LABORATORY EVALUATION OF FIRST, SECOND, FIFTH, AND TENTH *IN VITRO*-REARED GENERATIONS OF CATOLACCUS GRANDIS

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Abstract

The biological characteristics of the ectoparasitoid *Catolaccus grandis* (Burks) were evaluated after 1, 2, 5, and 10 generations of *in vitro*-rearing and compared to parasitoids reared in boll weevil (*Anthonomus grandis* Boheman) larvae. The weight of female pupae was not significantly affected after 10 successive generations reared on artificial diet. Fecundity of *in vitro* F_2 females was significantly higher than that of *in vitro* F_1 females. Fecundity of *C. grandis* was not significantly reduced after 5 generations of *in vitro*-rearing. However, the in vitroF showed a significantly lower fecundity than all other *in vitro* generations. Females reared on boll weevil larvae had a higher pupal weight and fecundity than females reared *in vitro*, but *in vitro*-reared females showed a significantly higher survival during the most actively reproductive ages.

Introduction

Extensive studies of the exotic ectoparasitoid *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) have shown its promise as a biological control agent of the boll weevil (*Anthonomus grandis grandis* Boheman) (Morales-Ramos & King 1991, Morales-Ramos & Cate 1992a, 1992b, 1992c, Summy et al. 1992, 1994, 1995, Morales-Ramos et al. 1994, 1995a). The effectiveness of inundative releases of this parasitoid to suppress boll weevil populations has been demonstrated in experimental fields in the Lower Rio Grande Valley of Texas (King and Coleman 1995, Summy et al. 1994, 1995).

However, the commercial application of *C. grandis* is greatly limited by the high costs of mass propagating this parasitoid. Mass propagation has been identified as the main constraint in commercialization of augmentative releases of natural enemies (King and Morrison 1984). The development of artificial diets for *in vitro*-rearing of natural enemies is considered essential to the commercial application of biological control by augmentation of natural enemies (King & Morrison 1984, King 1993).

Females of *C. grandis* reared on an artificial diet developed by Rojas et al. (in press) showed biological attributes comparable to those reared on boll weevil larvae (Rojas et al. 1995). Preliminary releases of *in vitro*-reared *C. grandis* in the Rio Grande Valley resulted in 39.4 to 55.6% parasitism of boll weevil third instars and pupae (Morales-Ramos et al. 1995b). However, we lack the knowledge on the effects that long term *in vitro*- rearing of *C. grandis* may have on its biological attributes. The objectives of this work were to study the effects of *in vitro*-rearing on the pupal weight, fecundity, survival, and net reproductive rate of the first, second, fifth, and tenth generations of *C. grandis*.

Materials and Methods

A colony of *C. grandis* was reared *in vitro* for 10 consecutive generations, but only the F_1, F_2, F_5, and F_{10} were studied. The pupal weight, fecundity, survival, and net reproductive rate were compared among the different *in vitro* generations and a control reared *in vivo*. The control group was reared using the Parafilm encapsulation method reported by Cate (1987).

In Vitro-Rearing

The parasitoids were reared *in vitro* using the Gamma diet developed by Rojas et al. (in press). The diet was dispensed under aseptic conditions in plastic disposable 128-well bioassay trays (Bio-Ba-128, C-D International, Pitman, NJ). One single *C. grandis* egg was deposited manually (with the aid of a fine brush) in each well.

The parasitoid eggs were obtained by stimulating the parasitoid female to oviposit into a petri dish covered with Parafilm®. Approximately 5 ml of the diet were deposited in a plastic sterile disposable petri dish (9 X 9mm), which was covered with Parafilm®. The Parafilm® cover was coated with a mixture of mashed weevil larvae and the liquid portion of the parasitoid diet. Then the mixture was allowed to dry. Each petri dish was exposed to a colony of 120 females of *C. grandis* over a 4-h period. These colonies were reared in cages made from 2.8-l Rubbermaid® containers (No. 6). The containers were modified by adding 4 water dispensers and a cotton sleeve to prevent wasps from escaping while changing the petri dishes containing the oviposition stimulant.

