El Colegio de la Frontera Sur

Competitividad sexual, supervivencia y dispersión en campo de *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) irradiadas a diferentes dosis

TESIS

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Contenido

I. Introducción

En México y otros países de América Latina, la principal limitante para la comercialización y movilización del mango es la mosca Mexicana de la fruta, *Anastrepha ludens* (Loew) y la mosca de las Indias Occidentales, *Anastrepha obliqua* (Macquart). La presencia en los huertos comerciales de estas dos especies es motivo de establecimiento de rigurosas restricciones por parte de los países compradores de fruta, como es el caso de Estados Unidos (USDA/APHIS 2006).

Debido a la importancia económica del mango en nuestro país, las moscas de la fruta son consideradas plagas de interés público, por lo que en 1992, el gobierno federal implementó la Campaña Nacional contra Moscas de la Fruta (CNCMF) aplicando un manejo con enfoque en áreas grandes para lograr establecer zonas libres y/o de baja prevalencia (Reyes et al. 2000). Para lograr dicha meta, se aplican diversos métodos de control, como es la aplicación de cebos tóxicos, recolección y destrucción de frutos, cosecha sanitaria, liberación de parasitoides y la técnica del insecto estéril (TIE) (NOM-075-FITO-1997).

La TIE es una estrategia de manejo de plagas que requiere de la cría masiva, esterilización y liberación constante de grandes cantidades de insectos en campo. Según la Convención Internacional de Protección de Plantas (IPPC, por sus siglas en inglés), la TIE es un método de control de plagas mediante liberaciones sistemáticas de insectos estériles en áreas con presencia de una determinada plaga para reducir la fertilidad de las poblaciones silvestres (FAO 2005), lo cual requiere del establecimiento de una relación estéril: silvestre óptima (Klassen 2005).

La eficacia de la TIE como estrategia de control depende del desempeño sexual de los machos estériles que son liberados (McInnis et al. 1994), ya que la disminución de la población silvestre está en función de los apareamientos que ocurran entre machos estériles y hembras silvestres. Se ha demostrado en varias especies de moscas de la fruta que los machos de cría masiva presentan bajo desempeño sexual en comparación con los machos silvestres, lo que disminuye la eficacia de la TIE (Cayol 2000, Rull et al. 2007).

Actualmente, para la irradiación de las pupas de *A. obliqua* se aplica una dosis de 80 Gy que induce una alta esterilidad en machos. Sin embargo, esta dosis reduce la competitividad sexual, sobrevivencia y dispersión en campo de los machos (Rull et al. 2012, Lance y McInnis 2005, Calkins y Parker 2005, Robinson et al. 2002). En estudios previos realizados por Toledo et al. (2004) se sugirió que *A. obliqua* cuando es irradiada a 60 Gy incrementa su desempeño sexual.

Por lo tanto, sigue siendo prioridad la optimización de la dosis para inducir la esterilidad deseada en los machos sin comprometer su desempeño sexual (Robinson et al. 2002).

La calidad de las moscas estériles cuenta con dos componentes principales, la competitividad sexual y la supervivencia de los insectos en campo. Por lo tanto, es recomendable realizar evaluaciones periódicas de la calidad de las moscas irradiadas en campo, entre ellas determinar el tiempo que sobreviven las moscas después de ser liberadas y la distancia que se dispersan (Moore et al. 1985. Hernández et al. 2007).

Es por ello que el objetivo de esta investigación fue evaluar el efecto de diferentes dosis de irradiación sobre la competitividad sexual, supervivencia y dispersión en campo de machos de *A. obliqua*.

El capítulo II es el manuscrito sometido al Journal of Economic Entomology para su posible publicación. Ahí se presentan y discuten los resultados obtenidos en esta investigación. En el capítulo III se establecen las principales conclusiones del trabajo haciendo énfasis en la aplicación de los resultados.

II. **Sexual Competitiveness, Field Survival and Dispersal of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) Fruit Flies Irradiated at Different Doses**

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Sexual Competitiveness, Field Survival and Dispersal of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) Fruit Flies Irradiated at Different Doses

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ABSTRACT. *Anastrepha obliqua* (Macquart) is a major pest of mango in the Neotropics. The sterile insect technique (SIT) is used in area-wide pest management programs. In this study sexual competitiveness, field survival and dispersal of *A. obliqua* irradiated at 0, 40, 50, 60, 70, and 80 Gy were evaluated. A dose of 60 Gy produced 98% sterility, whereas doses of 70 and 80 Gy produced 99% sterility. Sexual competitiveness was assessed in field cages, comparing males irradiated at 0, 50, 60, 70, and 80 Gy against wild males for mating with wild fertile females. Males irradiated at 50 and 60 Gy achieved more matings than those irradiated at 70 and 80 Gy. Wild males were more competitive than mass-reared males, even when these were not irradiated (0 Gy). There was no effect of irradiation on mating latency, yet wild males showed significantly shorter mating latency than mass-reared males. Female remating did not differ among those that mated with wild males and those that mated with irradiated males at different doses. The relative sterility index (RSI) increased from 0.25 at 80 Gy to 0.37 at 60 Gy. The Fried competitiveness index was 0.69 for males irradiated at 70 Gy and 0.57 for those irradiated at 80 Gy, which indicates that a 10 Gy reduction in the irradiation dose produces greater induction of sterility in the wild population. There were not significant differences in field survival and dispersal of flies irradiated at 70 or 80 Gy. Reducing the irradiation dose to 60 or 70 Gy could improve the performance of sterile males and the effectiveness of the SIT. Our results allowed to sorting out the effect of irradiation from the effects of mass-rearing on the performance of sterile males.

Key Words: West Indian fruit fly, sterile insect technique, irradiation, mass rearing.

Introduction

In Mexico, the West Indian fruit fly, *Anastrepha obliqua* (Macquart) is considered the second most important species affecting mango (*Mangifera indica* L.) production. This insect pest limits movement of fresh fruit both within the country and to export markets (Aluja and Mangan 2008). To avoid or minimize its harmful effects, an area-wide integrated pest management program has been implemented (Gutierrez 2010). This program includes different control methods such as the use of bait sprays, biological control through release of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) and application of the Sterile Insect Technique (SIT) (Reyes et al. 2000, Orozco et al. 2004). The SIT involves production, sterilization and systematic release of large quantities of sterile insects so that they mate with the wild ones and reduce their reproductive potential (Knipling 1955, Klassen 2005).

