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Author(s): Nicola Irvin, Javier Suarez-Espinoza, and Mark Hoddle

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# Functional Response of *Gonatocerus ashmeadi* and the “New Association” Parasitoid *G. tuberculifemur* Attacking Eggs of *Homalodisca vitripennis*

NICOLA IRVIN,<sup>1,2</sup> JAVIER SUAREZ-ESPINOZA,<sup>3</sup> AND MARK HODDLE<sup>1,4</sup>

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**ABSTRACT** The functional response of two egg parasitoids, *Gonatocerus tuberculifemur* (Ogloblin) and *G. ashmeadi* Girault (Hymenoptera: Mymaridae), to varying densities of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) eggs was studied in the laboratory. *G. tuberculifemur* showed a type II functional response where parasitism increased at a decreasing rate as host egg density increased from 5 to 40, reaching an asymptote of an upper limit of 9.3 host eggs within the 1-h exposure period. The instantaneous attack rate and handling time as estimated by the random predator equation were 2.28/h and 0.10 h, respectively. In contrast, *G. ashmeadi* showed a type I functional response where the number of hosts attacked followed a constant linear increase regardless of host density to an upper limit of 11.5 host eggs within the 1-h exposure period. Female *G. ashmeadi* parasitized on average 4.3 and 3.5 more eggs per hour when presented with 10 and 20 *H. vitripennis* eggs, respectively, compared with *G. tuberculifemur*. When 5 or 40 *H. vitripennis* eggs were offered, there was no significant difference in parasitism rates between parasitoid species. Percentage of female offspring was significantly higher (5%) for *G. ashmeadi* compared with *G. tuberculifemur*. Under the experimental conditions used in these evaluations, results suggest that *G. tuberculifemur* is an inferior parasitoid to *G. ashmeadi*.

**KEY WORDS** biological control, functional response, *Gonatocerus ashmeadi*, *Gonatocerus tuberculifemur*, *Homalodisca vitripennis*

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae: Proconiini [formally *H. coagulata*]), is native to the southeastern United States and northeastern Mexico. This invasive pest established in California in the late 1980s, where it has caused significant economic problems for producers of agricultural and ornamental commodities. *H. vitripennis* is a vector of a xylem-limited bacterium, *Xylella fastidiosa* Wells et al., which causes disease in a variety of economically important plants including Pierce's disease in grapes, almond leaf scorch, alfalfa dwarf, phony peach disease, and oleander leaf scorch (Blua et al. 1999, UCOP 2000, Varela et al. 2001). Pierce's disease is endemic in 28 Californian counties, and increased vectoring risk posed by *H. vitripennis* currently threatens grape production that is worth \$4.14 billion annually (CDFA 2006). The distribution of this pest increased in 2006 and new infestations were found in Fresno and Santa Clara Counties (CDFA 2006), suggesting *H. vitripennis* is continuing

its northward advance out of southern and central California.

In 2001, the California Department of Food and Agriculture (CDFA) implemented a classical biological control program against *H. vitripennis* that has involved importing, mass-producing, releasing and establishing, and evaluating biological control agents for the suppression of *H. vitripennis*. To date, a total of six species of mymarid egg parasitoids (Hymenoptera) have been released in 13 Californian counties in an attempt to provide effective classical biological control of *H. vitripennis* (CDFA 2006). One parasitoid species being considered for release and establishment in California is the “new association” (Hokkanen and Pimentel 1989) biological control agent *Gonatocerus tuberculifemur* (Ogloblin) (Mymaridae), a parasitoid from Argentina that attacks proconiine sharpshooters (the same tribe as *H. vitripennis*) in South America (León et al. 2008). This parasitoid was imported into quarantine in California in 2002 and has been successfully maintained on *H. vitripennis* egg masses with regular infusions of new genetic material from South America.

It is argued that new association biological control agents that have no evolutionary history with the target pest should be more effective natural enemies because the pest will not be able to adequately defend

<sup>1</sup> Department of Entomology, University of California, Riverside, CA 92521.

<sup>2</sup> Corresponding author, e-mail: nic.irvin@ucr.edu.

<sup>3</sup> Department of Statistics, University of California, Riverside, CA 92521.

