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

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ABSTRACT

Egg age preference, competitive ability, and behavior of *Gonatocerus tuberculifemur* ('new association' parasitoid) and *Gonatocerus ashmeadi* ('old association' parasitoid) were investigated in the laboratory to determine if one species exhibited competitive superiority. When searching concurrently for *Homalodisca vitripennis* egg masses, *G. ashmeadi* consistently outperformed *G. tuberculifemur* by parasitizing 25–53% 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 81–97% across all egg ages. *G. tuberculifemur* in control vials parasitized 60–66% of eggs 1 and 3 days old, and just 18% of eggs 5 days old. *G. ashmeadi* produced 5–16% more female offspring than *G. tuberculifemur* for all experimental conditions. In comparison to *G. ashmeadi*, *G. tuberculifemur* was observed off leaves with host eggs 20% more frequently and it oviposited 15% less frequently. *G. ashmeadi* and *G. tuberculifemur* when confined together allocated ~1% of behaviors to antennating or aggressively chasing competitors off egg masses, and up to 2% of behaviors to antennating host egg masses and/or ovipositing into eggs from the opposite side of the leaf. These latter behaviors did not occur when parasitoids were confined alone with host eggs.

1. Introduction

Controversy exists as to whether biological control agents that have not co-evolved with a pest will be effective natural enemies. A co-evolved natural enemy may be more efficient in finding and successfully attacking a target because it has evolved to exploit it (Messenger and van der Bosch, 1971). Alternatively, coevolution between pests and biological control agents leads to decreased effectiveness of natural enemies and increased resistance of the pest to attacks because the system works towards establishing a balance between natural enemy and pest (Pimentel, 1963). Consequently, it is therefore desirable to seek new association biological control agents which have no evolutionary history with the target pest 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 [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 1980's. This pest is a vector of a xylem-limited bacterium, *Xylella fastidiosa,* Wells et al., which causes disease in a number of important plants including grapes, almond, alfalfa, peach and oleander (Blua et al., 1999; UCOP, 2000; Varela et al., 2001). Considerable effort has been expended in California to develop a classical biological control program for this pest with egg parasitoids.

Gonatocerus tuberculifemur (Ogloblin) (specifically *G.* sp. near *tuberculifemur* "Clade 1" from Triapitsyn et al. (2008), referred to hereafter as *G. tuberculifemur*) (Hymenoptera; Mymaridae) is a common and widespread parasitoid that attacks Proconiini sharpshooters in Argentina and Chile in South America. It was imported from Argentina into quarantine in Texas in 2001, and into California in 2002, and reared on egg masses of *H. vitripennis*, (Triapitsyn

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et al., 2008). Although *G. tuberculifemur* has no evolutionary history with this pest, authorization for its release in California has received preliminary approval and final confirmation is pending (CDFA, 2005; D.J.W. Morgan, pers. comm.). If released into California, this would make *G. tuberculifemur* a new association biological control agent of *H. vitripennis*. The current parasitoid guild attacking *H. vitripennis* in California consists of eight species of egg parasitoids (CDFA, 2006), 70% of which is comprised of *Gonatocerus ashmeadi* (CDFA, 2006). Pilkington et al. (2005) reported that year round parasitism of *H. vitripennis* for all parasitoid species averages 15.5%. 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 et al., 1989; Briggs, 1993; Denoth et al., 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 consistently provide effective biological control of *H. vitripennis* populations. Pimentel (1991) reported that in more than 95% cases of successful biological control, it only took one natural enemy to suppress pest numbers to acceptable levels. It is possible that *G. tuberculifemur*, a new association natural enemy of *H. vitripennis,* could provide the sought after year round suppression of this pest in California.

To determine whether *G. tuberculifemur* should be introduced into California, research has been conducted to determine if it can outperform *G. ashmeadi* the dominant parasitoid of *H. vitripennis.* Functional response studies reported by Irvin et al. (2009) demonstrated that *G. tuberculifemur* failed to outperform *G. ashmeadi* over variable host densities. Additional research into the competitive ability of *G. tuberculifemur* was undertaken to determine whether *G. tuberculifemur* 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. tuberculifemur* when foraging simultaneously with *G. ashmeadi* on *H. vitripennis* egg masses, and aggressive interactions between these two parasitoid species when resources were contested. Collectively, the results from these studies, together with other completed work, should be used to guide the decision to release *G. tuberculifemur* from quarantine for liberation and establishment in California for new association biological control of *H. vitripennis*.

2. Materials and methods

2.1. Insect colonies and parasitoid preparation

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° ± 2 °C and 30–40% RH under a L14:10D 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 two 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-liter containers, and fertilized every two weeks with Miracle-Gro (20 ml/3.5 l of water, Scotts Miracle-Gro Products Inc., Marysville, OH). Female *G. tuberculifemur* were sourced from *G. tuberculifemur* colonies (*G.* sp. near *tuberculifemur* "Clade 1" as described in Triapitsyn et al. (2008)) maintained in the Insect and Quarantine building at UCR, California. These colonies had completed ~75 generations since arriving at UCR in September 2002 and were augmented periodically with new material collected from Argentina. *G. tuberculifemur* colonies were maintained in ventilated plastic cages (9 × 9 × 16 cm) and held at 24° ± 2 °C, 40–50% RH under a L14:10D photoperiod. Females were held with 50% honey–water for 2–3 days before exposure to *H. vitripennis* eggs laid on euonymus leaves (*Euonymus japonica* L.; Celastraceae) or 'Eureka' lemon leaves depending on the source of *H. vitripennis* eggs. Approximately 85% of *G. tuberculifemur* used in these studies had emerged from egg masses laid on 'Eureka' lemon leaves compared to 100% of *G. ashmeadi*. 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. tuberculifemur* colony cage. The bottom of the parasitoid colony cage was perforated with holes, and placed in a metal tray (20 × 20 × 45 cm) containing 2 cm of tap water which watered the foam pad holding leaves. *G. ashmeadi* and *G. tuberculifemur* colonies were provisioned with honey–water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA) and checked daily for parasitoid emergence.

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, Oakland CA) was supplied in droplets on the lid. This was repeated for *G. tuberculifemur.* Parasitoids were held in the laboratory for 24 h at 26° ± 2 °C and 30–40% RH under a L14:10D photoperiod prior to use in experiments. On days when parasitoids (~24–36 h of age) and host eggs were available, all experiments were set up between 10 am and 1 pm in the laboratory at 26° ± 2 °C and 30–40% RH under a L14:10D photoperiod with fluorescent lighting. Parasitoids were discarded if no eggs were available that day. Irvin and Hoddle (unpublished data) found that female-applied brochosomes cover 64% of *H. vitripennis* egg masses, and brochosomes interfere with parasitism (Velema et al., 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.

