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INFLUENCE OF RADIATION DOSE ON THE LEVEL OF F_1 STERILITY IN THE CACTUS MOTH, *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

We examined inherited sterility effects on the F_1 and F_2 generations of the cactus moth, *Cactoblastis cactorum* (Berg) after gamma sterilization. Our objectives were to identify the dose of gamma radiation that would fully sterilize F_1-generation moths and result in no viable offspring when F_1 males were inbred- or out-crossed to fertile females, and that would allow maximum production of F_1 sterile *C. cactorum* adults by irradiated males. Newly emerged adults of *C. cactorum* were exposed to increasing doses of gamma radiation and inbred or out-crossed to fertile counterparts. Inherited effects resulting from irradiation of males and females were expressed in the F_1 generation as reduced egg hatch, increased developmental time for the F_1 egg, and increased F_1 larval to adult mortality. These effects were most pronounced when parental adults were irradiated at 200 Gy. Survival of F_1-generation offspring originating from irradiated male × fertile female crosses was greatest at 200 Gy. In addition, inbred- and out-crosses of surviving F_1 adults, with 1 parent irradiated at 200 Gy, resulted in no F_2 adults. Maximum production of sterile F_1 adults at 200 Gy suggests this dose is the most appropriate dose for implementing the sterile insect technique (SIT)-F_1 sterility for control of *C. cactorum* in North America and for testing host suitability and potential geographical range in the field.

Key Words: cactus moth, weed biological control, F_1 sterility, sterile insect technique, invasive species

RESUMEN

Examinamos los efectos de esterilidad heredados en las generaciones F_1 y F_2 de la palomilla de cactus,*Cactoblastis cactorum* (Berg) después de ser irradiadas. Nuestros propósitos fueron para identificar la dosis de radiación gamma requerida para esterilizar completemente las palomillas de la generación F_1 que resultara en tener progenie no viable cuando los machos de la generación F_1 fueran cruzadas con hembras fertiles de su propria descendencia o con hembras de otras descendencias, con lo que permitirá la producción máxima de adultos esteriles de *C. cactorum* de generación F_1 por la irradiación de machos. Los adults de *C. cactorum* recién emergidos fueron expuestos al incremento de dosis de radiación gamma y fueron cruzadas con individuos fertiles de su propria progenie o de otra descendencia. Los efectos heredados como resulto de la irradiación de machos y hembras fueron expresados en la generación F_1 con una reducción en el número de huevos esclosionados, un aumento en el tiempo del desarrollo del huevo de F_1 y un aumento en la mortalidad de la larva y adulto de la generación F_1. Estos efectos fueron mas pronunciados cuando los adultos paternales fueron irradiados con 200 Gy. La sobrevivencia de la progenie de la generación F_1 fue la mas alta al nivel de la 200 Gy y en los cruces con machos irradiados × hembras fertiles. Los cruces con adultos sobrevivientes apareados con el sexo opuesto de su propria linea y con los de otra linea con un pariente expuesto a irradiación de 200 Gy resultaron en la no presencia de adultos en la generacion F_2. La producción máxima de adultos esteriles de F_1 expuestos a 200 Gy indica que esta dosis es la mas apropiada para la implementación de la técnica de insecto estéril (TIE)-F_1 esteril para el control de *C. cactorum* en America del Norte, asi como para probar si el hospedero es apropiado y el determinar su rango geográfico potencial en el campo.

Successful control of invasive *Opuntia* cacti in Australia and South Africa through importation and release of the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is one of the most cited success stories in biological control of weeds (Dodd 1940; Pettey 1948; Moran & Zimmerman 1984). In Australia, this effort resulted in the recovery of 90% of 24 million hectares of infested agricultural lands (Dodd 1940). As a consequence of this success, *C. cactorum* was released in other geographical areas for the control of unwanted cacti including in the Caribbean, Nevis Island, in

1957 (Simmonds & Bennett 1966). Many factors have been implicated in the accidental arrival of the cactus moth into Florida including unaided dispersal to other islands in the Caribbean (Zimmerman et al. 2001), *Opuntia* cactus commerce (Pemberton 1995), human-aided dispersal (Stiling & Moon 2001), and weather events (Stiling 2002).

