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REARING *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE) ON A FACTITIOUS MERIDIC DIET AT DIFFERENT TEMPERATURES AND LARVAL DENSITIES

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ABSTRACT

Cactoblastis cactorum (Berg) is an invasive pest that represents an economic and ecological threat to native cacti in the U. S. and Mexico and that is currently the object of an eradication/control program in both countries. One tactic used to mitigate the threat of this species involves the SIT (Sterile Insect Technique), in which large numbers of larvae are reared on artificial diet or cactus cladodes, preferably the former, and released as sterile adults to mate with wild individuals at or near the leading edge of invasion. We studied combinations of 3 different rearing temperatures (22, 26, and 29°C) and 3 different crowding levels (50, 215, and 500 eggs) to determine percent survival to the adult stage, pupal size of each gender, and fecundity of *C. cactorum* reared on a factitious meridic diet. Temperature and crowding levels affected survival. The lowest rearing temperature and the low and high crowding levels adversely affect survival. Female pupae were larger than male pupae, and pupal size of males, but not females, was affected by rearing temperature, with smaller male pupae produced at the lowest temperature. Length and width, but not weight, of female pupae were affected by crowding levels, with slightly larger pupae produced at the intermediate level. For both genders, there was significant regression of pupal weight on percent survival, with larger pupae produced from treatment combinations that produced higher percent survival to the adult stage. Number of eggs laid per female was positively correlated with weight of the female as a pupa.

Key Words: *Cactoblastis*, *Opuntia*, artificial diet, mass rearing, temperature, crowding, invasive organism

RESUMEN

Cactoblastis cactorum (Berg) es una plaga invasora que representa una amenaza económica y ecológica a los cactus en los Estados Unidos y México y actualmente es el objeto de un programa de radiación/control en ambos países. Una táctica usada para mitigar la amenaza de esta especie se trata de TIS (Técnica del Insecto Estéril), en cual altos números de larvas son criadas sobre una dieta artificial o sobre los cladodes del cactus, preferiblemente la anterior, y liberadas como adultos estériles para aparearse con individuos en, o cerca del borde delantero de la invasión. Nosotros estudiamos combinación de 3 temperaturas de cría diferentes (22, 26 y 29°C) y 3 diferentes niveles de hacinamiento (50, 215 y 500 huevos) para determinar el porcentaje de sobrevivencia en el estadio adulto, el tamaño de pupa de cada sexo y la fecundidad de *C. cactorum* criada en un medio sobre una dieta facticia. La temperatura y los niveles de hacinamiento afectaron a la sobrevivencia. La temperatura mas baja de criarlas y los niveles de hacinamiento mas bajos y altos afectaron adversamente la sobrevivencia. Pupas de las hembras fueron más grandes que las pupas de los machos, y el tamaño de las pupas de macho, pero no las hembras, fue afectado por la temperatura de cría, con las pupas más pequeñas del macho producido a las temperaturas mas bajas. La longitud y anchura, pero no el peso, de las pupas de las hembras fueron afectadas por el nivel de hacinamiento, con pupas ligeramente mas grandes producidas al nivel intermedio. Para ambos sexos, hubo una regresión significativa en cuanto el peso de la pupa y el porcentaje de sobrevivencia, con las pupas más grandes producidas de las combinaciones de tratamiento que también producían un mayor porcentaje de sobrevivencia hasta el estadio adulto. El número de huevos puestos por hembra fue correlacionado positivamente con el peso de la hembra en el estadio de pupa.

The South American cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) is celebrated for its role as a biological control agent for weedy *Opuntia* spp., but its unintended arrival in North America represents an economic and eco-logical threat to native *Opuntia* spp. in the U. S. and Mexico (Zimmermann et al. 2004). Implementation of *C. cactorum* as a biological control agent in Australia and South Africa required disseminating large numbers of eggs into the field

where invasive opuntias were present. This was largely accomplished by mass rearing *C. cactorum* in cages provisioned with excised *Opuntia* cladodes (Dodd 1940; Pettey 1948). To mitigate the economic and ecological threat of *C. cactorum* to native *Opuntia* spp. in North America, large numbers of *C. cactorum* have been reared in the laboratory on excised cladodes to support the development of survey tools such as lures (Heath et al. 2006), traps (Hight et al. 2002; Bloem et al. 2005a), control tactics such as insecticides (Bloem et al. 2005b) and the Sterile Insect Technique (SIT) (Carpenter et al. 2001; Hight et al. 2005). However, mass-rearing *C. cactorum* in numbers sufficient to fully implement the SIT requires a suitable artificial medium and efficacious laboratory rearing protocols.

