DIFFERENCES IN BIOLOGICAL PARAMETERS OF *CATOLACCUS GRANDIS* FROM SINALOA, MEXICO COMPARED TO A CROSSBRED COLONY FROM TABASCO, CHIAPAS, OAXACA MEXICO AND EL SALVADOR

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Abstract

A new colony of the boll weevil ectoparasitoid *Catolaccus grandis* was introduced from Guasave, Sinaloa, Mexico to improve genetic variability of a 12 year old laboratory reared stock in Weslaco, Texas. The biological characteristics of the introduced colony were compared to those of the Weslaco colony. Developmental time was not significantly different among the 2 colonies, but the preovipositional period of the Sinaloan females was 3 times as long. The fecundity, net reproductive rate (R_o), and intrinsic rate of increase (r_m) of females from Sinaloa were significantly lower than those of females from Weslaco. Generation time (G) and doubling time (DT) were significantly longer in the Sinaloan colony. These characteristics make the Sinaloa population disadvantageous for mass propagation and release to control boll weevil populations. Therefore it was recommended no to cross breed the introduced wild colony with the existing *C. grandis* stock.

Introduction

Augmentative releases of the exotic ectoparasitoid *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) have been proven to be effective to biologically control the boll weevil (*Anthonomus grandis grandis* Boheman) (Coleman et al. 1996, King et al. 1995, Morales-Ramos & King 1991, Morales-Ramos et al. 1994, 1995, Summy et al. 1992, 1993, 1994, 1995, Vargas-Camplis 1997). Rearing methods for this parasitoid have been perfected over the years (Cate 1987, Morales-Ramos et al. 1992, 1994, 1997, Palamara 1995, Roberson and Harsh 1993). But, the development of an artificial diet (Rojas et al. 1996, 1997) has been the most significant advance of the mass propagation technology of this parasitoid. The effectiveness of diet-reared *C. grandis* has been established by laboratory and field evaluations (Morales-Ramos et al. 1995, 1998, R. J. Coleman unpublished). All these advances have made the mass propagation of *C. grandis* economically feasible according to an economical analysis done in 1997 (Ellis et al. 1997).

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The process of colonization and mass rearing for long periods of time may be detrimental to the reared species (Barlett 1984). Initial loss of genetic variability and subsequent selection tend to induce domestication and adaptation to laboratory environments (Barlett 1984). After 12 years of constant laboratory rearing of *C. grandis*, there is no evidence of loss of searching capacity or field adaptation in this parasitoid; however, the potential for loss of genetic variability and subsequent adaptation to laboratory environments is a constant concern. Some methods to avoid such problems include pooling multiple-founder colonies, maintain variable laboratory environments, and regular infusion of wild genetic stock (Joslyn 1984).

The first method has been used with the colony of *C. grandis* that is currently maintained in Weslaco, Texas. This colony is the result of a systematic cross breeding among 3 distinct populations of *C. grandis* from Tabasco and Chiapas Mexico, and from El Salvador (Morales-Ramos unpublished) (see Materials and Methods). The second method is difficult to apply because of it requires large supplies of equipment, space, and personnel. These resources have not been available to the present research program. This study is an attempt to apply the third method by introducing wild *C. grandis* from Sinaloa, Mexico to restore genetic variability to the Weslaco colony. The objectives were to evaluate the biological characteristics of the newly introduced parasitoids to determine the desirability of cross breeding the wild population from Sinaloa with the Weslaco population.

Materials and Methods

Boll weevil larvae used in this study were reared on artificial diet at the Gast Insect Rearing Research Unit in Mississippi State, Mississippi (Roberson and Wright 1984). Rearing of *C. grandis* was as reported by Morales-Ramos et al. (1992) using the Parafilm^R encapsulation method developed by Cate (1987). Parasitoid colonies and experiments were held at constant 27 ± 1°C, 50 ± 10% R.H., and 14:10 (L:D) h photoperiod.