2.2. Parasitoid egg age preferences and competitive abilities

One mated female *G. ashmeadi* and *G. tuberculifemur* (~24–36 h old) were presented simultaneously to one *H. vitripennis* egg mass (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° ± 2 °C and 30–40% RH under a L14:10D photoperiod for three 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. tuberculifemur* could be identified because 4–7 days after oviposition the whole egg turns orange/red (Virla et al., 2005), 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.

2.2.1. Statistical analysis

All statistical analyses were conducted in SAS (1990). To determine whether the two parasitoid species foraging concurrently increased overall parasitism of *H. vitripennis* eggs compared with only one species, logistic regression was used to compare raw counts of the total number of parasitoid offspring independent of species per vial, to total number of eggs per vial, between the three experimental treatments (*G. ashmeadi* control, *G. tuberculifemur* control, and combined species treatment). Pair-wise contrast tests at the 0.05 level of significance were used to separate means, and results were considered significantly different if $p < 0.017$ (i.e., $p < 0.05/3$ treatments) (Agresti, 2002). Similarly, logistic regression and contrast tests were used to determine the effect of treatment on overall emergence of *H. vitripennis* nymphs. To compare the competitive ability of *G. ashmeadi* and *G. tuberculifemur* within the combined species treatment, a standard logistic regression model was used to compare parasitism rates between *G. ashmeadi* and *G. tuberculifemur.* 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).

To determine the effect of egg age on *G. ashmeadi* parasitism rates, logistic regression and contrast tests at the 0.05 level of significance were used on data obtained from *G. ashmeadi* control vials for each egg age and raw counts of *G. ashmeadi* offspring were compared to total number of host eggs between the three egg age classes. This analysis was repeated for *G. tuberculifemur* control vials. To determine the effect of interspecific competition on parasitism rates by *G. ashmeadi*, contrast tests were used to compare raw counts of *G. ashmeadi* offspring to total number of host eggs between *G. ashmeadi* control vials and the combined species treatments. This analysis was repeated separately for each egg age and then conducted on data pooled over all egg ages. For contrast tests comparing *G. ashmeadi* control vials to the combined species treatment, results were considered significantly different if $p < 0.025$ (i.e., $p < 0.05/2$ treatments). These analyses were repeated for *G. tuberculifemur.* 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 each species. Data were pooled over treatments and egg ages and a Chi-Square analysis at the 0.05 level was used to determine whether sex ratio significantly varied between the two parasitoid species. All percentage parasitism and nymph means presented in the results section are calculated as percentages of total number of *H. vitripennis* eggs.

2.3. Parasitoid behaviors and competitive abilities

One mated female *G. ashmeadi* and *G. tuberculifemur* (~24–36 h) was presented simultaneously to one *H. vitripennis* egg mass (4–8 eggs, 1–3 days of age an age category preferred by both *G. ashmeadi* and *G. tuberculifemur* [see results in Section 3.1]) 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). Exposure time was 15 min. 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° ± 2 °C and 30–40% RH under a L14:10D photoperiod for three weeks. The number 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. The control vials containing two females of the same species were used to determine whether competition between two different 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.

2.3.1. Statistical analyses

All statistical analyses were conducted in SAS (1990). To investigate the effect of treatment on total parasitism of *H. vitripennis* eggs, logistic regression was used to compare raw counts of total number of parasitoid offspring per vial, to total number of host eggs per vial, between treatments (vials containing one female *G. ashmeadi* and *G. tuberculifemur* [combined species treatment{AT}], vials containing one female *G. ashmeadi* only [A-control], vials containing one *G. tuberculifemur* only [T-control], vials containing two *G. ashmeadi* [AA-control], and vials containing two *G. tuberculifemur* [TT-control]). Pair-wise contrast tests at the 0.05 level of significance was used to separate means (results were considered significantly different if $p < 0.01$ [$p < 0.05/5$ treatments]). Similarly, logistic regression and contrast tests were used to determine the effect of treatment on parasitism by *G. ashmeadi*, parasitism by *G. tuberculifemur,* and emergence of *H. vitripennis* nymphs.

To compare the competitive ability of *G. ashmeadi* and *G. tuberculifemur* when *G ashmeadi* and *G. tuberculifemur* were concurrently foraging on the same *H. vitripennis* egg mass, a Chi-square test for equal proportions was used to compare the proportion of eggs parasitized between *G. ashmeadi* and *G. tuberculifemur* for the combined species treatment. To determine the effect of interspecific competition on parasitism by *G. ashmeadi*, contrast tests at the 0.05 level of significance were used to compare raw counts of *G. ashmeadi* offspring to total number of eggs, between the *G. ashmeadi* control vials (AA and A) and the combined species treatment (AT). For contrast tests comparing across A-controls, AA-controls and AT, results were considered significantly different if $p < 0.017$ ($p < 0.05/3$ treatments). These analyses were repeated for *G. tuberculifemur* controls and the combined species treatment. A one factor logistic regression model was used to determine the effect of treatment on sex ratio for each species. Data were pooled over treatments and a Chi-square test at the 0.05 level was used to determine whether sex ratio significantly varied between the two parasitoid species.

Behavior data were used to calculate the percentage of time spent in each behavioral event for each treatment. For the combined species treatment (AT), the behavior of each female was identified as being either *G. ashmeadi* (AT–A) or *G. tuberculifemur* (AT–T) (i.e., there were 6 treatments: A, AA, T, TT, AT–A, AT–T). Chi-square Test for Specified Proportions (i.e., Specified Multinomial Test)

(Agresti, 2002) was used to determine whether the percentage of observations allocated to each behavior significantly varied within each treatment. This test is suitable for dependant data which is the case when comparing behaviors within each treatment because the frequency of one behavior may affect the frequency of subsequent behaviors. The null hypothesis for this Chi-square for Specified Proportions test was that the percentage of observations allocated to each behavior were equal to 1/total number of behaviors occurring in each treatment. A significant difference between behaviors exists when $p < 0.05$ (Agresti, 2002). For behaviors with means greater than zero, the Chi-square for Specified Proportions test was used to separate means at the 0.05 level of significance. To detect significant differences between means $\geqslant$ zero, the percentage of observations allocated to each behavior was converted to binomial data (where no observations of that behavior equaled zero and the observed behavior equaled one) prior to conducting Fishers Exact Tests (McDonald, 2008) at the 0.05 level of significance. Logistic regression was used to determine the effect of treatment on the percentage of observations allocated to each behavior. Pair-wise contrast tests at the 0.05 level of significance were used to separate means. Contrast tests are suitable for independent data which is the case here when comparing between treatments. For these contrast tests, results were considered significantly different if $p < 0.008$ ($p < 0.05/6$ treatments).

2.4. 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. tuberculifemur* 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 et al., 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 as previously described, and exposed simultaneously to one mated female *G. ashmeadi* and *G. tuberculifemur* (~24–48 h old) for either 24 h or 5 days. Vials were held at 26° ± 2 °C and 30–40% RH under a L14:10D 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° ± 2 °C and 30–40% RH under a L14:10D photoperiod for three weeks as previously described. Twenty replicates were set up for each exposure time. The number of male and female *G. ashmeadi* and *G. tuberculifemur* offspring were recorded for each vial as previously described.