Despite the fact that the SIT is an efficient and safe pest control method, it has been observed that mass-rearing and irradiation process reduce the sexual competitiveness of sterile males compared to wild males (Wong et al. 1983, Lux et al. 2002a, Rull et al. 2012). This loss of sexual competitivity reduces the efficiency of the SIT (Cayol 2000, Cayol et al. 2002). Some authors suggest that the sterile males are limited in terms of sexual competitiveness with the wild males as a result of the irradiation dose applied (80 Gy) to induce high levels of sterility (Rull et al. 2012). In this way, a study evaluating doses of 40 and 80 Gy, suggest that *A. obliqua* could be irradiated at a lower dose (Toledo et al. 2004). However, the sexual competitiveness and dispersal ability of males irradiated at doses between 50 and 70 Gy remain unknown.

High irradiation doses increase sterility, but reduce insects' field performance (Calkins and Parker 2005). Optimization of the irradiation dose is necessary in order to diminish its effect on the sexual performance of sterile insects and induce the desired level of sterility (Robinson et al. 2002). The aim of this study was to evaluate the effect of different irradiation doses on the sexual performance and dispersal ability of sterile *A. obliqua* flies.

Materials and Methods

Biological Material. Wild and mass-reared insects were used in this study. Wild flies were obtained as larvae from infested mango fruits, collected around Tapachula city (14°50’56” N; 92°20’15” W) in Chiapas, Mexico. The collected fruits were taken to the laboratory, weighted and placed in plastic trays (60- by 52- by 20-cm) with a capacity for 12-kg, with moistened vermiculite. These trays were and maintained at 26 ± 1 °C for eight days. Once mature, the larvae were extracted from the fruits using entomological forceps and placed in plastic containers (10- by 10- by 10-cm) with moistened vermiculite to avoid dehydration and promote pupation.
When the pupae reached 14 d of development (approximately two days prior to adult emergence), they were separated from the vermiculite using a sieve (mesh size 18) and placed in a 100-mm by 20-mm plastic Petri dish lid, which was placed into 30- by 30- by 30-cm glass cages for adults emergence. The flies were maintained under laboratory conditions, at 25 ± 1 °C temperature and 80 ± 5% relative humidity until they reached sexual maturity, which were 15 d for wild and 8 d for mass-reared flies (Orozco et al. 2007).
Mass-reared flies, both sterile and fertile, were provided by the Moscafrut mass rearing and sterilization facility located in Metapa de Domínguez, Chiapas (Domínguez et al. 2010). They were provided as pupae two days prior adult emergence. Irradiation treatments were carried out at this stage, following the standard procedures used at the facility (Domínguez et al. 2010).
Three days after emergence adults were sorted by sex, placed in 30- by 30- by 30-cm glass cages and provided with a 3:1 sugar: enzymatically hydrolyzed yeast (MP Biomedicals ®) mixture.
Water was provided in tubes covered with cotton swabs.
Mass reared flies reach sexual maturity 8 d after emergence, which is earlier than wild flies. For

the biological dosimetry, we used 8 day-old flies. For the mating competitiveness test we used wild and mass reared virgin flies of 8 and 15 day-old, respectively. For the field survival and dispersal, we used 5 day-old old flies.

Biological Dosimetry. The estimation of the sterility induced by each irradiation dose, mass-reared males irradiated at doses of 0, 40, 50, 60, 70, and 80 Gy were mated with fertile laboratory females. Irradiation was performed in a Gamma Beam 127 irradiator with a Cobalt 60 source, with 18,000 Ci. Plastic containers with 80 ml of pupae in hypoxia were exposed to the gamma source for different times according to the desired dose. Once the flies reached sexual maturity (8 d after emergence), 20 sterile males were confined with 20 fertile females for each treatment in glass cages with food and water as described above. After 24 h, three 2-cm in diameter agar spheres, prepared by mixing 3 liters of water with 80-g of agar, dyed with green vegetal colorant (McCormick® México, S.A. de C.V. Mexico City) and wrapped in Parafilm® membrane (American National Can TM, Nena, Wi), were hung from the top of each cage to serve as oviposition devices (Freeman and Carey 1989).

These spheres were replaced every 24 h, from which a sample of 100 eggs per treatment (cage) were collected. This was repeated for five consecutive days. The eggs of each sample were placed on a black strip of cloth placed on a moistened sponge inside a Petri dish and incubated at 28 ± 1 °C for five days, after which larval hatch was determined. The proportion of eggs that hatched was used as a measure of fertility. Four replicates were evaluated for each dose.

Sexual competitiveness of sterile males. Sexual performance was evaluated in field cages of 3.0-m in diameter by 2.2-m in height. A mango potted tree of approximately 1.5-m height was placed inside each field cage. Five days before the test, males were marked with a small dot of acrylic paint (Baco, Mexico City, Mexico) on the thorax, using colors to identify each treatment. Twenty laboratory reared fertile males, 20 wild males and 20 sterile males per treatment (irradiated at 50, 60, 70, and 80 Gy) were released into each field cage with 60 wild females. This 2:1 male:female ratio allowed males to compete for females. Males were released first, at 07:00 h, and 30 minutes later females were released. As pairs were formed, they were removed from the cages and placed in vials covered with a cotton wool swab. The number of pairs formed were recorded in order to calculate the competitivity indices proposed by Cayol et al. (1999) and the relative sterility index (RSI) proposed by McInnis et al. (1996). In addition, the mating latency was determined by recording the time elapsed from the release of the females until the occurrence of copulation. The study consisted of six treatments (wild males, nonirradiated mass-reared males and mass-reared males irradiated at 4 irradiation doses). A total of 15 replicates were conducted over five days.

Once copulations were completed, mated females were placed individually into 250-ml plastic containers of 13.5-cm in height by 7.5-cm in diameter and provided with water and food. Each female was identified with the data of the male with which it had copulated. After seven days, a fertile laboratory male was placed from 7:30 to 16:00 h with each female, and whether females remated or not was recorded. Environmental conditions were 25 ± 1 °C temperature and 80 ± 5% relative humidity.