<sup>4</sup> Center for Invasive Species Research, University of California, Riverside, CA 92521.

itself from novel attacks. However, this strategy has been considered risky because natural enemy species that are most likely to produce effective new association biological control are those preadapted to using new hosts and therefore could pose high risk to non-target species because of this polyphagy (Roderick 1992). A potentially broad host range for *G. tuberculifemur* raises concerns about nontarget impacts on native U.S. proconiine sharpshooters. Environmental concerns could be even greater if *G. tuberculifemur* successfully left California and infiltrated the home range of *H. vitripennis*, which would put this upper trophic level organism in sympatry with a variety of other nontarget native proconiine sharpshooter species that *G. tuberculifemur* could likely use as hosts (e.g., *Oncometopia* sp.).

The *H. vitripennis* egg parasitoid guild in California comprises eight species (C DFA 2006) and is reported to inflict an average year-round parasitism of 15.5% (Pilkington et al. 2005). Low parasitism rates may be because of interference among members of the existing parasitoid guild, which is reducing the effectiveness of biological control (Myers et al. 1989, Denoth et al. 2002). Alternatively, low average parasitism may be indicative that an aggressive and efficacious natural enemy that can dominate the guild of *H. vitripennis* egg parasitoids has not been established in California and is needed for successful biological control. It has been proposed that the new association agent, *G. tuberculifemur*, could be a dominating efficacious parasitoid species that will provide control of *H. vitripennis* in California. However, it is unknown whether *G. tuberculifemur* can outperform *G. ashmeadi*, the dominant *H. vitripennis* parasitoid in California, to provide the desired level of control.

To measure the efficacy of *G. tuberculifemur* for processing *H. vitripennis* eggs for comparison to *G. ashmeadi*, the functional response of *G. tuberculifemur* and *G. ashmeadi* when exposed to different densities of *H. vitripennis* eggs was studied. The functional response defines the change in the number of hosts parasitized per parasitoid, per unit time, in relation to host density (Royama 1971) and has been used extensively in population ecology and foraging theory to study the potential for natural enemies to regulate host populations (Murdoch and Oaten 1975). The functional response type (i.e., type I, type II, type III) is characterized by the shape of the curve that describes attack rates (which are influenced by handling times and search efficiencies) of natural enemies over increasing host densities within a fixed exposure time (Juliano 1993). Quantification of parameters defining functional response curves for *G. tuberculifemur* and *G. ashmeadi* may provide insight into which natural enemy is likely to be more efficacious at processing varying densities of *H. vitripennis* eggs, thereby providing better biological control of this pest. Consequently, functional response parameters could be used as metrics to provide relative measures of competitiveness, which may help to determine which parasitoid species is likely to be the superior natural enemy in the field and to scientifically guide decisions

on whether or not to release *G. tuberculifemur* from quarantine for establishment in California. The results of functional response experiments for *G. ashmeadi* and *G. tuberculifemur* are reported here.

## Materials and Methods

**Insect Colonies and Laboratory Conditions.** 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  $26 \pm 2^\circ\text{C}$  and 30–40% RH under a L 14:10 D photoperiod and reared in cages (50 by 40 by 40 cm) 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* cultivar Eureka trees, ~2 yr 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 2 wk with Miracle-Gro (20 ml/3.5 liters of water; Scotts Miracle-Gro Products, Marysville, OH). Female *G. tuberculifemur* were obtained from *G. tuberculifemur* colonies (clade I as described in León et al. 2008) maintained in the Insectary and Quarantine Facility at UCR. 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 by 9 by 16 cm) and held at  $24 \pm 2^\circ\text{C}$  and 40–50% RH, under a L 14:10 D photoperiod. Females were held with 50% honey-water for 2–3 d 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 this functional response study had emerged from egg masses laid on Eureka lemon leaves compared with 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 by 20 by 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 parasitoids were collected and prepared for functional response experiments.

**Experimental Procedure for Functional Response Experiments.** Newly emerged (~24 h) naive 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 procedure was repeated for *G. tuberculifemur*. Parasitoids were held in the laboratory

