DIFFERENCES IN REPRODUCTIVE POTENTIAL OF TWO POPULATIONS OF *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE) AND THEIR HYBRIDS

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ABSTRACT

A new colony of the boll weevil ectoparasitoid *Catolaccus grandis* was introduced from Guasave, Sinaloa, Mexico to improve vigor 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 and a crossbreed of these 2 colonies. Developmental time was not significantly different among the 3 colonies, but the preovipositional period of the Sinaloa females was 3 times as long compared to the other 2 colonies. 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 and the hybrid colony. Generation time (G) and doubling time (DT) were significantly longer in the Sinaloa colony. These characteristics make the Sinaloa population less desirable for mass propagation and release to control boll weevil populations than the Weslaco colony. The biological and population parameters of the hybrid colony were not significantly different from those of the Weslaco population. The implications of the observed results on the mass propagation and release strategies against the boll weevil are discussed and recommendations are presented.

Key Words: Boll weevil, parasitoid, population, variability, biological parameters, biological control

RESUMEN

Una nueva colonia del parasitoide del picudo del algodonero *Catolaccus grandis* de Guasave, Sinaloa, México, fue introducida a Texas con el objetivo de mejorar el vigor de la colonia madre de este parasitoide. Las características biológicas de la colonia introducida fueron comparadas con aquellas de la colonia de Weslaco, Texas y el híbrido entre estas dos colonias. No hubo diferencias significativas en el tiempo de desarrollo entre las tres colonias, pero el período de preoviposición de las hembras de Sinaloa fue el triple del observado en las otras 2 colonias. La fecundidad, tasa reproductiva neta (R_o) y tasa intrinseca de crecimiento (r_m) de las hembras de Sinaloa, fueron significantemente bajas comparadas con las de las hembras hibrídas y de Weslaco. Los tiempos de generación (G) y de duplicación (DT) fueron significantemente mas largos en la colonia de Sinaloa. Estas características indican que el uso de la colonia de Sinaloa en un programa de propagación en masa y liberación contra el picudo del algodonero podria ser desventajoso. Sin embargo, las características biológicas de la colonia híbrida no fueron significativamente diferentes a las observadas en la colonia de Weslaco. Se discuten las implicaciones de estos resultados en las estrategias de propagación en masa y liberación contra el picudo del algodonero y se presentan recomendaciones.

Augmentative releases of the exotic ectoparasitoid *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) have proven effective at biologically controlling 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 et al. 1997). Rearing methods for this parasitoid have been improved over the years (Cate 1987, Morales-Ramos et al. 1992, 1994, 1997, Palamara 1995, Roberson & Harsh 1993). The development of an artificial diet (Rojas et al. 1996, 1997) significantly advanced the mass propagation technology for 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 economic analysis done by Ellis et al. (1997).

The process of colonization and mass rearing over long periods of time may have detrimental effects in reared species (Bartlett 1984). Initial loss of genetic variability and subsequent selection tend to induce domestication and adaptation to laboratory environments (Bartlett 1984, van Lenteren 1991, Wajnberg 1991, Lappla 1993). 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 (Morales-Ramos et al. 1995, Vargas-Camplis et al. 1997); however, the potential for loss of genetic variability and subsequent adaptation to laboratory environments is a constant concern. Some methods for avoiding such problems include 1) pooling multiple-founder colonies, 2) maintaining variable laboratory environments, and 3) 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, TX. This colony is the result of systematic cross breeding among 3 distinct populations of *C. grandis* from Tabasco and Oaxaca Mexico, and from El Salvador (J. A. M., unpublished data) (see Materials and Methods). The second method is difficult to apply because 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 prevent the loss of genetic variability from the Weslaco colony. The objectives were to evaluate the reproductive potential 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 USDA-ARS, Biological Control and Mass Rearing Research Unit at Mississippi State, Mississippi (Roberson & Wright 1984). Rearing techniques for *C. grandis* were as reported by Morales-Ramos et al. (1992) using the Parafilm® encapsulation method developed by Cate (1987). Parasitoid colonies and experiments were held at constant 27 ± 1°C, 50 ± 10% R.H., and a photoperiod of 14:10 (L:D).