Fried Test. In this experiment, the sexual competitivity of males irradiated at 80 and 70 Gy was evaluated, considering the degree of sterility induced by sterile males in wild females, following the Fried (1971) method. Four 20- by 20- by 20-cm plexiglass cages were setup: (1) “wild control” cages into which 15 wild males were released along with 15 wild females; (2) “sterile control” cages into which 15 sterile males were released along with 15 wild females; (3) “competitiveness 80 Gy” cages into each 15 sterile males and 15 wild males were released along with 15 wild females; (4) “competitiveness 70 Gy” cages into which 15 sterile males and 15 wild males were released along with 15 wild females (Orozco et al. 2007). Cages were kept under laboratory conditions and provided food and water. When the females reached 8 d of age, two agar spheres of 2 cm in diameter (described above), were placed in each cage. The spheres were replaced every 24 h; a sample of 100 eggs per day per treatment was collected during five consecutive days. Female fertility was assessed by observing larval hatching, as described above. Four replicates were done.

Field survival and dispersal. The fourth experiment evaluated field survival and dispersal of flies irradiated at 70 and 80 Gy (control). About 10,000 flies of each treatment were released in the center of a trapping grid at the "Maria Eugenia" orchard (14° 46' 3" N, 92° 16' 48" W), Tapachula, Chiapas, Mexico (Fig. 1). The pupae were marked with artificial colorant (Aurora Pink, Day-Glo Color Corp., Cleveland, OH, USA), with different colors according to treatment, alternated for each release. Five day old adults were transported to the study area where they were released following the Moscafrut standard packing and release procedure at a site located in the center of the orchard grid (Dector et al. 2016). In order to trap and retain the flies, five hours after release, 50 Multilure traps were set up distributed in concentric circles around the point of release, with approximately 15 to 45-m between traps. These were baited with Biolure® and to retain the attracted flies, 200-ml of propylene glycol were added. Figure 1 shows trap distribution within the orchard. The traps were serviced every 24 h for seven consecutive days and the trapped flies were placed in jars with 70% alcohol and transported to the laboratory for identification and quantification. Four releases were carried out 15 d apart, each of which was considered a replicate.

Data analysis. The egg eclosion variables were compared with a binomial analysis and means were compared using a Tukey test with a 95% degree of confidence. The number of copulations per treatment were added across the five days observed and were analyzed according to male treatment and replicate by a Generalized Linear Model (GLM) with Poisson distribution and log link function. Significant differences between male irradiation doses and between wild and mass-reared males were compared by post-hoc contrasts according to planned comparisons (Ruxton and Beauchamp 2008). Mating latency was averaged by male treatment and replicate and was analyzed using logistic regression. These two analyses were carried out in JMP version 9 (2010, SAS, Instititute, Inc.). The probabilities of rematings were analyzed using a Kruskal-Wallis test. The coefficient of competitivity was estimated with the Fried equation (Fried 1971, Hernández et al. 2010), considering values that range from 1 to 0. Values of 1 indicate equal competitivity between the two types of males and those tending towards 0 indicate dominance of the wild males (FAO-IAEA-USDA 2003). The RSI variable was compared with an analysis of variance (ANOVA) and means were compared using a Tukey with a 95% degree of confidence. In order to model the dispersion of the sterile males, considering the number of flies captured, and the location (coordinates) of each trap, the data were analyzed geostatistically, and with an ANOVA. Data were analyzed with the statistical software R for Windows (Version 3.2.0).

Results

Biological Dosimetry. The highest level of sterility was recorded for females that mated with males irradiated at 60, 70, and 80 Gy, achieving 98, 99, and 99% sterility, respectively. There were no significant differences among these three doses ($P > 0.05$). The sterility of females that mated with males irradiated at 50 and 40 Gy was 97 and 93%, respectively, which were significantly different from the higher doses ($P < 0.05$) and between them ($P < 0.001$) (Fig. 2).

Sexual competitiveness. There were significant differences among mating frequencies according to male treatment (χ^2= 39.112; d.f. = 5, 19; $P < 0.0001$), but not among replicates (χ^2=

14.604; d.f. = 14, 19; $P = 0.405$). Post-hoc contrasts revealed significantly higher frequency of matings for males irradiated at 60 Gy compared to 80 Gy, as well as 50 Gy compared to 80 Gy, while no significant difference between matings at 70 and 80 Gy (Fig. 3). Contrasts also revealed significantly higher frequency of matings by wild males compared to all other mass-reared males regardless of irradiation. Wild males had the highest number of copulations, while males irradiated at 70 and 80 Gy had the lowest (Fig. 3). Significant differences were observed in the relative sterility index (RSI) ($F = 2.675$; d.f. = 4; 70, $P < 0.05$). The RSI estimated from males irradiated at 80 Gy was 0.25%, while for males irradiated at 60 Gy it was 0.37%, with the differences between them proving to be significant (Table 1).

Mating latency, time elapsed since the release of males until mating, was significantly different among to treatments ($F = 5.291$; d.f. = 5; 66, $P = 0.0004$) and replicate ($F = 15.592$; d.f. = 14; 66, $P < 0.001$). However, post hoc Tukey´s test revealed no significant differences among the mating latency of males irradiated at any dose, or nonirradiated males. Only wild males had statistically significant shorter mating latencies than all other mass-reared males regardless of irradiation dose (Fig. 4).

Remating frequency was low (<15%) and no significant differences were observed. Females that mated with wild males, nonirradiated males or males irradiated at different doses showed the same low propensity to remate ($H = 6.125$; d.f. = 5; 48, $P = 0.294$) (Fig. 5).

Fried test. The level of sterility induced was 56.7 and 59.8% for males irradiated at 80 and 70 Gy, respectively. This resulted in a Fried competitiveness index of 0.58 for males irradiated at 80 Gy and 0.70 for males irradiated at 70 Gy (Table 2).

Dispersion. From the four releases performed, a total of 3236 flies from both treatments were captured, representing 4.04% of the total number of flies released. The average capture per release was 225 flies for those irradiated at 80 Gy and 313 flies for those irradiated at 70 Gy.