At present, separate colonies of *C. cactorum* at the USDA, CPMRU, facility in Tifton, GA, are reared on both artificial diet and *Opuntia* cladodes collected from the wild (Marti et al. 2008). Until a completely satisfactory artificial diet that meets all the requirements of the SIT program is developed, cactus cladodes will continue to be the food source for one of the CPMRU *C. cactorum* colonies. Research is presently underway to develop a suitable artificial diet that will allow a transition away from cladode-based rearing and permit mass-rearing of *C. cactorum* on a scale much larger than has been possible to the present. Problems with viral diseases (Marti et al, 2007), microsporidian infections, and possible stress-induced factors due to diet formulations that may be less than optimal, combine to present difficulties in mass rearing of this insect on artificial diet. To date, the only similar problems noted in the cladode-reared colony are occasional viral and microsporidian infections.

Because of the above considerations, research on factors unrelated to diet development, such as the effects of incubation temperature and larval crowding levels were, of necessity, conducted with our 2004 diet formulation, adapted from a diet used for rearing *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), the maize stem borer, in South Africa (Kfir 1992). This diet is somewhat different than the *C. cactorum* diet formulation currently in use and described by Marti et al. (2008) but we report the results here because the work involved a detailed study of the effects of different incubation temperatures and larval crowding levels on the survival of *C. cactorum* to adulthood and should provide knowledge useful for improving mass rearing regardless of the final version of the artificial diet. Furthermore, we also examined pupal size (weight, length, and width) of both sexes and mated pairs derived from the same treatment combinations to determine what factors, if any, produced differences in survival, size, and fecundity in this species when reared on a factitious meridic diet.

MATERIALS AND METHODS

Mature egg sticks of a US strain of *C. cactorum* reared on cactus (*Opuntia ficus-indica* L.) cladodes in 2004 (Marti et al. 2008) at CPMRU, Tifton, GA, were examined under low power microscopy to determine the number of eggs in each stick. Three temperatures (22, 26, and 29°C) and 3 larval crowding levels (low (mean = 51), medium (mean = 213), and high (mean = 502)), with 4 replications, were used in the experiment. Because individual eggsticks may comprise 20 to 80 or more eggs each, we used several eggsticks with various numbers of eggs to reach the required number in each replicate. Therefore, numbers of eggs varied slightly, from 49-53 at the low crowding level, 211-215 at the medium level, and 496-507 at the high level. Eggsticks were incubated at the intermediate temperature, 26°C, and then distributed randomly to the assigned treatments several days before the beginning of egg hatch.

Two separate rooms used in *C. cactorum* production in the rearing facility at CPMRU were maintained at 14:10 D/N photoperiod and 60-80% RH, one at 26°C and the other at 29°C, with a ±2°C daily variation. A laboratory environmental chamber maintained at 22°C with a ±2°C daily variation, 14:10 D/N photoperiod, and 60-80% RH was used for the third temperature treatment for comparative purposes, although *C. cactorum* is not currently reared at this temperature. Considerable temperature variation, particularly at the 2 higher temperatures, was the norm in these rearing rooms and we did not attempt to limit temperature variations or to alter rearing practices that were in place.

This work was conducted while the *Cactoblastis* diet was under development. Indeed, the diet is subject to constant improvement as more is learned about the nutritional requirements of the insect and about cost-effective and efficient methods to prepare it. The diet used in this study was adapted from a diet developed in South Africa for *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), the maize stalk borer, by Kfir (1992), and initially evaluated for *C. cactorum* by Zimmermann (2003). The diet was composed of the following: 2.5 L water, 45 g agar, mold inhibitor (15 mL of a solution consisting of 418 mL propionic acid, 42 mL phosphoric acid, and 540 mL water), ascorbic acid 9.6 g, sorbic acid 4.2 g, sucrose 100 g, methyl paraben 6 g, Brewer's yeast 186 g, and 630 g of white kidney beans ground in a hammer mill with a 0.238- cm screen.

