PARASITISM OF THE GLASSY-WINGED SHARPSHOOTER: FUNCTIONAL RESPONSES AND SUPER-PARASITISM BY THE EGG PARASITOID *GONATOCERUS ASHMEADI.*

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ABSTRACT
The functional responses and super-parasitism by the egg parasitoid, *Gonatocerus ashmeadi*, on *Homalodisca coagulata* eggs were related to host age and density when studied under laboratory conditions. Parasitism of Glassy-winged Sharpshooter (GWSS) eggs, 1-, 3-, 5-, 7- and 9-d-old, was measured at 22 ± 1ºC and under 10L:14D regime. For each host age, 10-60 eggs were exposed to an individual parasitoid for 24 h. The functional responses for the parasitoids to host eggs of all age groups most closely fit the type II and III models of Hollings (1959) and Hassell (1978) which relate to the elapsed time for accomplishing the behavioral events associated with parasitism of the host as modified by host density. The instantaneous attack rate by parasitoids on 1-d-old host eggs, as specified in the type III model, was significantly greater from that of the other ages. This rate was also greater in the type II model but was not statistically significant. The total number of host eggs parasitized varied significantly with host density and age of the eggs, but not when analyzed by a host x density interaction. Host age and density, as well as the host x density interaction, contributed significantly to the differences found in length of development time of *G. ashmeadi* within host eggs. The wasps exhibited a tendency towards super-parasitism at relatively high parasitoid-to-host ratios. The maximum number of parasitoid eggs found in a single host egg was 18. The development time and eclosion of the parasitoids had no correlation with parasitoid-to-host ratios. Frequencies of super-parasitism for *G. ashmeadi* displayed an aggregated distribution over all observed host densities.

INTRODUCTION
The effectiveness of parasitoids in regulation of a pest population is highly dependent on their ability to search for and handle hosts in a varying ecosystem. This effectiveness has been traditionally related to the functional response of a parasite or predator (Hassell 1978, Fujii *et al.* 1986). The functional response is defined as the relationship between the numbers of prey taken by the predator as a function of prey density (Holling 1959). The functional response is an essential component of the dynamics of host-parasitoid relationship, and is an important determinant of the stability of the system (Oaten and Murdoch 1975). Functional response analyses are commonly used to help predict the potential for parasitoids to regulate host population (Solomon 1949, Oaten and Murdoch 1975). Successful parasitoids have the ability to discriminate among parasitized eggs, avoid super-parasitism and minimize the waste of time and energy associated with their searching and parasitizing behaviors (Godfray 1994). However, under certain circumstances, superparasitism might be adaptive (van Alphen & Visser 1990). Further, when mass-rearing solitary parasitoids for use in an augmentative release program, super-parasitism represents a waste of the production colony's potential output. This report presents the progress on investigations determining certain aspects of the functional responses and super-parasitism by the parasitoid, *G. ashmeadi*.

OBJECTIVES
1. Investigate the response of *G. ashmeadi* to GWSS eggs of different ages and determine the effects of host egg age on functional response parameters and parasitism.
2. Determine effect of host densities and ages with respect to developmental time of wasps.
3. Investigate relationship between super-parasitism by the wasp at different host densities and effect of super-parasitism on wasp emergence and development time.

RESULTS
Functional Responses
There was a significant increase in the numbers of *H. coagulata* eggs of different ages parasitized by egg parasitoid, *G. ashmeadi,* with an increase in host density (Table 1). At the host densities of 40, 50, and 60, the numbers of eggs parasitized were significantly higher than that of relatively low densities of 10 and 20 over all host ages. The number of 1-d-old eggs parasitized was slightly greater than that of 5-, 7- and 9-d–old-eggs. A two-way ANOVA, with age and density as factors, revealed that the number of eggs parasitized varied significantly with host age (F = 3.64, df = 4,299, P = 0.007) as well as host densities (F = 88.43, df = 5,299, P < 0.0001). There was no significant effect of age × density interaction on the number of host eggs parasitized (F = 0.44, df = 20, 299, P = 0.899).

The functional responses of *G. ashmeadi* parasitizing host eggs at the various ages showed that the shape of the functional response curves were affected by differences in the parasitization rates of *G. ashmeadi*. At all host ages, the *G. ashmeadi* functional response data most closely fit the type II and III models. Coefficients of determination (r^2 values) for type II and III curves were very similar (Table 2). The instantaneous attack rates (a) and handling time (T_h) estimated from type II functional response models varied slightly but were not significantly different among host ages (Table 3). The *a* value for 1-d-old eggs was slightly higher than that for other ages when data were fit to a type II functional response model. The estimate for handling time (time spent on eggs) by the wasps for all host egg ages did not vary significantly. When the data were fitted to a type III functional response model, the *a* value estimated for 1-d-old eggs was significantly higher than that for host eggs of 3-, 5-, 7- and 9-d-old. However, the handling time of *G. ashmeadi* for all egg ages was similar, ranging from the value of 0.032 to that of 0.040.

