INHERITED STERILITY IN *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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Abstract

Newly emerged male and female adult cactus moths, *Cactoblastis cactorum* (Berg), were treated with increasing doses of gamma radiation, and the moths were outcrossed to fertile counterparts. Fecundity of the moth pairs was not affected by increasing doses of radiation. The minimum dose at which treated females were found to be 100% sterile when mated to untreated males was 200 Gy. Fertility of treated males declined with increasing doses of radiation to approach 0% near 500 Gy. Inherited effects resulting from irradiation of P males and females were expressed in the F_1 generation as increased developmental time from oviposition to larval eclosion, increased egg mortality, and increased neonate to adult stage mortality. A shift in the F_1 sex ratio in favor of males was not observed.

Key Words: cactus moth, inherited sterility, sterile insect technique, invasive species

Resumen

Adultos recientemente emergidos de ambos sexos de *Cactoblastis cactorum* (Berg) fueron tratados con varias dosis de radiación gamma y apareados con individuos fértiles del sexo opuesto. La fecundidad de estas parejas no fue afectada por las dosis de radiación utilizadas. La dosis mínima que causó esterilidad completa en las hembras tratadas fue 200 Gy. La fertilidad de los machos tratados decreció a medida que la dosis de radiación aumentó hasta alcanzar esterilidad completa con la dosis de 500 Gy. Los efectos heredados al irradiar a los machos y hembras de la generación P fueron expresados en la generación F_1 como una reducción en la eclosión de huevecillos, un desarrollo mas lento durante la embriogenesis de los huevecillos y un aumento en la mortalidad observada durante el periodo larvario. No se observaron distorciones de la tasa sexual en favor de la progenie de sexo masculine.

The control of exotic *Opuntia* cacti in Australia and South Africa by the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is one of the classic success stories in biological control (Dodd 1940; Pettey 1948). However, the recent unintentional arrival of *C. cactorum* in Florida from the Caribbean has raised concerns for the well being of native *Opuntia* in the southern United States and in Mexico (Habeck & Bennett 1990; Pemberton 1995; Johnson & Stiling 1998; Zimmerman et al. 2001).

Cactoblastis cactorum is a pyralid moth native to northern Argentina, Uruguay, Paraguay, and southern Brazil (Mann 1969). Larvae of *C. cactorum* are phytophagous, feeding on many species of *Opuntia* cactus, primarily from the subgenus *Platyopuntia* (Mann 1969). The female lays eggs in a vertical chain extending from the cactus surface or the tip of a cactus spine. Known as egg sticks, these chains average 50-80 eggs and each female can lay an average of three to four egg sticks in its lifetime (Dodd 1940; Myers et al. 1981; Robertson 1987). Eggs take four to five weeks to develop. Upon eclosion, the larvae crawl down from the egg sticks and burrow into a cactus pad. The larvae are gregarious internal feeders and will move to a new pad when the current feeding site is destroyed (Dodd 1940). Approximately four pads are needed to support the complete development of a cohort of larvae from an average egg stick (Monro 1967; Meyers et al. 1981). Fully developed larvae usually leave the plant to spin cocoons in the litter or the bark of nearby trees. Occasionally pupation occurs within the skeletonized pad. The number of generations per year in Florida is unknown, however, in Australia (Dodd 1940) and South Africa (Robertson & Hoffmann 1989) *C. cactorum* has two generations per year, with a possible third. Laboratory observations of *C. cactorum* collected in Georgia show the life cycle to be approximately 90 days from oviposition to adult stage at 26-27°C (authors, pers. obs.).

No satisfactory method of control, including pesticides, pathogens and biological control, is currently known for *C. cactorum*. However, the use of the sterile insect technique and the phenomenon of inherited (F_1) sterility in Lepidoptera offer some potential for managing the spread of this insect in North America (see Carpenter et al. 2001 for a discussion of the potential applications of F_1 sterility for researching and managing *Cactoblastis* and other invasive lepidopterans).