Three 1-L flasks of tap water were autoclaved for 10.5 min at 124.2 kPa in the chamber and jacket at a temperature of 250°C. After autoclaving, 2.5 L of water was poured into a 4-L blender. Agar was added first and stirred into the hot water; then the other ingredients were mixed, with the ground kidney beans added last. The mixture

was stirred and blended for 1 min with the lid in place. The hot diet was poured to a depth of 2 cm in a cookie tray lined with aluminum foil and allowed to harden, and then cut into 4 × 4-cm squares with a knife or cookie cutter.

This diet differs from the *Chilo* diet principally in the omission of fishmeal and the use of a larger amount of ground white kidney beans. Although the protein content of fishmeal is higher than that of kidney beans, the substitution was made because *C. cactorum* larvae were found to grow faster on diet containing kidney beans. The modified *Chilo* diet differs from the current diet developed for *C. cactorum* (Marti et al. 2008) in that organic acids added to reduce the pH to about 4.1 were not included, and methyl paraben later became an optional ingredient used only if mold growth in the frass became a problem.

Diet squares were dipped into molten paraffin to provide a thin waxy coat simulating a wax-covered cactus cladode. Eggsticks were placed on the surface of a diet cake in a 20 × 30 × 10-cm plastic container fitted with a ventilated lid. Each container (n = 36) housed the larvae and diet from 1 treatment × crowding × replicate combination. After 1 week, eggsticks were removed and unhatched eggs were counted and recorded. A fresh diet cake was provided in all containers before the previous cake was completely consumed. Frass, silk, and uneaten diet were removed weekly, and twice weekly as the larvae matured. Dead larvae were counted and removed. This species is not known to be cannibalistic. Any live larvae remaining on old diet were transferred manually to fresh diet cakes. Because of differences in rearing conditions, larvae matured, spun cocoons, and molted to the pupal stage at different rates over a period of several weeks. In order to collect data on pupal gender, weight, length, and width, a sample of cocoons spun within a 5-d period was collected from most of the 36 containers. Ten to 60 (median = 30) cocoons were collected from 28 of 36 containers. Because of unfavorable treatment combinations, percent survival varied from 0-97% and few or no cocoons were collected from several containers. None were collected from larvae reared at 22°C at the lowest crowding level, although there were small numbers of survivors. Pupae were allowed to harden for 3 d, and then placed in a small sieve and desilked in undiluted Clorox® (5.25% sodium hypochlorite) for 18 s, rinsed 2 min in running tap water, and allowed to dry on a paper towel (Marti & Carpenter 2008).

Pupae were weighed on a Mettler® AJ100 electronic balance and sexed and measured under a Nikon Optiphot® stereoscopic microscope fitted with a reticle in an ocular of the microscope. Length and width of each pupa in reticular units were recorded and converted to centimeters. Because percent survival in individual containers varied widely, percent survival was analyzed by ANOVA and subsequent analyses were conducted with regression analysis. Means were separated (P < 0.05) by Duncan's multiple range test and Student's *t*-test (StatSoft, 2004). Means ± standard errors are presented unless stated otherwise.

RESULTS AND DISCUSSION

Rearing temperature significantly affected percent survival of *C. cactorum* larvae (F = 16.147, df = 2, 27, P < 0.001). Mean percent survival to the adult stage at 22, 26, and 29°C was 31.54 ± 7.81, 65.39 ± 5.87, and 77.38 ± 6.61, respectively. There was no significant temperature × crowding interaction on percent survival (F = 2.164, df = 4, 27, P = 0.1002). Percent survival at 22°C was less than survival at 26 or 29°C, (P < 0.05).

Crowding level significantly affected percent survival (F = 4.036, df = 2, 27, P = 0.029), with crowding levels of 50, 215, and 500 having survival percentages of 54.06 ± 10.29, 71.48 ± 6.22, and 48.77 ± 8.46, respectively. Low and high crowding levels unfavorably affected percent survival compared to the intermediate level. *Cactoblastis cactorum* is a gregarious species and larvae typically feed together in large numbers as long as food remains available. Survival at the high crowding level was less (P < 0.05) than that at the intermediate level. Although there was no significant interaction between incubation temperature and crowding levels, comparison of the means of the 9 temperature × crowding combinations showed that percent survival at low and high crowding levels at 22°C, 14.38% and 16.66%, respectively, were lower (P < 0.01) than those of the other 7 combinations, which varied from 55-82%, but were not different (P > 0.05) (Fig. 1). At 22°C, the low and high crowding levels were clearly unfavorable to larval survival. Yet at the warmer incubation temperatures of 26 and 29°C, percent survival was not affected by crowding level.

