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Performance improvement through quality evaluations of sterile cactus moths, *Cactoblastis cactorum* (Lepidoptera: Pyralidae), mass-reared at two insectaries

Stephen D. Hight[1,] and James E. Carpenter[2]*

Abstract

A bi-national program was established by Mexico and the United States to mitigate the threat of the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae)—an invasive herbivore from South America—to native *Opuntia* spp. (Caryophyllales: Cactaceae) biodiversity and *Opuntia*-based industries. Mass-rearing, sterilization, and transport and release technologies assisted with the development of several control tactics including the sterile insect technique. Following the successful eradication of *C. cactorum* from Mexico and the elimination of *C. cactorum* from Alabama barrier islands, the bi-national program established an additional mass-rearing insectary for the production of sterile moths. Laboratory and field bioassays were conducted on sterile moths from both insectaries. Bioassays and assessments included moth mass, moth longevity, percentage of female moths mated at time of collection from the insectary, percentage of female moths mated 24 h after collection, flight ability, percentage recaptured after release in the field, and mean distance dispersed from release site. Data from the quality assessments and comparisons between the 2 insectaries were used as feedback mechanisms to make protocol changes in both rearing and handling that improved sterile moth quality and performance.

Key Words: *Opuntia*; invasive insects; quality control**;** mass-rearing; sterile insect technique; SIT

Resumen

Un programa bi-nacional fue establecido por México y los Estados Unidos para mitigar la amenaza de *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae)—un herbívoro invasivo de América del Sur—a la biodiversidad de las especies de *Opuntia* (Caryophyllales: Cactaceae) nativas y las industrias basadas en el *Opuntia.* La cria masiva, la esterilización y la tecnología del transporte y liberación ayudaron con el desarrollo de varias tácticas de control, incluyendo la técnica del insecto estéril. Tras el éxito en la erradicación de *C. cactorum* de México y la eliminación de *C. cactorum* de islas barreras del estado de Alabama, el programa bi-nacional estableció un insectario adicional de cría en masa para la producción de polillas estériles. Se realizaron bioensayos de laboratorio y de campo en las polillas estériles de ambos insectarios. Los bioensayos y evaluaciones incluyeron la masa y la longevidad de las polillas, el porcentaje de las polillas hembra apareadas en el momento de su recolección en el insectario, el porcentaje de las polillas hembra apareadas 24 horas después de la recolección, la capacidad de vuelo, el porcentaje recapturadas después de la liberación en el campo, y el promedio de la distancia del sitio de la liberación de las polillas capturadas. Los datos de las evaluaciones de la calidad y ls comparación entre los 2 insectarios fueron utilizados como una mecanismo de retroalimentación para hacer cambios en el protocolo, tanto en la cría como en el manejo que mejoran la calidad de la polilla estéril y el rendimiento.

Palabras Clave: *Opuntia*; insectos invasivos; control de calidad; cría en masa; técnica del insecto estéril; TIE

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), gained renowned success as a biological control agent against weedy *Opuntia* spp. (Caryophyllales: Cactaceae) in Australia (Dodd 1940), South Africa (Pettey 1948), and other locations where exotic *Opuntia* spp. had become an invasive pest (Julien & Griffiths 1998). Subsequently, *C. cactorum* was introduced in the Caribbean island of Nevis to control native *Opuntia* spp. that had become invasive due to overgrazing of rangeland (Simmonds & Bennett 1966). As the moth spread naturally or by human-assisted introductions throughout the Caribbean (García-Turudi et al. 1971), the reputation of *C. cactorum* as the "poster child" of weed biological control (Stiling 2002) began to give way to the growing concern that *C. cactorum* could become an invasive pest. These concerns were confirmed when populations of *C. cactorum* were first observed in the Florida Keys in 1989 (Habeck & Bennett 1990; Dickle 1991) and then spread throughout most of the Florida peninsula and along the Atlantic Coast to South Carolina and the Gulf Coast to Louisiana (Hight et al. 2002; Hight & Carpenter 2009). Because of the rapid geographical expansion and its reputation as a voracious *Opuntia* feeder, *C. cactorum* was regarded as a serious economic and ecological threat to native and cultivated *Opuntia* spp. in the United States and Mexico (Soberón et al. 2001; Vigueras & Portillo 2001; Zimmermann et al. 2004).

In 2000, an assessment and planning workshop on *C. cactorum* was held in Tampa, Florida, to address the needs for research, education and outreach, risk assessment and regulatory issues, and international collaboration (Mahr 2001). Subsequently, a consultants meeting was hosted by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) to review

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and evaluate the threat of *C. cactorum* to international agriculture and biodiversity (IAEA 2003). The United States and Mexico developed a Bi-National Cactus Moth Program to be implemented with funding from both countries. The most important elements of the bi-national plan included monitoring, regulatory and awareness strategies, and the development of the sterile insect technique (SIT) and other control tactics to be used in an area-wide approach for containment and eradication of *C. cactorum* (Bloem et al. 2007; Hernández et al. 2007). Research supported by the bi-national program included sex pheromone identification (Heath et al. 2006), dispersal studies (Hight et al. 2002; Bloem et al. 2005a; Sarvary et al. 2008), developmental biology (Mclean et al. 2006), insecticidal susceptibility (Bloem et al. 2005b), mass-rearing on artificial diet (Marti & Carpenter 2008a,b; Marti et al. 2008; Carpenter & Hight 2012), and development of the SIT (Carpenter et al. 2001a; Hight et al. 2005; Carpenter et al. 2009). Operations supported by the Bi-National Cactus Moth Program were directed at the leading edge of the geographical range along the gulf coast of the United States and at outbreaks in Quintana Roo, Mexico, where incursions of *C. cactorum* in 2006 and 2007 had been detected in Isla Mujeres and Isla Contoy, respectively. Operational tactics, which included survey, host plant removal, host plant sanitation, and the release of sterile moths from the USDA insectary in Tifton, Georgia (TIF insectary), were successful in significantly reducing *C. cactorum* populations along the Gulf Coast of Florida, Alabama, and Mississippi, and eradicating the *C. cactorum* populations in Mexico (Carpenter et al. 2008; NAPPO 2009).