Cactoblastis cactorum was first reported on Big Pine Key, Florida in 1989 which raised concerns for the survival of endemic cacti in the southeastern United States (Habeck & Bennett 1990; Pemberton 1995; Johnson & Stiling 1998; Zimmerman et al. 2001) and for the perceived negative impacts of this information on biological control of weeds (Mahr 2001; Hight et al. 2002; Stiling 2002). Since its arrival in Florida the cactus moth has quickly dispersed northward and westward (Stiling & Moon 2001; Hight et al. 2002). By 2003, Hight et al. (2003) reported populations from as far north as Folly Island, near Charleston, South Carolina and as far west as Pensacola Beach, Florida. The current westward limit of *C. cactorum* distribution is at Dauphin Island, Alabama (S. Hight, pers. comm.).

Rapid expansion of the geographical range of *C. cactorum* poses a heightened threat to conservation of *Opuntia* diversity in North America and the Caribbean as well as to the safety and survival of wild and cultivated *Opuntia* in the southwestern United States and Mexico (Strong & Pemberton 2000; Pérez-Sandi 2001; Soberón et al. 2001). Mexico has approximately 3 million hectares of wild *Opuntia* and 250,000 hectares of cultivated *Opuntia*, with a cash crop value of approximately USD $50 million per year (Soberón et al. 2001; Stiling 2002).

Several control tactics have been evaluated against the cactus moth. Biological control through the actions of generalist parasitoids has resulted in population reductions of 5-30% in South America (Zimmermann et al. 1979; Pemberton & Cordo 2001a). However, importation and release of generalist parasitoids in North America is problematic because of potential non-target effects on other native cactophagous Lepidoptera (Habeck & Bennett 1990; Pemberton & Cordo 2001a, b). Use of entomopathogens is not recommended because of lack of host specificity (Pemberton & Cordo 2001a; Bloem et al. 2003) and low infection rates (Pemberton & Cordo 2001b). Insecticides were used effectively to control infestations of *C. cactorum* in *Opuntia* plantations in South Africa (Burger 1972; Pretorius et al. 1986; Pretorius & Van Ark 1992). In addition, laboratory data on several insecticides registered for use in ornamentals in the United States indicated high mortality of eggs and neonates of *C. cactorum* (Bloem et al. 2005). As such, insecticides may have potential use in nurseries and urban settings to control *C. cactorum* (Bloem et al. 2005). Nevertheless, widespread use of insecticides is not recommended in sensitive ecosystems (Mahr 2001; Leibee & Osborne 2001; Hight et al. 2005), and insecticide use alone would not be adequate to stop the westward spread of *C. cactorum* (Carpenter et al. 2001a; Stiling 2002; Bloem et al. 2005).

Autocidal control methods (i.e., sterile insect technique (SIT) and F_1 sterility) represent the best option for preventing further westward spread of *C. cactorum* into the *Opuntia*-rich ecosystems of the Western United States and Mexico (Carpenter et al. 2001a; Stiling 2002). Data on the effects of gamma radiation on the fecundity and fertility of *C. cactorum* were published by Carpenter et al. (2001b). These authors suggested further studies at doses between 100 and 200 Gy were warranted in order to select a dose that would allow for maximum production of partially sterile F_1 adults while inducing full sterility in the F_1 generation.

Applications of F_1 sterility to the study of *C. cactorum* populations were discussed by Carpenter et al. (2001b) and include (1) the elucidation of the potential host range of the cactus moth on key native *Opuntia* species from across the U.S.A., (2) the prediction of its potential geographic range in the U.S.A. and Mexico, and (3) the delineation of the impact of native natural enemies on the spread of *C. cactorum*. In each of these cases, research protocols would involve release of irradiated adults beyond the leading edge of *C. cactorum* distribution and would required 100% reproductive sterility of F_1 progeny produced by irradiated *C. cactorum*. Although a dose of 100-200 Gy was suggested for use in SIT to prevent the westward spread, additional studies are needed to determine the level of sterility of the progeny of irradiated adults when they are inbred or out-crossed to insure that only reproductively inactivated insects would be released beyond the current leading edge of distribution. Herein, we report results from these additional studies and comment on their relevance to the development and use of the sterile insect technique for mitigating the threat of *C. cactorum* in North America.