The parasitoid eggs were collected from the inner wall of the Parafilm® cover, where the female parasitoids glued them. Once placed in the diet filled bioassay wells, the parasitoid eggs were allowed to develop to the pupal stage at a constant 27 ± 1ºC. The pupae were collected and allowed to complete development in emergence cages. The adults were then placed in groups of 120 females inside cages previously described to collect eggs for the next generation of *in vitro* reared parasitoids. This procedure was repeated 10 times until the F_{10} *in vitro* was obtained.

Pupal Weight

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Two to 3-d old female parasitoid pupae of each *in vitro* generation and the control were weighed in groups of varied numbers on a Mettler PM100 balance. The weights of the different groups were analyzed in a regression model using the GLM procedure of SAS software (SAS Institute 1988). This procedure generates least square means for the different generations and provides two types of hypothesis test (*T* and *F*). Then, male and female parasitoid pupae were collected and placed inside an emergence cage until they completed development.

Fecundity

Once the parasitoids completed development, 72 females from each *in vitro* generation and 72 reared in encapsulated boll weevils were individually placed in plastic petri dishes (9 X 9mm) at a constant 27 ± 1°C. The sample size used was adequate to estimate the population mean (μ) of eggs/female and eggs/female/day within a confidence interval (E) of 20 and 1.5, respectively, with α=0.05. This was determined by using the equation:

$$n = ((Z_a2)^2\ \sigma^2)/E2$$

where *n* is the sample size, $Z_a/2)^2$=1.96 (from tables), σ is the population standard deviation (estimated from sample '*s*'), and *E* is the confidence interval (Ott 1984).

Each female was provided with a male, water and honey. Three days after emergence, each female parasitoid was exposed to 12 encapsulated boll weevil larvae for 24 h during 15 d. Dead females were not replaced, but dead males were replaced during the first 13 d. Each day, the Parafilm$^{\Gamma}$ capsules enclosing the parasitized weevils were opened to count the number of eggs oviposited per female. Then, they were resealed and returned to the environmental chamber for parasitoid development. Nine days later, the Parafilm$^{\Gamma}$ capsules were reopened to count and sex the parasitoid pupae. The number of eggs oviposited per female per day and the number and sex of developing progeny were recorded for the 15-d period.

The total number of eggs oviposited by each female during the 15-d period and the mean number of eggs oviposited per day were used to compare the fecundity of females from each of the *in vitro* generations tested (F_1, F_2, F_5, and F_{10}) and the control. The sex of each of the female's progeny was recorded and the sex ratio of the progeny was calculated. The GLM procedure of SAS software (SAS institute 1988) was used to analyze the data of fecundity and sex ratio.

Net Reproductive Rate and Survival

Life tables were calculated for each of the *in vitro* generations studied and the control with the data obtained from the 72 females from each generation during the 18 day experimental period (3 d of preoviposition and 15 d of oviposition). The 'm_x' (female progeny produced per female) was estimated by multiplying the mean number of eggs produced per female of age '*x*' by the mean proportion of developing females (= 1–(1/sex ratio)) at age '*x*'. The 'l_x' (proportion surviving from birth to age '*x*') at age 18 was compared between the different treatments and the control. The net reproductive rate (R_o) was calculated as:

$$R_o = \sum_{x=0}^{n} l_x m_x$$

where n is the oldest age (18 in this study)(Krebs 1985).

The l_x values of the different *in vitro* generations and the control group at 5, 10, and 18 days after emergence were compared using the *Z*-Test at α = 0.05. The *Z* statistic is designed to test proportional data with values between 0 to 1 (Ott 1984). This statistic was calculated by the formula:

$$Z = \frac{\pi_1 - \pi_2}{\sigma\pi 1 - \pi 2} \quad \text{for } H_o: \pi_1 - \pi_2 = 0$$

where $$\sigma\pi 1 - \pi 2 = \left[\pi(1 - \pi) \left[\frac{1}{n1} + \frac{1}{n2} \right] \right]^{1/2},$$

π1 and π2 are the observed proportion of successes of treatment 1 and 2 respectively; π is the proportion of successes common to both treatments; n1 and n2 are the sample sizes of treatments 1 and 2 respectively (Ott 1984).