Biological Materials Origin

A *C. grandis* colony was established in Guasave, Sinaloa, Mexico using parasitoids isolated from boll weevil infested cotton squares and bolls. However, a substantial number of adult *C. grandis* females were captured in the field using insect nets. The infested cotton material and adult parasitoids were collected from a commercial cotton field located near Guasave. The colony was maintained and increased using the encapsulation method described above with boll weevils collected from the field.

Two shipments of *C. grandis* were received at the APHIS quarantine facility located at the Biological Control Center in Mission, Texas (importation permit No. 31352). The shipments consisted of 120 females and 136 males in

August 1st and 144 females and 106 males in August 8. The *C. grandis* colony was held in quarantine for one generation while being screened for purity and microbial contamination. The Sinaloa colony of *C. grandis* (Sinaloa population) was released from quarantine in October 25, 1996 (permit No. 31669) to the USDA-ARS Subtropical Agricultural Research Center in Weslaco, Texas.

The mother colony maintained at Weslaco, Texas (Weslaco population) originated in 3 different localities in Southeast Mexico and one locality from El Salvador. These localities were Cardenas, Tabasco from *Hampea nutricia* (29 males and 13 females); La Ventosa, Oaxaca from *Cienfuegosia rosei* (1 male and 1 female); Tehuantepec, Chiapas from *Cienfuegosia rosei* (6 females), Mexico; and The University of El Salvador, El Salvador from cultivated and wild cotton (2 males and 2 females). The samples, which consisted of plant fruits infested by boll weevil, were ship to the quarantine facility in Texas A & M University at College Station, Texas. These colonies were reared independently for 3 years in the Department of Entomology Texas A & M University from 1985 to 1988.

A cross bred colony was produced in 1989 from a systematic cross between all 3 Mexican populations. This was accomplished by placing 300 newly emerged females from one colony and 300 newly emerged males from another in a new cage. A total of 6 combinations were initially created. The progeny of all the 6 combinations was then mixed and reared as a single colony. This colony was successfully transferred to Weslaco, TX in February 1990 and it has been in constant culture until the present day. Several permits have been issued to release this particular population in the state of Texas. The most recent one was issued in September 21, 1994 (permit No. 944483).

Pupal Weight

A total of 72 female parasitoid pupae of each population (3-d–old) were weighed individually on a Mettler H51 precision balance. The weights of the different groups were analyzed by t–test using SigmaStat software. Then, the female parasitoid pupae were placed individually in plastic Petri dishes (9 X 9mm) where they completed development at the conditions described above.

Fecundity and Progeny Sex Ratio

Once the parasitoids completed development, two males were placed in each of the Petri dishes to ensure fertilization. Each female was provided daily with 12 encapsulated boll weevils, water and honey. Dead females were not replaced, but dead males were replaced during the first 15-d. Each day, the Parafilm^R 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^R 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 a 45-d period.

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:

$$n = ((Z_{\alpha/2})^2\ \sigma^2)/E^2$$

where n is the sample size, $Z_{\alpha/2}$=1.96 (from tables), σ is the population standard deviation (estimated from sample 's'), and E is the confidence interval (Ott 1984).

The total number of eggs oviposited by each female during the 45-d period and the mean number of eggs oviposited per day during the fecundity plateau period were used to compare the fecundity of females from each of the two populations studied. The starting age of the fecundity plateau period was determined according the criteria used by Morales-Ramos and Cate (1992). The sex of each of the female's progeny was recorded and the sex ratio of the progeny was calculated. The t-student test was used to compare fecundity and progeny sex ratio between the two populations studied using SigmaStat software.