2.4.1. Statistical analyses

All statistical analyses were conducted in SAS (1990). Paired *t*-tests at the 0.05 level of significance were used to compare percentage parasitism (calculated as a percentage of total number of parasitized eggs) between species for each exposure time. Wald Chi-square tests at the 0.05 level of significance were used to determine the effect of exposure time on percentage parasitism for each species. A one factor logistic regression model was used to determine the effect of exposure time on sex ratio for each species. Data were pooled over exposure times and a Chi-Square test at the 0.05 level of significance was used to determine whether sex ratio significantly varied between the two parasitoid species.

3. Results

3.1. Parasitoid egg age preferences and competitive abilities

There was a significant effect of treatment on total percentage parasitism ($\chi^2 = 118$, $df = 2$, $p < 0.0001$) and the overall percentage of *H. vitripennis* nymphs emerging from exposed egg masses ($\chi^2 = 101$, $df = 2$, $p < 0.0001$). Vials containing one female *G. tuberculifemur* resulted in significantly less parasitism (i.e., 37–42% lower) and 32–35% higher percentage of *H. vitripennis* nymph emergence when compared with the *G. ashmeadi* control vials and mixed vials containing one female of each species (Fig. 1). Total percentage parasitism and overall percentage nymph

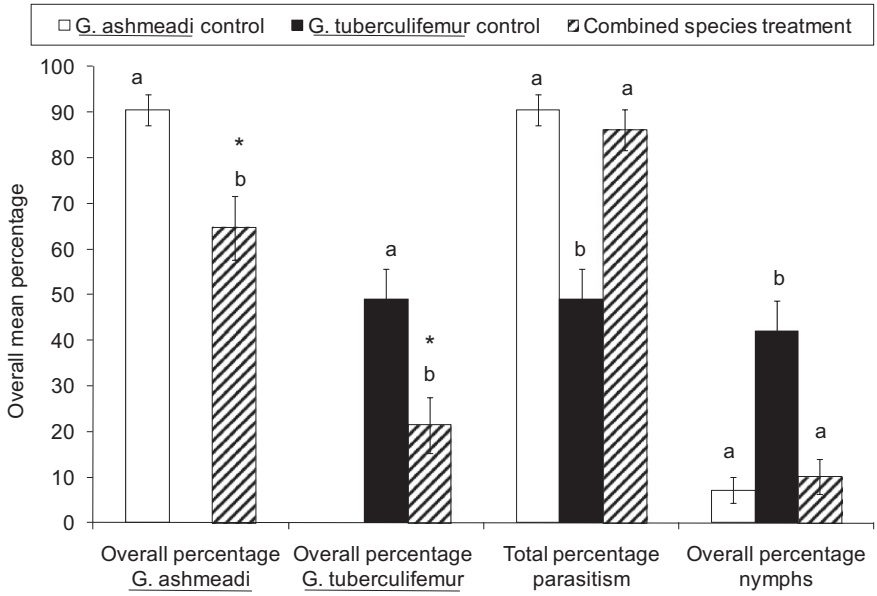

Fig. 1. Overall percentage parasitism by *G. ashmeadi*, percentage parasitism by *G. tuberculifemur*, total percentage parasitism, and percentage nymphs emerging when *H. vitripennis* egg masses were exposed to three parasitoid treatments (vial containing one female *G. ashmeadi* only [*G. ashmeadi* control], vial containing one *G. tuberculifemur* only [*G. tuberculifemur* control], vial containing both *G. ashmeadi* and *G. tuberculifemur* [combined species treatment]) for 1 h at 26 °C (error bars indicate ± 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. tuberculifemur* for the combined species treatment).

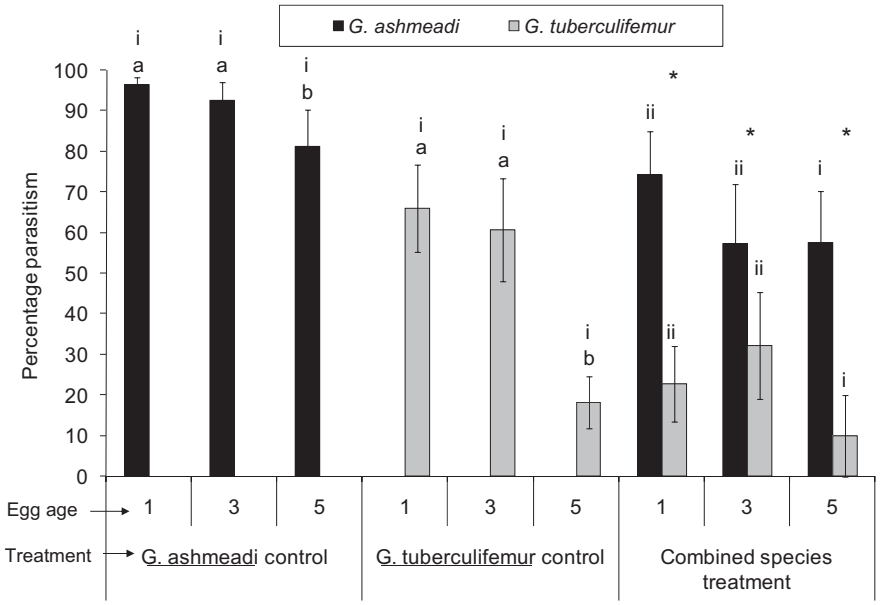

Fig. 2. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when *H. vitripennis* egg masses aged 1, 3, and 5 days of age were exposed to three parasitoid treatments (vial containing one female *G. ashmeadi* only [*G. ashmeadi* control], vial containing one *G. tuberculifemur* only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

emergence was statistically equivalent between *G. ashmeadi* control vials and the combined species treatment (Fig. 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. tuberculifemur* for 1 h showed that overall parasitism by *G. ashmeadi* was 43% higher compared to *G. tuberculifemur* ($\chi^2 = 16.68$, $df = 1$, $p < 0.001$) (Fig. 1). The effect of egg age on the difference in parasitism between species was not significant ($\chi^2 = 4.58$, $df = 2$, $p = 0.10$) thereby indicating that parasitism by *G. ashmeadi* was consistently and significantly higher (i.e., by 25–51%) than *G. tuberculifemur* for all three egg ages (Fig. 2).

There was a significant effect of treatment on overall percentage parasitism by *G. ashmeadi* ($\chi^2 = 29.03$, $df = 1$, $p < 0.0001$) and overall percentage parasitism by *G. tuberculifemur* ($\chi^2 = 44.92$, $df = 1$, $p < 0.0001$) (Fig. 1). Interspecific competition between *G. ashmeadi* and *G. tuberculifemur* (AT treatment) reduced overall parasitism by *G. ashmeadi* and *G. tuberculifemur* by 26% and 27%, respectively, compared with control vials with single females.