Regression of pupal weight on percent survival in containers (n = 27) from which pupae were collected was significant for both male (F = 15.061, df = 24, 463, P < 0.001) and female pupae (F = 7.369, df = 24, 356, P < 0.001). Mean weight of female pupae (0.1078 ± 0.023 g) was greater than that of the males (0.0733 ± 0.016 g) and the difference was significant (t = 26.22, df = 867, P < 0.001). Sarvary et al. (2008) also reported a marked sexual dimorphism in *C. cactorum*, with female pupae weighing 80% more than males.

Length and width of pupae were greater in females than males. Mean length of female pupae was 1.354 ± 0.077 cm and that of the males was 1.216 ± 0.086 cm (t = -22.48, df = 867, P < 0.001). Width of female pupae was 0.3889 ± 0.032 cm and that of the males was 0.3379 ± 0.030 cm (t =
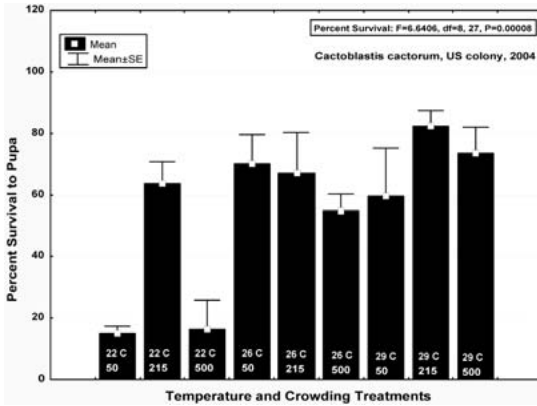
Fig. 1. Percent survival of *C. cactorum* to the pupal stage following rearing larvae on artificial diet in 9 combinations of incubation temperature × crowding level.

-24.35, df = 867, P < 0.001). Only about 9.2% (35/381) of females had a pupal width less than the mean of the males. Only about 4.7% (23/488) of males had a pupal width greater than the mean of the females (Fig. 2, Table 1). Although the gender difference in pupal width was very small, only about 0.05 cm, it may be enough to allow mechanical separation of pupae by gender with a suitably engineered device.

We envision that a device consisting of declined pairs of metal rollers with a gradually increasing gap from 0.24 to 0.50 cm and rotating in opposite directions away from the gap would allow pupae with the smallest width, mostly males, to fall through first and that the wider pupae, mostly females, would tend to be collected later. A series of containers beneath the rollers could be arranged to collect pupae based on desired differences in width.
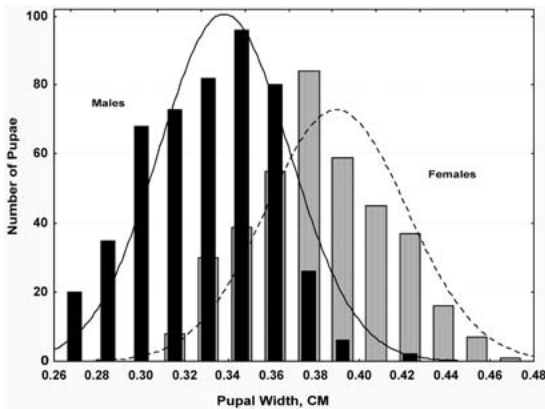

Fig. 2. Distribution of pupal widths (cm) of male and female *C. cactorum* reared on artificial diet.