Survival was similar in both treatments. The greatest captures were in the first two days after the release, representing ~80% of the total number of flies (Fig. 6). The average distance of dispersal was 29.70 and 29.95-m for irradiated at 80 Gy and 70 Gy males, respectively; and the average distance of dispersion of irradiated females was 33.92-m at 80 Gy and 34.88-m at 70 Gy (Fig. 7). The difference between these values was not significant (F = 0.622; d.f. = 1; 10, P > 0.05).

Discussion

Results from the biological dosimetry show that males treated at 60 Gy, induced high levels of sterility that were not significantly different from those obtained at doses of 70 and 80 Gy. These results agree with those reported by Rull et al. (2007), who found that *Anastrepha ludens* (Loew) females mated with males irradiated at 60 Gy had no significantly different sterility than females mated with males irradiated at 80 Gy. Likewise, Toledo et al. (2004) reported that fertile females of *A. obliqua* that had mated with males irradiated at 60 Gy recorded high percentages of sterility. Our results show 1.0% fertility when females mated with 80 Gy irradiated males, similar to previous reports of 1.6% hatching when females mated with males irradiated at this same dose (Rull et al. 2012).

Field cage tests demonstrated that the sexual performance of males irradiated at 70 and 80 Gy was lower than that of the males irradiated at lower doses. This is important as reducing the irradiation dose to 60 Gy will not reduce the level of sterility and will improve the mating performance of the sterile males. Wild males had higher sexual competitiveness compared to all mass-reared males, including nonirradiated males. Most likely this was the effect of mass-rearing conditions (Cayol 2000, Lux et al. 2002a, Rull et al. 2005) and not due to irradiation. Thus, irradiation is not the only or main factor affecting the performance of sterile flies.

The effect of irradiation dose on the number of matings obtained, was confirmed with the estimation of the RSI. Males irradiated at 60 Gy were more competitive than those irradiated at 80 Gy, presenting indices of 0.37 and 0.25, respectively. This finding indicates that males irradiated at 60 Gy exhibited a better sexual performance. The Fried competitiveness index showed that the sterile males can compete successfully with the wild males and that there is a negative relationship with dose. The competitiveness index observed in males irradiated at 80 Gy was similar to that previously reported in both *A. obliqua* and *A. ludens* (Toledo et al. 2004, Orozco et al. 2007).

The current high dose (80 Gy) applied to *A. obliqua* flies in order to obtain minimum fertility (< 1%) produced a low induction of sterility in the wild populations due to the reduced sexual performance of these irradiated males (Bakri et al. 2005, Calkins and Parker 2005). However, using a dose of 70 Gy and even reducing to 60 Gy, will give better results since this would increase sexual competitiveness. Reducing the irradiation dose could improve performance of sterile insects to a certain level, but the effects of mass-rearing seem to bear a greater weight. Previous studies have suggested that lowering the dose of radiation by 10 Gy can contribute to improved results by achieving a better balance between sterility and sexual competitivity in the sterile males (Rull et al. 2014, Rull et al. 2012, Cáceres et al. 2007). Toledo et al. (2004) reported that males irradiated at 60 Gy presented a better sexual performance compared to wild males. In the males irradiated at 70 and 80 Gy, the capacity for mating was reduced almost twofold relative to the wild males, which coincides with that reported for *Ceratitis capitata* (Wiedemann) males (Lux et al. 2002b).

The greater mating success of wild males was also reflected in their shorter latency to mate compared to mass-reared males. Again irradiation had no effect on the speed at which males found mates, but mass-rearing did. In contrast, sterile males of *Bactrocera tryoni* (Froggatt) and *A. ludens* have been found to have shorter mating latencies than wild males (Pérez-Staples et al.

2009, Abraham et al. 2016). Both of these species call and mate at dusk, in contrast to *A. obliqua*, which is a diurnal species. In similar field cage studies with other tephritid species, discrepancies between the onset of sexual activity (calling) between sterile and wild males have also been observed (reviewed in Pérez-Staples et al. 2013). However, this does not always result in lower sexual competitivity for the sterile males (e.g. Orozco et al. 2007). Further studies on the effects of irradiation and mass-rearing on circadian rhythms and sexual activity are needed. Here for *A. obliqua*, lowering the irradiation dose will increase sexual competitivity, while further improvements to mass-rearing conditions and/or providing post-teneral treatments previous to release of sterile males could help reduce this discrepancy in time taken to obtain matings between mass-reared and wild males.

Another important finding here was that irradiation dose had no effect on a male´s ability to inhibit female remating, as there were no differences in remating frequency of females that initially mated with males irradiated at different doses, nonirradiated males or wild males. Similarly, in *Anastrepha fraterculus* (Wiedemann) and *A. ludens*, sterile males were as capable of inhibiting female remating as wild males (Abraham et al. 2013, 2014). However, this is not always consistent across tephritids, as studies on *Anastrepha serpentina* (Wiedemann) found sterile males were less likely to inhibit mass-reared female remating than fertile mass-reared males (Landeta-Escamilla et al. 2016), and in *C. capitata* females previously mated with wild males remated less frequently than those that had mated with mass-reared males, or sterile males, both in the laboratory and under the field cage conditions (Vera et al. 2002, Vera et al. 2003, McInnis et al. 2002a, Gavriel et al. 2009).

In the release and recapture field test, there was no effect of irradiation doses. No difference was observed in the number of recaptured of flies treated at 70 and 80 Gy. The percentage of recaptured flies (4.04%) was similar to previous studies (Hernández et al. 2007, Dector et al.

2016). More females than males were recaptured. Various studies have demonstrated that *A. obliqua* females are more attracted to the synthetic foods lures used than males (Díaz-Fleischer et al. 2009, Arredondo et al. 2014, Dector et al. 2016). The capture of adults in both treatments was higher in the first two days after release, and represented ~80% of the total number of flies captured (Fig. 6). In this sense, Peck et al. (2005) state that the flies present such a short flight range because of the favorable conditions of the release site, where the trees provide adequate refuge and food. Thomas and Loera-Gallardo (1998) found that the life expectancy of *A. ludens* flies under field conditions in North-East Mexico was 9.85 d. However, Hernández et al. (2007) found that the life expectancy *A. obliqua* under the field conditions in Southern Mexico was four days, which agrees with our results. We found no effect of irradiation dose on field survival and dispersal (Fig. 6, and 7). It would be of interest to examine the field dispersion of flies treated at 60 Gy.