Following the successful eradication of *C. cactorum* from Mexico and the elimination of *C. cactorum* from Alabama barrier islands, the Bi-National Cactus Moth Program established an additional mass-rearing insectary at the Florida Department of Agriculture and Consumer Services, Department of Plant Industry (DPI), Biocontrol Rearing Facility in Gainesville, Florida (DPI insectary). Rearing at DPI was designed to be high-density in 5 L containers using eggs from the low-density TIF insectary. Sterile moths from the 2 insectaries were shipped to different release sites along the leading edge of the infestation. Program workers released the sterile moths and recorded the percentage of fliers at the time of release. Workers also recorded the number of sterile moths from each release and each insectary that were recaptured in pheromone baited traps that had been deployed at each release site to survey for wild *C. cactorum* males. Although the moths from each insectary were not released at the same site or the same time, and although the trap number and distribution pattern varied at each release site, recorded data indicated that the percentage of DPI fliers at the time of release was lower than the percentage of TIF fliers at the time of release. Also, trapping data indicated that percentage recapture of released DPI males was lower than percentage recapture of released TIF males. These empirical data suggested to program managers that the moth quality and performance from the DPI insectary needed to be improved. Therefore, we examined the quality and performance of sterile moths from both insectaries using laboratory bioassays and field release/recapture bioassays. The goal was to identify production and handling protocols that might negatively impact moth quality, make changes to the protocols where indicated, and repeat the laboratory and field assessments to see if protocol changes improved quality and performance.

Materials and Methods

TEST INSECTS AND HANDLING

All *C. cactorum* used in this study originated from the laboratory colony at the USDA-ARS Crop Protection and Management Research Unit Laboratory, Tifton, Georgia. This TIF colony was established from multiple collections of *C. cactorum* larvae from infested *Opuntia* spp. along the Florida Gulf Coast during 2002 and 2004, and from nearly 10,000 eggs collected from *Opuntia* spp. plantations near Craddock, South Africa, and shipped to Tifton, Georgia in 2002. Insects were reared continuously on an artificial diet (Carpenter & Hight 2012) using the protocols described by Marti & Carpenter (2008a). However, because the presence of virus and microsporidia in the *C. cactorum* population (Marti et al. 2007) increased the risk of mortality as the larval rearing density increased, a type of filter rearing system (FRS) (Parker 2005) was established for disease abatement of the TIF colony. Briefly, the FRS protocols required low-density rearing in 600 mL diet rearing containers each infested with only the eggs from one eggstick (approximately 70 eggs), and required that any container with diseased larvae was discarded. Only moths from the FRS were used to supply eggs for larval rearing. Surplus moths produced from the FRS were used for sterile insect releases in the Bi-National Cactus Moth Program. In order to increase the supply of sterile moths for the Bi-National Cactus Moth Program, larval rearing of *C. cactorum* was established at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI colony) during 2007–2008. Rearing protocols at DPI were the same as the Tifton protocols (Marti & Carpenter 2008a), except the DPI colony was designed to be high-density in 5 L containers using eggs from the low-density TIF colony.

At each insectary, as larvae pupated, they were removed from their larval rearing containers, transferred to screened emergence cages (30 × 60 × 60 cm), and held on moth scale collection stands in walk-in environmental chambers (26 ± 0.5 °C, 45–60% RH, a photoperiod of 14:10 h L:D). Emerged moths were collected daily after transferring emergence cages to cold rooms to lower their activity. At each insectary, adult moths were chilled and 200–250 were placed in disposable 100 × 20 mm Petri dishes where they remained chilled during the irradiation process wrapped in small flexible freezer packs. TIF moths were exposed to gamma radiation using a Co^{60} Gamma Cell 220 irradiator (J. L. Shepherd & Associates, San Fernando, California; dose rate of approximately 5.9 Gy/min). DPI moths were irradiated by a Varian L-1000A Linear Accelerator electron beam (Varian Medical Systems, Inc., Palo Alto, California) with a current of 42 mA, an energy of 5.2 MeV, and a conveyor belt speed of 4.57 cm/s in the Florida Accelerator Services and Technology facility located adjacent to the DPI insectary. Routine dosimetry was performed at each irradiator with radiochromic film (Gafchromic® HD-810, ISP Technologies, Inc., Wayne, New Jersey) placed in the center and edges of the load. Dosimeters were read with a FWT-92 Radiachromic® reader (Far West Technology, Inc., Goleta, California) at 600 nm. Dose variation was ± 5%. All moths for this study were irradiated with a dose of 200 Gy which causes 100% sterility in adult females, about 50–60% sterility in adult males and 100% F_1 sterility (Carpenter et al. 2001b). This 200 Gy was used as the operational dose of the SIT component for the Bi-National Cactus Moth Program.

