MORTALITY INDUCED ON BOLL WEEVIL LARVAE BY *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE) FEMALES AT DIFFERENT DEGREES OF CONDITIONING

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Abstract

Studies were conducted to determine the effects of the different conditioning of exotic ectoparasitoid *Catolaccus grandis* (Burks) females on their oviposition activity and mortality of the host [the boll weevil, *Anthonomus grandis grandis* (Boheman)]. Eggs are laid and a feeding tube is formed only by conditioned (post-preovipositional period and exposed to the host for a 2-d period) or unconditioned (post-preovipositional period but no exposure to host) parasitoid females. Feeding-tube formation without oviposition occurs when newly emerged (ca. 10 h post-emergence) females discover their first host larvae. *Catolaccus grandis* conditioned females are oviposited more actively in time of attacked boll weevil larvae than unconditioned females (66.7% versus 29.2%). Laboratory studies showed that reproductive mature *C. grandis* females induced significantly higher levels of mortality on boll weevil larvae than newly emerged females. The highest survival of parasitoids to the pupal stage was observed in host larvae where the *C. grandis* females oviposited (60.0%). Venom extracts from newly emerged females were significantly less effective paralyzing and killing host larvae than those from mature conditioned or unconditioned females. This may indicate that the venom activity on newly emerged females is not completely developed. The venom extracts of conditioned and unconditioned mature females were equally effective inducing paralysis and death on boll weevil larvae. The injection of venom to paralyze the host and the oviposition of *C. grandis* females are, in many cases, independent processes.

Introduction

The exotic ectoparasitoid *Catolaccus grandis* (Burks) has been reported as an effective biological control agent against the boll weevil, *Anthonomus grandis grandis* (Boheman), on cotton (King et al. 1993). *Catolaccus grandis* has been reared in the laboratory on 3rd instar boll weevils (Cate 1987, Morales-Ramos et al. 1992, Roberson and Harsh 1993). Augmentative releases of this parasitoid have suppressed boll weevil populations in small plots in Lower Rio Grande Valley and in commercial cotton fields of Texas (Summy et al. 1992, 1994, 1995; King et al. 1995; Coleman et al. 1996). The economic feasibility of implementing augmentative releases for biological control of the boll weevil is dependent, in part, on developing suitable mass propagation technology for it. Efficient mass rearing of *C. grandis* requires definition of the optimal conditions for growth, development, and reproduction. The further improvement for the mass propagation of *C. grandis* was based on studies of its biological parameters (Morales-Ramos and Cate 1992 a, b, c, 1993; Morales-Ramos et al. 1996; Greenberg et al. 1994, 1995 a, b; 1996 a, b), its venom and role in parasitoid development and host regulation (Morales-Ramos et al. 1995), and the development of an artificial diet (Rojas et al. 1995, 1996 a,b).

According to existing technology, newly emerging parasitoid females are maintained in the laboratory for 5 days before releasing in the field (ca. 3-d preovipositional period plus ca. 2- d exposure to the host for conditioning). These procedures make the system more complex and the final product more expensive. However, experimental data regarding mortality induced by *C. grandis* females on boll weevil larvae during the preovipositional and conditioning periods are incomplete. Information regarding the initiation of venom production in the emerging females, and when the females begin to paralyze the host, is incomplete.

The objectives of this study were to determine (1) the influence of age and conditioning of *C. grandis* females on the degree of parasitism and mortality of the host; and (2) when parasitoid females first produce venom and begin to paralyze the host.

Materials and Methods

Catolaccus grandis was reared on 3rd instar boll weevils enclosed in Parafilm® capsules. The capsules were constructed by impressing the surface of a Parafilm® sheet between 2 aluminum moulds, one having 0.8-cm diameter holes, and the other with 1.4-cm diameter aluminum pegs. This process resulted in Parafilm sheets, each containing 120-132 capsules. One host larva was placed inside each capsule; after this, the sheet containing the larva was sealed with a flat Parafilm® sheet (Cate 1987). The parasitoids were reared as reported by Morales-Ramos et al. (1992). The parasitoids were held at 27±1°C, 65±5%, and a photoperiod of 12:12 (L:D) h. These conditions were used in all of the experiments.