In conclusion, our results suggest that the irradiation dose can be reduced to 60 Gy, a level that produces sterile males that exhibit better sexual performance, with no negative effects on mating latency and remating and no expected negative effects on field survival and dispersal. This dose will not compromise the level of sterility. Mass-rearing seems to have a greater effect than irradiation on the performance of sterile insects. Post-teneral treatments, such as food, aromatherapy or hormones (Pereira et al. 2013), as well as colony management systems (McInnis et al. 2002b, Rull and Barrera-Landa 2007, Quintero et al. 2016, Sánchez-Rosario et al. 2017) represent alternatives to overcome the negative effects of mass-rearing and improve SIT efficacy.

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Table 1. Relative sterility index (RSI) of wild fertile *A. obliqua* males or mass-reared irradiated males at different doses (Gy) mating with wild females in field cages.

Treatment Doses (Gy)	Relative sterility index (RSI ± E.E)
80	0.25 ± 0.05 a
70	0.33 ± 0.04 ab
60	0.37 ± 0.05 b
50	0.41 ± 0.03 b
0	0.41 ± 0.03 b

*Averages ± E.E. in the same column with the same letter are not significantly different ($P > 0.05$) in accordance with the ANOVA.

Table 2. Fried competitiveness index obtained from Fried´s equation (Fried´s test) between mass-reared irradiated and wild males.

Type of males	Hatching (%)			
Doses (Gy)	Wild	Sterile	Competition	Fried
70	68.0	0.4	40.2	0.699
80	68.0	0.5	43.3	0.578

Figures caption

Fig. 1. Map of spatial distribution of trap in a mango cv. Ataulfo orchard to assess the dispersal of *A. obliqua* males.

Fig. 2. Percentage of egg sterility from *A. obliqua* females mated with males irradiated at different doses (Gy).

Fig. 3. Number of matings observed in field cages between wild and mass-reared *A. obliqua* males irradiated at different doses (Gy). Asterix above columns indicate significant differences after post-hoc contrasts. Wild male matings were significantly higher than all other treatments.

Fig. 4. Mating latency of wild and mass-reared *A. obliqua* males irradiated at different doses (Gy).

Fig. 5. Percentage of *A. obliqua* female rematings with fertile mass-reared males after initially mating with wild or irradiated mass-reared males in field cages.

Fig. 6. Field survival of *A. obliqua* females (A) and males (B) irradiated at different doses and released in a mango cv. Ataulfo orchard.

Fig. 7. Dispersal of *A. obliqua* females and males irradiated at different doses and released from a central point in a mango cv. Ataulfo orchard.