After irradiation at either insectary, moths were transported back to the cold room within their chilled Petri dishes and packaged in a cardboard wrapped Styrofoam insulated shipping box (53 × 35 × 50 cm) (KoolTemp™ GTS-89 Shipping System, Cold Chain Technologies, Holliston, Massachusetts). The Petri dishes containing irradiated insects were placed in a small cardboard box (21.5 × 21.5 × 21.5 cm), wrapped with a flexible ice pack sheet, and placed in the middle of the shipping box. Six foam refrigerant bricks were placed around the perimeter of the shipping box. A temperature data logger was strapped to the Petri dish stack—and after insect release in the field—evaluated to insure that the temperature next to the sterile insects maintained within the targeted range of 1–4 °C.

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BIOASSAYS

Laboratory bioassays were conducted at both the DPI and TIF insectaries on the same days and using the same methods in environmentally controlled rooms (27 ± 0.5 °C, 70–75 % RH, a 14:10 h L:D photoperiod). Field bioassays were conducted at the University of Florida research farm in Citra, Florida. Laboratory bioassays included moth weight by gender, moth longevity by gender, the ability/propensity of moths to mate while in small cages, and the ability/propensity of male moths to fly from a flight cylinder. Field bioassays measured the ability of male moths released in a small plot with nearly 200 plants in 4 rows of *Opuntia ficus-indica* (L.) Miller to engage in pheromone-mediated flight and locate a pheromone source, i.e., to be captured in a pheromone-baited trap. Each day that releases were made in the field, laboratory bioassays were conducted using moths from the same cohort. This study consisted of 2 trials. During the first trial, bioassays and field releases were conducted on 23, 25, 28 Jun, and 1 Jul 2010. After the results from the first trial were examined and the indicated remediation made to the rearing and handling protocols in the DPI colony, a second trial was performed with bioassays and releases conducted on 16, 18, 23, and 25 Aug 2010. Field releases during both trials were made in the middle to late afternoon (14.00–18.00 h).

Moth Weight. A random sample of 20 male and 20 female moths within 24 h of adult emergence from each insectary was taken from each cohort of moths collected for bioassays. Mean weight for each gender was recorded.

Moth Longevity. A random sample of moths (30 males and 30 females) within 24 h of adult emergence from each insectary was taken from each cohort of moths collected for bioassays. After irradiation, moths were placed individually in 30 mL plastic cups and their mortality was recorded daily. Neither water nor a food source was added to the cups since adult *C. cactorum* have reduced mouthparts and do not feed.

Mating Cage. A random sample of 30 female moths within 24 h of adult emergence from each insectary was taken from each cohort of moths collected for bioassays. These females were dissected and the presence of a spermatophore in the bursa copulatrix of the female was used to determine the percentage of females that had mated (Ferro & Akre 1975) prior to collection. At each insectary, mating ability/propensity of moths following the radiation treatment was examined by placing 10 females and 10 males in each of 4 screened cages (30 × 30 × 30 cm) for 48 h. The mean percentage increase in mating during the mating bioassay was calculated by subtracting the mean percentage of mated females at the time of collection from the insectary from the mean percentage of mated females after the end of the mating cage bioassay.

Flight Cylinders. Flight cylinders (Carpenter et al. 2012) were produced by cutting polyvinyl chloride (PVC) irrigation pipe (color = light blue; diam = 16 cm) into pieces that were 16 cm tall. The inside surfaces of the cylinders were coated with unscented talc to ensure that the moths used flight to escape the cylinders. Irradiated male moths within 24 h of adult emergence from each insectary were placed in 4 of 5 cylinders (10 males per cylinder) that were inside a large screen cage (460 × 460 × 760 cm). The cylinder without moths was randomly selected and served as a measure of the number of flying insects that fell back into cylinders during the experiment. The number of males per cylinder was recorded after 24 h and again after 48 h.

Field Release/Recapture. Sixty two Pherocon® 1-C Wing traps (Trécé, Adair, Oklahoma), each baited with synthetic female sex pheromone lure (Scentry Biologicals, Billings, Montana) and mounted on top of a 1.5 m pole were placed in a grid around a small cactus plantation (46 × 8 m). The plantation consisted of 4 rows of *O. ficus-indica* (approximately 50 plants/row). Sixty two traps were arranged in 7 rows of 9 traps (the center trap was removed to serve as a release point). Each trap was placed 5 m apart within and between rows. The center row ran down the middle of the 4-row plantation. Irradiated male moths within 24 h of adult emergence from DPI and TIF insectaries were prepared and packaged in the same manner as those for the operational program (see description above), and transported to the study site and released in the center of the cactus plantation. Approximately 1,000 sterile male *C. cactorum* were released from each insectary during each of 8 release dates, i.e., 4 releases per trial. Each trap was marked with a unique number and its distance from the release point was measured. Moths were dusted with a unique color of fluorescent powder to distinguish each release and each insectary. Traps were checked daily and trap bottoms that caught irradiated male moths were removed, replaced, and returned to the laboratory so that male moths could be examined with an UV light to distinguish the color of the powder on each moth. Data were calculated and recorded for percentage males recaptured by release date, by number of days following the release (1–7), and by the distance males were from the release site when captured.

DATA ANALYSIS

Data for each variable from the first trial were examined with the Shapiro-Wilk's *W* test and the hypothesis that each distribution was normal could not be rejected ($P > 0.05$). Therefore, data were initially analyzed with a paired *t*-test (PROC TTEST, SAS Institute 1989) to identify any differences between insectaries for the following variables: percentage of females that had mated by the time they were collected from the insectary, percentage of females that mated after the mating bioassay, percentage increase of female mating after the mating bioassay, male longevity, female longevity, percentage of males that flew out of the flight cylinders within 24 h, percentage of males that flew out of the flight cylinders within 48 h, male weight, female weight, percentage recapture of released males, and mean recapture distance from the release site. Data from each of these variables were also analyzed using a stepwise general linear model (PROC STEPWISE, SAS Institute 1989) to examine subsets of predictor variables (using the max R-square method) that most adequately predicted moth quality and performance, defined by the dependent variable percentage recapture. The findings from these analyses resulted in a careful examination of the differences between the 2 insectaries regarding the rearing and handling protocols. After procedural changes were made at the DPI insectary and enough time had elapsed for changes to be effective, a second trial was conducted and data from both trials were combined to compare the performance of moths from the 2 insectaries.