Biological Materials Origin

A *C. grandis* colony was established in Guasave, Sinaloa, Mexico in early June, 1996 using parasitoids isolated from boll weevil infested cotton squares and bolls and from net captures of adult *C. grandis* females. The infested cotton material and adult parasitoids were collected from a commercial cotton field located near Guasave. The colony was maintained and increased using Cate's (1987) method of encapsulation.

Two shipments of *C. grandis* were received at the APHIS quarantine facility located at the Biological Control Center in Mission, TX (importation permit No. 31352). The ship-

ments consisted of 120 females and 136 males on August 1st and 144 females and 106 males on August 8, 1996. 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 on October 25, 1996 (permit No. 31669) to the USDA-ARS Subtropical Agricultural Research Center in Weslaco, TX. The parasitoids used for this study were from the 2nd generation after introduction.

The mother colony maintained at Weslaco, TX (Weslaco population) originated from 2 different localities in Southeast Mexico and one locality from El Salvador. The localities in Mexico were Cardenas, Tabasco from *Hampea nutricia* (29 males and 13 females); La Ventosa, Oaxaca from *Cienfuegosia rosei* (1 male and 7 females); and in El Salvador, The University of El Salvador, from cultivated and wild cotton (2 males and 2 females). Samples of plant buds and fruits infested by boll weevil were hand-carried to the quarantine facility at Texas A&M University in College Station, TX. These colonies were reared independently for 3 years at the Department of Entomology, Texas A&M University, from 1985 to 1988.

A crossbred colony was produced in 1989 from a systematic cross between the 3 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 were 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 since (approximately 153 generations). Several permits have been issued to release this particular population in the state of Texas. The most recent one was issued on September 21, 1994 (permit No. 944483).

The third *C. grandis* colony tested was a crossbreed (hybrid) of the Sinaloa (12th generation) and Weslaco (generation 169) populations. The hybridization was accomplished by placing 100 female pupae of each population in separated cages made of 2.8-liter Rubbermaid® containers (No. 6 Rubbermaid, Wooster, OH). The pupae were allowed to emerge at 27 ± 1°C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D). One d after emergence, the 100 females from the Weslaco population received 50 newly-emerged males from the Sinaloa population. Similarly, the 100 females from the Sinaloa population were provided with 50 newly emerged males from the Weslaco population. Each cage was provided with distilled water, honey and 120 encapsulated boll weevil larvae, which were replaced daily for a period of 20 d. The parasitized boll weevils were maintained at the conditions described above for 10 d to allow the parasitoids to develop to mid-age pupae. Eighteen female parasitoid pupae were randomly selected from weevils parasitized by each of the 2 groups when the females were 8 d old. This procedure was repeated again using weevils parasitized when the females were 12 d old to obtain a total of 72 hybrid *C. grandis* pupae.

Pupal Weight

A total of 72 female parasitoid pupae of each population (3 d-old) were weighed individually using a Mettler H51 precision balance. The weights of the different groups were analyzed by Analysis of variance (ANOVA) and the means were compared by Tukey's test using SigmaStat® software (Jandel Corporation 1995). The female parasitoid pupae were placed individually in plastic square Petri dishes (9 × 9 cm) where they completed development at the conditions described above.

Fecundity and Progeny Sex Ratio

Once the female parasitoids completed development and emerged, two males from the same population were placed in each of the Petri dishes to ensure fertilization.

Each female was provided daily with 12 encapsulated boll weevils, water and honey according to evaluation methods reported by Morales-Ramos and Cate (1992). Dead females were not replaced, but dead males were replaced during the first 15 d. Each day, the Parafilm® capsules enclosing the parasitized weevils were opened to count the number of eggs oviposited per female. They were resealed and returned to the environmental chamber for parasitoid development. Nine d later, the Parafilm® 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 over a 45-d period.