Effect of Host age on Parasitoid Development Time

The development time of *G. ashmeadi* within host eggs varied significantly with host density and host age (Table 4). Within the 1-, 3-, 5-, 7- and 9-d-old host eggs, the mean development time (± SE) of the parasitoid was 16.0 ±1.0 d (n = 1435), 18.9 ± 1.8 d (n = 996), 18.3 ± 1.5 d (n = 1181), 17.6 ± 1.2 d (n = 961) and 17.8 ± 1.5 d (n = 1254), respectively. The parasitoid within 1-d-old sharpshooter eggs developed significantly faster than that within other ages (F = 766.41, df = 5, 5826, P < 0.0001). A two way ANOVA further showed that host age (F= 999.47, df = 4, 5826, P < 0.0001) and density (F = 58.26, df = 5, 5826, P < 0.0001) contributed significantly to the development time of *G.ashmeadi*. The significant interactive effect on development time occurred between host age and density (F = 62.82, df = 20, 5826, P < 0.0001).

Super-parasitism.

Maximum number of parasitoid eggs in one host egg was 18. The level of super-parasitism of *G. ashmeadi* (Table 5) varied significantly with increasing host density (F = 225.17, df = 5,549, P < 0.0001). The mean number of parasitoid eggs per sharpshooter egg at 1:1 parasitoid-to-host ratio is significantly greater than that at other ratios. When the parasitoid-to-host ratio increased to > 1:15, host eggs pooled from each host density were almost all parasitized. There was a significant positive correlation between the number of parasitoid eggs per host egg and parasitoid-to-host ratio (F = 1231.69, df = 548, r = 0.8319, r^2 = 0.692, P < 0.0001). *G. ashmeadi* is a solitary parasitoid and normally only one wasp emerges from each egg of its host. In treatments with high host densities such as at 1:1 and 1:5 parasitoid ratios, the percentage of parasitoid eclosion was significantly higher than in low-density treatments (F = 3.996, df =4,243, P = 0.004)(Table 5). However, there is no correlation between parasitoid-to-host ratio and percentage of parasitoid eclosion (F = 3.29, df = 242, r = 0.1140, r^2 = 0.013, P = 0.071). Although there was a significant statistical difference in development time of the parasitoid within the host egg among different parasitoid-to-host ratios (F = 46.851, df = 4, 1862, P < 0.0001), the maximum difference was only about 0.7d.

For *G. ashmeadi*, x^2 goodness-of-fit analyses of parasitoid egg numbers per host egg revealed that frequencies of super-parasitism were significantly different from the expected Poison distribution over all host densities (x^2 = 231.291, df=4, P < 0.0001). The relationship between the variances (S^2) and means (m) was described by Taylor's power law (Taylor 1961) as: log S^2 = -0.4384 + 1.0288 log$m$ (r^2= 0.604, df = 28, F = 42.78, P < 0.0001, where b = 1.0288 > 1, indicating an aggregated distribution of super-parasitism for *G. ashmeandi* over all experimental parasitoid-to-host ratios.

CONCLUSIONS

The studies on the functional responses of *G. ashmeadi* to GWSS eggs of different ages and densities in the laboratory have improved our understanding of the interactions between the parasitoid and host egg. Because this parasitoid fits the II and III functional response models in relation to different host ages, it further confirms that the wasp has the capacity of effectively parasitizing eggs throughout most of the embryonic development of the GWSS. Further, studies on super-parasitism of *G. ashmeadi* provide valuable information for the mass-rearing and field release of this parasitoid. Our results indicate that super-parasitism occurs when the parasitoid-to-host ratio is greater than 1:15. Super-parasitism results in a waste of the reproductive potential of this species because *G. ashmeadi* is a solitary-developing wasp and usually only one parasitoid emerges from one GWSS egg.

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Table 1. Parasitism by *G.ashmeadi* on *H. coagulata* eggs of different ages at varying densities.

	Mean No. Parasitized (SE)				
Density	**1d**	**3d**	**5d**	**7d**	**9d**
10	9.5(1.3) a	8.7(2.2) a	8.9(1.6) a	9.0(2.2) a	9.1(1.1) a
20	18.1(1.6) b	15.5(3.2) b	14.8(3.4) b	14.6(3.9) ab	14.7(3.3) ab
30	22.9(3.0) c	17.9(8.4) b	22.0(5.8) c	19.8(7.7) bc	18.7(4.1) b
40	26.5(4.7) cd	22.2(9.8) bc	25.1(7.3) cd	22.7(5.6) c	25.8(6.2) c
50	30.3(7.5) d	25.6(10.0) cd	29.4(5.1) de	23.9(11.9) cd	29.5(13.1) c
60	34.8(4.7) e	30.7(6.9) d	32.2(4.5) e	29.9(7.3) d	30.1(3.4) c
	$F = 43.12$ df = 5,59 $P < 0.0001$	$F = 11.02$ df = 5,59 $P < 0.0001$	$F = 31.69$ df = 5,59 $P < 0.0001$	$F = 10.59$ df = 5,59 $P < 0.0001$	$F = 16.96$ df = 5,59 $P < 0.001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 2. Coefficients of determination for functional response regression models of *G. ashmeadi* to *H. coagulata* eggs of different ages.