Laboratory and field data from both trials were combined and each variable was examined with the Shapiro-Wilk's *W* test for normality ($P \leq 0.05$). Three variables, percentage of females mated at the time they were collected from the insectary, mean distance recaptured from the release site, and percentage recapture, were not normally distributed and normality could not be sufficiently improved by transforming the data. These data were analyzed using the Chi Square (χ^2) statistic (GLZ procedure, StatSoft 2004) with percentage of females mated at the time they were collected from the insectary as the dependent variable and insectary and trial as sources of variation, and with the mean distance recaptured from the release site and percentage recapture as the dependent variables and release date, day of capture after release, and trial as sources of variation. Data from the laboratory bioassays were analyzed using multi-factor analysis of variance (PROC GLM, SAS Institute 1989) with percentage of females mated after mating bioassay, percentage increase of female mating after mating bioassay, male longevity, female longevity, percentage of males that flew out of the

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flight cylinders within 24 h, percentage of males that flew out of the flight cylinders within 48 h, male weight, and female weight as dependent variables and insectary and trial as sources of variation. All interactions were included in the statistical models to test the null hypotheses of independent effects of the different sources of variation. When the analysis indicated a significant effect, means were separated by the Tukey-Kramer statistic at $P = 0.05$ (SAS Institute 1989).

Results

Data from trial 1 indicated that there was no difference between the DPI and TIF insectaries with respect to the mean weights of moths of the same gender. However, laboratory bioassays during trial 1 detected many differences between the moths reared at the 2 insectaries. The mean percentage of females that had mated in the insectary before they were collected for irradiation and released was significantly greater for DPI (28.3) than for TIF (1.7) (Table 1). In contrast, the percentage of females that were mated after 48 h in the mating cages was significantly greater for TIF (71.6) than for DPI (26.0) (Table 1). Further, there was no increase in mating of DPI females after being collected from the insectary, whereas the mating for TIF females increased significantly during the 48 h mating bioassay (Table 1). These data suggest that the rearing and handling protocols at the DPI insectary were different than those at the TIF insectary, allowing the DPI females the opportunity to mate before being collected for irradiation and release. But the data also suggests that DPI females were of lower quality because the overall incidence of mating was significantly lower than the mating of the TIF females. Other indications of lower quality in the DPI moths compared with the TIF moths were evident in significantly reduced longevity for both DPI males and females, and smaller numbers of DPI males that flew from the flight cylinders (Table 1). Field bioassays during trial 1 also revealed reductions in quality and performance of the DPI males compared with the TIF males. The percentage of released DPI males that were recaptured (1.1 %) was significantly lower than the percentage of released TIF males that were recaptured (10.4 %) (Table 1). Nevertheless, of the males that were recaptured, the mean distance from the release site that moths were trapped was not significantly different between DPI and TIF males.

A stepwise general linear model was constructed with trial 1 data to identify predictor variables (using the max R-square method) that most adequately predicted moth quality and performance as defined by the percentage recapture of released males. None of the individual predictor variables were significantly correlated with the percentage recapture of released males; consequently, a significant model was not obtained until 5 predictor variables were included (Table 2). The model indicated that the greater incidence of mating that occurred in the DPI insectary before moths were collected was negatively related to the performance of the males released in the field. The model also indicated that other factors independent from the mating differences, such as female longevity, flight ability, and male weight, were importantly related to male moth performance in the field. The findings from these analyses were used in a careful examination of the differences between the 2 insectaries regarding the rearing and handling protocols.

Procedural changes that were made at the DPI insectary to harmonize the rearing and handling protocols with those of the TIF insectary included (1) collecting the moths before the end of the scotophase during which they emerged to prevent mating, (2) changing the photoperiod during larval rearing from 12:12 h. L:D to 14:10 h L:D so that developing larvae would synchronize during development with the increased photophase during emergence, and (3) reducing the temperature from 8 °C to 2 °C in the collection room and from 8 °C to 6 °C during the irradiation procedure to prevent moth mobility and movement. Specific changes to the DPI insectary to adjust the scotophase consisted of shifting the time the lights turned on in the emergence room so cages could be moved to the cold room before the lights came on and stimulated mating. Also red lights were added to both emergence and cold rooms to allow enough light for manipulating cages in the dark without turning on overhead white lights. Additional caution was taken to keep adults chilled by reducing the temperature in the cold room to 2 °C before cages containing newly emerged moths were transferred and by adding an additional chill plate to the rack of Petri dishes during their transport to and from the irradiator facility and during irradiation. After allowing enough time for these changes to become effective, a second trial (trial 2) was conducted and data from both trials were combined for each insectary for the analysis.

Table 1. Comparison of initial quality and performance of *Cactoblastis cactorum* from 2 insectaries as measured by laboratory and field assessment parameters (trial 1).