Experiment 1. Probing and oviposition activity

Three groups of mated *C. grandis* females were used. Group 1. Newly emerged females (6 replications), which were in their preovipositional period (ca. 10-h old). Group 2. Unconditioned females (6 replications), which were in post-preovipositional period but no expose to host (3-d old). Group 3 (control). Conditioned females (5-d old, 4 replications), which were in post-preovipositional period (3-d) and daily exposed to host for a 2-d period (10 third instar

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boll weevils per female). Ten females were used per replication.

Parasitoid females were daily provided with distilled water and honey. Once females from each group reached the right age and conditioning, each female was placed in a square Petri dish and provided with 10 encapsulated third instar boll weevils for 24 h. At the end of this period the Parafilm® capsules were opened and inspected under microscope. The number of capsules with *C. grandis* eggs or only with ovipositor punctures were counted and recorded.

Experiment 2. Induced boll weevil mortality

Encapsulated third instar boll weevils were exposed for 24 h to 3 groups of *C. grandis* females as described above (660 weevils for 132 parasitoid females per each group). After exposure, the parasitoid eggs from the capsules were removed. A control group consisting of the same number of encapsulated weevils was maintained without contact to parasitoid females. The number of live boll weevils (to pupal stage), number of paralyzed by *C. grandis* females, and number of dead weevils were counted and recorded. The total number of boll weevil larvae inspected was 2,640.

Boll weevil larvae classified as live were those able to move their body, continued development, and reached the pupal stage after 3-d. Larvae were classified as paralyzed, even though capable of some movement when touched by a dissecting pin, if they failed to continue development and became flaccid by the third day after contacted by the parasitoid females. Host-feeding events were identified by the presence of feeding tubes or by wounds (black spots) on the weevil integument, or both. Most of these larvae did not change their colour but they became wrinkled and flaccid.

Experiment 3. Survival of *C. grandis* on healthy and stung boll weevil larvae

Three groups of encapsulated boll weevils (120 third instars and 5 replications per group) were used: group 1, the boll weevil larvae from capsules with *C. grandis* eggs; group 2, the boll weevil larvae from capsules with *C. grandis* ovipositor punctures; and group 3 (control), boll weevil larvae without contact to parasitoids. Host larvae from each group and parasitoid eggs (at the beginning of hatching) were manually transferred to a new Parafilm® capsules (host larva and parasitoid egg per capsule). Thereafter, the boll weevil larvae of the different groups with accompanying parasitoid eggs were sealed, and parasitoid larvae were allowed to develop (at 27 ± 1°C) to the dark yellow-colored pupal stage (Morales-Ramos and Cate 1992a). Finally, the number of live hosts and parasitoid pupae were counted and recorded.

Experiment 4. Effect of abdomen extracts of different groups of *C. grandis* females on the host survival

Water soluble components were extracted from samples of 300 *C. grandis* females per treatment. The contents were homogenized for 2 min in 25-ml water using a Virtishear-10 micro-homogenizer at a speed setting of 70 (15,000 RPM). Then homogenates were centrifuged at a speed of 7,500 RPM for 5-min using a Serval centrifuge. Finally the extracts were tested for toxicity to 3-rd instar boll weevil larvae. A micro-applicator (ISCO, Lincoln, NB) was used to inject the boll weevil larvae. The experimental treatments were as follows:

1. Control
   1.1. Third instar boll weevils injected with distilled water (10 replications);
   1.2. Third instar boll weevils without application of distilled water (10 replications).
2. Third instar boll weevils injected with extract from the abdomen of *C. grandis* conditioned, unconditioned, and newly emerged females (18 replication per each group of females).

Ten 3rd instars was used per replication. After the applications, the boll weevil larvae were allowed to develop to pupal stage. The number of live, paralyzed, or dead weevils were counted and recorded.

Statistical analyses were conducted using analysis of variance (ANOVA) and the Tukey studentized range honestly significant difference (HSD) test a = 0.05 level of significance (SAS Institute 1988).