Length, width, and weight of male pupae were affected by rearing temperature. Pupae of male larvae reared at 22, 26, and 29°C weighed 0.0676 ± 0.002, 0.0766 ± 0.001, and 0.0724 ± 0.001g, respectively (F = 12.25, df = 2, 485, P < 0.00001). These means differed from each other by Duncan's test (P < 0.02). Length of male pupae reared at 22, 26, and 29°C measured 1.186 ± 0.008, 1.227 ± 0.06, and 1.220 ± 0.07 cm, respectively, and were different (F = 8.349, df = 2, 485, P = 0.0003). Male pupae reared at 22°C were shorter (P < 0.001) than those reared at 26 or 29°C but those reared at 26 and 29 ºC did not differ (P > 0.05) by Duncan's test. Pupae of male larvae reared at 22, 26, and 29°C differed in width (F = 15.783, df = 2, 485, P < 0.0001). Width of pupae reared at 26 and 29 C did not differ (P > 0.05) but width of pupae reared at 22°C differed from those reared at 26 and 29°C (P < 0.001) by Duncan's test.

Rearing temperature had no significant effect on the weight of female pupae (F = 1.181, df = 2, 378, P = 0.31). At 22, 26, and 29°C female pupae weighed 0.1048 ± 0.02, 0.1084 ± 0.002, and 0.1092 ± 0.002g, respectively. The effect of temperature on length of female pupae was not significant (F = 1.367, df = 2, 378, P = 0.26). Mean lengths of female pupae at 22, 26, and 29 C were 1.347 ± 0.010, 1.347 ± 0.008, 1.364 ± 0.008, respectively. The effect of temperature on width of female pupae was significant but slight (F = 4.465, df = 2, 378, P = 0.012). Mean female pupal widths at 22, 26, and 29°C were 0.3833 ± 0.003, 0.3863 ± 0.003, and 0.3948 ± 0.003. Female pupal width at 29°C was different from those at 22 and 26°C (P < 0.05), according to Duncan's test.

The effect of crowding on female pupal weight was not significant (F = 1.452, df = 2, 378, P = 0.23). At crowding levels of 50, 215, and 500, female pupal weights were 0.1058 ± 0.003, 0.1104 ± 0.002, and 0.1065 ± 0.02g. However, crowding levels significantly affected female pupal length (F = 5.451, df = 2, 378, P = 0.0046). Mean pupal lengths at 50, 215, and 500 crowding levels were 1.3488 ± 0.012, 1.3744 ± 0.008, and 1.3402 ± 0.007, respectively, but only the means at the two higher crowding levels were significantly different (P < 0.05) from each other. Crowding level also significantly affected width of female pupae (F = 4.813, df = 2, 378, P < 0.01). Mean female pupal width at crowding levels of 50, 215, and 500 were 0.3870 ± 0.004, 0.3956 ± 0.003, and 0.3846 ± 0.002. Only the means at the 215 and 500 crowding levels were significantly different (P < 0.05).

Incubation temperature was only weakly correlated with pupal length (r = 0.08, P = 0.013) and width (r = 0.14, P = 0.0001), compared to no correlation to weight (r = 0.06, P = 0.052), and good correlation with percent survival (r = 0.58, P = 0.00001). Correlations with crowding level were all negative: pupal length (r = -0.13, P = 0.0001), pupal width (r = -0.13, P = 0.0001), pupal weight

TABLE 1. SEPARATION OF *C. CACTORUM* PUPAE BY GENDER BASED ON DIFFERENCES IN WIDTH (CM) FROM MEASUREMENTS OF 869 PUPAE.

Factor	Males	Females	Total
n	488	381	869
Mean width	0.3376	0.3889	—
n < mean male width	244	35	279
% < mean male width	87.5	12.5	100
n > mean male width	244	346	590
% > mean male width	41.4	58.6	100
n > mean female width	23	190	213
% > mean female width	10.8	89.2	100
n < mean female width	465	191	656
% < mean female width	70.9	29.1	100

($r = -0.06$, $P = 0.07$), and percent survival $r = -0.54$, $P = 0.0001$). Percent survival, pupal length, width, and weight were positively correlated with incubation temperature and negatively correlated with crowding level.

Significant regression of pupal weight on percent survival at each of the 3 different crowding levels was found only at the intermediate level (215 larvae) and was significant for both male and female pupae. At a crowding level of 215, and across all 3 temperature levels, the regression of male pupal weight on percent survival was significant ($r^2 = 0.45$, $r = 0.67$, $P < 0.0001$). For female pupae, the regression of pupal weight on percent survival was also significant ($r^2 = 0.24$, $r = 0.49$, $P < 0.0001$). Crowding levels at 50 and 500, for both genders, and at all 3 temperatures were not significant ($P > 0.05$).