Parameter	Insectary*	Mean ± SD	df	*t*	χ^2	*P*
% females mated at time of collection for mating bioassay	DPI	28.3 ± 9.6	1		114.9	0.001
	TIF	1.7 ± 1.9				
% females mated after mating bioassay	DPI	26.0 ± 12.4	6	−5.53		0.0016
	TIF	71.6 ± 10.9				
% change in mating from before to after mating bioassay	DPI	−2.3 ± 20.6	6	−6.17		0.0008
	TIF	69.9 ± 11.2				
Male longevity (d)	DPI	6.2 ± 0.8	6	−4.62		0.0036
	TIF	8.5 ± 0.6				
Female longevity (d)	DPI	5.2 ± 0.8	6	−5.09		0.0022
	TIF	7.4 ± 0.4				
% flown from flight cylinder after 24 h	DPI	22.5 ± 7.4	6	−10.42		<0.0005
	TIF	78.1 ± 7.7				
% flown from flight cylinder after 48 h	DPI	26.3 ± 10.1	6	−8.60		0.0001
	TIF	80.0 ± 7.4				
% recapture of released males	DPI	1.1 ± 0.8	6	−2.87		0.029
	TIF	10.4 ± 6.5				

*DPI = Department of Plant Industries, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. TIF = Tifton, Georgia, United States Department of Agriculture.

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Table 2. A stepwise general linear model constructed of predictor (dependent) variables (using the max R-square method) from trial 1 laboratory bioassays that most adequately predicted moth quality and performance as defined by the percentage recapture of *Cactoblastis cactorum* males released in the field. (R^2 = 0.9809; df = 5, 7; F = 20.58; $P > F$ = 0.047).

Variable	Parameter estimate	SE	Type II SS	F	$P > F$
Intercept	−43.9	11.5	42.4	14.60	0.062
% females mated at time of collection	−0.8	0.1	113.2	39.02	0.025
% females mated after mating bioassay	−1.0	0.2	96.2	33.18	0.023
Female longevity (d)	8.8	2.6	32.9	11.35	0.078
% flown from flight cylinder after 24 h	0.1	0.05	22.8	7.88	0.107
Male weight	1,121.0	187.7	103.4	35.66	0.027

Moth Weight and Longevity. Male and female moth weights were not significantly influenced by either the insectary or the trial. Overall mean (± SD) weight was 0.0427 (± 0.00442) g for male moths and 0.0833 (± 0.01078) g for female moths. However, an interaction between trial and insectary significantly influenced male (F = 30.044; df = 1,12; P = 0.00014) (Fig. 1) and female (F = 50.395; df = 1,12; P = 0.00001) longevity. Longevity for both genders was significantly shorter for DPI moths than for TIF moths in trial 1 (Table 1); however, after procedural changes had been made at the DPI insectary, the longevity of DPI male moths (trial 2) increased significantly and was significantly longer than that of the TIF moths (Fig. 1).

Mating. The mean percentage of females that were mated when collected from the insectary was significantly influenced by trial (χ^2 = 138.8; df = 1,110; $P < 0.0001$) and insectary (χ^2 = 100.6; df = 1,110; $P < 0.0001$) (Fig. 2). Procedural changes made at the DPI insectary requiring emerged moths to be collected before the end of the scotophase significantly reduced the incidence of mating in collected moths. In the mating cage bioassay, the mean percentage mating for the TIF moths at the time of collection (1.28 ± 1.8) was significantly less than the mean percentage mating at the time of collection for the DPI moths (14.17 ± 16.4), and the mean percentage mating for collected moths in trial 1 (15.00 ± 15.6) was significantly greater than the mean percentage mating for collected moths in trial 2 (0.45 ± 1.3). The increase in the percentage mating during the mating bioassays was significantly influenced by an interaction between trial and insectary (F = 5.32; df = 1,12; P = 0.0397) (Fig. 3) because fewer females from the DPI insectary had mated before being collected during trial 2.

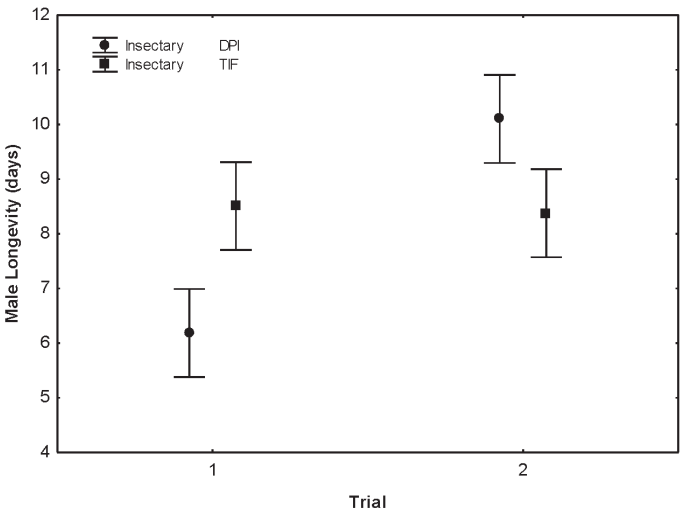


Fig. 1. The mean longevity (d) of *Cactoblastis cactorum* males as influenced by the insectary (DPI or TIF) and the trial conducted before (trial 1) and after (trial 2) quality improvements were made to the rearing and handling protocols at the DPI insectary. Vertical bars denote 0.95 confidence intervals.