Materials and Methods

A laboratory colony originating from populations collected along the coast of Georgia near Jekyll Island was used in this study. Larvae were reared on cladodes of *Opuntia stricta* (Haworth) Haworth inside rectangular plastic containers (34 × 24 × 13 cm) at 26°C, 70% relative humidity (R.H.), and a photoperiod of 12:12 (L:D) as described in Carpenter et al. (2001b). Pupae were collected 2-3 d after initiation of pupation and cocoon silk was removed by treatment with a 5% solution of sodium hypochlorite (NaOCl). Pupae were sorted by gender, held separately in screen cages (30.5 × 30.5 × 30.5 cm) and allowed to emerge at room temperature (23 ± 1°C).

Parental Crosses

Newly emerged (≤24 h) adults were irradiated in a Cobalt^{60} Gammacell 220 irradiator with a dose rate of 20.3 Gy/min at doses of 0, 25, 50, 75, 100, 125, 150, 175, and 200 Gy. Dose calibration by Fricke dosimetry indicated a dose error of approximately ± 5%. Treated males and females were paired with either a non-irradiated adult (normal) or with a treated adult of the opposite gender in Solo® plastic containers (473 mL), modified for ventilation (Table 1). A small piece of cactus was provided as an oviposition surface and cups were provided with moisture. Containers were placed in an environmental chamber at 26°C, 70% relative humidity (R.H.), and a photoperiod of 14:10 (L:D).

Containers were checked daily. If a dead moth was found, gender and date of death was recorded to determine adult longevity. Mating was confirmed by the presence of a spermatophore in the bursa copulatrix of the female (Ferro & Akre 1975). When an egg stick was found, date of oviposition and total number of eggs per egg stick were recorded. Egg sticks were incubated at 26°C, 70% relative humidity (R.H.), and a photoperiod of 12:12 (L:D) for 25-30 d. A piece of cactus was added to each cup when eggs were near eclosion. Percentage egg hatch (larval eclosion) was calculated. F_1 progeny from each parental cross was reared as explained below. Percent pupation and F_2 adult emergence were recorded to estimate sterility of the F_1 generation.

F_1 Crosses

F_1 progeny from each parental cross and dose combination were reared on cladodes of *O. stricta* in plastic containers (34 × 24 × 13 cm) at 25-27°C. F_1 pupae were collected and separated by gender as above. All emerged F_1 adults were either inbred or out-crossed to non-irradiated adults of the opposite gender (Table 1). Pairs were allowed to mate and egg sticks were collected and incubated as described above. The following parameters were recorded for each pair: adult longevity, female fecundity and fertility, time to first pupation (in days), total number of pupae and emerged adults, and time to first F_2 adult emergence. Sterility in the F_1 generation was calculated based on fertility, pupation, and adult F_2 emergence data.

Statistical Analyses

Data from parental-generation (p-generation) crosses were analyzed with ANOVA (PROC MIXED, SAS Institute 1990). Type of cross and dose of radiation were treated as fixed effects and insect pair was treated as a random effect. The effects of cross and dose were evaluated on adult longevity, fecundity, egg developmental time, number of F_1 larvae and percentage F_1 larval eclosion (fertility), number of F_1 pupae, percentage pupation, number and percentage of F_1 adults emerging, and total developmental time.