for 24 h at  $26 \pm 2^\circ\text{C}$  and 30–40% RH under a L 14:10 D photoperiod before use in functional response experiments. Host densities presented to parasitoids were 5, 10, 20, and 40 *H. vitripennis* eggs per female, and each experimental egg density was replicated 13–18 times for each parasitoid species. Host density range and exposure time were selected based on previous studies with *G. ashmeadi* and *G. tuberculifemur*. The upper host density was chosen because female *G. ashmeadi* are synovigenic, emerging with  $\approx 30$  mature eggs in their ovaries (Irvin and Hoddle 2008). Pilkington and Hoddle (2006) provided individual *G. ashmeadi* with 40 host eggs per day in their life table studies, which was surplus to what female parasitoids could use in the 24-h exposure period. Exposure time in this study was limited to 1 h to ensure that an upper limit to the functional response was detected, because studies by N.I. and M.H. (unpublished data) had shown that, on average, female *G. ashmeadi* parasitize eight eggs within 20 min of detecting a patch. Furthermore, a 1-h exposure time is likely to be more realistic in a field situation compared with a 24-h enclosure with one host patch. Functional response experiments were conducted between 1000 and 1300 hours on days when parasitoids and host eggs were available. *H. vitripennis* eggs laid on Eureka lemon leaves were <48 h old, an age shown to be successfully used by both *Gonatocerus* spp. (Irvin and Hoddle 2005; N.I. and M.H., unpublished data). N.I. and M.H. (unpublished data) found that brochosomes cover 64% of *H. vitripennis* egg masses; therefore, in this study, all egg masses were gently wiped with a damp paper towel before experiments to remove any brochosomes that could interfere with parasitism attempts (Velema et al. 2005), thereby standardizing egg mass quality within and across treatments. For each replicate, the petioles of leaves containing egg masses were placed through holes drilled through the lid of a 130-ml plastic vial that held water. Leaf number was standardized to four leaves per vial by including lemon leaves without *H. vitripennis* egg masses. A second 130-ml plastic vial with ventilation (three 2-cm holes [one on the bottom, and one on each of two sides] covered with mesh netting [80  $\mu\text{m}$ ; Jelliff, Southport, CT]) was inverted and attached to the lid of the vial holding the water and lemon leaves. One mated and honey-water fed female parasitoid (<48 h old) was introduced into each vial and left to forage for 1 h, after which leaves containing egg masses exposed to parasitoids were placed into petri dishes (3.5 by 1 cm; Becton Dickinson Labware; Becton Dickinson and Co., Franklin Lakes, NJ) lined with moist filter paper (4.25 cm; Whatman International, Maidstone, United Kingdom). Petri dishes were labeled with replicate number, density, and species and held at  $26 \pm 2^\circ\text{C}$  and 30–40% RH under a L 14:10 D photoperiod for 3 wk to allow parasitoids and *H. vitripennis* nymphs to emerge.

Experiments were conducted in the laboratory at  $26 \pm 2^\circ\text{C}$  and 30–40% RH under a L 14:10 D photoperiod with fluorescent lighting. Premature drying of leaves sometimes occurred, which occasionally pre-

vented successful insect emergence. Therefore, unemerged eggs were dissected and the numbers of unemerged male and female parasitoid pupae were also recorded. Presence or death of parasitoid eggs and larvae were not determined. Female replicates that resulted in zero parasitism (*G. ashmeadi* = 11% of replicates; *G. tuberculifemur* = 14%) within the experimental hour were labeled “incompetent” and removed from analyses. Unemerged eggs that did not contain identifiable parasitoid pupae were recorded as “unemerged *H. vitripennis* nymphs” and contributed to “nymph mortality.” A set of 12–16 controls were set up for each experiment host egg density and held at  $26 \pm 2^\circ\text{C}$  and 30–40% RH under L 14:10 D. These vials did not contain a parasitoid and were used to determine naturally occurring mortality of *H. vitripennis* eggs under the experimental conditions and exposure time. Control mortality was calculated as the proportion of unemerged *H. vitripennis* nymphs from the total number of experimental eggs.

**Statistical Analysis.** Functional response data for each species was analyzed in two phases using SAS (SAS Institute 1990). First, the shape of the functional response curve was determined by logistic regression of the proportion of *H. vitripennis* eggs parasitized as a function of initial density (Trexler et al. 1988). Second, the random predator equation was fitted to data after the functional response type was determined (Juliano 1993). The random predator equation accounts for host depletion without replenishment over the course of the experimental period, which was the design in these experiments. This equation was also used in *G. ashmeadi* functional response studies conducted by Chen et al. (2006).

The polynomial function from Juliano (1993) was used to fit data on the proportion of *H. vitripennis* eggs parasitized:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)} \quad [1]$$

where  $N_e/N_0$  is the probability of being parasitized,  $N_e$  is the number of host eggs parasitized, and  $N_0$  is the initial host density. Maximum likelihood estimates of parameters  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  were obtained by applying logistic regression (Proc Logistic) in SAS (SAS Institute 1990) to a dichotomous variable that equaled one for the egg being parasitized or zero if the host egg was not parasitized. The positive or negative signs of the linear ( $P_1$ ), quadratic ( $P_2$ ), and cubic ( $P_3$ ) coefficients from equation 1 can be used to distinguish the shape of the functional response curve from experimental data (Juliano 1993). Linear terms not significantly different from 0 indicate a type I functional response, a significant negative linear coefficient indicates a type II response, and a significant positive linear term indicates a type III response (Juliano 1993). To determine the significance of the linear term from polynomial equation 1, the log likelihood-ratio test was used to determine whether equation coefficients differed significantly from 0 (Trexler and Travis 1993). In this test, the difference in log-likelihoods is a  $\chi^2$  with 1 df.