F_1 sterility takes advantage of two unique genetic phenomena in Lepidoptera (LaChance 1985). First, females are generally more sensitive to radiation than are males of the same species. This allows one to adjust the dose of radiation used to treat the insects such that treated females are completely sterile and the males are only partially sterile. Second, when partially sterile males are outcrossed with wild fertile females the radiation-induced deleterious effects are inherited by the F_1 generation. As such, larval eclosion is reduced and the (F_1) offspring produced are more sterile than the irradiated parent and predominately male. The lower dose of radiation used in this technique increases the quality and competitiveness of the released moths as compared to fully sterile moths (North 1975). In addition, because F_1 sterile progeny are produced in the field, the release of partially sterile insects offers both greater suppressive potential than the release of fully sterile insects (LaChance 1985) and the potential to make developmental and behavioral observations under actual field conditions without concern for establishing a breeding population.

Nothing is currently known about radiation effects on *C. cactorum*. However, F_1 sterility has been demonstrated in a number of economically important Lepidoptera (Bloem & Carpenter 2001), including other Pyralidae such as the European corn borer, *Ostrina nubilalis* (Hübner) (Nabors & Pless 1981), the almond moth, *Cadra cautella* (Walker) (Brower 1980, 1982), and the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Walker & Quintana 1968a, b; Sanford 1976, 1977). In this paper, we examine the effect of various doses of gamma radiation on the fecundity and fertility of *C. cactorum*. In particular, we were interested in determining the minimum dose at which females are 100% sterile when mated to fertile males and in verifying that the F_1 sterility effects are manifested in this species.

Materials and Methods

Test Insects

Cactus moths used in this study came from field-collected larvae found infesting stands of *Opuntia stricta* (Haworth) Haworth along the causeway connecting the Georgia mainland with Jekyll Island, GA. Two collections were made in the same general location on two different dates (1 & 10 March, 2001). Several hundred larvae of mixed age and additional uninfested cactus pads were collected, and the material returned to the USDA-ARS-CPMRU Laboratory in Tifton, GA. Infested pads were placed in rectangular plastic containers (34 × 24 × 13 cm) on top of a thin layer of sterilized sand. Uninfested pads of *O. stricta* were added to the containers to serve as additional food for developing larvae. Containers were kept in a growth chamber at 26.5°C, 70% relative humidity (R.H.) and a photoperiod of 12L:12D and checked every few days for the presence of cocoons. Most pupae formed under the container lids, although a few pupated under the cactus pads. Cocoons were collected 2-3 days after initiation of pupation. Pupae were carefully extracted from the silken cocoons, sorted by gender, and male and female pupae were held separately in 475 ml plastic cups at the above conditions. Prior to adult eclosion, male and female pupae were placed inside separate screen cages (30.5 × 30.5 × 30.5 cm) and allowed to emerge at room temperature (23 ± 1°C).

Effect of Gamma Radiation on Adult Moth Sterility

Newly emerged (<24 h old) virgin adult male and female cactus moths were exposed to gamma radiation in groups of 1-5 moths in 30 ml plastic cups. A Cobalt^{60} gammacell 220 irradiator with a dose rate of 20.3 Gy/min was used to administer doses of 0, 50, 100, 200, 300, 400, and 500 Gy. After irradiation, each treated (T) moth was placed in a 475 ml plastic container with a non-treated (N) adult of the opposite sex. N♀ × N♂ crosses served as controls. Each mating container was provisioned with a pad of *O. stricta* as an ovipositional substrate and a small petri dish with a cotton wick soaked in 10% sugar solution to provide nourishment to the moth pairs. The moths were allowed to mate and lay eggs at 26°C, 70% R.H. and a photoperiod of 14L:10D until the females died. Adult longevity was recorded for females and males. Females were dissected to ascertain whether a spermatophore was present in the spermatheca, thus confirming their mating status (Ferro & Akre 1975).

Egg sticks deposited by each female were collected and held separately (one cup for each oviposition event per female) in small 30 ml plastic cups at the above conditions. Egg sticks were incubated for approximately 30 days to allow for complete egg development and larval eclosion. The total number of eggs produced and the total number of larvae that eclosed were recorded for each female at each treatment dose. Sterility was expressed as the percentage of eggs from which no larva eclosed. Seven to 13 pairs of T♀ × N♂ or N♀ × T♂ were used per treatment dose.