There was a significant effect of egg age on percentage parasitism in the *G. ashmeadi* control vials ($\chi^2 = 11.96$, $df = 2$, $p < 0.01$) and the *G. tuberculifemur* control vials ($\chi^2 = 36.02$, $df = 2$, $p < 0.001$). Results from the *G. ashmeadi* control vials indicated that *G. ashmeadi* parasitism ranged from 81% to 97% across all egg ages. Percentage parasitism was significantly higher (i.e., 11–15%) when *G. ashmeadi* were presented with *H. vitripennis* eggs aged 1 and 3 days of age when compared with eggs 5 days of age (Fig. 2). Results from the *G. tuberculifemur* control vials indicated that 60–66% of eggs 1 and 3 days of age were successfully parasitized by *G. tuberculifemur* (Fig. 2). Eggs 5 days of age were less suitable for *G. tuberculifemur* development and resulted in just 18% parasitism.

Interspecific competition between *G. ashmeadi* and *G. tuberculifemur* significantly reduced *G. ashmeadi* parasitism by 22–35% compared with *G. ashmeadi* control vials for 1 ($\chi^2 = 9.53$, $df = 1$, $p < 0.01$) and 3 ($\chi^2 = 15.54$, $df = 1$, $p < 0.0001$) day old eggs (Fig. 2). For eggs 5 days of age, there was no significant ($\chi^2 = 4.67$, $df = 1$, $p = 0.03$) difference in *G. ashmeadi* parasitism rates when compared to *G. ashmeadi* control vials and the combined species treatment (Fig. 2). For *G. tuberculifemur*, interspecific competition between *G. ashmeadi* and *G. tuberculifemur* significantly reduced *G. tuberculifemur* parasitism by 28–43% compared with *G. tuberculifemur* control vials when females were presented eggs aged 1 ($\chi^2 = 32.67$, $df = 1$, $p < 0.0001$) and 3 ($\chi^2 = 11.87$, $df = 1$, $p < 0.001$) days of age (Fig. 2). For eggs 5 days of age, there was no significant ($\chi^2 = 2.07$, $df = 1$, $p = 0.15$) difference in *G. tuberculifemur* parasitism between *G. tuberculifemur* control vials and the combined species treatment (Fig. 2).

There was no significant effect of treatment, egg age, or their interaction on sex ratio for *G. ashmeadi* (treatment: $\chi^2 = 0.39$, $df = 1$, $p = 0.53$; egg age: $\chi^2 = 2.18$, $df = 2$, $p = 0.34$; interaction: $\chi^2 = 0.45$, $df = 2$, $p = 0.79$) or *G. tuberculifemur* (treatment: $\chi^2 = 0.29$, $df = 1$, $p = 0.59$; egg age: $\chi^2 = 1.68$, $df = 2$, $p = 0.43$; interaction: $\chi^2 = 0.74$, $df = 2$, $p = 0.69$). Overall sex ratio did not significantly differ between species ($\chi^2 = 2.34$, $df = 1$, $p = 0.13$), at 76 ± 10% and 71 ± 6% for *G. ashmeadi* and *G. tuberculifemur*, respectively.

3.2. Parasitoid behaviors and competitive abilities

There was a significant effect of treatment on total percentage parasitism ($\chi^2 = 126$, $df = 42$, $p < 0.0001$) and percentage of *H. vitripennis* nymphs emerging from exposed egg masses ($\chi^2 = 128$, $df = 4$, $p < 0.0001$). Vials containing one or two female *G. tuberculifemur* resulted in significantly less total parasitism (i.e., 35–47% lower) and 31–52% more *H. vitripennis* nymphs emerging when compared to the *G. ashmeadi* controls and the combined species treatment (Fig. 3). Total percentage parasitism was statistically equivalent between both *G. ashmeadi* controls and the combined species treatment (Fig. 3). Vials containing two female *G. ashmeadi* resulted in the lowest *H. vitripennis* nymph emergence (i.e., 14% emergence) and was significantly lower than the remaining treatments (Fig. 3).

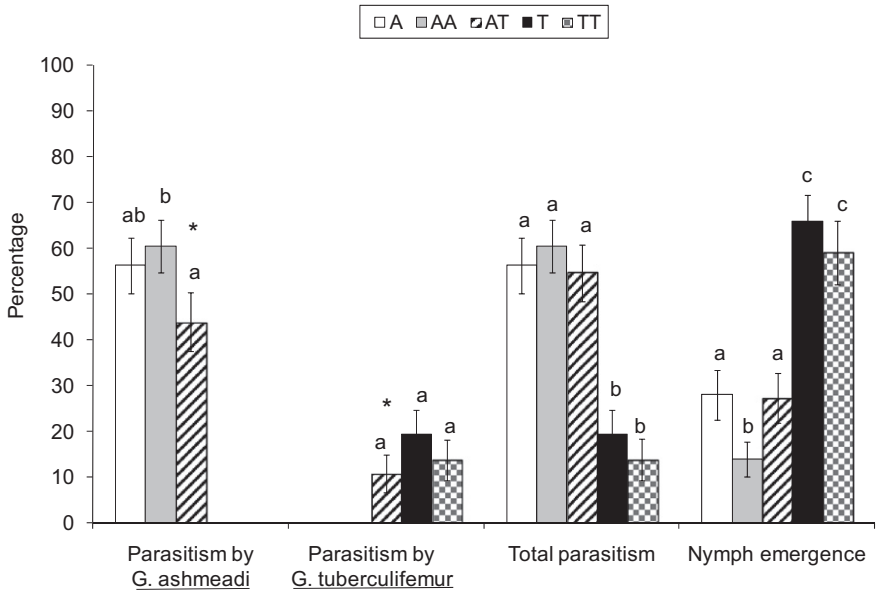

Fig. 3. Percentage parasitism by *G. ashmeadi,* percentage parasitism *G. tuberculifemur*, total percentage parasitism, and percentage nymphs resulting when *H. vitripennis* egg masses were exposed to parasitoids either alone or with intraspecific or interspecific competition for 15 min at 26 °C (A = control vial containing one female *G. ashmeadi*; AA = control vial containing two female *G. ashmeadi*; AT = one female *G. ashmeadi* and *G. tuberculifemur*, TT = two female *G. tuberculifemur*; T = one female *G. tuberculifemur*; 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. tuberculifemur* for the combined species treatment).