Figure 1

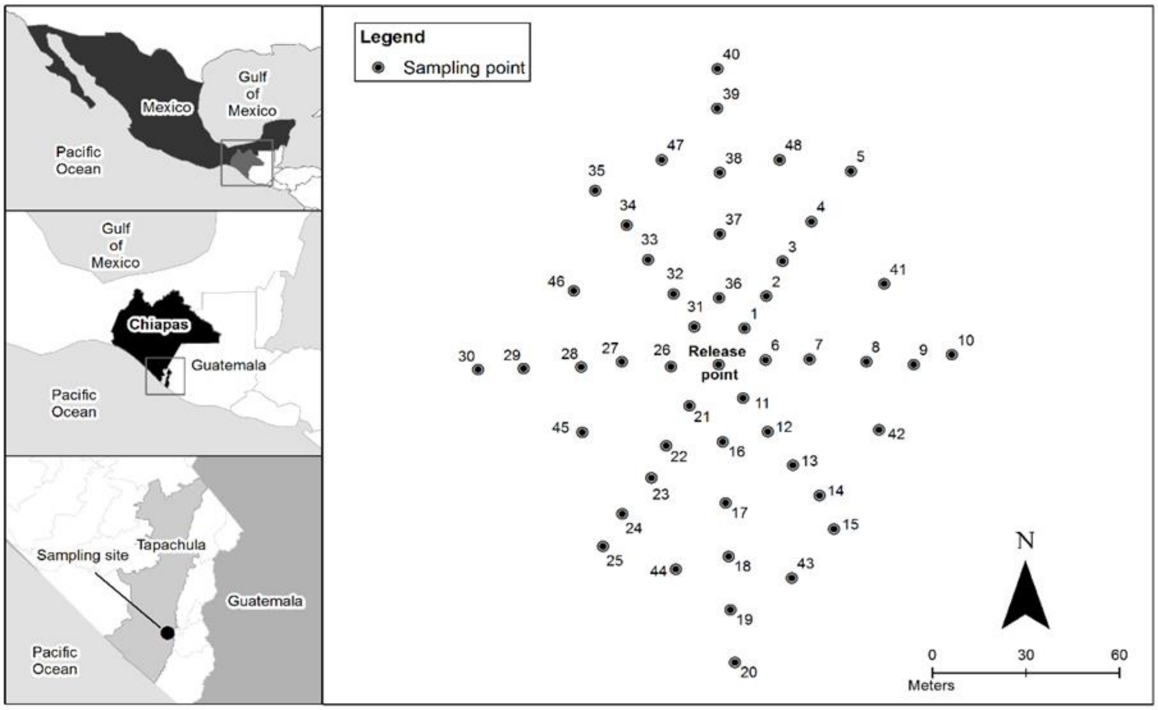
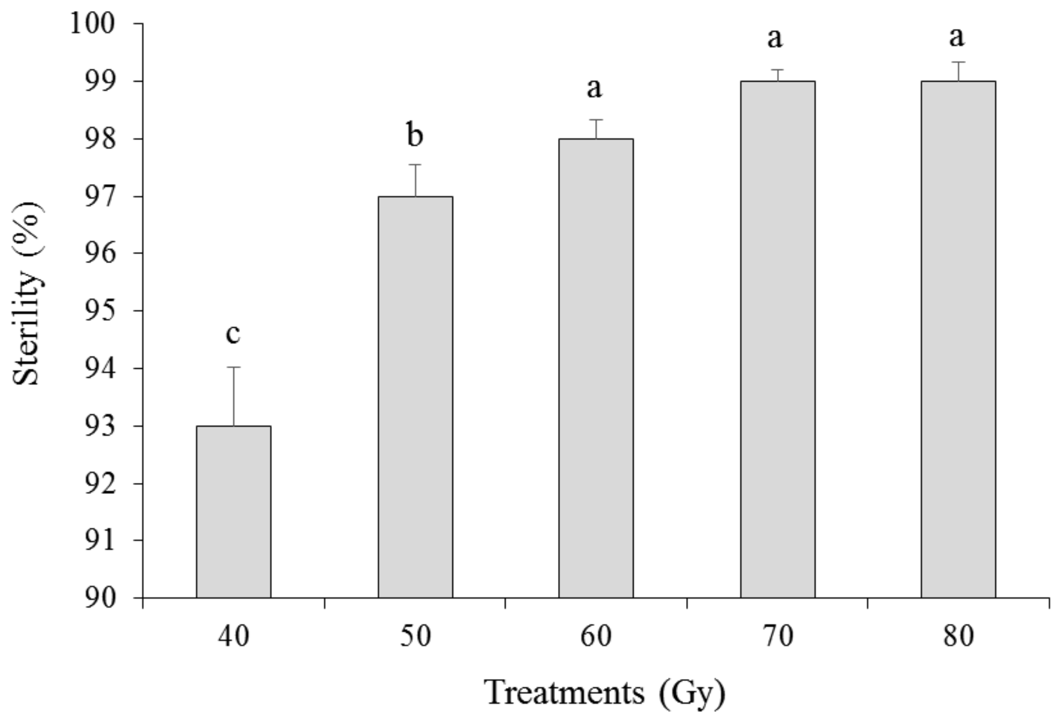
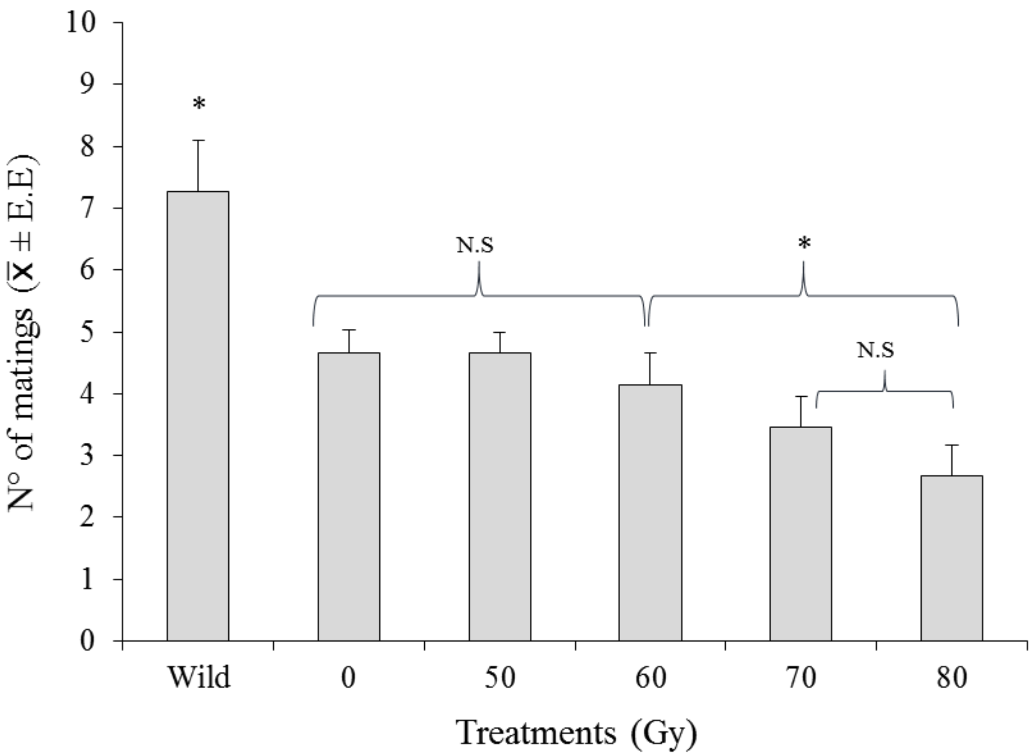

Gulf
of
Mexico
Mexico
Pacific
Ocean
Gulf
of
Mexico
Chiapas
Guatemala
Pacific
Ocean
Tapachula
Sampling site
Guatemala
Pacific
Ocean
Legend
Sampling point
Release
point
N
0
30
60
Meters

Figure 2



100
99
98
97
96
95
94
93
92
91
90
Sterility (%)
c
b
a
a
a
40
50
60
70
80
Treatments (Gy)

Figure 3



N° of matings (X̄ ± E.E)
10
9
8
7
6
5
4
3
2
1
0
*
N.S
*
N.S
Wild
0
50
60
70
80
Treatments (Gy)