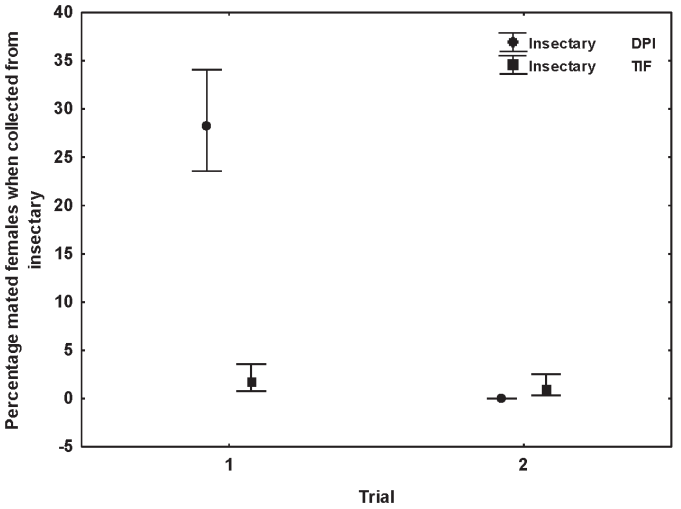


Fig. 2. The mean percentage of *Cactoblastis cactorum* females that were mated at time of collection from the insectaries (DPI or TIF) and the trial conducted before (trial 1) and after (trial 2) quality improvements were made to the rearing and handling protocols at the DPI insectary. Vertical bars denote 0.95 confidence intervals.

Flight Cylinders. The mean percentage of male moths flying out of the flight cylinders after 24 h (both trials combined) was significantly (F = 43.92; df = 1,14; $P < 0.0001$) less for DPI (33.4%) than for TIF (78.1%). Moth escape by flight during trial 1 and trial 2 was not significantly

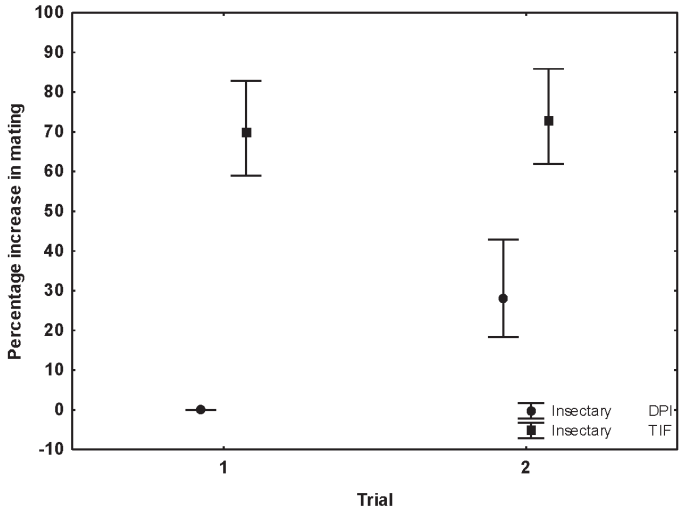


Fig. 3. The mean percentage increase in mating of *Cactoblastis cactorum* females in mating cage bioassays as influenced by the insectary (DPI or TIF) and the trial conducted before (trial 1) and after (trial 2) quality improvements were made to the rearing and handling protocols at the DPI insectary. Vertical bars denote 0.95 confidence intervals.

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different for the 2 insectaries, but there was some evidence of an interaction between trial and insectary (F = 4.522; df = 1,12; P = 0.0549). The percentage escape by flight for DPI was 22.5% in trial 1 and 44.4% in trial 2, whereas the percentage escape by flight for TIF was 79.4% in trial 1 and 76.9% in trial 2. Similar to the flight response after 24 h, the mean percentage of male moths (data combined for both trials) flying out of the flight cylinders after 48 h was significantly (F = 80.26; df = 1,13; P < 0.0001) less for DPI (35.94%) than for TIF (83.13%). There also was a significant (F = 7.13; df = 1,13; P < 0.019) trial effect on flight after 48 h with the percentage escape by flight greater (66.6%) in trial 2 than the percentage escape by flight (52.5%) in trial 1.

Field Release/Recapture. The mean percentage of moths recaptured during the field release of irradiated moths was significantly influenced by an interaction between insectary and trial (χ^2 = 7.365; df = 2,109; P < 0.0067) (Fig. 4), and significantly influenced by the day following a release that capture occurred (χ^2 = 101.2; df = 6,105; P < 0.0001) (Fig. 5). For both trials, the percentage recapture of TIF males was higher on the first day after the release, followed by the second and third days. In trial 1, very few DPI males were recaptured for all days and recapture was significantly less than recapture of TIF males on the first day after release (Table 1). However, after the procedural changes at the DPI insectary in rearing and handling protocols, the increased quality in the DPI males was evident. In trial 2 there was no significant difference in the mean percentage recapture of DPI (4.8%) and TIF (6.9%) males and there was a significant increase in the mean percentage recapture of DPI males as compared with trial 1 (Fig. 4).

The mean distance moths were captured from the release site was significantly influenced by an interaction among insectary, trial, and day of capture after release (χ^2 = 101.2; df = 3,108; P < 0.0001) (Fig. 6). As the number of days following a release increased, there was a decrease in the mean distance from the release site that moths were captured. In trial 1, there was a more rapid rate of decline in the mean distance by day of recapture for the DPI moths than for the TIF moths. The difference between the 2 insectaries in this rate of decline was reduced slightly in trial 2, suggesting that the procedural changes at the DPI insectary in rearing and handling protocols might have improved the flight distance to some degree. Comparing the overall mean (± SD) distance TIF males (10.16 ± 8.1 m) and DPI males (6.18 ± 7.8 m) were captured from the release site, TIF males were recaptured nearly 4 m farther from the release site than DPI males. Although it seems intuitive that the mean distance from the release site that a male is captured would increase with lapsed time from when the release is made because a male would have had time to fly farther, observations in the field indicated that many released moths remained within 1 m of