One *H. vitripennis* egg mass exposed simultaneously to one *G. ashmeadi* and *G. tuberculifemur* for 15 min resulted in parasitism by *G. ashmeadi* that was 33% higher compared to *G. tuberculifemur* ($\chi^2 = 39.22$, $df = 1$, $p < 0.001$) (Fig. 3). There was a significant effect of treatment on percentage parasitism by *G. ashmeadi* ($\chi^2 = 8.88$, $df = 2$, $p < 0.01$) (Fig. 3). Interspecific competition between *G. ashmeadi* and *G. tuberculifemur* reduced *G. ashmeadi* parasitism by 17% compared with AA-control vials. There was no significant difference in parasitism by *G. ashmeadi* between the combined species treatment and the A-control (Fig. 3). Treatment had no significant effect on percentage parasitism by *G. tuberculifemur* ($\chi^2 = 6.27$, $df = 2$, $p = 0.04$) (Fig. 3). There was no significant effect of treatment on sex ratio for *G. ashmeadi* ($\chi^2 = 0.35$, $df = 2$, $p = 0.84$) and *G. tuberculifemur* ($\chi^2 = 2.47$, $df = 2$, $p = 0.29$). Overall sex ratio was significantly higher (17%) for *G. ashmeadi* (mean = 76 ± 2%) compared with *G. tuberculifemur* (59 ± 6%) ($\chi^2 = 7.14$, $df = 1$, $p < 0.01$).

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, AA-control vials and the combined species treatment allocated up to 42% more observations to oviposition compared with all remaining behavioral events (Table 1). By contrast, female *G. tuberculifemur* in the T-control, TT-control vials and the combined species treatment spent up to 43% of observations off the leaf compared with all remaining behavioral events. For the A-controls and T-controls where there was only one female present, no observations of females searching the egg mass from the opposite side of the leaf or ovipositing through the leaf were recorded (Table 1). By contrast, when a competitor was present (i.e., AA-controls, AT-treatment, and TT-controls) female *G. ashmeadi* and *G. tuberculifemur* spent up to 2% of observations searching for and ovipositing into host eggs on the opposite side of the leaf. However, there was one exception; female *G. tuberculifemur* in the TT-control were not observed to oviposit from the opposite side of the leaf. Additionally, the presence of a competitor resulted in *G. ashmeadi* and *G. tuberculifemur* spending approximately 1% of observations antennating competitors and aggressively chasing competitors off *H. vitripennis* egg masses (Table 1). Aggressive chasing involved running directly at the other female and often concluded with physical contact. Although aggressive behavior was demonstrated by *G. ashmeadi* and *G. tuberculifemur*, 43 observations (or 9% of 465 observed behavioral events) of simultaneous oviposition by both females on the same egg mass was observed in 14 of 31 replicates (i.e., 45%).

Comparisons between parasitoid treatments indicated that the percentage of observations allocated to searching the leaf, off the leaf, oviposition, and grooming significantly varied between treatments (Table 1). There was no significant effect of treatment on frequency of observations allocated to searching egg masses, resting, chasing competitors, antennating competitors, searching egg masses from the opposite side of the leaf, oviposition from the opposite side of leaves, and drinking water (Table 1). When *G. ashmeadi* and *G. tuberculifemur* were presented simultaneously with *H. vitripennis* egg masses, *G. ashmeadi* allocated 15% more observations to ovipositing and 20% fewer observations were recorded off the leaf compared with *G. tuberculifemur* (Table 1). There was no significant difference between the frequency *G. ashmeadi* and *G. tuberculifemur* were observed in the remaining behaviors for this treatment. The frequency that *G. ashmeadi* was observed off the leaf was statistically equivalent between the A-controls, AA-controls and combined species treatment. Similarly, female *G. tuberculifemur* in the T-controls, TT-controls and combined species treatment were equally observed off the leaf suggesting that the presence of a competitor did not affect the frequency females were observed off the leaf.

The frequency that female *G. ashmeadi* were observed searching the leaf was 8–10% higher in the control vials containing two females of the same species and the combined species treatment, compared with the control vials containing one female (Table 1). *G. ashmeadi* were observed ovipositing in the A-controls 10% more frequently compared with the AA-controls, whereas, there was no significant difference in the frequency of ovipositing behavior between A-controls and the combined species treatment (Table 1). Results for *G. tuberculifemur* demonstrated that the addition of a *G. tuberculifemur* or *G. ashmeadi* competitor significantly decreased

Table 1
The frequency (mean [± SEM] percentage) of eleven behaviors observed once every 1 min when *G. ashmeadi* and *G. tuberculifemur* were exposed to one *H. vitripennis* egg mass for 15 m under five parasitoid treatments (A = control vial containing one female *G. ashmeadi*; AA = control vial containing two female *G. ashmeadi*; AT = one female *G. ashmeadi* and *G. tuberculifemur*, TT = two female *G. tuberculifemur*; T = one female *G. tuberculifemur*).

Behavior	Parasitoid Treatment						
	A	AA	AT		T	TT	Between treatments test statistics[a]
			G. ashmeadi	*G. tuberculifemur*			
Off leaf	19.0 ± 4.3 A, i	21.1 ± 2.9 A, i	19.1 ± 3.4 A, i	38.9 ± 6.5 A, ii	42.9 ± 6.2 A, ii,iii	39.4 ± 4.2 A, iii	χ^2 = 178.67, df = 5, p < 0.0001
Searching Leaf	8.1 ± 2.2 B, i	18.0 ± 2.2 B, ii	16.1 ± 3.6 A, ii	18.1 ± 4.3 B, ii	8.0 ± 2.0 B, i	8.0 ± 2.7 B, ii	χ^2 = 48.43, df = 5, p < 0.0001
Searching egg mass	11.0 ± 1.7 C	8.4 ± 1.2 C	9.9 ± 1.6 B	7.7 ± 1.5 C	6.7 ± 1.9 C	6.8 ± 1.2 C	
Oviposition	42.1 ± 5.6 D, i	32.5 ± 2.6 D, ii	37.4 ± 4.6 C, i,ii	22.8 ± 4.8 D, iii	29.1 ± 6.2 D, ii	14.0 ± 2.8 D, iii	χ^2 = 165.08, df = 5, p < 0.0001
Resting	10.8 ± 4.2 C	10.1 ± 2.7 E	8.8 ± 3.2 B	5.6 ± 2.6 E	7.6 ± 3.1 B	11.0 ± 2.9 E	
Grooming	8.3 ± 3.2 B, i	4.4 ± 1.2 F, ii,iii	4.1 ± 1.9 D, iii	3.8 ± 1.4 E, iii	5.8 ± 2.0 C, i,ii	8.3 ± 1.5 F, i	χ^2 = 24.51, df = 5, p = 0.0002
Chasing Competition		0.1 ± 0.1 G	1.3 ± 0.7 E	0.2 ± 0.2 F		0.3 ± 0.3 G	
Anntenaeing Competitor		1.1 ± 0.4 H	0.6 ± 0.4 E	1.3 ± 0.4 G		0.9 ± 0.3 J	
Searching egg top side	0 ± 0 E	1.1 ± 0.7 H	0.9 ± 0.5 E	0.4 ± 0.3 F	0 ± 0 D	0.2 ± 0.2 G	
Oviposition top side	0 ± 0 E	2.3 ± 1.4 I	1.3 ± 1.1 E	0.9 ± 0.7 G	0 ± 0 D	0 ± 0 I	
Drinking	0.6 ± 0.5 E	0.7 ± 0.4 H	0.4 ± 0.2 E	0.2 ± 0.2 F	0 ± 0 D	1.0 ± 0.5 J	
Between behaviors test statistics[b]	χ^2 = 361.87 df = 6 p < 0.0001	χ^2 = 1181 df = 10 p < 0.0001	χ^2 = 672.52 df = 10 p < 0.0001	χ^2 = 797.96 df = 10 p < 0.0001	χ^2 = 314.43 df = 5 p < 0.0001	χ^2 = 1183.07 df = 9 p < 0.0001	

[a] Logistic regression was used to determine the effect of treatment on the frequency of observations allocated to eleven behaviors for each behavior. Pair-wise contrast tests were used to separate means. For these tests, there was a significant (p < 0.05) difference between treatments when p < 0.008. Different roman numerals (i, ii, iii) indicate significant differences between behaviors within each parasitoid treatment.

