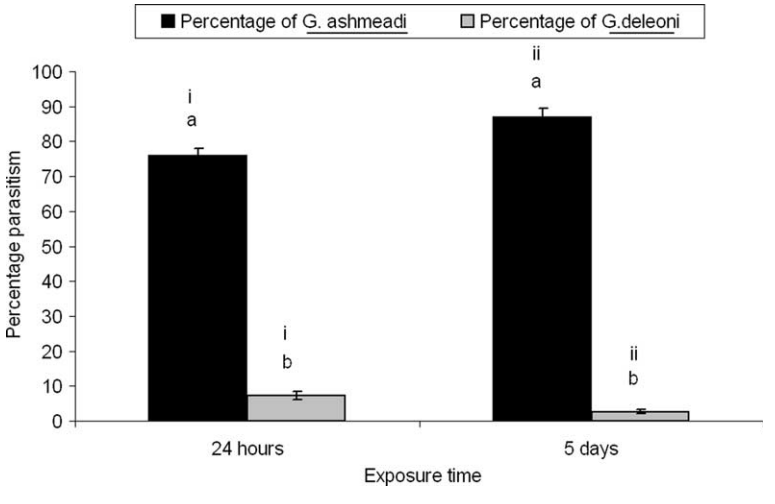

Figure 4. The mean percentage of *G. ashmeadi* and *G. deleoni* offspring emerging when 50 *H. vitripennis* eggs were exposed simultaneously to one mated female *G. ashmeadi* and *G. deleoni* for 24 h or 5 days in the laboratory at 26°C (error bars indicate ± SEMs; different letters – a, b, indicate significant, $P < 0.05$, differences in percentage parasitism between parasitoid species within each exposure time; different roman numerals – i, ii, indicate significant, $P < 0.05$, differences in percentage parasitism between exposure times within each parasitoid species).

(11%) when *H. vitripennis* eggs were exposed for 5 days compared with 24 h ($\chi^2 = 26.91$, df = 1, $P < 0.001$). In contrast, parasitism by *G. deleoni* was significantly lower (5%) when *H. vitripennis* eggs were exposed for 5 days compared with 24 h ($\chi^2 = 5.64$, df = 1, $P < 0.01$) (Figure 4).

When eggs were exposed to parasitoids for 24 h, treatment had a significant effect on total parasitism ($\chi^2 = 507.61$, df = 2, $P < 0.001$) and *H. vitripennis* nymph emergence ($\chi^2 = 448.54$, df = 2, $P < 0.001$). The combined species treatment resulted in significantly higher (11–71% higher) parasitism of *H. vitripennis* eggs and significantly lower nymph emergence (43–49% lower) compared with either species alone (Figure 5). Parasitism by *G. ashmeadi* ranged from 72 to 76% at exposure time 24 h and was statistically equivalent between treatments ($\chi^2 = 2.97$, df = 1, $P = 0.09$) (Figure 5). In contrast, parasitism by *G. deleoni* was low, ranging from 7 to 12%. The presence of *G. ashmeadi* significantly reduced *G. deleoni* parasitism by 5% compared with the *G. deleoni*-control vials ($\chi^2 = 9.13$, df = 1, $P < 0.01$) (Figure 5).

Offspring sex ratio comparisons

For parasitoid egg age preferences and competitive abilities experiment, there was no significant effect of treatment, egg age, or their interaction on sex ratio of *G. ashmeadi* offspring (treatment: $\chi^2 = 0.05$, df = 1, $P = 0.82$; egg age: $\chi^2 = 0.30$, df = 1,