Figure 4

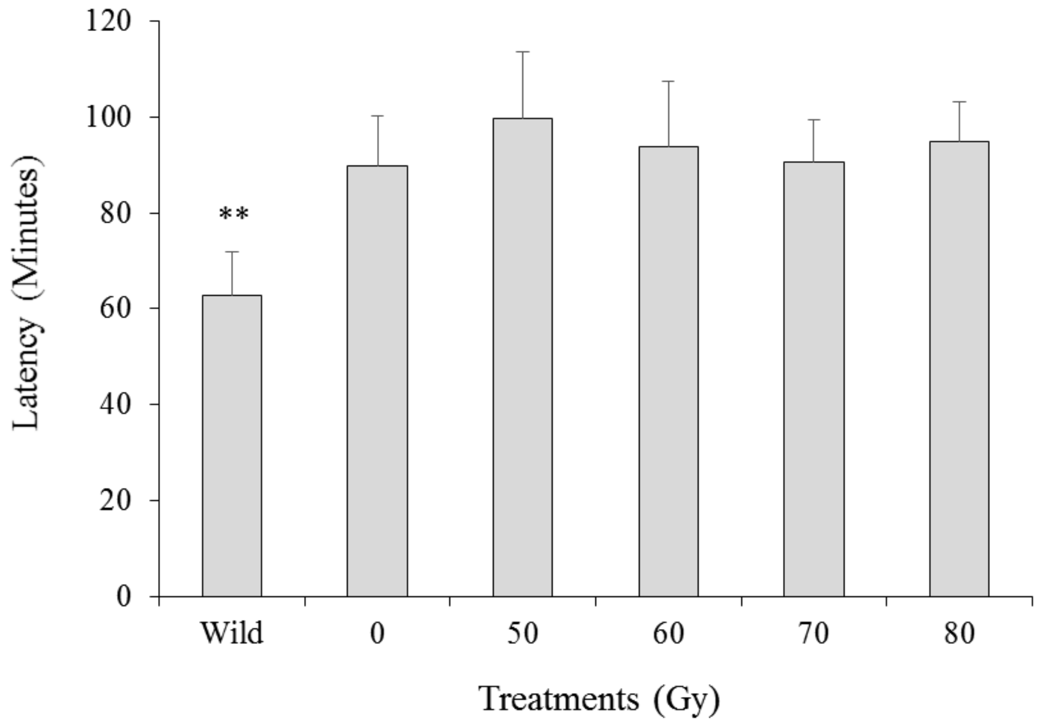

120
100
80
60
40
20
0
Latency (Minutes)
**
Wild
0
50
60
70
80
Treatments (Gy)