Cactus Moth F_1 Progeny Follow-Up

F_1 neonates from each cross were carefully placed on pads of *O. stricta* and reared at 25-27°C in plastic containers (34 × 24 × 13 cm). Days to pupation were recorded for all treatments. Pupae were collected and separated by gender as above, and all emerging adults were outcrossed to fertile (unirradiated) counterparts of the opposite sex. Moths were allowed to mate and lay eggs as above. Egg sticks were collected and incubated as above and the sterility in the F_2 generation was calculated. Longevity for the F_1 pairs was recorded, and mating status of the F_1 females was verified as above.

Statistical Analysis

Data collected from both the parental (irradiated) and the developing F_1 generations were analyzed using a two-factor analysis of variance and regression analysis, with dose used and gender irradiated as sources of variation (PROC GLM) (SAS Institute 1989). The longevity of irradiated moths, number of eggs laid, percentage larval eclosion, percentage of neonates surviving to pupation, percentage of neonates surviving to adulthood, sex ratio (% male) of F_1 adults, developmental time from oviposition to larval eclosion, and developmental time from neonate to adult were the dependant variables. When significant ($P \leq 0.05$) interaction was detected between dose used and gender irradiated, the effect of dose within each gender was examined using polynomial regression (PROC GLM).

Data collected for the F_1 generation were analyzed using a three-factor analysis of variance and regression analysis, with dose, F_1 gender and parental gender irradiated as sources of variation (PROC ANOVA) (SAS Institute 1989). The longevity of F_1 moths, number of eggs laid, egg development time, percentage larval eclosion, and percentage of larvae surviving to the second instar were the dependent variables. When significant ($P \leq 0.05$) interaction was detected between dose, F_1 gender and parental gender irradiated, the effect of dose within each F_1 gender and gender irradiated was examined using polynomial regression (PROC GLM).

Results and Discussion

Fecundity (= total # eggs laid) and adult longevity of the *C. cactorum* parental generation were not significantly affected by radiation dose or by the gender irradiated. However, there was a significant ($F = 6.23$; df = 1, 55; $P = 0.0156$) difference in the longevity of male (13.0 ± 3.5 d) and female moths (11.3 ± 2.6 d) irrespective of treatment. During this study, an average of 50% of the moth pairs mated in the laboratory. The mean (±SD) number of eggs laid by mated females was 119.8±68.9.

The percentage of eggs from which larvae eclosed and the developmental time from oviposition to larval eclosion for the parental generation of *C. cactorum* were significantly affected by the gender irradiated and by the dose of radiation. For each gender, the percentage of larval eclosion declined significantly as the dose of radiation increased (Fig. 1). This dose effect was greater for irradiated females ($y = 76.4 - 10.9x + 0.56x^2$; $F = 23.89$; df = 4, 30; $P < 0.0001$) than for irradiated males ($y = 98.3 - 4.5x + 0.01x^2$; $F = 33.29$; df = 4, 28; $P < 0.0001$). The dose of radiation also had a significant effect on the time from oviposition to larval eclosion for both genders (Fig. 2). Again, this dose effect was greater for irradiated females ($y = 27.1 + 0.22x$; $F = 14.22$; df = 4, 11; $P < 0.0031$) than for irradiated males ($y = 27.2 + 0.09x$; $F = 3.42$; df = 4, 21; $P < 0.0265$).

For the F_1 generation, the percentage of neonates that pupated declined significantly as the dose of radiation administered to the parent increased ($F = 5.81$; df = 10, 29; $P < 0.0001$). In addition, the percentage of F_1 neonates that emerged as adults was significantly affected by the gender irradiated and by the dose of radiation administered to the parent. For each gender, the percentage of F_1 neonates that emerged as adults declined significantly as the dose of radiation increased. This dose effect was greater for irradiated females ($y = 47.9 - 8.27x$; $F = 98.97$; df = 2, 6; $P < 0.0001$) than for irradiated males ($y = 49.5 - 1.64x$; $F = 3.51$; df = 3, 13; $P < 0.0465$). The mean number of adult F_1 progeny produced from irradiated *C. cactorum* reflects the total mortality in the F_1 generation and is presented in Fig. 3. The effect of radiation on F_1 mortality was greater when the female was irradiated. The developmental time from F_1 neonate to adult and the sex ratio of the adult F_1 was not significantly affected by dose of radiation or gender irradiated.