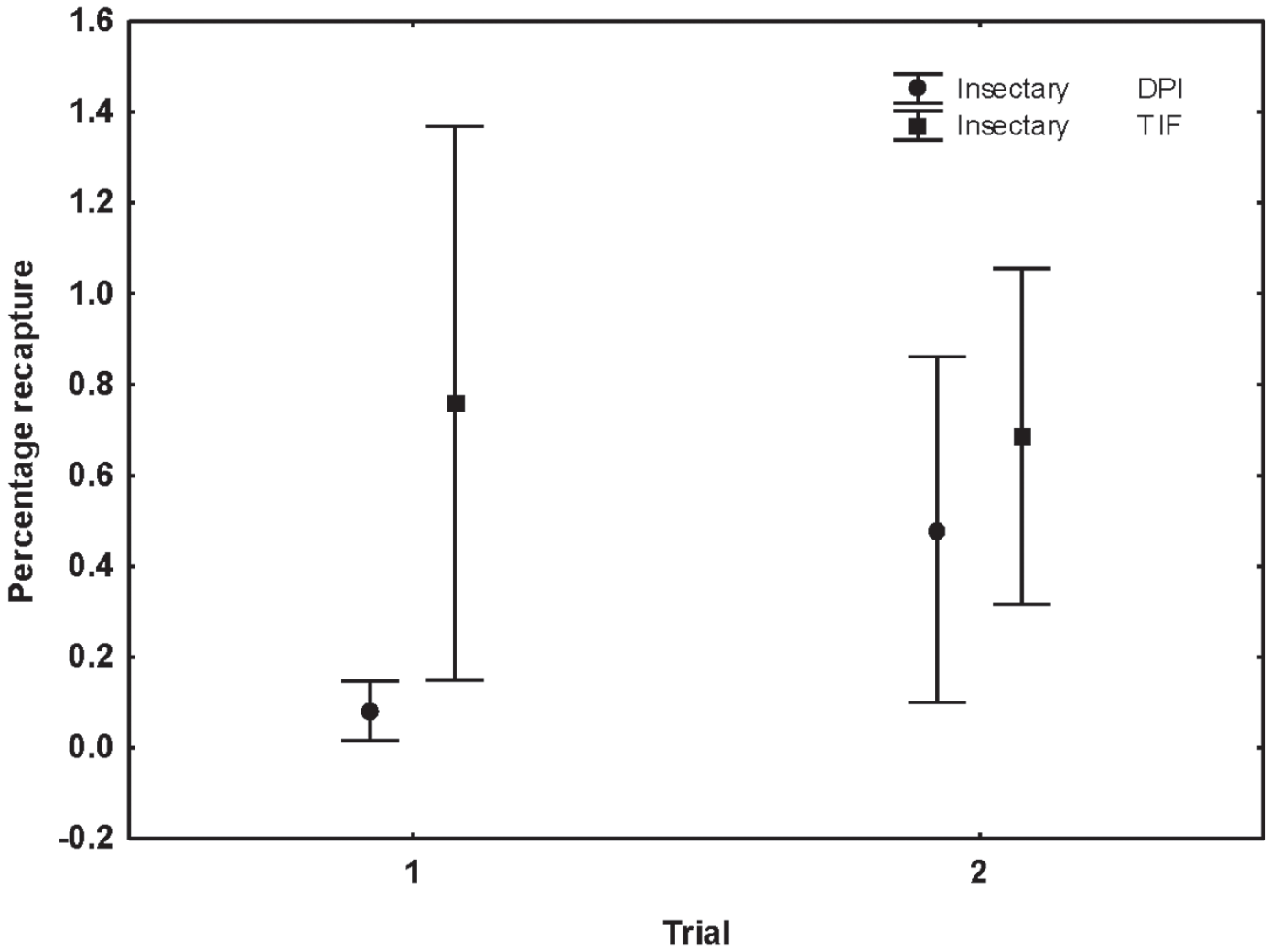


Fig. 4. The mean percentage recapture of *Cactoblastis cactorum* males released in the field as influenced by the insectary (DPI or TIF) and the trial conducted before (trial 1) and after (trial 2) quality improvements were made to the rearing and handling protocols at the DPI insectary. Vertical bars denote 0.95 confidence intervals.

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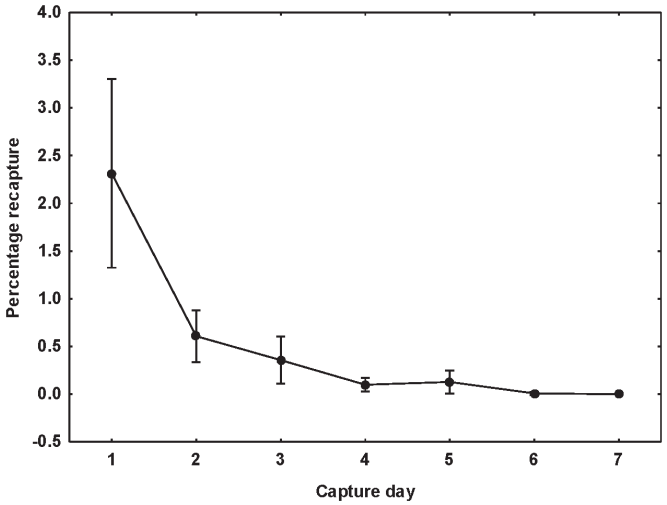


Fig. 5. The relationship between the mean percentage recapture of *Cactoblastis cactorum* males released in the field as influenced by the day that released males were recaptured. Males recaptured include individuals from both DPI and TIF insectaries. Vertical bars denote 0.95 confidence intervals.

the release site perched on vegetation for several days. It is likely that these inactive males were of poor quality and accounted for the males captured in the traps near the release site on days 4, 5, and 6 following a release.