[b] Chi-square Test for Specified Proportions was used to determine whether the frequency of the eleven behaviors significant varies within each treatment. Different letters (A, B, C) indicate significant differences between parasitoid treatments within each behavior.

frequency of oviposition by *G. tuberculifemur* by up to 15%, when compared with the T-controls (Table 1). Finally, when comparing the frequency of grooming between treatments, results showed that *G. ashmeadi* were observed significantly less often (i.e., 4%) grooming when a competitor was present, when compared with the A-controls (Table 1). *G. tuberculifemur* were observed to groom significantly less (i.e., 2–5%) when competing with *G. ashmeadi* for egg masses, compared with the T- and TT-controls (Table 1).

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

Mean parasitism by *G. ashmeadi* was significantly higher (44–53%) than *G. tuberculifemur* for both exposure times (24 h: $t = 9.31$, $df = 21$, $p < 0.0001$; 5 days: $t = 13.30$, $df = 16$, $p < 0.0001$) (Fig. 4). There was no significant effect of exposure time on parasitism by *G. ashmeadi* ($\chi^2 = 3.46$, $df = 1$, $p = 0.06$). Parasitism by *G. tuberculifemur* was significantly higher (5%) when *H. vitripennis* eggs were exposed for 24 h compared with 5 days ($\chi^2 = 9.89$, $df = 1$, $p < 0.001$) (Fig. 4). There was no significant effect of exposure time on sex ratio for *G. ashmeadi* ($\chi^2 = 1.81$, $df = 1$, $p = 0.18$) or *G. tuberculifemur* ($\chi^2 = 0.64$, $df = 1$, $p = 0.42$). Overall sex ratio was significantly higher for *G. ashmeadi* (mean = 87 ± 1%) compared with *G. tuberculifemur* (79 ± 4%) ($\chi^2 = 11.94$, $df = 1$, $p < 0.001$).

4. 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. tuberculifemur*. *G. ashmeadi* consistently outperformed *G. tuberculifemur* by parasitizing more *H. vitripennis* eggs, it demonstrated an ability to exploit a larger host egg age range, and produced more female offspring when compared with *G. tuberculifemur*. The ability to parasitize more host eggs over a wider range of host egg age categories is a favorable trait because it increases the probability that *H. vitripennis* eggs of varying ages will be successfully parasitized upon discovery and may enable *G. ashmeadi* to outcompete *G. tuberculifemur*.

Several reasons may exist for the observed inferiority of *G. tuberculifemur* in these laboratory tests. First, female *G. tuberculifemur* may be less efficient at host searching (Paust et al., 2008), or second, parasitizing eggs (Li et al., 2008). Third, *G. tuberculifemur* may require a longer post-oviposition period before recommencing oviposition. Fourth, a higher proportion of *G. tuberculifemur* larvae may die from host defense mechanisms or host unsuitability since *G. tuberculifemur* did not co-evolve with *H. vitripennis* and it cannot readily circumvent these defenses. Fifth, *G. tuberculifemur* larvae may be less efficient at interspecific competition against *G. ashmeadi* larvae in host eggs. *G. ashmeadi* larvae possess enlarged mandibles (Irvin et al., 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. tuberculifemur* larvae possess specialized structures for larval combat. Other possible characteristics that may enable *G. ashmeadi* larvae to be intrinsically superior to *G. tuberculifemur* are that *G. ashmeadi* larvae may hatch more quickly and grow more rapidly than immature *G. tuberculifemur* (DeMoraes et al., 1999), kill *G. tuberculifemur* via starvation (Lawrence, 1988), or physiologically change the host to suppress the growth of competitors (Salt, 1961; Lawrence, 1988; Mackauer, 1990). Sixth, *G. ashmeadi* may conduct ovicide, the deliberating killing of a competitor's eggs by a superparasitizing female with her ovipositior (Netting and Hunter, 2000).

4.1. Potential impact of G. tuberculifemur on H. vitripennis

To reduce uncertainty about the reliability of competitive experiments, the current study included two types of controls containing one parasitoid species. For the observational 'parasitoid behaviors' experiment containing one leaf in a Petri dish, the percentage of nymphs that emerged from egg masses in the AA-control was lower compared to the A-control indicating that two female *G. ashmeadi* increased mortality of *H. vitripennis* eggs. By contrast, *H. vitripennis* emergence was equivalent between A-control and the combined species treatment (AT) suggesting that there was no additional benefit by *G. tuberculifemur* on parasitism of *H. vitripennis*. There was no significant difference in parasitism between vials containing one *G. ashmeadi* (A), two *G. ashmeadi* (AA), or one *G. ashmeadi* and *G. tuberculifemur* (AT). This may be attributable to the presence of a competing female which increased

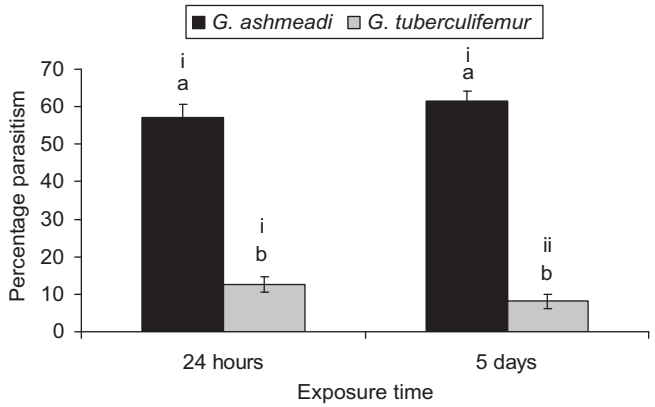

Fig. 4. The mean percentage of *G. ashmeadi* and *G. tuberculifemur* offspring emerging when 50 *H. vitripennis* eggs were exposed simultaneously to one mated female *G. ashmeadi* and *G. tuberculifemur* for 24 h or 5 d in the laboratory at 26 °C (error bars indicate ± SEMs; different letters [a, b] 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).

non-ovipositional behaviors such as aggressive chasing or antennating of competitors. Collectively, these behaviors which were only observed under competition conditions, reduced the time available for host searching and oviposition.