The sample size needed to estimate the population mean (μ) of eggs/female and eggs/female/d for confidence intervals (E) of ± 20 and 1.5, respectively, with $\alpha = 0.05$, 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), and σ is the population standard deviation (estimated from sample 's'). This equation determines the adequate sample size for a given value of E based on the standard deviation of a preliminary samples from each population (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 (as defined by Morales-Ramos and Cate 1992) were used to compare the fecundity of females from each of the 3 populations studied. The starting age of the fecundity plateau period was determined according to 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 Analysis of variance was used to compare fecundity and progeny sex ratio between the three populations studied and Tukey's test was used to compare the means using SigmaStat® software.

Results and Discussion

Biological Parameters

Developmental time of *C. grandis* females was not significantly different among the Sinaloa and Weslaco populations and their hybrid (Table 1). The preovipositional period, on the other hand, was significantly longer (16 days) in the Sinaloa population than in the Weslaco and hybrid populations (4.3 and 3.6 d respectively) ($F = 64.4$, $df = 2, 126$, $P < 0.001$) (Table 1). The difference observed between the Weslaco and hybrid populations was not significant, showing that the hybrids tend to resemble more the Weslaco population for this parameter instead of showing an intermediate value between the Weslaco and Sinaloa populations as expected.

A longer preovipositional period is considered to be an undesirable trait. In many parasitoid species, the females do not respond to host cues during this period (Vinson 1981, 1984). Evidence from field studies on searching capacity indicated that this may be the case in *C. grandis*; no parasitism of boll weevil larvae was observed in an experimental field in Ricardo, TX up to 5 d after the release of newly emerged females (J. A. M. unpublished data). 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. M., unpublished data).

No significant differences in female longevity and pupal weight were observed among the Sinaloa and Weslaco populations and their hybrids (Table 1). Parasitoid

TABLE 1. BIOLOGICAL PARAMETERS OF 3 *C. GRANDIS* POPULATIONS.

Parameter	Population		
	Weslaco	Hybrid	Sinaloa
Developmental time (females)[1]	13.4 ± 0.6	13.5 ± 0.7	13.8 ± 1.3
Preovipositional period[1]	4.3 ± 3.4b	3.6 ± 4.3b	16.0 ± 10.1a
Longevity[1]	43.2 ± 20.7a	44.1 ± 16.4a	35.9 ± 22.6a
Fecundity			
Total eggs	554.0 ± 247a	600.1 ± 253a	242.0 ± 300b
Eggs/F./d[2]	22.0 ± 8.8a	21.6 ± 9.8a	14.5 ± 13.5b
Pupal weight[3]	6.4 ± 1.1a	6.3 ± 1.0a	6.6 ± 1.5a
Progeny sex ratio[4]	5.1 ± 4.4a	4.5 ± 3.6a	4.6 ± 4.3a

X ± SD, means with the same letter are not significantly different after ANOVA Tukey test $\alpha = 0.05$.
[1]In days.
[2]During the fecundity plateau period.
[3]In mg.
[4]In females per male.

females from the Weslaco and hybrid populations oviposited a significantly higher number of eggs (554 and 600 respectively) than that of females from the Sinaloa population (242) ($F = 37.6$, $df = 2, 213$, $P = 0.001$) (Table 1). Total fecundity was not significantly different between the Weslaco and hybrid populations, again, showing resemblance of the hybrids to the Weslaco population.

Parasitoids from the Weslaco and hybrid populations produced most of their progeny earlier in their life cycle than those of the Sinaloa population (Fig. 1A). The fecundity plateau period (period of highest fecundity) started earlier in the Weslaco and hybrid populations (9 and 8 d of age, respectively) than in the Sinaloa population (23 d of age). The daily oviposition of Weslaco and hybrid parasitoids during the fecundity plateau period was significantly higher (22.0 and 21.6 eggs/d, respectively) than that of parasitoids from the Sinaloa population (14.5 eggs/d) ($F = 95.3$, $df = 2, 2103$, $P < 0.001$) (Table 1). The daily oviposition rate during this period was not significantly different between the Weslaco and hybrid populations.

A reduced oviposition rate is another undesirable trait. The effectiveness of *C. grandis* in 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, and at more than twice the age.