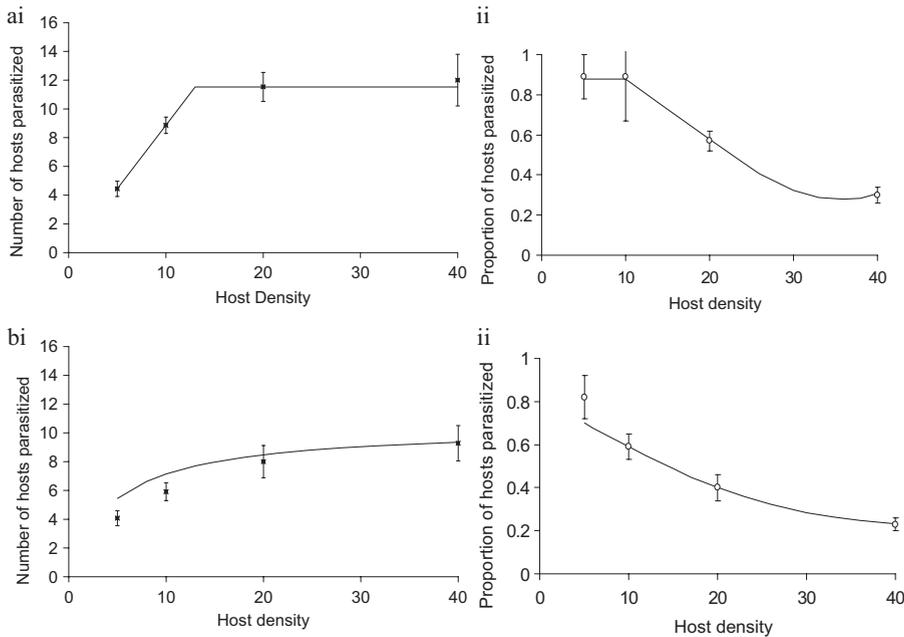


Fig. 1. Functional responses of (a) *G. ashmeadi* and (b) *G. tuberculifemur* at 26°C. Mean number ( $\pm$ SE) of *H. vitripennis* eggs parasitized (i) and the mean ( $\pm$ SE) proportion of host eggs parasitized (ii).

Where the cubic equation resulted in a nonsignificant cubic parameter, in the interest of simplicity, the model was reduced by eliminating the cubic term and the other parameters were retested (Juliano 1993).

Once the functional response type was determined from logistic regression and log likelihood-ratio tests, the functional response data were fitted. For *G. tuberculifemur*, the parameters of a type II model were estimated using the iterative nonlinear least squares regression to fit the random predator equation 2 (Rogers 1972):

$$N_a = N_0[1 - \exp[a(T_h N_a - T)]] \quad [2]$$

where  $N_a$  is the number of parasitized hosts,  $N_0$  equals the initial host density,  $a$  is the attack constant,  $T_h$  equals the handling time, and  $T$  is the total time available for parasitism.

The logistic regression and log likelihood-ratio test results suggested that *G. ashmeadi* demonstrated a type I functional response (see Results). To ensure a type II model was not a more appropriate model for predicting the functional response of *G. ashmeadi*, a type II functional response was fitted to the number of eggs parasitized at each density using both the random predator equation 2 (Rogers 1972) and the random parasitoid equation 3 (Royama 1971, Kafle et al. 2005).

$$N_a = N_0[1 - \exp(-aT/1 + aT_h N_0)] \quad [3]$$

It was hypothesized that the random parasitoid equation may be better at detecting a significant type II model, if one existed, because female *G. ashmeadi* may not discriminate between unparasitized and recently

parasitized hosts and could therefore engage in self-superparasitism at low host egg densities (Chen et al. 2006). The significance of each constant ( $a$  and  $T_h$ ) was determined using 95% confidence intervals (Juliano 1993).