Life Table Analysis

Life tables were calculated for each of the two populations studied with the data obtained from the 72 females from each population during the 45 day experimental period. 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 the beginning of the fecundity plateau period was compared between the two populations. 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 generation time (G), which is equivalent to the mean period elapsing between the birth of parents and the birth of offspring was calculated as:

$$G = \frac{\sum_{x=0}^{w} x\, l_x\, m_x}{R_o}$$

where w is the oldest of the age classes (Carey 1993). The intrinsic rate of population increase (r_m) was calculated by two methods. The first method used was by iteration in the Lotka's (1907) equation:

$$\sum_{x=0}^{w} \exp(-r_m\, G)\, l_x\, m_x = 1$$

(Carey 1993). The doubling time (*DT*), defined as the time required for the population to double its size, was calculated by the formula:

$$DT = \frac{\ln(2)}{r_m}$$

(Krebs 1985). The reproductive value (V_x) is defined as the contribution to the future population that an individual female of age *x* will make (Krebs 1985). The calculation of reproductive values for every age class was done by:

$$V_x = \sum_{t=x}^{w} (l_t / l_x)\, m_t$$

where *x* is the base age class, *w* is the oldest age class, and *t* is any age class between *x* and *w*.

A modification of Tukey's Jackknife technique (Efron 1982, Roff 1992) was used to estimate variability of the parameters *G*, *DT*, R_o, and r_m. This method involved the estimation of 36 values of the life table parameters using the data obtained from the 72 females of each population after deletion of 12 randomly selected observations. Thirty six series of 12 random numbers were produced by a computer random number generator. These numbers were used to decide which of the 72 original data points (numbered) would be deleted. The life table parameters were estimated using the remaining 60 data points. Then, the data set was restored to its original 72 data points and the next series of 12 random numbers was used to determine the next deletions. This procedure was repeated 36 times to obtain 36 different estimates of the life table parameters. The t-student test was used to compare the life table parameters between the two populations tested using SigmaStat software.

Results and Discussion

Biological Parameters

Developmental time of females of the Sinaloa population was slightly longer, but not significantly different compared to that of females of the Weslaco population (Table 1). The preovipositional period, on the other hand, was significantly longer (16 days) in the Sinaloa population than in the Weslaco population (3.2 days) (t = 9.57, df = 105, P < 0.001) (Table 1).

A longer preovipositional period is considered to be a disadvantageous trait. In many parasitoid species, the females do not respond to host cues during this period (Vinson 1981, 1984). There is some evidence supporting the notion that this may be the case in *C. grandis*. In searching capacity field studies of *C. grandis* no parasitism of boll weevil larvae was observed in experimental fields in Ricardo, Tx up to 5 d after the release of newly emerged females (J. A. Morales-Ramos unpublished). However, a release of 5-d old parasitoids produced high rates of parasitism during the same period of time in the same experimental field (J. A. Morales-Ramos unpublished).

As a consequence of this experimental evidence, *C. grandis* is released after the females had passed the preovipositional period. As standard procedure, females are released not earlier than 5 d after emergence. A longer preovipositional period would increase the length of the holding period increasing the needs for space and labor in a mass propagation effort. Females of the Sinaloa population would need to be held for at least 17 d before they could be released. This would more than triplicate the needs for space and maintenance labor to mass produce effective parasitoids.

Longevity of Sinaloan females was significantly longer (36 d) than that of females from the Weslaco population (27.5 d) (t = 2.11, df = 142, P = 0.037). However, The parasitoid females from Weslaco oviposited a significantly higher number of eggs (363.1) during their life than those from the Sinaloa population (241)(t = 2.21, df = 142, P = 0.029) (Table 1). This, despite the fact that Sinaloan female pupae were significantly heavier (6.6 mg) than those from Weslaco (5.9 mg) (t = 2.9, df = 142, P < 0.004) (Table 1).

Parasitoids from the Weslaco population produced most of their progeny earlier in their life cycle than those from the Sinaloa population (Fig. 1 A). The fecundity plateau period (period of highest fecundity) started earlier in the Weslaco population (9 d of age) than in the Sinaloa population (23 d of age). The daily oviposition of Weslaco parasitoids during the fecundity plateau period was significantly higher (26.3 eggs/d) than that of parasitoids from the Sinaloa population (14.5 eggs/d) (t = 15.77, df = 1135, P < 0.001) (Table 1).