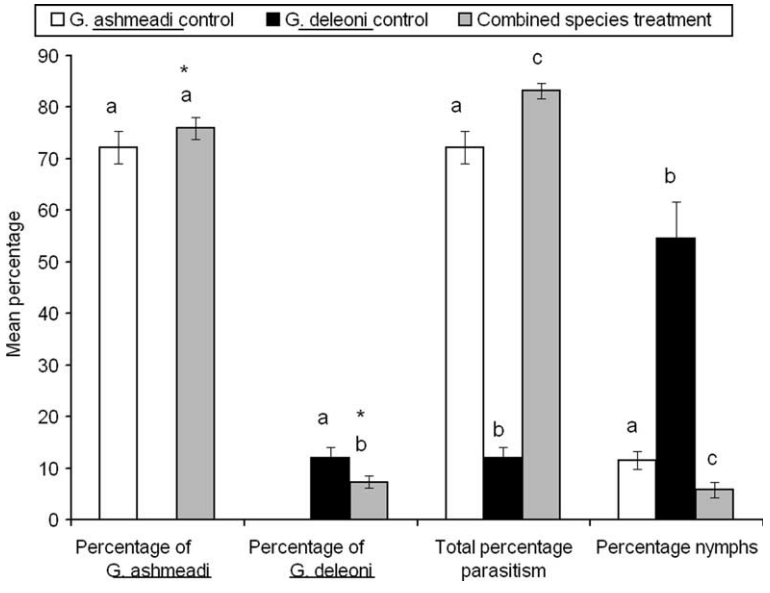

Figure 5. Mean percentage parasitism by *G. ashmeadi,* percentage parasitism *G. deleoni*, total percentage parasitism, and percentage nymphs resulting when *H. vitripennis* egg masses were exposed to three parasitoid treatments (vial containing one female *G. ashmeadi* only – *G. ashmeadi* control; vial containing one *G. deleoni* only – *G. deleoni* control; vial containing both *G. ashmeadi* and *G. deleoni* – combined species treatment) for 24 h at 26°C (error bars indicate ± SEMs; different letters indicate significant, $P < 0.05$, differences between treatments for each percentage category; asterisks indicate a significant difference, $P < 0.05$, in parasitism between *G. ashmeadi* and *G. deleoni* for the combined species treatment).

$P = 0.59$; interaction: $\chi^2 = 0.04$, df = 2, $P = 0.83$). Overall offspring sex ratio did not significantly differ between species (Table 3). However, *G. ashmeadi* had a significant female bias in offspring sex ratio, whereas, *G. deleoni* offspring sex ratio was not significantly greater than 50% female (Table 3).

For the parasitoid behavior and competitive abilities experiment, there was no significant effect of treatment on offspring sex ratio for *G. ashmeadi* ($\chi^2 = 0.80$, df = 2, $P = 0.67$). Overall sex ratio was statistically equivalent between *G. ashmeadi* and *G. deleoni* (Table 3). The overall sex ratio of *G. ashmeadi* offspring was significantly female biased, whereas *G. deleoni* offspring sex ratio was not significantly greater than 50% (Table 3).

For the experiment involving different exposure times and host densities, there was no significant effect of exposure time on offspring sex ratio for *G. ashmeadi* ($\chi^2 = 1.44$, df = 1, $P = 0.22$) or *G. deleoni* ($\chi^2 = 0.40$, df = 1, $P = 0.53$). Sex ratio of *G. ashmeadi* offspring was significantly lower in the combined species treatment (mean = $90 \pm 0.5\%$), compared with the *G. ashmeadi*-controls ($94 \pm 0.5\%$; $\chi^2 = 4.71$, df = 1, $P < 0.05$). There was no significant effect of treatment on sex ratio of *G. deleoni* offspring ($\chi^2 = 0.00$, df = 1, $P = 0.97$). Overall offspring sex ratio was 37% higher for *G. ashmeadi* compared with *G. deleoni* (Table 3). The overall sex ratio of *G. ashmeadi* offspring was significantly female biased, whereas, *G. deleoni* offspring sex ratio was statistically equivalent to 50% (Table 3).

Discussion

The results from multiple laboratory experiments reported here indicate that *G. ashmeadi* is a superior parasitoid of *H. vitripennis* eggs when compared to *G. deleoni*. *G. ashmeadi* consistently outperformed *G. deleoni* by parasitizing up to 89% more *H. vitripennis* eggs under three experimental designs that had varying host densities, egg ages, and exposure times. Unlike *G. deleoni*, female *G. ashmeadi* also consistently produced female biased sex ratios. This is a favorable attribute because faster parasitoid population growth results which may increase the likelihood of pest control and greater numerical domination by this parasitoid.