Based on the results presented here, we suggest that there may be no advantage to releasing *G. tuberculifemur* from quarantine, unless it can be demonstrated that this new association agent can either: (1) 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 parasitizing *H. vitripennis* egg masses early in the spring when *G. ashmeadi* parasitism levels are extremely low, over-wintering *G. ashmeadi* are uncommon, and host eggs are relatively scarce (Triapitsyn et al., 2003). The ability of *G. tuberculifemur* to fill this spring-time niche may depend on its over-wintering phenology, in particular, the exploitation of eggs of alternative host species should they be available when *H. vitripennis* eggs are scarce or absent. Alternatively, *G. tuberculifemur* could possess an ability to diapause over winter and have an ability to quickly generate a large spring population when *H. vitripennis* eggs are relatively uncommon by efficiently exploiting host eggs when they occur at very low densities; (2) perform better on host plants that were untested here or that *G. ashmeadi* fails to utilize. We are unaware of the existence of such plants in Southern California. However, Krunger et al. (2008) reported that *G. ashmeadi* was less attracted to volatiles emitted from grapevine compared to lemon and crape myrtle. Krunger et al. (2008) did not investigate parasitism rates of *G. ashmeadi* on different host plants so it is unknown whether lower attraction led to reduced parasitism rates; (3) be more efficient than *G. ashmeadi* at locating hosts over larger areas and lower densities than were tested here; (4) be more efficient at parasitizing brochosome covered egg masses compared to *G. ashmeadi* (brochosomes were removed from hosts in the current study) or (5) perform better under the prevailing field conditions in California where *H. vitripennis* is problematic and *G. ashmeadi* is not the most common parasitoid species (such as some coastal and desert areas [D.J.W. Morgan, pers. comm.]).

4.2. 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 significantly from the field environment where, for example, fluctuations in temperature and humidity exist, eggs are laid on a variety of different intact host plants, and female parasitoids need to search large areas for hosts. It is unknown whether *G. tuberculifemur* 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 et al., 1980; Andow and Prokrym, 1990; Murray and Rynne, 1994; Gingras et al., 2003; Amalin et al., 2005; Carrillo et al., 2008). In California, citrus is a highly preferred host plant for *H. vitripennis* and it is the most common reproductive and over-wintering host for this pest (Blua et al., 1999). Bioassays conducted by Krunger et al. (2008) demonstrated that naïve female *G. ashmeadi* are highly attracted to odors emitted from lemon plants (used in these studies) when compared with grapevines. Because *H. vitripennis* oviposits on over 100 different host plants in the field (CDFA, 2009) it is possible that *G. tuberculifemur* may have performed better if different plants were used in bioassays. Learning and memory have been documented in the preimaginal and post-emergence period of insects (Hérard et al., 1988; Tully et al., 1994; Gandolfi et al., 2003) and host plant emergence experience influences searching times and parasitism rates (Bjorksten and Hoffmann, 1998). Therefore, it is possible that *G. tuberculifemur* used in these studies that emerged from euonymus (15%) may have been less sensitive to volatiles from experimental lemon leaves, therefore reducing searching efficiency and *G. tuberculifemur* parasitism. This effect, if it existed, was likely to be minor because of the low number of parasitoids used that were reared from euonymus and used in experiments.

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. tuberculifemur* field performance based on laboratory studies. Irvin and Hoddle (2005) documented that *G. ashmeadi* outperformed *G. triguttatus* and *G. fasciatus* across egg age utilization studies, parasitism rates, 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. Establishment and recovery of *G. triguttatus* and *G. fasciatus* has been inconsistent. For example, of the 69,474 *G. triguttatus* that were released in 2008, only ~33 parasitized *H. vitripennis* egg masses were recovered for this species in 11 out of 65 release sites (17%). Similar poor results have resulted for *G. fasciatus* and mass production and release of this parasitoid was discontinued due to poor recovery rates (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)). Consequently, the results from the current work also suggest that *G. tuberculifemur* is inferior to *G. ashmeadi* and may experience difficulty in establishing, especially in areas where *G. ashmeadi* is dominant.

4.3. Behavioral comparisons

Parasitism by *G. tuberculifemur* was 33% lower than *G. ashmeadi* in the combined species treatment. The poor performance of *G. tuberculifemur* was attributed to these females spending 20% more observations off the leaf and 15% fewer observations depositing eggs compared with *G. ashmeadi*. Parasitism by *G. tuberculifemur* was statistically equivalent between the combined species treatment and the T-control, therefore the presence of *G.*

ashmeadi had no effect on *G. tuberculifemur* performance. At the beginning of each behavioral trial, female *G. tuberculifemur* spent more time off the leaf and took longer to locate experimental leaves containing hosts, compared with *G. ashmeadi* (N. A. Irvin, personal observation). Krunger et al. (2008) demonstrated that naïve female *G. ashmeadi* are attracted to volatiles emanating from lemon plants and prefer the volatiles from lemon plants infested with *H. vitripennis,* compared with plants that had no *H. vitripennis* oviposition. The higher percentage of observations *G. tuberculifemur* spent off the leaf compared with *G. ashmeadi* may suggest that female *G. tuberculifemur* were not attracted to the plant surface, volatiles from the host plant, or the host egg mass. This lack of association to the host and host plant may be because *G. tuberculifemur* did not evolve with *H. vitripennis* and does not readily recognize volatile chemical profiles associated with *H. vitripennis* egg masses.

Interestingly, *G.* sp. near *tuberculifemur* "Clade 1" (i.e., the *G. tuberculifemur* used in this study) has been associated with eggs of two sharpshooter species (*Tapajosa rubromarginata* [Signoret] and *Anacuerna centrolinea* [Melichar]; both Proconiini) laid on broad bean (*Vicia fava* L.), quinoa (*Chenopoium quinoa* Willd) (Logarzo et al., 2006), Johnson grass (*Sorghum halepense* [L.]) and maize (*Zea mays* L.) (Virla et al., 2005). *G.* sp. near *tuberculifemur* "Clade 1" has also emerged from sentinel eggs of *T. rubromarginata* on citrus (Triapitsyn et al., 2008). These data indicate that this parasitoid is capable of foraging successfully on diverse plant species for different sharpshooter host species, which suggests host plant effects on host location and attack may not be overly important.

Female *G. ashmeadi* and *G. tuberculifemur* foraging for *H. vitripennis* eggs in the presence of a competitor (i.e., treatments AA, AT and TT) allocated up to 2% of behavioral observations to antennating the leaf surface directly under the egg mass, on the opposite side of the leaf, and/or inserting their ovipositor into an egg from the opposite side of the leaf. These behaviors were not observed when female parasitoids foraged individually. These results indicate 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. This behavior has also been observed when single female *G. ashmeadi* foraged on *H. vitripennis* egg masses that were heavily covered by brochosomes, a white chalky substance deposited by female sharpshooters on egg masses, which has been demonstrated to reduce parasitism (Velema et al., 2005).