A reduced oviposition rate is another undesirable trait. The effectiveness of *C. grandis* controlling the boll weevil depends on the rate at which the females find and parasitize host larvae. females from the Sinaloa population seem to parasitize at nearly half the rate as females from the Weslaco population do and at more than twice the age.

Life Table Parameters

The pattern of age-dependent survival (l_x) of adult females differed among the 2 populations of *C. grandis* (Fig. 1 B). Females of the Sinaloa population tend to live longer. However, the significant delay on the onset of oviposition and the significantly reduced fecundity of the Sinaloan females compared to that from the Weslaco population, significantly affected most of the population parameters. Generation time and doubling time were significantly longer in the Sinaloa population (38.2 and 5.4 d respectively) as compared to the Weslaco population (25.5 and 3.4 d respectively) (t = 115.35 and 96. 74, df = 70, P < 0.001) (Table 2).

The length of these parameters indicate the mean period of time required to start producing progeny and to double the

population size respectively (Carey 1993). In a mass propagation effort, smaller values of these parameters mean more production in a sorter period of time. The mass production of the Sinaloa population would be less efficient than that of the Weslaco population.

The net reproductive rate (R_o) was significantly lower in the Sinaloa population (132.8) compared to the Weslaco population (363.1) ($t = 21.47$, $df = 70$, $P < 0.001$). Similarly, the intrinsic rate of increase (r_m) was significantly lower in the Sinaloa population (0.128) than in the Weslaco population (0.206) ($t = 126.31$, $df = 70$, $P < 0.001$) (Table 2). This is another indication that mass propagating the Sinaloa colony would be significantly less efficient. The parameter R_o is the mean number of females produced per female. The value of R_o of the Sinaloa colony is half of that of the Weslaco population. It would require twice as many breeding females of the Sinaloa population to produce a given number of females for release. Again, this would increase the needs for space and labor for the maintenance of the breeding colony.

The results of the life table analysis and the biological parameters can be summarized by the reproductive values (V_x). This value provides the reproductive potential of a female at any given age (Krebs 1985). Females of the Weslaco population had higher values of V_x than those of Sinaloan females. In addition, the V_x values peak earlier in life in the Weslaco population (Fig. 1 C). This indicate that Weslaco females are more successful colonizers than Sinaloan females.

Summary and Conclusions

The Sinaloa population of *C. grandis* shows many undesirable biological traits as compared to the Weslaco population. Sinaloan females have a considerable longer preovipositional period and tend to require longer time to acquire full fecundity. As a consequence, for an effective parasitoid release, the holding time of the parasitoids previous to their release would have to be extended considerably (3 fold). Sinaloan females oviposit at nearly half the rate of the Weslaco females. This may reduce their effectiveness in the field because the weevils would be parasitized at a reduced rate.

The population parameters of the Sinaloa population indicate a significantly slower maturation time (higher *G* value) and population growth (higher *DT* and lower R_o and r_m values). These would make the mass propagation process significantly less efficient and more expensive. The Sinaloa population of *C. grandis* would require a larger breeding stock (twice as large) and the parasitoids would have to be held for a longer period of time (3 times as long) before their release. These would increase the needs for space and labor of the mass propagation efforts.

On base of the results presented in this paper, our recommendation is not to introduce Sinaloan individuals into the Weslaco colony. The undesirable characteristics observed in this population outweigh the potential benefits that could be gained by the introduction of new genetic variability in the Weslaco colony. Additional studies should be conducted to measure the impact of the cross breeding of these two populations before discarding the Sinaloa colony. The efforts on the introduction of wild genetic stocks to the Weslaco colony will continue with careful monitoring of the introduced populations.