Several reasons may exist for the observed inferiority of *G. deleoni* in these laboratory tests. Female *G. deleoni* may be less efficient at host searching (Paust et al. 2008), or parasitizing host eggs (Li, Luo, Zhou, Zhou, and Zhang 2008). *G. ashmeadi* may be more efficient in finding and successfully attacking *H. vitripennis* because it has evolved to exploit it (Messenger and van der Bosch 1971). *G. deleoni* appears to be highly host specific in Argentina as it has been recorded attacking only *Tapajosa rubromarginata* (Signoret) (Cicadellidae: Proconiini) and not eggs of Cicadellini (Triapitsyn et al. 2008), and this may result in *H. vitripennis* being a poor host for *G. deleoni*. It is possible that a high proportion of *G. deleoni* larvae may die from host defense mechanisms or host unsuitability because *G. deleoni* did not co-evolve with *H. vitripennis* and it cannot readily circumvent these defenses. Additionally, *G. deleoni* larvae may be less efficient at interspecific competition against *G. ashmeadi* larvae in host eggs. *G. ashmeadi* larvae possess enlarged mandibles (Irvin, Hoddle, and Morgan 2006) possibly indicating that this species fights within hosts to physically eliminate competitors (Salt 1961; Mackauer 1990; Tillman and Powell 1992). It is unknown whether *G. deleoni* larvae possess specialized structures for larval combat. Other possible characteristics that may enable *G. ashmeadi* larvae to

Table 3. Overall *G. ashmeadi* and *G. deleoni* offspring sex ratio and correlating test statistics resulting from each of three experiments.

Experiment	*G. ashmeadi* offspring sex ratio	*G. deleoni* offspring sex ratio	Test statistics for difference between species	Test statistics for whether sex ratio is female bias (>50%)	
				G. ashmeadi	*G. deleoni*
Parasitoid egg age preferences and competitive abilities	81 ± 1%	70 ± 10%	$\chi^2 = 1.56$, df = 1, $P = 0.21$	$\chi^2 = 95.67$, df = 1, $P < 0.001$	$\chi^2 = 1.67$, df = 1, $P = 0.19$
Parasitoid behaviors and competitive abilities	74 ± 3%	67 ± 20%	Fishers Exact test, $P = 0.69$	$\chi^2 = 16.41$, df = 1, $P < 0.001$	Fishers Exact test, $P = 1.00$
Assessing the effect of longer exposure times and higher *H. vitripennis* densities on parasitism rates	91 ± 0.4%	54 ± 5%	$\chi^2 = 203.51$, df = 1, $P < 0.001$	$\chi^2 = 950.93$, df = 1, $P < 0.001$	$\chi^2 = 0.52$, df = 1, $P = 0.47$

be intrinsically superior to *G. deleoni* are that *G. ashmeadi* larvae may hatch faster and grow more rapidly than immature *G. deleoni* (DeMoraes, Cortesero, Stapel, and Lewis 1999), kill *G. deleoni* via starvation (Lawrence 1988), or physiologically change the host to suppress the growth of competitors (Salt 1961; Lawrence 1988; Mackauer 1990). Abiotic factors in the laboratory may have impeded performance by *G. deleoni*. This parasitoid, from the desert areas of Mendoza, Argentina may be more efficient at higher temperatures than those used in the current study. The current study was conducted at 26°C which is similar to temperatures (25°C) used in studies conducted by Jones et al. (2005) investigating biology and non-target effects of *G. deleoni*. Pilkington and Hoddle (2006) demonstrated that total progeny produced by *G. ashmeadi* varies across a temperature range of 20–33°C. Therefore it is conceivable that parasitism by *G. deleoni* may also be affected by higher temperatures.