In a subset of pupae that were eventually paired as adults ($n = 219$ pairs) from containers having >22% survival (deleting pupae from 1 container with lowest survival), regression (Fig. 3) of pupal weight on percent survival was significant for both male ($r^2 = 0.51$, $P < 0.0001$) and female pupae ($r^2 = 0.23$, $P = 0.0124$). In mated females which laid eggs (Fig. 4), regression of number of eggs laid on weight of the female as a pupa was significant ($r^2 = 0.35$, $P < 0.0001$). Deleting 3 outliers (arrows in Fig. 4) representing females that laid fewer than 20 eggs, resulted in an increase to 0.45 in the r^2 value. In any case, the regression of eggs on the pupal weight of females that laid eggs was highly significant. The equation for the regression relating eggs and pupal weight, Eggs = -12.3026 + 776.3855 × Pupal Weight, showed that each 10-mg increase in pupal weight of a female that eventually laid eggs resulted in an increase of 7.76 eggs laid. Over the range of female pupal weights, from 0.07 g to 0.15 g, this represents a difference of about 62 eggs.

Insect body mass is often expressed as dry weight and compared with other parameters such as length, as was done by Rogers et al. (1976), who found a highly significant linear relationship be-
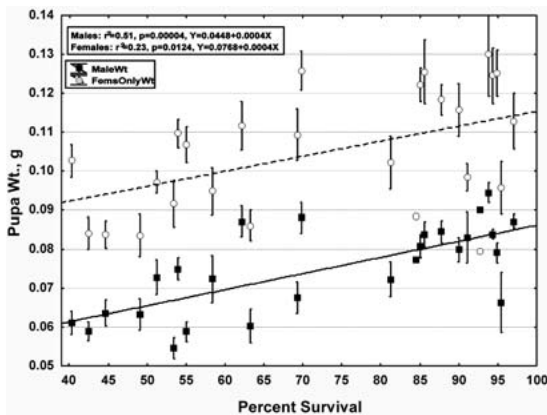

Fig. 3. Regression of male and female *C. cactorum* pupal weights on percent survival. Data from 1 container in which survival was < 22% has been deleted.
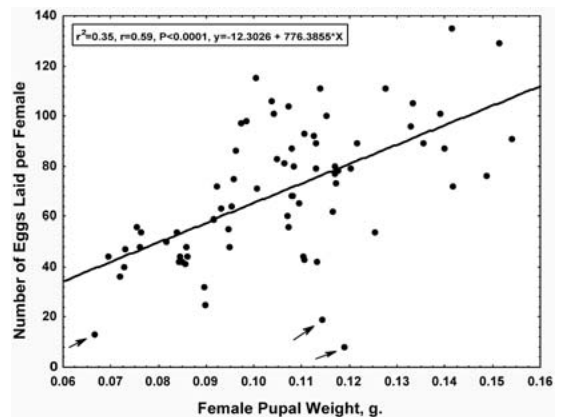

Fig. 4. Regression of number of eggs laid per female *C. cactorum* on pupal weight. Three outliers (arrows) representing fewer than 20 eggs are not included in the regression line.

tween dry weight and body length ($r^2 = 0.94$) across 8 orders of insects. If adult live weight is taken as 0.6 of pupal weight (Honek 1993), then estimation of egg production in individual *C. cactorum* females can be made from either pupal length or weight. Since fecundity is closely related to pupal weight, one objective of a *C. cactorum* rearing program should be to develop a diet or management regime which tends to maximize pupal size. One unsolved problem, however, is that there is as yet no good explanation why some otherwise normal females fail to mate or why some mated females fail to lay their eggs.

Legaspi & Legaspi (2007) reported that pupal weights declined with increasing temperature and were always lower in males than females. We also found consistently lower pupal weights for males. However, we found no significant effect on pupal weight due to temperature ($F = 0.32$, $df = 1$, 373, $P = 0.57$) or crowding ($F = 3.50$, $df = 1$, 373, $P = 0.062$) on female pupae. In addition, there was no significant temperature x crowding interaction ($F = 0.906$, $df = 3$, 373, $P = 0.438$). In the case of male pupae, however, there was a significant effect of both temperature ($F = 7.217$, $df = 1$, 480, $P = 0.007$) and crowding ($F = 15.195$, $df = 1$, 480, $P = 0.0001$), but there was no interaction ($F = 0.905$, $df = 3$, 480, $P = 0.438$). Although we had no data for the treatment combination of the lowest temperature with the lowest crowding level, the general trend was for male pupal weights to be highest at the intermediate temperature (26°C) and lowest at the greatest crowding level (500). No similar trends could be detected in female pupae.