There was no significant difference in progeny sex ratio among the 3 populations (Table 1). This indicates that the rates of fertilization and host acceptance were not affected by hybridization.

These results indicate that the undesirable characteristics of the Sinaloa population were not manifested after hybridization with the Weslaco population. However, we recommend caution in introducing the Sinaloa strain to the mother colony (Weslaco). The detrimental characteristics observed in the Sinaloa population may resurface in subsequent generations in the hybrid population. Since the only apparent potential advantages of introducing the Sinaloa strain to the mother colony is gain of genetic variability, we recommend the use of a different strain to achieve this purpose.

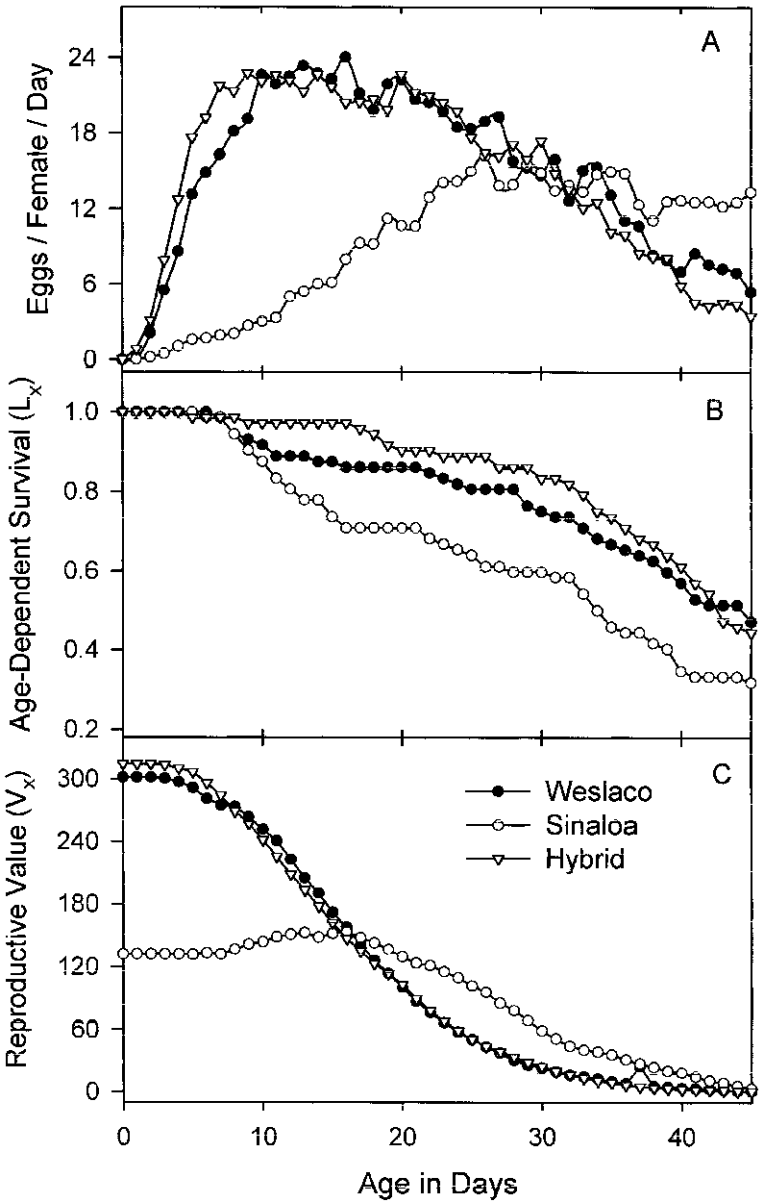

Fig. 1. Age-dependent fecundity and survival of females of 2 *C. grandis* populations and their hybrid. A) Age-dependent fecundity expressed as eggs oviposited per female per day; B) age-dependent survival expressed as l_x which is the proportion of individuals surviving from eclosion to the beginning of age x (in days); and C) Age-dependent reproductive value (V_x), which is the potential female progeny of a female of age x (in days).

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