Experimental design and application of results to the field

These studies were conducted under artificial laboratory conditions involving *H. vitripennis* egg masses laid on lemon leaves placed in vials and the parasitoids confined in small spaces. Such conditions differ substantially from the field environment where, for example, fluctuations in temperature and humidity exist, eggs are laid on various different intact host plants, and female parasitoids need to search large areas for hosts. It is unknown whether *G. deleoni* exhibits preferences for certain host plants when searching for host eggs. Host plants can influence host location and parasitism rates due to differences in leaf thickness, production of plants volatiles, and physical complexity (Ables, McCommas, Jones, and Morrison 1980; Andow and Prokrym 1990; Murray and Rynne 1994; Gingras, Dutilleul, and Boivin 2003; Amalin, Pena, and Duncan 2005). In California, citrus is a highly preferred host plant for *H. vitripennis* and it is the most common reproductive and overwintering host for this pest (Blua et al. 1999). Because *H. vitripennis* oviposits on over 100 host plant species in the field (CDFA 2009) it is possible that *G. deleoni* may have performed better if other plants were used in bioassays. As mentioned, *G. deleoni* parasitizes only eggs of *T. rubromarginata* in its native range, a sharpshooter species that has a variety of host plants (Virla, Luft Albarracin, Logarzo, and Triapitsyn 2005b; Virla, Cangemi, and Logarzo 2007), including citrus (Toledo, Virla, and Humber 2006), therefore host plant effects may not be overly important. The experimental procedure used in this study involved rinsing leaves under cold water and gently wiping brochosomes from eggs with a soft paper towel prior to presentation to parasitoids. It is possible that this process removed chemical oviposition attractants and/or cues which may have impeded the ability of female *G. deleoni* to locate hosts.

Extrapolating laboratory findings to field situations is inherently difficult because experimental design can produce results that favor different parasitoid species (Irvin et al. 2006). Laboratory studies similar to these presented here involving *G. triguttatus* and *G. fasciatus* may offer insight into predicting *G. deleoni* field performance based on laboratory studies. Irvin and Hoddle (2005) documented that *G. ashmeadi* outperformed *G. triguttatus* and *G. fasciatus* across egg age use studies, parasitism levels, adult parasitoid longevity and competitive ability. Mass releases of *G. triguttatus* and *G. fasciatus* in California where *G. ashmeadi* is present,

began in 2001 and 2002, respectively, and establishment and recovery of *G. triguttatus* and *G. fasciatus* has been low or non-existent. Consequently, mass production and release of *G. fasciatus* was discontinued due to poor recovery levels (CDFA 2008).

Laboratory studies predicted that *G. fasciatus* and *G. triguttatus* were inferior to *G. ashmeadi* and low parasitism rates, reduced longevity, and interspecific competition may have contributed to poor establishment. Climatic mis-match, at least for *G. triguttatus* in southern California, seems unlikely, as year round conditions should be favorable for this parasitoid (Pilkington and Hoddle 2007). Similarly, the results from the work presented here suggest that in ways similar to *G. triguttatus* and *G. fasciatus*, *G. deleoni* is inferior to *G. ashmeadi* and may experience difficulty in establishing in areas where *G. ashmeadi* is dominant.

Potential effect of G. deleoni *on* H. vitripennis

To reduce uncertainty about the reliability of competitive experiments, the current study included two types of controls containing one parasitoid species. When high densities of *H. vitripennis* eggs were exposed to one female of each species (the AD treatment), total parasitism and emergence of *H. vitripennis* nymphs was significantly 11% higher and 6% lower, respectively, compared with vials containing one female *G. ashmeadi*. This demonstrates that the addition of *G. deleoni* significantly increased parasitism of *H. vitripennis* eggs under these laboratory conditions. Interestingly, the 11% increase in parasitism was almost identical to the 12% parasitism produced in vials containing one female *G. deleoni*. However, it is unknown whether the combined species treatment reached maximum parasitism of *H. vitripennis* and it is likely that adding a second female *G. ashmeadi* (i.e., AA-control which was not conducted for the last experiment) may have resulted in similar or higher levels of parasitism compared with the addition of *G. deleoni*.