Legaspi & Legaspi (2007) determined the reproductive parameters for *C. cactorum*, including R_0, the net reproductive rate. To calculate the R_0, the product of the probability of a female living to each age and the number of female eggs at each of those ages are summed. Our study was not designed to determine reproductive parameters. We did, however, obtain the total egg output from mated females which produced eggs and we used that as a means to estimate the R_0 and to compare it with the results obtained by Legaspi & Legaspi (2007). In our study, R_0 at incubation temperatures of 22, 26, and 29°C, was 30.7, 38.1, and 30.9, respectively, and at crowding levels of 50, 215, and 500, R_0 was 38.8, 33.8, and 33.3, respectively. The overall R_0 was 34.7. Our results are in good agreement with those of Legaspi & Legaspi (2007), who found R_0 highest (49.20) at the intermediate temperature of 26°C and lower at the extremes.

Regression of egg number (>20 eggs) on the pupal weight of the males mated to the females was significant ($r = 0.31$, $r^2 = 0.09$, $P < 0.05$) but much more variable than the females, as shown by the lower r value. However, the apparent significance of male pupal weight on number of eggs laid by females was due to significant correlation between male and female pupal weights. Covariance analysis showed that after removing the effects of female pupal weight, the male weight effect was not significant ($P = 0.74$), whereas the female weight effect was highly significant ($P < 0.0001$) for females that produced eggs. The combined weight of the male and female pupae was a significant predictor of egg production ($r = 0.57$, $r^2 = 0.33$, $P < 0.00001$), though not as good as the female pupae alone. While egg production in females which produced >0 eggs was highly correlated with weight of the female as a pupa, there was no correlation ($P > 0.05$) between number of eggs produced and the incubation temperature or crowding level at which the female was reared in the larval stage. Others have also reported a similar relationship between number of eggs laid and body size of the female (Tammaru et al. 1996).

Regression of pupal weight on pupal length at each level of temperature and crowding were nearly identical. In each case, the regression was significant ($P < 0.0001$), with r^2 values of 0.65 or higher. Comparison of females which laid eggs with those that did not showed that the regression equations were parallel. We therefore conclude that failure of some adults to mate was not related to incubation conditions experienced in the larval stage. We also found, by subtracting the numbers of dead larvae and pupae and the numbers of surviving pupae from the number of hatched eggs in each container at the start of the experiment, that many larvae were missing and presumed dead from unknown causes. After submitting dead larvae for electron microscopical examination, viral disease was implicated in the death of many of these larvae (Marti et al. 2007)

Although 869 pupae were weighed in the study, only 240 individuals of each gender were paired within treatment combinations to determine fecundity. Of these 240 pairs, 69 produced eggs while 171 failed to produce eggs. This proportion was significant ($\chi^2 = 29.49$, $P < 0.001$). Because of unequal distribution of pairs by treatments, we used χ^2 to examine treatment effects on egg production in these 240 pairs. At each of the 3 incubation temperatures and at the 2 highest crowding levels, the proportions of pairs that laid no eggs was significantly ($P < 0.05$) greater than those which produced eggs. At 26 and 29°C, χ^2 increased as crowding level increased and was significant ($P < 0.05$) at the highest crowding level. Plotting on a 3D grid the proportion of pairs failing to produce eggs at each temperature x crowding combination showed that failure to produce eggs was associated with high incubation temperature in the larval stage. The association of crowding on the proportion of pairs not producing eggs was very slight, with lower crowding levels somewhat more favorable than high levels. Dissection of females showed that a spermatophore was absent from many of them and that, therefore, they had failed

to mate. Failure to mate, failure of some mated females to lay eggs, and the presence of pathogens have been major obstacles thus far in the development of a mass rearing program for *C. cactorum*. Research into these problems, as well as development of an artificial diet to replace cactus cladodes collected in the wild, are currently in progress.