Data collected from F_1-generation crosses also were analyzed with ANOVA (PROC MIXED, SAS Institute 1990). As above, cross and dose were treated as fixed effects and pair was treated as a random effect. A significant interaction between type of cross and dose of radiation was observed; consequently, these data were analyzed by cross (PROC MIXED, SAS Institute 1990). When a significant dose effect was observed on F_1 longevity,

TABLE 1. PARENTAL- AND F_1-GENERATION CROSSES OF NORMAL AND IRRADIATED *CACTOBLASTIS CACTORUM* MOTHS

Parental generation crosses (♀ × ♂)	♀	♂
♀N*♂N	Normal	Normal
♀N*♂T	Normal	Irradiated
♀T*♂N	Irradiated	Normal
♀T*♂T	Irradiated	Irradiated

	F_1-♀		F_1-♂	
F_1-generation crosses (♀ × ♂)	Parental-♀	Parental-♂	Parental-♀	Parental-♂
♀(N × N) × ♂(N × N) = NN × NN	Normal	Normal	Normal	Normal
♀(N × N) × ♂(T × N) = NN × TN	Normal	Normal	Irradiated	Normal
♀(N × N) × ♂(N × T) = NN × NT	Normal	Normal	Normal	Irradiated
♀(T × N) × ♂(N × N) = TN × NN	Irradiated	Normal	Normal	Normal
♀(N × T) × ♂(N × N) = NT × NN	Normal	Irradiated	Normal	Normal
♀(T × T) × ♂(N × N) = TT × NN	Irradiated	Irradiated	Normal	Normal
♀(N × N) × ♂(T × T) = NN × TT	Normal	Normal	Irradiated	Irradiated
♀(T × T) × ♂(T × T) = TT × TT	Irradiated	Irradiated	Irradiated	Irradiated

N, Normal.
T, Irradiated.

fecundity, egg developmental time, percentage egg hatch (fertility), percentage larval pupation, percentage F_2 adult emergence, and total developmental time, data were analyzed by polynomial regressions (PROC GLM, SAS Institute 1990). Degrees of freedom were adjusted by the Satterthwaite approximation method when PROC MIXED procedures were used. Differences between means were separated with Tukey-Kramer mean separation procedures. Data were log transformed when standard deviations were proportional to the mean (heteroscedasticity) and distributions were skewed. In addition, arcsine transformations were applied to percentage larval eclosion, larval pupation, and adult emergence when necessary to normalize data.

RESULTS

Parental Crosses

Neither type of cross nor dose of radiation significantly affected the fecundity of parental crosses. However, both cross ($F = 13.1$, $df = 2, 227$, $P < 0.0001$) and dose ($F = 6.5$, $df = 7, 225$, $P < 0.0001$) significantly affected the fertility of the cross; no significant interaction between cross and dose was observed. In addition, no significant interaction was observed on percentage pupation, however, significant cross ($F = 17.1$, $df = 2, 224$, $P < 0.0001$) and dose ($F = 7.1$, $df = 7, 222$, $P < 0.0001$) effects were observed that were due to gender of irradiated moths. No significant interaction between cross and dose effects was observed on percentage adult emergence. Conversely, significant cross ($F = 14.3$, $df = 2, 225$, $P < 0.0001$) and dose ($F = 6.8$, $df = 7, 223$, $P < 0.0001$) effects were observed on percentage adult emergence.

F_1 Crosses

Significant interaction between type of cross (parental-gender or genders irradiated) and dose of radiation were observed on fecundity, percentage larval eclosion, percentage pupation, and percentage adult emergence (Table 2). No significant differences in fecundity were observed among crosses ($F = 1.82$, $df = 6, 183$, $P = 0.10$). In contrast, significant differences in percentage larval eclosion were observed among crosses ($F = 4.76$, $df = 6, 148$, $P = 0.0002$). Radiation dose given to the parental generation significantly affected fecundity ($F = 10.7$, $df = 7, 185$, $P < 0.0001$) and fertility (percentage larval eclosion) ($F = 59.3$, $df = 6, 150$, $P < 0.0001$) of F_1 generation adults. Reductions in F_2 larval eclosion due to increased dose were heightened in crosses where the parental male (P-generation) was irradiated.