*Gonatocerus ashmeadi* showed a type I functional response (see Results). To estimate the parameters for a type I model, piecewise regression (Ryan and Porth 2007) was used. The upper limit was visually detected using Fig. 1ai (see results), and piecewise regression (Ryan and Porth 2007) was performed on the number of eggs parasitized at densities that resulted in parasitism below the upper limit (densities < 13). Linear regression was used to confirm that the second part of the type I response (for densities above the upper limit [densities 13–40]) approximated a straight line and the slope did not differ significantly from zero.

If two parasitoid species have a type II functional response curve, the attack constant ( $a$ ) and handling time ( $T_h$ ) can be compared using Proc NLIN and separation of statistically different parameter estimates made using 95% confidence intervals (Juliano 1993). However, results derived here suggested that *G. ashmeadi* had a type I response curve, whereas *G. tuberculifemur* had a type II response curve (see results). In this case, parameters cannot be compared between species because there is no  $T_h$  in the type I model, and comparing the attack constant from a type II and slope from a type I has questionable biological significance (S. A. Juliano, personal communication).

Consequently,  $t$ -tests were performed in SAS (SAS Institute 1990) to compare the mean number of *H. vitripennis* eggs parasitized by *G. ashmeadi* and *G.*

**Table 1.** Results of logistic regression analyses of the proportion of *H. vitripennis* eggs parasitized by *G. ashmeadi* or *G. tuberculifemur* compared with the initial host numbers offered in the laboratory at 26°C

Parameter	Estimate ± SE	df	$\chi^2$ value	P
<i>G. ashmeadi</i>				
Intercept	-0.235 ± 1.450	1	0.03	0.87
Linear	0.474 ± 0.291	1	2.66	0.10
Quadratic	-0.032 ± 0.016	1	4.26	0.04
Cubic	0.0005 ± 0.0002	1	4.67	0.03
<i>G. tuberculifemur</i>				
Intercept	1.391 ± 0.382	1	13.26	<0.01
Linear	-0.115 ± 0.036	1	10.37	<0.01
Quadratic	0.001 ± 0.001	1	3.40	0.06
Cubic	—	—	—	—

*tuberculifemur* for each host egg density to determine whether significant differences in parasitism between parasitoid species at each host density existed. One-way analysis of variance (ANOVA) was used to determine the effect of density on the proportion of mortality for controls. Finally, percentage of female offspring data was transformed (Box Cox 3) to normalize distribution, and transformed data were compared between species using *t*-test in SAS (SAS Institute 1990). Means presented here are back-transformed.

## Results

**Functional Response Determination for *G. ashmeadi*.** *Gonatocerus ashmeadi* exhibited a type I functional response because results from logistic regression and log likelihood-ratio tests showed that quadratic and cubic coefficients were significantly different from 0, whereas the linear term was not significant (Table 1) (Juliano 1993). The proportion of *H. vitripennis* eggs parasitized by *G. ashmeadi* remained constant for host egg densities 5 and 10 and then decreased (Fig. 1a<sub>ii</sub>). This observed trend is consistent with a type I functional response (Juliano 1993). The instantaneous attack rate (*a*) and handling time ( $T_h$ ) for *G. ashmeadi* as estimated by the random predator equation was 7.22/h and 0.08 h, respectively (Table 2). Handling time was significantly different from zero, whereas attack rate (*a*) was not significantly different from zero. The latter result,  $a \approx 0$ , confirmed that type II is not an appropriate model for describing the functional response of *G. ashmeadi* under these experimental conditions. Furthermore, when the random parasitoid equation (equation 3) was used to estimate handling time and instantaneous attack rate for *G. ashmeadi*, the iterative methods failed to converge, and no results were obtained for these parameters with this model, thereby further confirming the appropriateness of the type I functional response model.

Linear regression analyses for host egg densities <13 was significant ( $y = 0.88x + 0.02$ ,  $F = 26.90$ ,  $df = 1, 23$ ,  $P < 0.0001$ ,  $R^2 = 0.55$ ), confirming that type I is the best model for predicting the functional response of *G. ashmeadi* within this range. Piecewise regression indicated that a breakpoint (upper limit) of 13 hosts

**Table 2.** Attack rate (*a*) and handling time ( $T_h$ ) of the functional response of female *G. ashmeadi* and *G. tuberculifemur* to densities of *H. vitripennis* eggs conducted in the laboratory at 26°C