For the observational 'parasitoid behaviors' experiment containing one leaf in a Petri dish, total parasitism and percentage of nymphs that emerged from egg masses was equivalent between the A-control, AA-control, and combined species treatment indicating that the addition of female *G. ashmeadi* or *G. deleoni* did not increase parasitism of *H. vitripennis* eggs in these time limited studies. For the AA-control, this may be attributable to the presence of a competing female *G. ashmeadi* which increased non-ovipositional behaviors such as antennating of competitors and subsequent grooming. These behaviors reduced the time available for host searching and oviposition. In contrast, female *G. deleoni* in the combined species treatment did not engage in competitor antennating or increase grooming activity in comparison to *G. deleoni*-controls.

The poor performance of *G. deleoni* in the current study demonstrates marked similarities to *G. fasciatus* reported in Irvin and Hoddle (2005). For example, *G. fasciatus* allocated 40% of observations to being off the leaf and attacked 65% fewer *H. vitripennis* than *G. ashmeadi* in mixed species competition studies conducted by Irvin and Hoddle (2005). These results were similar to the behavioral experiment of the current study where *G. deleoni* allocated 73% of observations to being off the leaf and attacked 81% fewer *H. vitripennis* eggs compared with *G. ashmeadi*. The studies on *G. deleoni* presented here and previously for *G. fasciatus* (Irvin and Hoddle 2005) have demonstrated that an overlap in egg age preference exists between these two

species when competing with *G. ashmeadi*, indicating that interspecific competition for eggs may occur in the field. The poor field recovery rates and discontinuation of mass release of *G. fasciatus* as a biological control agent for *H. vitripennis* may illustrate what could be expected for the new association biological control agent, *G. deleoni*, in California should it be released.

Based on the results presented here, we suggest that there may be no advantage to releasing *G. deleoni* from quarantine, unless it can be demonstrated that this new association agent can either fulfill a niche in the field where competition with *G. ashmeadi* is reduced (i.e., parasitizing *H. vitripennis* egg masses in areas of California where *G. ashmeadi* is less dominant (see below) or it has a strong ability to pass through the winter in large numbers when host eggs are scarce. Low host availability over winter and spring is considered the major impediment for natural enemies of *H. vitripennis* in California (Triapitsyn et al. 2003). The ability of *G. deleoni* to fill this spring-time niche may depend on its overwintering phenology, either by exhibiting diapause or through the exploitation of eggs of alternative host species should they be available when *H. vitripennis* eggs are scarce or absent. Alternatively, *G. deleoni* may perform better on host plants that were untested here or that *G. ashmeadi* fails to use, however, we are unaware of the existence of such plants in southern California. Additionally, experiments could be conducted to determine whether *G. deleoni* is more efficient than *G. ashmeadi* at locating hosts over larger areas and lower densities than were tested here. These studies would be incredibly difficult to complete in quarantine. Alternatively, biological parameters (e.g., developmental times and fecundity estimates) could be used to estimate if *G. deleoni* has the potential to perform better in some coastal and desert areas where *H. vitripennis* is problematic and *G. ashmeadi* is not the most common parasitoid species (D.J.W. Morgan, pers. comm.).

Behavioral comparisons

Parasitism by *G. deleoni* was 61% lower than by *G. ashmeadi* in the combined species treatment, and this poor performance was attributed to these females spending 66% more observations off the leaf and 52% fewer observations ovipositing when compared with *G. ashmeadi*. Parasitism by *G. deleoni* was statistically equivalent between the combined species treatment and the D-control, therefore the presence of *G. ashmeadi* had no effect on *G. deleoni* performance. At the beginning of each behavioral trial, female *G. deleoni* spent more time off the leaf and took longer to locate experimental leaves containing hosts, compared with *G. ashmeadi* (N.A. Irvin, personal observation). The higher percentage of observations *G. deleoni* spent off the leaf compared with *G. ashmeadi* may suggest that female *G. deleoni* were not attracted to the plant surface, volatiles from the host plant, or the host egg mass. This may have occurred because *G. deleoni* has a very narrow host range and did not evolve with *H. vitripennis*, so it does not readily recognize volatile chemical profiles associated with *H. vitripennis* egg masses.