Adult longevity of *C. cactorum* during the F_1 generation and the ability of F_2 neonates to establish on cactus pads were not significantly affected

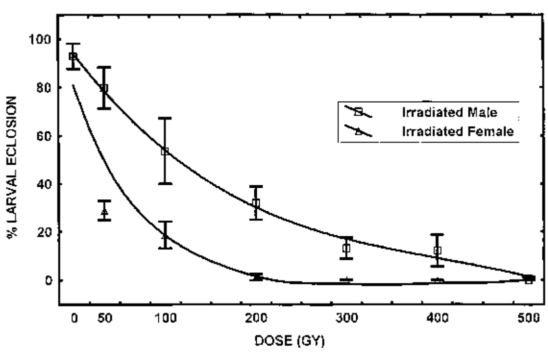

Fig. 1. Percentage larval eclosion obtained when *Cactoblastis cactorum* adults were treated with 0, 50, 100, 200, 300, 400, and 500 Gy of gamma radiation and outcrossed with untreated *C. cactorum*. Bars represent ±1 SD.

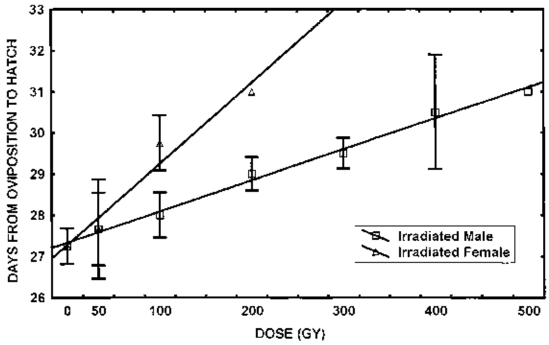

Fig. 2. Relationship between the dose of gamma radiation administered to *Cactoblastis cactorum* adults and the number of days from oviposition to larval eclosion for the F_1 generation eggs. Males and females were treated with 0, 50, 100, 200, 300, 400, and 500 Gy of gamma radiation and outcrossed to untreated counterparts. Bars represent ±1 SD.

by radiation dose, the gender irradiated or the gender of the F_1 adult. Fecundity of F_1 adults was not significantly affected by radiation doses of 50 and 100 Gy administered to the parent generation. However, no eggs were laid by F_1 moths descending from matings (n = 7) between normal females and males irradiated with 200 Gy (females irradiated with 200 Gy produced no adult progeny).

The percentage of larvae eclosing from eggs laid by the F_1 adults was significantly affected by the dose of radiation applied to the parental generation ($F = 15.94$; df = 2, 40; $P < 0.0001$) and the P gender irradiated ($F = 4.67$; df = 1, 40; $P = 0.0368$) (Fig. 4). In addition, we found a significant interaction between the gender irradiated and the gender of the F_1 adult descending from an irradiated parent ($F = 4.24$; df = 1, 40; $P = 0.0460$). The percentage of larvae that eclosed was significantly reduced with an increasing dose of radia-

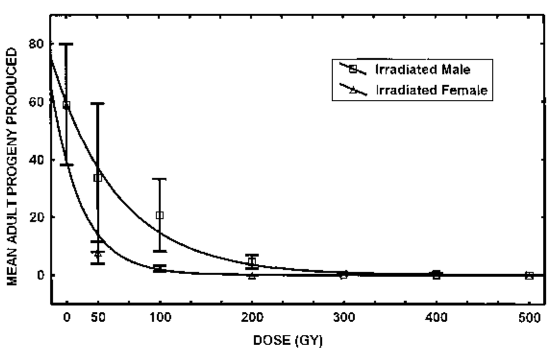

Fig. 3. Mean number of adult F_1 progeny produced per mating pair when *Cactoblastis cactorum* adults were treated with 0, 50, 100, 200, 300, 400, and 500 Gy of gamma radiation and outcrossed with untreated *C. cactorum*. Bars represent ±1 SD.