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Table 1. Biological parameters of two *Catolaccus grandis* populations, one from a 12 year old culture in Weslaco, Texas and the other from a new introduction from Sinaloa, Mexico.

Biological parameter	Weslaco	Sinaloa
Developmental time (females)[a]	13.4 ± 0.6 A	13.8 ± 1.3 A
Preovipositional period[a]	3.2 ± 2.7 B	16.0 ± 10.1 A
Longevity[a]	27.5 ± 25.2 B	35.9 ± 22.6 A
Fecundity		
Total eggs	363.1 ± 350.7 A	242.0 ± 300.1 B
Eggs/F./d[b]	26.3 ± 10.5 A	14.5 ± 13.5 B
Pupal weight[c]	5.9 ± 1.3 B	6.6 ± 1.5 A
Progeny sex ratio[d]	71.9 ± 28.7 A	70.9 ± 36.5 A

X ± S, means with the same letter are not significantly different after t–Student test $\alpha = 0.05$.
[a]In days.
[b]During the fecundity plateau period.
[c]In mg.
[d]In percent females.

Table 2. Life table parameters of two *Catolaccus grandis* populations, one from a 12 year old culture in Weslaco, Texas and the other from a new introduction from Sinaloa, Mexico.

Parameter	Weslaco	Sinaloa
Net reproductive rate (R_o)	189.5 ± 11.5 A	132.8 ± 10.5 B
Generation time[a]	25.5 ± 0.2 B	38.2 ± 0.6 A
Doubling time[a]	3.4 ± 0.04 B	5.4 ± 0.12 A
Intrinsic rate of Increase (r_m)	0.206 ± 0.002	A0.128 ± 0.003 B

X ± S, means with the same letter are not significantly different after t–Student test $\alpha = 0.05$, n = 36.
[a]In days.

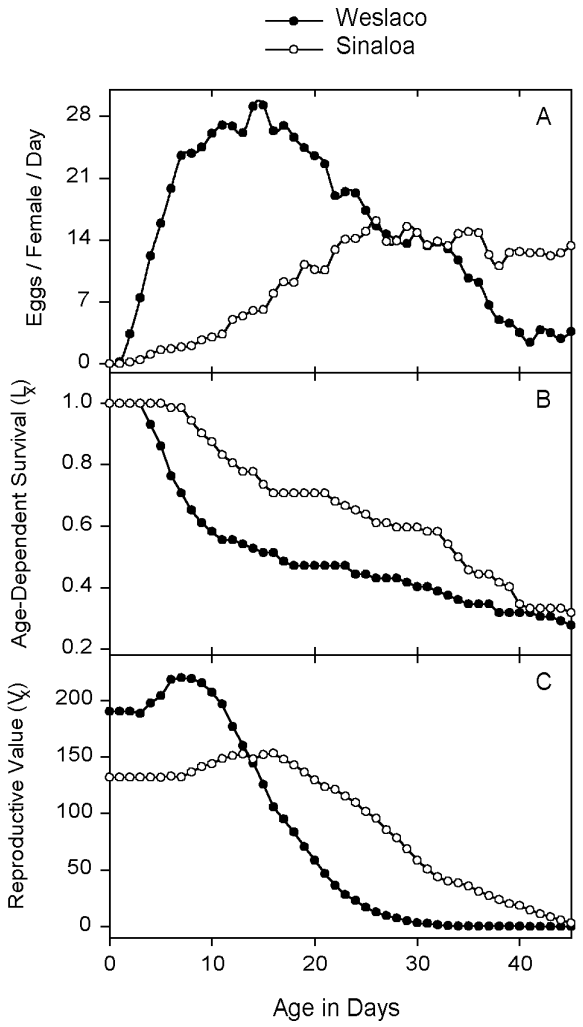

Figure 1. Age-dependent fecundity and survival of 2 populations of *Catolaccus grandis.* A) Mean eggs per female per day, B) adult female survival expressed as Lx, C) reproductive value expressed as number of potential female progeny per female.