Results and Discussion

Pupal Weight

The pupal weight of the females from the control group (reared *in vivo*) was significantly higher (F = 83.11, df = 1, 48, P = 0.0001) than the weight of female pupae of all generations reared *in vitro* (Table 1). These results are consistent with those observed by Rojas et al. (1995) on the evaluation of different diets for *C. grandis*. No significant difference in pupal weight was observed (F = 1.98, df = 3, 34, P = 0.135) among females of the different generations reared *in vitro* (Table 1). These results indicate that the pupal weight of *C. grandis* females is not affected after 10 generations of *in vitro* rearing.

Fecundity

The fecundity of females reared in boll weevil larvae (control) was significantly higher (F = 89.6, df = 1, 289, P = 0.0001) than that of *in vitro*- reared parasitoids of all generations (Table 1). This result is consistent with previous studies (Rojas et al. 1995) and with the notion that fecundity is correlated with pupal weight of *C. grandis* females (Greenberg et al. 1995).

Significant differences in fecundity were observed among the different *in vitro* reared generations of *C. grandis*. Females from the *in vitro* F2 produced a significantly

higher number of eggs than those from the *in vitro* F1 (F = 8.38, df = 1, 289, P = 0.004) (Table 1). In fact, the fecundity of *in vitro* F2 females was significantly higher than that of females from all the other generations (Table 1). This may be the result of an initial adaptation of *C. grandis* to the artificial diet. Females from the *in vitro* F_{10} produced significantly lower number of eggs than all other *in vitro* generations (F = 16.85, df = 1, 289, P = 0.0001) (Table 1). No significant differences in fecundity were observed between the *in vitro* F1 and F5 (F = 0.27, df = 1, 289, P = 0.6) (Table 1). The pattern of the age-dependent fecundity was not affected by the successive *in vitro*-rearing of *C. grandis* (Fig. 1).

The differences observed in fecundity among the *in vitro* generations can not be explained by differences in size because no differences were observed in pupal weight among the different *in vitro* generations. Consequently, we concluded that the differences in fecundity observed indicate physiological changes induced by nutritional deficiency. However, the results show that the fecundity of *C. grandis* females is not affected after 5 generations of *in vitro*-rearing. Because the *in vitro* F_6 to F_9 were not evaluated, the maximum number of generations that can be reared *in vitro* without affecting fecundity is unknown. Nevertheless, the differences in fecundity observed between the *in vitro* F_1 and F_{10} are marginally significant (F = 5.05, df = 1, 289, P = 0.025). Therefore, the fecundity of some of the *in vitro* generations between F_5 and F_{10} may not be significantly affected.

Net Reproductive Rate and Survival

The net reproductive rate (R_o) of the control group was higher than that of all *in vitro* generations (Table 2). No significant differences were observed in progeny sex ratio among the *in vitro* generations and the control (F = 1.04, df 4, 288, P = 0.38). Therefore, the differences observed in the Ro values among the treatments and the control are mainly the result of differences observed in fecundity.

Survival of *C. grandis* females 5 d after emergence was significantly higher in the *in vitro* F10 than in the other generations and the control ($|Z| > 2.49$, $\alpha = 0.05$) (Table 2). Apparently, rearing *C. grandis* in artificial diet for 10 generations increases female survival during the early ages. Adult survival 10 and 18 d after emergence did not differ significantly among the different *in vitro* generations. However, survival of the control females 10 d after emergence was significantly lower than the *in vitro* F_1 ($|Z|$ = 2.54) and F10 ($|Z|$ = 3.03) and survival 18 d after emergence was significantly lower than all *in vitro* generations ($|Z| > 2.31$, $\alpha = 0.05$) (Table 2). These results show that rearing *C. grandis* in artificial diet improves the survival of the adult females during the most actively reproductive ages (from 8 to 15 d old) (Morales-Ramos and Cate 1992a, 1992b).