H. vitripennis lay individual eggs side by side to form an egg mass, which is deposited on the lower leaf surface in a slit cut with the ovipositor between the epidermis and parenchyma (Irvin and Hoddle 2004). Irvin and Hoddle (2010) speculated that oviposition through the upper leaf surface may provide females with a way of countering direct competition on the lower leaf surface where *H. vitripennis*

eggs were present because this behavior was only recorded in treatments containing a competitor. However, female *G. ashmeadi* caged individually allocated 6% of observations to oviposition through the upper leaf surface which was 4–6% higher than the AA-controls and combined species treatment. These results demonstrate that under laboratory conditions, oviposition through the upper leaf surface may not be confined to avoiding direct competition on the lower leaf surface or avoiding brochosomes (a white chalky substance deposited by female sharpshooters on egg masses that reduces parasitism) as discovered by Velema et al. (2005). When female *G. ashmeadi* and *G. tuberculifemur* were in the presence of a competitor they allocated approximately 0.1–1.3% of behaviors to aggressively chasing competitors off contested egg masses (Irvin and Hoddle 2010). In the current study, no accounts of aggressive behavior were recorded between congenerics or conspecifics. This may have attributable to *G. deleoni* spending 73% of observations off the leaf which reduced direct competition between congenerics, compared with 39% of observations female *G. tuberculifemur* spent off the leaf (Irvin and Hoddle 2010).

Risk assessment for introduction of G. deleoni

The decision to introduce the new association parasitoids *G. deleoni* or *G. tuberculifemur* (Irvin and Hoddle 2010) into California may be influenced by the estimated benefits on suppressing *H. vitripennis* populations, the anticipated host range of these agents, the value placed on potential non-target species, the risk that new parasitoid species may disrupt the efficacy of resident parasitoids that co-evolved with *H. vitripennis* (e.g., *G. ashmeadi*) and the estimated damage (economic or ecological) of alternative actions to suppress *H. vitripennis*, including the costs of doing nothing (Van Driesche and Hoddle 1997; Moeed, Hickson, and Barratt 2006). *G. deleoni* was considered a more promising biological control agent of *H. vitripennis* because it has a narrower host range and restricted native range in comparison to *G. tuberculifemur* (Triapitsyn et al. 2008). However, when comparing the efficacy of *G. deleoni* and *G. tuberculifemur* as biological agents of *H. vitripennis, G. deleoni* parasitized up to 42% fewer *H. vitripennis* eggs when compared with *G. tuberculifemur* in similar studies to those carried out here (Irvin and Hoddle 2010). This may indicate that *G. deleoni* is less efficient at parasitizing *H. vitripennis* eggs when compared to *G. tuberculifemur*, possibly due to it being more host specific (Triapitsyn et al. 2008).

The results outlined here are significant factors when considering the benefits of introducing the 'new association' *G. deleoni* for control of *H. vitripennis*. The current laboratory studies have demonstrated that *G. deleoni* was an inefficient parasitoid of *H. vitripennis* and failed to outperform *G. ashmeadi*, the dominant resident parasitoid attacking *H. vitripennis* eggs in California. Based on the results presented here we speculate that: (1) *G. deleoni* may have difficulties establishing in areas where *G. ashmeadi* is present and (2) the potential effect of releasing *G. deleoni* in California may be negligible unless *G. deleoni* can occupy and provide substantial benefit a niche in the field not currently occupied by *G. ashmeadi*. Additional research will be needed to address this point, if 'new-association' biological control of *H. vitripennis* with *G. deleoni* is to be justified and significant resources are expended on the mass rearing, releasing, and monitoring of this biological control agent. Such studies could investigate host finding abilities on whole plants, levels of parasitism on species of

host plants not tested here, characterization of optimal and sub-optimal temperature requirements for development, effects of competition with other *Gonatocerus* parasitoid species prevalent in coastal and desert areas where *G. ashmeadi* is less common, the phenology and over-wintering biology of *G. deleoni* in areas of its native range that are most similar to California, and a comprehensive non-target risk assessment.

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