Age of Eggs (d) [a]	**Type I (r^2)**	**Type II (r^2)**	**Type III (r^2)**
1	0.7776	0.9729	0.9727
3	0.4979	0.8993	0.8992
5	0.7260	0.9607	0.9608
7	0.4783	0.9038	0.9036
9	0.5872	0.9280	0.9280

[a] *G. ashmeadi* targeted host densities ranged from 10 to 60 sharpshooter eggs per experimental container. Type I functional response model was evaluated using SAS PROC GLM whereas Type II and III models were evaluated using SAS PROC NILN to generate r^2 values indicating best fit.

Table 3. Type II and III functional response parameters of *G. ashmeadi* when parasitizing *H. coagulata* eggs of different ages.

Functional response model	**Host age (d)**	**Instantaneous attack rate (a ± SE)**[a]	**Handling time (T_h ± SE)**[a]
Type II	1	0.5782 ± 0.0626 a	0.0300 ± 0.0004 a
	3	0.4544 ± 0.0959 a	0.0315 ± 0.0105 a
	5	0.5013 ± 0.0640 a	0.0286 ± 0.0058 a
	7	0.5064 ± 0.1088 a	0.0377 ± 0.0099 a
	9	0.4831 ± 0.0849 a	0.0296 ± 0.0082 a
Type III	1	2.8131 ± 2.2011 a	0.0342 ± 0.0056 a
	3	1.0137 ± 0.5410 b	0.0333 ± 0.0117 a
	5	1.4394 ± 0.6301 b	0.0316 ± 0.0067 a
	7	1.3858 ± 0.9508 b	0.0403 ± 0.0113 a
	9	1.2495 ± 0.6620 b	0.0322 ± 0.0094 a

[a] Instantaneous attack rate (*a*) and handling time (T_h) estimated by SAS PROC NLIN and pairwise compared among host ages using indicator variable (0 or 1) for age.

Table 4. Development time of *G. ashmeadi* within *H. coagulata* eggs of different ages when parasitized at varying densities.

Density	Development time (SE) at age:				
	1d	3d	5d	7d	9d
10	15.9(0.6) d	21.0(2.1) a	17.9(1.6) c	15.7(1.4) e	17.6(1.4) c
20	16.5(0.8) a	18.5(1.6) c	18.0(1.1) c	18.3(0.9) a	18.3(0.8) b
30	16.5(0.7) b	18.6(2.2) c	18.8(1.3) b	18.1(0.9) ab	19.1(1.2) a
40	16.1(0.8) c	18.3(2.2) c	17.8(1.5) c	18.0(1.0) bc	16.9(1.6) d
50	16.0(1.4) cd	18.6(1.5) c	19.5(1.2) a	17.8(0.6) c	17.4(1.4) c
60	15.5(0.7) e	19.4(1.0) b	17.4(1.0) d	17.2(1.2) d	18.1(1.2) b
	$F = 45.39$ df = 5,1434 $P < 0.0001$	$F = 37.00$ df = 5,995 $P < 0.0001$	$F = 88.13$ df = 5,1180 $P < 0.0001$	$F = 84.08$ df = 5,960 $P < 0.0001$	$F = 73.93$ df = 5,1253 $P < 0.0001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 5. Number (mean ±SE) of G. ashmeadi eggs per host egg, percentage of emergence and development time at different parasitoid-to-host egg ratios.

Parasitoid-host ratio	No. parasitoid / host		% Emergence		Development time	
	N_1	Mean ±SE	N_2	Mean ±SE	N_3	Mean ±SE
1:1	50	10.40 ± 4.86 a	NA	NA	NA	NA
1:5	100	3.02 ± 1.69 b	11	97.6 ± 1.7 a	141	18.02 ± 0.07a
1:10	100	2.24 ± 1.16 c	15	98.9 ± 0.6 a	136	18.20 ± 0.06 b
1:15	100	1.66 ± 0.89 d	77	93.6 ± 1.5 b	490	18.30 ± 0.04 b
1:20	100	1.20 ± 0.59 d	70	91.7 ± 1.0 b	263	18.25 ± 0.04 b
1:25	100	1.15 ± 0.58 d	71	90.0 ± 1.7 b	833	18.77 ± 0.03 c

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan). N_1 represents the number of dissected host eggs, N_2 represents the number of egg masses observed, and N_3 is the number of parasitoid emerging from host eggs.

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