Parameter	Estimate ± SE	Approximate 95% confidence intervals	
<i>G. ashmeadi</i>			
<i>a</i> ( $h^{-1}$ )	7.217 ± 5.240	-3.319	17.754
$T_h$ (h)	0.078 ± 0.008	0.060	0.095 <sup>a</sup>
<i>G. tuberculifemur</i>			
<i>a</i> ( $h^{-1}$ )	2.280 ± 1.105	0.058	4.500 <sup>a</sup>
$T_h$ (h)	0.096 ± 0.014	0.069	0.123 <sup>a</sup>

<sup>a</sup> Means show significance at the 0.05 level.

was significant for the type I response curve exhibited by *G. ashmeadi* ( $t = -3.33$ ,  $P < 0.01$ ). Piecewise regression above the upper host limit of 13 eggs was confirmed to be a straight line ( $t = 0.25$ ,  $df = 1$ ,  $P = 0.81$ ). These results indicate that the number of *H. vitripennis* eggs parasitized by *G. ashmeadi* in 1 h increased linearly to a maximum of 11.5 at a density of 13 hosts and then remained constant (Fig. 1a<sub>i</sub>). Consequently, it was estimated that, on average, each female *G. ashmeadi* had the potential to attack a maximum of 11.5 *H. vitripennis* eggs over the 1-h period regardless of whether available host egg densities exceeded 13 hosts.

**Functional Response of *G. tuberculifemur*.** *Gonatocerus tuberculifemur* exhibited a type II functional response because the linear term of equation 1 was both negative and significantly different from 0, indicating that the proportion of *H. vitripennis* eggs parasitized decreased significantly as egg density increased (Table 1; Fig. 1b<sub>ii</sub>) (Juliano 1993). The number of *H. vitripennis* eggs attacked and parasitized by *G. ashmeadi* increased at a decreasing rate until reaching an upper plateau with increasing *H. vitripennis* density (Fig. 1b<sub>ii</sub>). Each female *G. tuberculifemur*, on average, had the potential to attack 9.3 *H. vitripennis* eggs over the experimental 1-h exposure period. The instantaneous attack rate (*a*) and handling time ( $T_h$ ) for *G. tuberculifemur* as estimated by the random predator equation was 2.28/h and 0.10 h, respectively, and these parameters were significantly different from zero (Table 2).

**Comparing Mean Parasitism and Percentage Female Offspring Between Parasitoid Species.** Comparing the mean number of *H. vitripennis* eggs parasitized between species showed that female *G. ashmeadi* parasitized on average 4.3 and 3.5 more eggs per hour when presented with 10 and 20 eggs, respectively, compared with *G. tuberculifemur* (Table 3). When 5 or 40 *H. vitripennis* eggs were presented to parasitoids, there was no significant difference in mean parasitism rates between species (Table 3). One-way ANOVA indicated that there were no significant differences in *H. vitripennis* egg mortality in control vials with varying egg densities that lacked exposure to parasitoids (mean range = 28–31%;  $F = 0.05$ ,  $df = 3, 53$ ,  $P = 0.99$ ). Percentage of female offspring was significantly 5% higher for *G. ashmeadi* (mean =  $84 \pm 2\%$ ) compared with *G. tuberculifemur* ( $79 \pm 2\%$ ;  $t = 2.14$ ,  $df = 96$ ,  $P < 0.05$ ).

**Table 3.** Mean no.  $\pm$  SE of *H. vitripennis* eggs parasitized by female *G. ashmeadi* and *G. tuberculifemur* when offered four different host densities in the laboratory at 26°C

Density	<i>G. ashmeadi</i>	<i>G. tuberculifemur</i>	<i>t</i> -value <sup>a</sup>	df	<i>P</i>
5	4.44 $\pm$ 0.53	4.07 $\pm$ 0.52	0.48	20	0.64
10	8.87 $\pm$ 0.57	4.52 $\pm$ 0.62	3.44	24	0.01
20	11.53 $\pm$ 1.01	8.00 $\pm$ 1.12	2.33	28	0.03
40	12.00 $\pm$ 1.79	9.29 $\pm$ 1.23	1.29	21	0.21

<sup>a</sup> Test statistics reported from *t*-tests conducted to compare parasitism between species at each density.