REFERENCES CITED

BLOEM, S., S. HIGHT, J. E. CARPENTER, AND K. A. BLOEM. 2005a. Development of the most effective trap to monitor the presence of the cactus moth *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 88: 300-306.

BLOEM, S., R. F. MIZELL, K. A. BLOEM, S. D. HIGHT, AND J. E. CARPENTER. 2005b. Laboratory evaluation of insecticides for control of the invasive *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 88: 395-400.

CARPENTER, J. E., K. A. BLOEM, AND S. BLOEM. 2001. Applications of F1 sterility for research and management of *Cactoblastis cactorum* (Lepidiptera: Pyralidae). Florida Entomol. 84(4): 531-539.

DODD, A. P. 1940. The Biological Campaign Against Prickly Pear. Commonwealth Prickly Pear Board, Brisbane, 1-177.

HEATH, R. R., P. E. A. TEAL, N. D. EPSKY, B. D. DUEBEN, S. D. HIGHT, S. BLOEM, J. E. CARPENTER, T. J. WEISSLING, P. E. KENDRA, J. CIBRIAN-TOVAR, AND K. A. BLOEM. 2006. Pheromone-based attractant for males of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Environ. Entomol. 35: 1469-1476.

HIGHT, S. D., J. E. CARPENTER, K. A. BLOEM, S. BLOEM, R. W. PEMBERTON, AND P. STILING. 2002. Expanding geographical range of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Florida Entomol. 85: 527-529.

HIGHT, S. D., J. E. CARPENTER, S. BLOEM, AND K. A. BLOEM. 2005. Developing a sterile insect release program for *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae): Effective overflooding ratios and release-recapture field studies. Environ. Entomol. 34: 850-856.

HONEK, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66: 483-492.

KFIR, R. 1992. A simple artificial diet for mass rearing the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). J. Entomol. Soc. South Africa. 55: 283-284.

LEGASPI, J. C., AND B. C. LEGASPI, JR. 2007. Life table analysis for *Cactoblastis cactorum* immatures and female adults under five constant temperatures: implications for pest management. Ann. Entomol. Soc. America 100: 497-505.

MARTI, O. G., AND J. E. CARPENTER. 2008. Chemical desilking of *Cactoblastis cactorum (*Berg) pupae. J. Entomol. Sci. 43: 344-347.

MARTI, O. G., R. E. MYERS, AND J. E. CARPENTER. 2008. Rearing *Cactoblastis cactorum* (Lepidoptera: Pyralidae) on artificial diet and *Opuntia* cladodes. J. Entomol. Sci. 43: 95-106.

MARTI, O. G., E. L. STYER, R. E. MYERS, AND J. E. CARPENTER. 2007. Viruses in a laboratory-reared colony of the cactus moth, *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 90: 274-277.

PETTEY, F. W. 1948. The Biological Control of Prickly Pear in South Africa. Science Bulletin, Department of Agriculture of the Union of South Africa 271: 1-163.

ROGERS, L. E., W. T. HINDS, AND R. L. BUSCHBOM. 1976. A general weight vs. length relationship for insects. Ann. Entomol. Soc. America 69: 387-389.

SARVARY, M. A., K. A. BLOEM, S. BLOEM, J. E. CARPENTER, S. D. HIGHT, AND S. DORN. 2008. Diel flight pattern and flight performance of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) measured on a flight mill: influence of age, gender, mating status, and body size. J. Econ. Entomol. 101: 314-324.

TAMMARU, T., P. KAITANIEMI, AND K. RUOHOMÄKI. 1996. Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): Relation to body size and consequences to population dynamics. Oikos 77: 407-416.

ZIMMERMANN, H. G. 2003. Costs and Efficacy of Mass-rearing *Cactoblastis cactorum* in South Africa. Interim Progress Report: October 2002-March 2003 for the International Atomic Energy Agency, Vienna, Austria, 12 pp.

ZIMMERMANN, H. G., S. BLOEM, AND H. KLEIN. 2004. Biology, History, Threat, Surveillance and Control of the Cactus Moth, *Cactoblastis cactorum*. pp 1-40. IAEA/FAO-BSC/CM, Vienna.