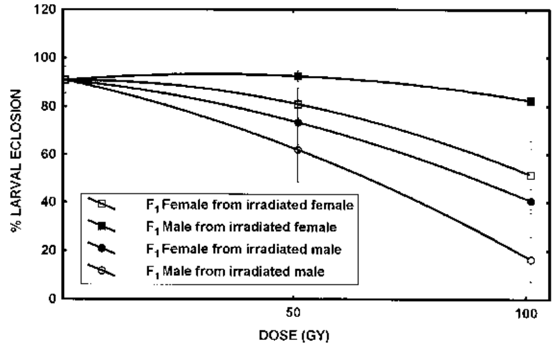

Fig. 4. Effect of the dose of gamma radiation used, the parental gender irradiated, and the gender of the F_1 adult on percentage larval eclosion for F_2 generation eggs of *C. cactorum*. Bars represent ±1 SD.

tion, and was lowest when the P gender irradiated was male. For F_2 larvae that did hatch, the developmental time from oviposition to larval eclosion ranged from 28.3-33.3 d.

Attributes that are common to inherited sterility in Lepidoptera include (1) F_1 males and females are more sterile than the irradiated parental generation, and (2) more F_1 male progeny than female progeny are produced (LaChance 1985). Other attributes that have been reported include delayed developmental times and reduced sperm quality in F_1 progeny. We found that *C. cactorum* females are more radiosensitive than are males. These findings agree with those reported for other lepidopteran species (North 1975; Carpenter et al. 1986; Bloem et al. 1999). Our results also showed that irradiated *C. cactorum* share some attributes that are common to other irradiated lepidopterans. We demonstrated that F_1 progeny from irradiated males were more sterile than their irradiated parent. For example, at 100 Gy, irradiated P males were approximately 45% sterile when outcrossed to untreated females (Fig. 1), while their male progeny were approximately 82% sterile and the female progeny were approximately 60% sterile (Fig. 4). In addition, the F_1 progeny from irradiated females were more fertile than F_1 progeny from irradiated males (Fig. 4) (North 1975; LaChance 1985; Carpenter et al. 1986; Bloem et al. 1999). We also found that increasing radiation dose significantly delayed the developmental time (between oviposition and larval eclosion) for F_1 eggs. However, we did not detect a skewed sex ratio in favor of males for the F_1 adults. Although atypical, other researchers also have reported minimal effects of radiation on sex ratio of F_1 adults (LaChance et al. 1973). In this study, the failure to detect a distortion in the sex ratio may be the result of high F_1 mortality or reduced number of adults available for study at the radiation doses most likely to induce this sex ratio distortion (i.e., 200 Gy).

Carpenter et al. (2001) suggest that F_1 sterility for control of *C. cactorum* might be appropriate for (1) eradication of *C. cactorum* from areas of new introductions or from isolated and/or environmentally sensitive areas, (2) establishment of a barrier through the release of irradiated moths along the leading edge of the *C. cactorum* geographical range, and (3) provisioning sterile *C. cactorum* as hosts in the field to increase the initial survival and establishment of released natural enemies. Based on the results of this study, moths irradiated with a dose between 100-200 Gy might be suitable to address these control strategies. When moths are irradiated at 100 Gy, full sterility and/or mortality does not occur in the F_1 generation. The regression lines in Fig. 4 suggest that full sterility might be reached at 200 Gy for the progeny of irradiated males. However, the mean adult progeny produced from males treated with 200 Gy is quite low (Fig. 3), and no eggs were laid by the surviving F_1 adults. Therefore, we suggest that further studies involving larger test populations and at doses between 100 and 200 Gy are warranted in order to select a dose that would allow for maximum production of F_1 adults while inducing full sterility in the F_1 generation.

Carpenter et al. (2001) also discuss several ways in which F_1 sterility might be useful for studying *C. cactorum*. Examples include: (1) elucidation of the potential host range of *C. cactorum* for key native *Opuntia* species from across the U.S., (2) prediction of the potential geographic range of *C. cactorum* in the U.S. and Mexico, and (3) delineation of the potential impact of native natural enemies on the spread of *C. cactorum*. In each of these cases where the research protocol would involve the release of irradiated moths beyond the leading edge of the *C. cactorum* geographical range, 100% reproductive sterility of the F_1 progeny of irradiated *C. cactorum* would be mandatory. As stated above, our data suggest that the optimum dose of radiation for this purpose would be between 100-200 Gy. In addition, studies would be needed to determine the reproductive rate when progeny of irradiated parents are inbred ($F_1 \times F_1$ crosses) because only irradiated moths and their progeny would be found beyond the leading edge of the geographical range.

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