Regression analyses also revealed significant effects on the relationship of fecundity and fertility with dose in crosses where the parental male was irradiated. A significant quadratic relationship between fecundity and increasing dose of radiation was observed when the F_1 male had an irradiated father (NN**x**NT, $y = 58.0 + 2.4x - 0.34x$; $F = 19.7$, $df = 2, 35$, $P < 0.0001$), and a significant linear relationship when the F_1 female had an irradiated father (NT**x**NN, $y = 59.6 - 2.0x$; $F = 9.7$, $df = 1, 41$, $P = 0.003$) (Fig. 1A). Fertility and dose also had significant linear relationships when the F_1 male had an irradiated father (NN**x**NT, $y = 89.9 - 4.7x$; $F = 203.0$, $df = 1, 35$, $P < 0.0001$) and when the F_1 female had an irradiated father (NT**x**NN, $y = 94.9 - 4.7x$; $F = 208.7$, $df = 1, 36$, $P < 0.0001$) (Fig. 1B).

Significant differences in F_2-generation percentage larval pupation ($F = 2.32$, $df = 6, 148$, $P = 0.04$) and adult emergence ($F = 3.00$, $df = 6, 148$, $P = 0.008$) were observed among crosses (Table 2). Percentages of pupating larvae ($F = 20.4$, $df = 6, 149$, $P < 0.0001$) and percentages of moth emergence ($F = 5.14$, $df = 6, 149$, $P < 0.0001$) were significantly different among doses. Radiation dose effects were more evident in crosses where the parental male was irradiated.

Relationships between percentage larval pupation and percentage adult emergence with dose of radiation also were significantly affected in offspring (F_2) from F_1 moths (males and females) with an irradiated father. Percentage larval pupation and increasing dose had a linear relationship in crosses where the F_1 male had an irradiated father (NN**x**NT, $y = 47.0 - 3.1x$; $F = 29.3$, $df = 1, 26$, $P < 0.0001$) and where the F_1 female had an irradiated father (NT**x**NN, $y = 48.1 - 2.48x$; $F = 13.3$, $df = 1, 28$, $P = 0.001$) (Fig. 2A). Percent (F_2) adult emergence and increasing dose also had a linear relationship when the F_1 male had an irradiated

TABLE 2. ANOVA OF INTERACTION (CROSS AND RADIATION DOSE) EFFECTS ON FECUNDITY AND FERTILITY OF F_1 GENERATION MOTHS OF *CACTOBLASTIS CACTORUM* AND PERCENTAGE PUPATION AND ADULT EMERGENCE OF RESULTING F_2 OFFSPRING.

Variable	Effect	*df*	*df*	*F* Value	Pr > *F*
Fecundity	Cross*Dose	8	183	2.01	0.05
Fertility	Cross*Dose	8	148	4.25	0.0001
Percentage pupation	Cross*Dose	8	148	2.73	0.01
Percentage adult emergence	Cross*Dose	8	148	2.88	0.01

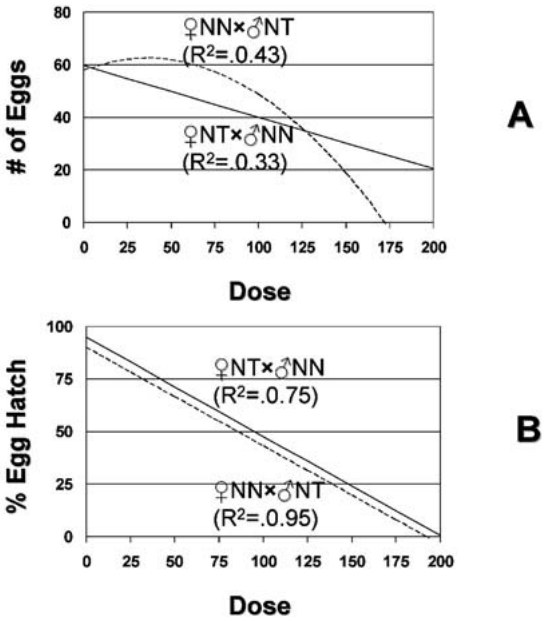

Fig. 1. Effect of dose of radiation on fecundity (A) and fertility (B) of F_1 generation adults of *Cactoblastis cactorum* from an irradiated father. Cross (NN**x**NT) is the cross of normal female with a F_1 male offspring from parental cross of a normal female and irradiated male. Cross (NT**x**NN) is the cross of a normal male with F_1 female offspring from parental cross of a normal female and irradiated male.