Summary

Pupal weight of *C. grandis* females was not affected by successively rearing this parasitoid in artificial diet for 10 generations. The fecundity of *in vitro* F_2 females was significantly higher than that of *in vitro* F_1 females. The fecundity of *C. grandis* was not significantly affected after 5 generations of *in vitro*-rearing. However, *C. grandis* females showed a significant reduction in fecundity after 10 generations of being reared in artificial diet. Survival of *in vitro*-reared *C. grandis* during the reproductive age as compared to *in vivo*-reared parasitoids was significantly improved. *In vitro* F_{10} *C. grandis* females had significantly higher survival during early ages than all other *in vitro* generations and the controls.

The results presented in this paper show that it is possible to rear *C. grandis in vitro* for consecutive generations throughout a cotton-growing season. Eight weekly *C. grandis* releases are the maximum recommended for control of the boll weevil during a cotton season (Morales-Ramos et al. 1996) and this number of releases can be produced with no more than 5 generations of *C. grandis* in culture. However, permanent culture of *C. grandis* may require alternate *in vivo* and *in vitro*-rearing to preserve the quality of the parasitoid females. Nevertheless, the use of artificial diet for mass propagation of *C. grandis* during the release season is economically significant in the commercial application of this biological control technology.

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Table 1. Comparison of pupal weight and fecundity among different generations of *Catolaccus grandis* females reared in artificial diet.

	n	Pupal Weight[a]	n	Fecundity[b]
Control	224	5.54 ± 0.52a	49	294.20 ± 54.44a
F_1	204	4.15 ± 0.34b	62	217.21 ± 49.11c
F_2	155	4.20 ± 0.12b	65	243.03 ± 45.84b
F_5	130	4.12 ± 0.10b	58	222.02 ± 54.28c
F_{10}	95	3.96 ± 0.15b	60	196.77 ± 47.05d

Mean ± Standard Deviation, means with the same letter are not significantly different after GLM's F-Test, $\alpha = 0.05$.

[a]In mg, df 1, 48.

[b]In eggs per female during a 15 d period, df 1, 289.

Table 2. Comparison of survival and net reproductive rate among different generations of *Catolaccus grandis* reared in artificial diet.

		Survival from Emergence to Age[a]			
	n	5	10	18	R_0[b]
Control	79	0.886b	0.747b	0.608b	143.66
F_1	73	0.918b	0.904a	0.849a	116.36
F_2	81	0.852b	0.827ab	0.802a	117.48
F_5	73	0.863b	0.849ab	0.781a	125.51
F_{10}	72	1.000a	0.931a	0.833a	109.79

[a]In days. Values with the same letter within the same column are not significantly different after *Z*-Test at $\alpha = 0.05$.

[b]Net reproductive rate up to 17 d old adult females.

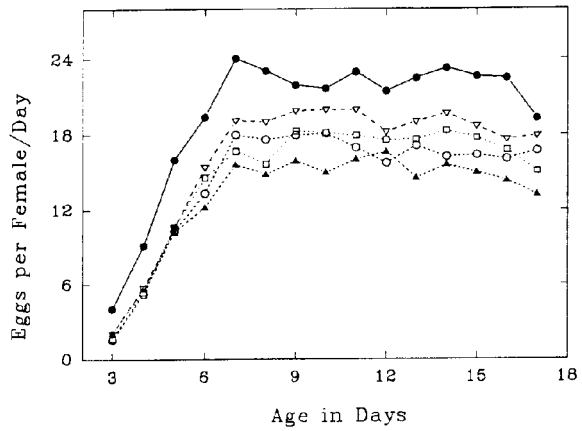

Figure 1. Age-dependent fecundity of *Catolaccus grandis* —●— reared in boll weevil larvae, and reared *in vitro* for ···○··· 1, ···▽··· 2, ···□··· 5, and ···▲··· 10 generations.