Results and Discussion

Probing and oviposition activity

Conditioned *C. grandis* females deposited more eggs on or near boll weevil larvae than did unconditioned or newly emerged females (Fig. 1). When females were conditioned, the percentage (66.7 ± 7.4%) of encapsulated weevils with parasitoid eggs was significantly higher than that obtained from the other two groups of females - unconditioned (29.2 ± 9.4%) or newly emerged (0.0%) ($F=115.1$; df= 2, 13; $P=0.0001$). When females were unconditioned, we observed that 49.0 ± 6.4% of the Parafilm® cells had only ovipositor punctures (but without parasitoid eggs) compared with 29.7 ± 5.5% of cells with ovipositor punctures from conditioned and 23.8 ± 6.6 from newly emerged *C. grandis* females ($F=26.0$; df=2, 13; $P=0.0001$). On the other hand, conditioned females showed a significantly lower number of cells without parasitoid eggs or punctures (3.6 ± 4.1%) compared with 21.8 ± 3.5% unconditioned and 76.8 ± 6.6% to newly emerged females ($F=301.3$; df=2, 13; $P=0.0001$).

Host mortality

When conditioned *C. grandis* females deposited eggs on or near boll weevil larvae, 54.4% of them were paralyzed, 18.7% died due to host-feeding by parasitoid females, 22.2% of the host larvae died from other factors, and only 4.7% remained survival and developed to the pupal stage. It was also observed that when conditioned females only punctured the Parafilm® cells, the percentage (9.8%) of paralyzed host larvae was significantly lower than when

females oviposited (T=6.0; df=11; P=0.0002). When conditioned females only punctured Parafilm® cells, 39.6% died due to host-feeding, the percentage of dead weevils from other factors was 18.9%, and the percentage (31.7%) of live host was significantly higher than when females oviposited (T=2.6, df=11, P=0.024; T=3.5, df=11, P=0.005; and T=5.5, df=11, P=0.0001, respectively). A similar situation was observed with unconditioned females which oviposited on the host larvae or only punctured the encapsulated Parafilm® cells. When third instars were attacked by newly emerged of *C. grandis* females, we did not observe paralyzed host larvae. The weevil mortality from other factors was 24.6%, 22.1% died due to host-feeding, and 53.3% of them survived. In the control group (weevils without contact to parasitoid females) 73.6% of the host larvae reached the pupal stage and 26.4% died (Table 1).

On basis of the obtained data from experiments 1 (oviposition activity of the different conditioning of parasitoid females) and 2 (mortality of the host on which conditioned or unconditioned females oviposited, and mortality of the host from the encapsulated cells only with ovipositor punctures by conditioned, unconditioned, or newly emerged parasitoid females), we calculated the percentage of total mortality of the weevils induced by *C. grandis* females (paralysis + host-feeding). In summary, the weevils mortality induced by conditioned females was 68.3%, unconditioned females was 43.1%, and by newly emerged females was 5.3%.

Survival of parasitoid

When *C. grandis* developed on third instars, on which parasitoid oviposited eggs, we observed significantly higher number of parasitoids completing development (60.0%) compared with the control group (42.5%). However, when *C. grandis* developed on weevils from cells with ovipositor punctures, the number of live parasitoids (50.0%) was not significantly different than when compared with the control group or the group of larvae from cells on which the parasitoids oviposited eggs (F=13.3, df=2, 12, P= 0.001). The female pupal weight was not significantly different among the different groups of boll weevil larvae tested (F=0.592, df=2, 89, P=0.556) (Table 2). These results are consistent with those reported by Morales-Ramos et al. (1995).

Effect of abdomen extracts from conditioned, unconditioned, and newly emerged *C. grandis* females on host survival

The injected third instar boll weevil larvae with extract from abdomen of conditioned females paralyzed 35.0% of weevils compared with 23.3% paralyzed larvae when treated with the extract from abdomen of unconditioned females and 10.0% from newly emerged females. Venom extracts from newly emerged females were significantly less effective paralyzing host larvae than those from mature conditioned or unconditioned females (F=13.0, df=2, 15, P=0.001). This may indicate that the venom activity on newly emerged females is not completely developed. The percentage of live weevils in the control group after injection with distilled water was 50.0% compared with 75.0% without treatments (F=12.7, df=1, 18, P=0.0001) (Table 3). This can be an explanation of a bigger percentage of dead weevils in experimental groups.