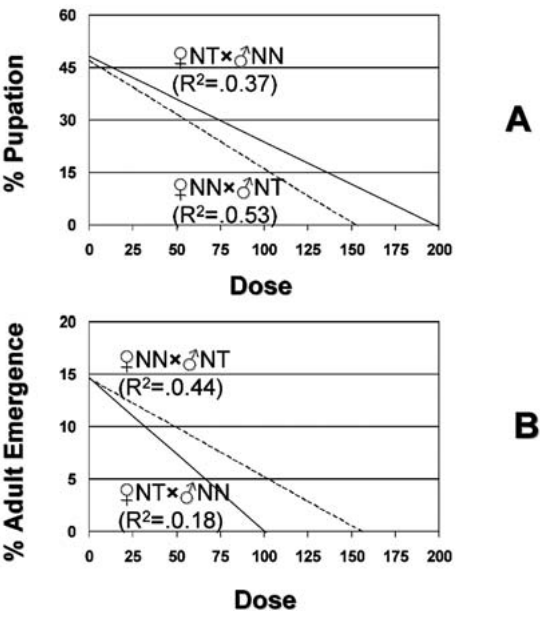

Fig. 2. (A) Effect of dose of radiation on percentage pupation of *Cactoblastis cactorum* and (B) adult emergence of F_2 offspring from F_1 generation adults from an irradiated father. Cross (NN**x**NT) is the cross of normal female with a F_1 male offspring from parental cross of a normal female and irradiated male. Cross (NT**x**NN) is the cross of a normal male with F_1 female offspring from parental cross of a normal female and irradiated male.

father (NN**x**NT, y = 14.5 – 0.94x; $F = 20.11$, $df = 1, 26$, $P = 0.0001$), but not when the F_1 female had an irradiated father (NT**x**NN, y = 15.4 – 0.46x; $F = 3.4$, $df = 1, 28$, $P = 0.07$) (Fig. 2B).

Discussion

Practical application of SIT with full sterility is problematic in Lepidoptera because these insects are more resistant to radiation than some other orders of insects (e.g., Diptera) (Bloem & Carpenter 2001; Bakri et al. 2005). Successful application of SIT in Lepidoptera is maximized by finding a dose of radiation that fully sterilizes females while only partially sterilizing males. Thus, detrimental effects due to radiation are minimized, and production of F_1 sterile adults is possible (North 1975; LaChance 1985; Bakri et al. 2005; Carpenter et al. 2005). In this study, we evaluated several doses to fully sterilize F_1-generation moths of *C. cactorum* which would result in no viable offspring when F_1 males were inbred- or out-crossed to fertile females and also allow for maximal production of F_1 sterile adults by irradiated males.

Carpenter et al. (2001b) reported full sterility (no F_1 progeny) in females irradiated at 200 Gy and in males at 500 Gy. At lower doses, partially sterile females produced F_1 progeny that were more fertile than their mother. In contrast, partially sterile males irradiated at doses from 125-200 Gy produced F_1 progeny that were more sterile than their irradiated father. At doses ≤125 Gy F_1 progeny retained some residual fertility. In our studies, we induced full sterility in parental females, partial sterility in parental males, and full sterility in any resulting F_1 progeny at 200 Gy that suggests 200 Gy is the appropriate dose to induce F_1 sterility in *C. cactorum*. Verification that 200 Gy is the most appropriate dose of radiation for inducing full sterility in F_1 moths while maximizing production of F_1 moths also will allow researchers to study this insect in a natural setting (Carpenter et al. 2001a) and implement a SIT-F_1 sterility program against *C. cactorum* (Carpenter et al. 2005).

The current USDA-APHIS strategic plan includes release of *C. cactorum* moths irradiated at 200 Gy in the SIT-F_1 sterility component of the program to achieve its stated objectives. These may include, but are not limited to, eradication of *C. cactorum* from newly infested and isolated sensitive areas, establishment of a barrier to impede further migration westward, and augment populations of native or released natural enemies of *C. cactorum* beyond the leading geographical edge (Carpenter et al. 2001b).