## Discussion

**Response Curves for Individual Species.** *Gonatocerus tuberculifemur* showed a type II functional response where parasitism increased at a decreasing rate as host density increased, reaching an asymptote that reflected an upper processing limit of 9.3 hosts/h. In contrast, *G. ashmeadi* showed a type I functional response where the number of hosts attacked followed a constant linear increase regardless of host density below the upper limit of 11.5 hosts processed per hour. The main difference separating a type I and type II functional response is the handling time of the natural enemy across varying host densities. Handling time refers to host processing time and includes time spent searching, antennating, drilling, ovipositing, resting, and grooming. For natural enemies showing a type II response, handling time increases across increasing host densities, which reduces the time available for searching (Hassell 1978). In contrast, a type I functional response implies that, as host densities increase, natural enemy handling time is constant and is not significantly affected as patch size increases (Hassell 1978). In the laboratory at 26°C, visual observations of female *G. ashmeadi* foraging for *H. vitripennis* egg masses indicated that parasitoids spend  $\approx$ 100–300 s ovipositing and preparing for the next oviposition event (N.I. and M.H., unpublished data). The random predator equation used in this study estimated handling time for female *G. ashmeadi* as being 281 s, which is within the observed range for *G. ashmeadi* under experimental conditions.

Mills and Lacan (2004) noted that, for *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) egg parasitoids and the whitefly parasitoids *Eretmocerus* sp. (Hymenoptera: Aphelinidae) that exhibit type I functional responses, the following characteristics are shared: (1) these natural enemies search for hosts by walking, (2) they attack exposed and immobile hosts, and (3) they can use behavioral mechanisms to minimize superparasitism. Additionally, the parasitoid's type I search strategy must be independent of host density and therefore unchanged by experience and physiological status (Mills and Lacan 2004). *G. ashmeadi* shares the first two characteristics listed above (N.I., unpublished data). Chen et al. (2006) showed that superparasitism by *G. ashmeadi* occurred at all host densities ranging from ratios of 10 females:10 hosts to 10:250 within a 48-h exposure period and therefore suggested that *G. ashmeadi* lacked

the ability to discriminate between unparasitized and hosts previously parasitized by conspecifics. However, given that the egg load of newly emerged *G. ashmeadi* is  $\approx$ 30 eggs (Irvin and Hoddle 2008) and the 10:250 ratio used in Chen et al. (2006) is equivalent to 12.5 hosts per female in 24 h, it is conceivable that females may have been forced to superparasitize during prolonged exposure to parasitized hosts, even if they possess the ability to discriminate between parasitized and unparasitized hosts. In this study, female *G. ashmeadi* were caged singly for a short period of time (i.e., 1 h), and it is unknown whether female *G. ashmeadi* can discriminate between unparasitized hosts and self-parasitized hosts when given a choice between host eggs in either of these two states.

Both *G. ashmeadi* (type I functional response) and *G. tuberculifemur* (type II) showed an upper limit to host processing either because of limited numbers of eggs for oviposition or they are unable to attack more hosts because of handling time limitations within the 1-h search period. Mymarid egg parasitoids are considered to be proovigenic where females emerge from hosts with a full complement of mature eggs (Jervis et al. 2001). However, Irvin and Hoddle (2008) recently showed that *G. ashmeadi* is synovigenic. Dissection of female *G. ashmeadi* immediately on emergence indicates that females have  $\approx$ 30 mature eggs in their ovaries for oviposition (Irvin and Hoddle 2008). This complement of  $\approx$ 30 mature eggs on birth would suggest that female *G. ashmeadi* used in this study only oviposited 60% of their egg load within the experimental 1-h time period. Therefore, in this study, females may have been time limited, unless self-superparasitism by female *G. ashmeadi* resulted in the oviposition of more eggs into hosts than the number of offspring recorded indicated. Chen et al. (2006) reported that superparasitism at low host densities may cause functional response studies to underestimate oviposition and attack rates because all of the oviposited eggs are not accounted for because only one parasitoid emerges from a superparasitized host egg that contains more than one parasitoid egg. *G. ashmeadi* superparasitized 25% of *H. vitripennis* egg masses at ratios 10 females:250 *H. vitripennis* when given a 48-h exposure time (Chen et al. 2006). Although these ratios are similar to the 1 *G. ashmeadi*:20 *H. vitripennis* eggs and 1:40 ratios used in this study, superparasitism may be less likely in this study because hosts were exposed to parasitoids for only 1 h.