Eggs are laid and feeding tubes are formed by conditioned or unconditioned *C. grandis* females. Feeding-tube formation without oviposition occurs by newly emerged females. Similar change in behavior characterizing females with different rates of oviposition readiness, can be seen in a description by Edwards (1954) of the activities in the pteromalid female *Nasonia vitripennis* (Walker).

According by our observations the movement by the boll weevil larvae is a stimulus for oviposition by *C. grandis* females (unpublished data). The same phenomenon as described by Bryden and Bishop (1945) on braconid *Perilitus coccinellae* (Schrank) and by Smith (1952) on braconid *Microctonus vittatae* Muesebeck.

Venom production is in all groups of *C. grandis* females tested. But *C. grandis* females began the injection of venom to paralyze the host only after preovipositional period. The study of Beard (1952) on the venom of *Bracon hebetor* Say is the first indepth investigation of the subject. He calculated that as one part of venom in 200 X 10^6 parts of host blood was sufficient to cause permanent paralysis. In many parasitic Hymenoptera the injection of venom to paralyse the host and the act of oviposition are two distinct operations. According to Tamashiro (1971) *B. hebetor* first paralyses all the host individuals and then it begins to lay eggs on any of them. Guerra et al. (1993) observed that *Bracon mellitor* Say also paralyses its host by injecting venom before parasitization. In many cases *C. grandis* females do not paralyze host prior to oviposition. In our experiment an average of 47.8% (calculated from Table 2) host third instars received parasitoid eggs but were not paralyzed. This is in agreement with Morales-Ramos et al. (1995). According to them *C. grandis* females paralyzed boll weevil larvae during the process of host-feeding. The percentage of unparalyzed hosts after parasitism by *C. grandis* was positively correlated with the age of *C. grandis* females and negativily correlated with the number of eggs oviposited by parasitoids on each host larva (superparasitism).

Summary

Eggs are laid and a feeding tube is formed only by conditioned or unconditioned parasitoid females. Newly emerged females formed tubes for host-feeding but did not oviposit. Conditioned females of *C. grandis* deposited more eggs on or near boll weevil larvae than unconditioned females. Movement by boll weevil larvae was a necessary stimulus for oviposition by *C. grandis*. Total mortality

(paralyzed + host-feeding) of host larvae in the presence of conditioned parasitoid females was 68.3% compared with 43.1% by unconditioned females and 5.3% by newly emerged females. The highest survival of parasitoids was observed on host larvae where the female oviposited. Venom that paralyzed the weevils are produced by conditioned, unconditioned, and newly emerged females. But *C. grandis* females began to paralyze the hosts only post-preovipositional period. The injection of venom to paralyze the host and oviposition of *C. grandis* females are in many cases independent processes.

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Table 1. Influence of different groups of *C. Grandis* females on mortality of boll weevil larvae

Groups of third instar BWL exposed to different groups of parasitoid females	Percentage of boll weevil larvae				
	Complet-ed develop-ment	Failed to complete development			
	Alive	Para-lyzed	Host-feeding	Total Para-lyzed + host feeding	Other factors
Control					
BWL without contact to parasitoid females	73.6 ±15.7a	0	0	0	26.4 ±15.7a
Conditioned females :					
-BWL on which females oviposited	4.7 ± 8.0b	54.4 ±15.6a	18.7 ±12.9a	73.1 ±10.9a	22.2 ±7.4a
-BWL from punctured by females encapsulated cells	31.7 ± 9.6c	9.8 ± 10.3b	39.6 ±7.0b	49.4 ±6.9b	18.9 ±3.8a
Unconditioned females:					
-BWL on which females oviposited	5.1 ± 6.5b	50.1 ± 8.8a	20.2 ±7.8a	70.3 ±11.1a	24.6 ±13.5a
-BWL from punctured by females encapsulated cells	28.4 ± 5.8c	8.8 ± 13.1b	37.6 ±10.7b	46.4 ±6.6b	25.2 ±3.2a
Newly emerging females:					
-BWL from punctured by females encapsulated cells	53.3 ± 6.5a	0	22.1 ±6.8a	22.1 ±6.8c	24.6 ±2.1a

Mean ± SD. Values in each column followed by different letters are significantly different at the 5% level, as determined by the Tukey studentized range test.