When female *G. ashmeadi* and *G. tuberculifemur* were in the presence of a competitor they allocated approximately 1% of behaviors to antennating the other female or aggressively chasing her off contested egg masses. Direct contact between parasitoids may have caused a reduction in searching efficiency and oviposition since frequency of oviposition was up to 15% less when a competitor was present compared to the one species controls. For both experiments involving one *H. vitripennis* egg mass, parasitism by *G. ashmeadi* and *G. tuberculifemur* were lower in the combined species treatment compared with the controls containing one female. Lower parasitism rates may have been attributable to aggressive behavior interrupting oviposition, a reduction in time spent ovipositing, or interspecific competition between parasitoid larvae (Salt, 1961; Mackauer, 1990; Tillman and Powell, 1992; DeMoraes et al., 1999). Interactions between parasitoids competing for the same host can have important effects on the success of biological control (Hassell and Varley, 1969).

Although *G. ashmeadi* and *G. tuberculifemur* demonstrated aggressive behavior when concurrently searching for *H. vitripennis* egg masses, 42 accounts (30% of total oviposition events) of simultaneous oviposition by both species on the same egg mass was recorded during observation experiments. Simultaneous oviposition by females of two different *Gonatocerus* species has been observed for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* (Irvin and Hoddle, 2005; Irvin et al., 2006). These results demonstrate that female *Gonatocerus* can co-exploit patches and they can encounter one another without initiating aggression. Reduction of aggressiveness allowing patch co-exploitation may occur if females are of similar size (Petersen and Hardy, 1996) as is the case with all of these parasitoids (Triapitsyn, 2006; Triapitsyn et al., 2008).

4.4. Risk assessment for introduction of G. tuberculifemur

The decision to introduce *G. tuberculifemur* to California may be influenced by the estimated benefits on suppressing *H. vitripennis* populations, the anticipated host range of *G. tuberculifemur,* the value placed on potential non-target species, the identification of other potential risks and their likelihood (e.g., introducing *G. tuberculifemur* in California 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 et al., 2006). The results outlined here are significant factors when considering the benefits of introducing the 'new association' *G. tuberculifemur* for control of *H. vitripennis*. The current laboratory studies have demonstrated that *G. tuberculifemur* failed to outperform *G. ashmeadi*, the dominant resident parasitoid attacking *H. vitripennis* eggs in California. Based on the results presented here and in Irvin et al. (2009) we speculate that: (1) *G. tuberculifemur* may have difficulties establishing in areas where *G. ashmeadi* is present, and (2) the potential impact of releasing *G. tuberculifemur* in California may be negligible unless *G. tuberculifemur* performs better under field conditions not examined in these studies, or can fulfill a niche in the field which is not currently dominated by *G. ashmeadi*. Additional research is recommended to address this second point, before 'new-association' biological control of *H. vitripennis* with *G. tuberculifemur* is implemented in California and significant resources are expended on the mass rearing, releasing, and monitoring of this biological control agent. Such investigations should determine host finding abilities on whole plants, levels of parasitism on other species of host plants, 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. tuberculifemur* in areas of its host range that are similar to California, and a comprehensive non-target risk assessment.

In earlier times, some natural enemies were deliberately chosen for biological control programs against arthropod pests because they were not highly host specific and could maintain high populations on alternative hosts in the absence of the target pest. However, there has been a marked shift towards using highly host specific predators or and parasitoids in biological control programs to reduce risks to non-target species, and to arguably increase the efficacy of control (Barratt et al. 2010). Conversely, it should be noted that there are examples of polyphagous natural enemy species, including egg parasitoids, which have been successful biological control agents with minimal effects on non-targets. One well studied system is seasonal augmentative releases of *Trichogramma* spp. into agroecosystems in Europe (Orr et al. 2000).

'New-association' biological control of *H. vitripennis* with *G. tuberculifemur* raises concerns about potential unwanted impacts on native non-target species of sharpshooters. Simberloff and Stiling (1996) argued that if a natural enemy and pest have not co-evolved then the probability is increased that non-target species will be affected at least as much as the target. In no-choice studies, *G. tuberculifemur* successfully parasitized eggs of two native US non-target sharpshooter species, *H. liturata* Ball and

Oncometopia sp. (both Cicadellinae: Proconiini) (Jones et al., 2005a). Investigation of the field host range in Argentina demonstrated that *G. tuberculifemur* successfully parasitized at least five species of Cicadellini, a tribe to which *H. vitripennis* does not belong (Jones et al., 2005b). Together, these laboratory and field results suggest that *G. tuberculifemur* is polyphagous, and may successfully exploit native non-target sharpshooter species in the tribes Proconiini and Cicadellini. Choice and no-choice assay designs would need to be conducted to determine if similar levels of polyphagy would be observed in areas where releases of *G. tuberculifemur* would be considered (i.e., California) or unintended incursion could occur (i.e., the Southeast USA and Northeast Mexico where *H. vitripennis* is native and sympatric with other sharpshooter species). *G. ashmeadi* also parasitizes both species of Proconiini mentioned above (Triapitsyn, 2006) and host range testing for *G. ashmeadi* has not been specifically conducted for Cicadellini. Interestingly, *G. ashmeadi* has not been reared from Cicadellini in it's native range despite extensive collection of egg masses of a variety of different sharpshooter species (S. V. Triapitsyn, pers. comm.).

Van Driesche and Hoddle (1997) proposed a general model of non-target testing that included no-choice and choice laboratory testing, assembling host records from previous studies, gathering records of non-target species known to safely coexist with the biological control agent in other locations, and an assessment of field host ranges. Ecological and biological data can also be useful in predicting field host ranges. For example, in French Polynesia, the risk of introducing *G. ashmeadi* for *H. vitripennis* control, on non-target native cicadellids was assessed using the phylogenetic relationship between native cicadellids and known hosts for *G. ashmeadi*, and biological traits of native cicadellids such as body size, egg laying biology, and ecology Grandirard et al. (2007). Wright et al. (2005) proposed a method of risk assessment that uses precision trees estimating the probability that a biological control agent will exceed certain densities in various habitats and the proportion of non-target species that will be exposed at each density. This method utilizes host range data and ecological and dispersal information from the home range of the biological control agent. However, it may be difficult to determine whether *G. tuberculifemur* could inadvertently spread into areas outside of California based on ecological data from its home range, because a likely avenue for dispersal is via interstate commerce of ornamental plants bearing parasitized *H. vitripennis* egg masses on leaves. The significance of any unwanted incursion, associated foodweb perturbations, and ecosystem impacts caused by *G. tuberculifemur* to native sharpshooters and their associated egg parasitoids outside of California is unknown, and could cause concern for ecologists and taxonomists studying native ecosystems.

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