In contrast to results presented here, Chen et al. (2006) described a type II functional response for *G. ashmeadi* attacking *H. vitripennis* eggs over a 24-h period at 22°C. However, similar to this study, Chen et al. (2006) also reported that the linear coefficient resulting from fitting the polynomial function to data for eggs 1 d of age was not significantly different from zero, suggesting that a type II functional response may not be the most appropriate model for describing the functional response of *G. ashmeadi*. Furthermore, Chen et al. (2006) did not report whether the attack constants for *G. ashmeadi* were significantly different from zero. In light of results obtained in this study, we

suggest that the data presented in Chen et al. (2006) portrays the linear part of a type I functional response and that host egg densities used in experiments were not high enough to detect the upper processing limit for this parasitoid when offered a 24-h exposure time. In this study, inclusion of a greater range of host densities between 0 and the upper limit of 11.5 hosts would have increased the number of data points describing the attack trajectory as it approached saturation.

**Comparing Efficiency Between Species.** Comparing the mean number of *H. vitripennis* eggs parasitized between parasitoid species showed that *G. ashmeadi* significantly outperformed *G. tuberculifemur* at host densities of 10 and 20 eggs but not at 5 or 40 host eggs, even though a higher proportion of eggs were attacked by *G. ashmeadi*. Female *G. ashmeadi* also produced a higher percentage (5%) of female offspring compared with *G. tuberculifemur*. Additionally, functional response data indicated that female *G. ashmeadi* exhibited an upper oviposition limit of 11.5 host eggs in a 1-h search interval, making this species more effective than *G. tuberculifemur*, which showed an upper parasitism limit of 9.3 host eggs in 1 h ( $\approx 24\%$  fewer eggs attacked compared with *G. ashmeadi*). Consequently, under the experimental conditions used in these evaluations, results suggest that *G. tuberculifemur* is likely an inferior parasitoid to *G. ashmeadi*. Hosts and parasitoids were spatially confined in this study, which removed the parasitoid's need to locate the host plant. These conditions are highly conducive to parasitism and may not represent the field environment (Legaspi et al. 1996). The temperature range over which the parasitoid must find hosts also affects functional response (Kafle et al. 2005, Kalyebi et al. 2005, Zamani et al. 2006). Furthermore, these functional response studies failed to directly assess patch residency times, egg load, host egg age preference, and aggressive behavior, all of which may affect parasitoid efficacy and competitiveness. However, completed studies investigating host patch behaviors have shown *G. ashmeadi* to be a more aggressive competitor when foraging concurrently with *G. tuberculifemur* (Hoddle and Irvin 2007). Aside from these limitations, functional response studies may provide a useful first step for comparing the efficiency of different species (Overholt and Smith 1990).

**Conclusions on Using *G. tuberculifemur* as a New Association Biological Control Agent for *H. vitripennis* Control in California.** There is substantial uncertainty whether *G. tuberculifemur* could provide significant additional control of *H. vitripennis* if established in California. Furthermore, "new-association" biological control of *H. vitripennis* with *G. tuberculifemur* raises concerns about potential unwanted impacts on native nontarget species of sharpshooters that are sympatric with *H. vitripennis* in invaded areas and the home range of this pest. In studies carried out in the United States, *G. tuberculifemur* successfully parasitized eggs of two native nontarget sharpshooter species *H. liturata* Ball and *Oncometopia* sp. (both Cicadellinae: Proconiini).

Study of the field host range in the area of origin of *G. tuberculifemur* in Argentina showed that this parasitoid successfully parasitized at least five species of Cicadellini, a tribe to which *H. vitripennis* does not belong (Jones et al. 2005). These laboratory and field results suggest that *G. tuberculifemur* is polyphagous and may successfully exploit nontarget sharpshooter species in the tribes Proconiini and Cicadellini if susceptible host species are encountered.

Should *G. tuberculifemur* be established in California, there is a possibility that *G. tuberculifemur* could inadvertently spread into areas outside of this state, possibly through interstate commerce of ornamental plants bearing parasitized *H. vitripennis* egg masses on leaves. The significance of the potential incursion, the magnitude of foodweb perturbations, and the ecosystem impacts posed by *G. tuberculifemur* to native sharpshooters and their associated egg parasitoids outside of California is completely unknown but could cause considerable concern for ecologists and taxonomists studying native ecosystems and documenting nontarget impacts resulting from biological control programs.

In conclusion, this study showed that *G. tuberculifemur* failed to outperform the dominant resident *H. vitripennis* parasitoid, *G. ashmeadi*. Laboratory testing studying aspects of host egg age preferences, the competitive ability of *G. tuberculifemur* when simultaneously foraging with *G. ashmeadi* on *H. vitripennis* egg masses in simple and complex environments, and aggressive behavior between these two species when competing for host eggs has been completed and will provide additional information as to whether *G. tuberculifemur* should be considered a new association biological control agent of *H. vitripennis*.

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