Table 2. Effect of the host attacked by *C. grandis* on parasitoid survival

Groups of third instar BWL + parasitoid eggs	Percentage of alive		Pupal weight of parasitoid females, mg
	Host	Parasitoid	
Control	35.7 ± 5.0a	42.5 ± 5.4a	5.57 ± 0.53a
BWL from cells on which parasitoid females oviposited	17.5 ± 5.4b	60.0 ± 6.3b	5.63 ± 0.51a
BWL from punctured by females encapsulated cells	27.5 ± 6.3ab	50.0 ± 4.2ab	5.71 ± 0.46a

Mean ± SD. Values in each column followed by different letters are significantly different at the 5% level, as determined by the Tukey studentized range test.

Table 3. Effect of the abdomen extracts of different groups of *C. grandis* females and form presentation on host survival

Treatments	Percentage of third instar BWL		
	alive	dead	paralyzed
1. Control:			
1.1. injection of distilled water	50.0 ± 11.5a	50.0 ± 11.5a	0
1.2. without application of distilled water	75.0 ± 10.8b	25.0 ± 10.8bc	0
2. Injection of abdomen extract from females:			
2.1. conditioned	25.0 ± 10.5c	40.0 ± 8.9ab	35.0 ± 5.5a
2.2. unconditioned	31.7 ± 7.5ac	45.5 ± 15.2ab	23.3 ± 10.3a
2.3. newly emerged	43.3 ± 10.3a	46.7 ± 8.2ab	10.0 ± 8.9b

Mean ± SD. Values in each column followed by different letters are significantly different at the 5% level, as determined by the Tukey studentized range test.

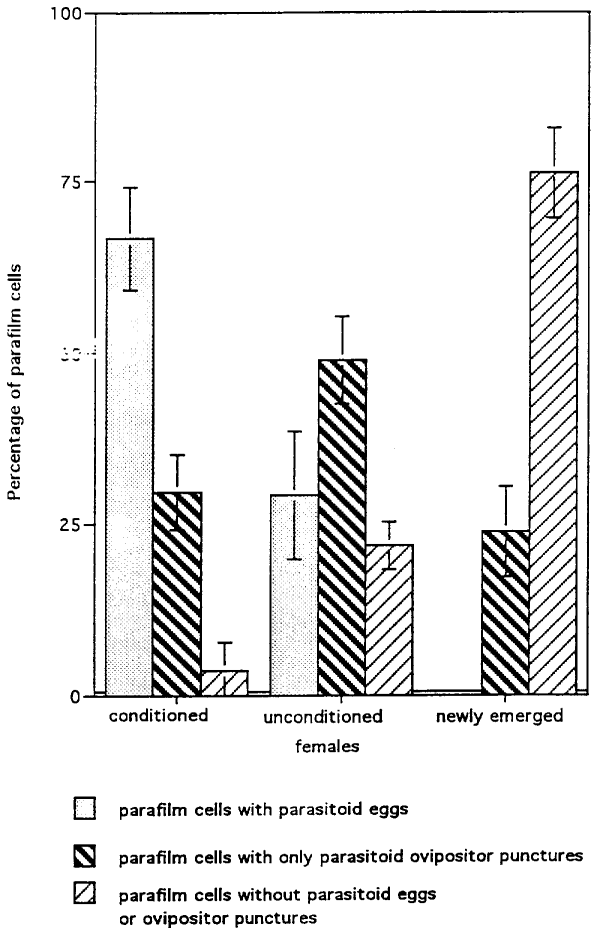

Figure 1. Host handling by parasitoid females after undergoing different levels of conditioning.