Along the Gulf Coast, eliminating populations of *C. cactorum* in newly infested and isolated sensitive areas is a priority. Cactus distribution in this area is clumped and is often separated by long distances, which could result in a number of small infestations over a large area. These small infestations could be suppressed or eradicated by removal of infested cactus cladodes and pupae, and releases of irradiated moths. In Florida, excluding *C. cactorum* from endemic populations of *O. corallicola* (Small) Werdermann, *O. triacantha* (Wildenow) Sweet, and *O. cubensis* Britton & Rose, on the Florida Keys (Johnson & Stiling 1998) also is desirable. Potentially, environmentally sensitive and protected areas composed of species like *O. corallicola*, *O. triacantha*, and *O. cubensis* would be prime candidates for application of SIT-F_1 sterility. Irradiated moths could be released in these areas to mitigate the threat *C. cactorum* without posing a risk to these environments.

Additionally, confirming 200 Gy as the appropriate dose for SIT-F_1 sterility provides immediate opportunities in host range and geographical range verification of *C. cactorum*. SIT-F_1 sterility with 200 Gy will allow host range testing in natural settings without risk of permanent establishment, because progeny from released fertile females and irradiated males would be reproductively sterile. This would allow researchers to evaluate the acceptability of *Opuntia* species not present in the Southeast, but likely to be impacted if *C. cactorum* establishes in the southwestern United States and Mexico. Results could allow researchers to categorize these species according to risk which would allow resources to be allocated to high risk areas.

Successful control of *C. cactorum* with SIT-F_1 sterility could serve as proof of concept for use of reproductively inactivated insects in risk assessment of potential biological control for invasive weed species (Greany & Carpenter 2000). Use of reproductively inactivated potential weed biological control agents could allow field evaluations of development and behavior. This would alleviate concerns about indirect and direct effects on nontarget species and unwanted biological control agent establishment that could have negative impacts on the fauna and ecology in pest plant habitats. Currently, host range testing for weed biological control agents is conducted through caged no-choice or choice laboratory tests (Marohasy 1998; Withers et al. 1998). These tests have been widely used to evaluate host range of herbivorous insects (Edwards 1999; Sheppard 1999) and other arthropods (Barratt et al. 1999; Sands & Coombs 1999; Sands & Van Driesche 2003).

However, efficacy of these laboratory (choice and no-choice) tests can vary (Hill 1999; Palmer 1999) depending on arena physical features (size, lighting, etc.), experimental design, and physiology of the agent being evaluated which can influence host preference (Sands & Van Driesche 2003). Variations in insect response to caged environments often results in overestimating (Marohasy 1998) and sometimes underestimating host range (Sands & Van Driesche 2003). Acceptance or rejection of hosts inconsistent with field host preference in cage experiments are often the result of insect behavior disruption (Field & Darby 1991; Sands 1993; Sands & Papacek 1993). Bernays (1998) suggested that behavior may influence host selection more than morphology or physiology and Schoonhoven et al. (1998) suggested that the sequence at which behavior occurs also may be important in host selection. Extrinsic and intrinsic factors influencing host range, plasticity of oviposition (Bernays & Chapman 1994), and absence of long-range oviposition behavior in cage studies (Wiklund 1975; Thompson 1993) suggest open-field tests in natural settings as more appropriate for predicting the host range of biological control candidates (Clement & Cristofaro 1995).

Currently, open-field testing is conducted in the native home of the agent (Clement & Cristofaro 1995). These tests examine the behavioral preference and mobility of the agent, host plant density, and spatial distribution (Rizza et al. 1988; Dunn & Campobasso 1993; Briese 1999). We suggest incorporation of reproductively inactivated moths into open-field testing protocols would allow safe evaluation of potential biological control agents in newly infested areas and allow researchers to understand how extrinsic and intrinsic factors in release-site environments could affect host preference (Bernays & Chapman 1994).

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