Discussion

Successful SIT programs rely on mass-reared insects and require reliable production and delivery of insects of high quality and performance (Calkins & Parker 2005). To ensure quality control and success of these SIT programs, accurate methods for monitoring potential quality degradation during each step of production, handling, and release are crucially important (Huettel 1976; Singh & Ashby 1985; Calkins & Parker 2005). Complex behaviors and abilities, such as mating and sperm transfer, response of males to calling females or pheromone traps, dispersal, adult longevity, and infusion of sterility in the wild population are important to the success of the SIT. Elemental to these more complex behaviors are moth mobility, flight propensity, and flight ability (Calkins & Parker 2005; Vreysen 2005; Simmons et al. 2010). Therefore, bioassays for mobility, flight propensity, and flight ability should be fundamental to monitoring the performance of sterile moths released in the field (Carpenter et al. 2012). Actographs or locomotion activity meters (Bloem et al. 2006a,b; Keil et al. 2001; Gu et al. 2006; Brown et al. 2016), flight mills (Huettel 1976; Schumacher et al. 1997), and flight cylinders (Carpenter et al. 2012) have been used successfully to examine factors that affect moth mobility, flight propensity, and flight ability. Other bioassays such as mating cages (Carpenter et al. 2012; Saour 2016; Woods et al. 2016), wind tunnels (Suckling et al. 2007), and release/recapture of marked adults (Butt et al. 1970; Bloem et al. 1998, 2004) have examined moth ability to perceive and respond to semiochemicals that induce flight. Although many of these bioassays are comparatively complicated requiring advanced expertise, specialized equipment, have a low throughput—which limits sample size—and have limited portability between the field and laboratory, Carpenter et al. (2013) found that both a laboratory flight bioassay and a field cage bioassay successfully detected quality and performance differences that were relevant to moth performance in the field. However, they reported that the field cage bioassay was a better predictor of the daily performance of males that had been released in the orchard than the laboratory flight bioassay. Conversely, the flight cylinder bioassay was more sensitive in detecting daily fluctuations in the quality of moths caused by factors within the mass-rearing facility. Therefore, Carpenter et al. (2013) suggested that both laboratory and field bioassays may be required to provide feedback on quality and performance of mass-reared moths in a SIT program.

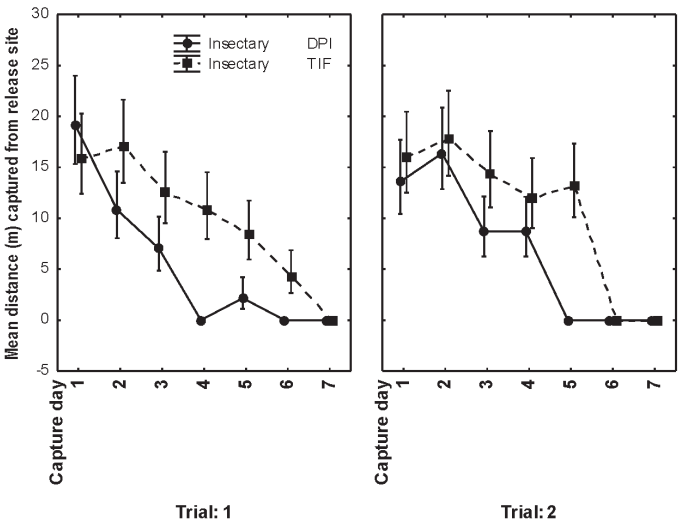


Fig. 6. The mean distance (m) from the field release site that *Cactoblastis cactorum* males were recaptured was significantly influenced by the insectary (DPI or TIF), the day that released males were recaptured, and the trial conducted before (trial 1) and after (trial 2) quality improvements were made to the rearing and handling protocols at the DPI insectary. Vertical bars denote 0.95 confidence intervals.

In the present study with *C. cactorum*, comparisons were made between 2 different insectaries that used eggs from the same insect colony and reared on the same meridic diet with the same rearing protocols. Quality and performance differences between moths from the 2 insectaries were detected by both laboratory and field bioassays. The results from trial 1 indicated significant loss of quality and performance of the DPI sterile moths, confirming the empirical field observations by workers in the Bi-National Cactus Moth Program. Parameters that indicated lower quality in DPI moths included longevity, percentage mated females at time of collection from the insectary, percentage females mating after collection, percentage of male moths flying out of the flight cylinders, percentage males recaptured following release in the field, and distance males were captured from the release site. A careful examination of the differences between the 2 insectaries regarding the rearing and handling protocols provided guidance in selecting the most appropriate procedural changes that were made at the DPI insectary. The scotophase was adjusted to insure that newly emerged adults remained in darkness to reduce their activity and mating before they were chilled for collection and transport to the irradiator. Adult activity was also reduced by lowering the temperature an additional 6 °C during adult collection and by yet another 2 °C during the transport and irradiation phase. The third adjustment made at the DPI insectary was to synchronize the photophase of the larval rearing period with the emergence period. While the best way to understand how each procedural change might affect moth quality would have been to conduct assessments after the implementation of each change, it was important to improve the quality and performance of DPI moths expeditiously to maximize the success of the ongoing Bi-National Cactus Moth Program. Nevertheless, relatively simple rearing and handling protocol changes at DPI increased moth longevity, the ability of moths to mate following collection from the insectary, the ability of male moths to fly

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from the flight cylinders, and the percentage of male moths recaptured in the field after release. At the termination of this study, however, DPI moths still demonstrated lower quality and performance than the TIF moths in the ability to mate after being collected from the insectary, the ability to fly from the flight cylinders, and the distance captured from the release site. Continuous quality monitoring and additional research are required to identify further procedural changes that would enhance moth quality and performance.

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