Figure 5

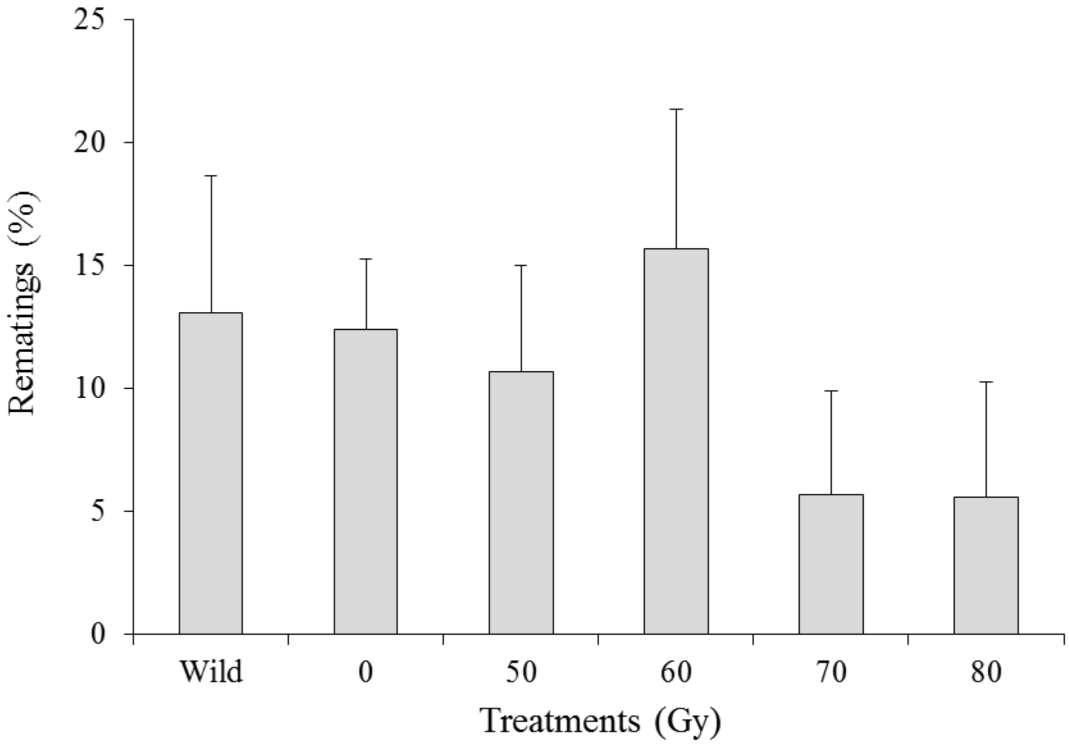


Figure 6

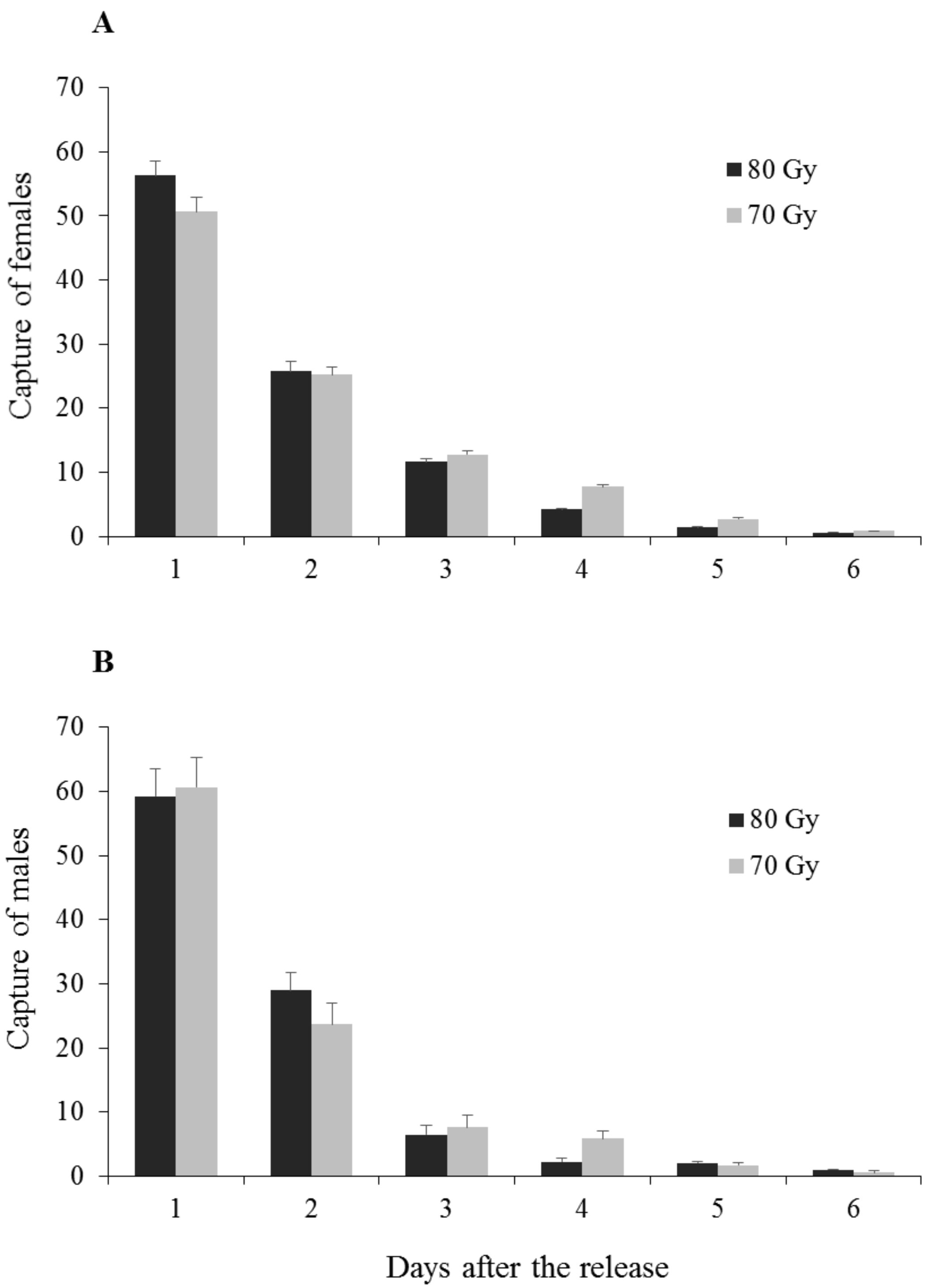
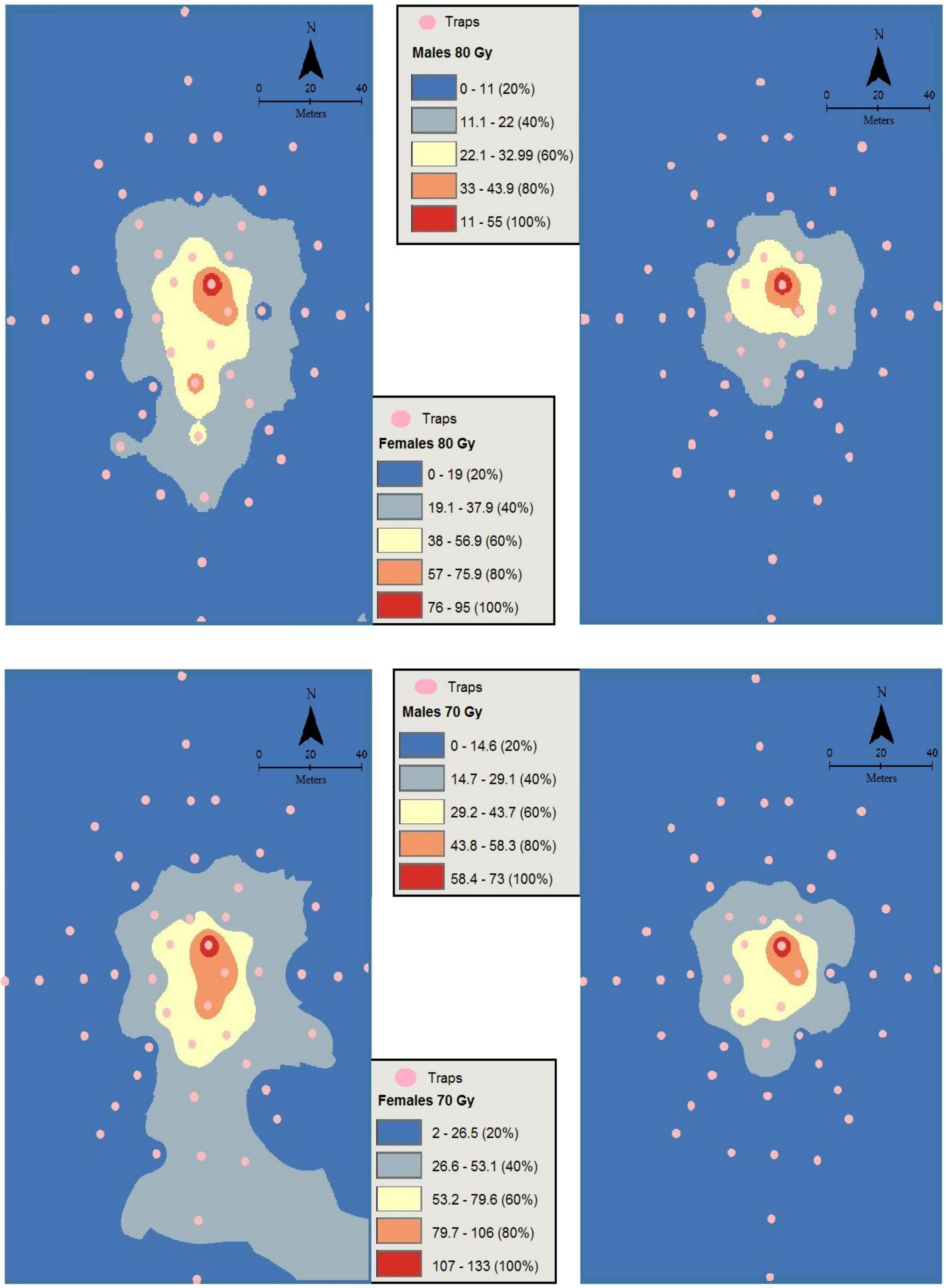

A
Capture of females
80 Gy
70 Gy
0
10
20
30
40
50
60
70
1
2
3
4
5
6
B
Capture of males
80 Gy
70 Gy
0
10
20
30
40
50
60
70
1
2
3
4
5
6
Days after the release

Figure 7


Traps
Males 80 Gy
0 - 11 (20%)
11.1 - 22 (40%)
22.1 - 32.99 (60%)
33 - 43.9 (80%)
11 - 55 (100%)
Traps
Females 80 Gy
0 - 19 (20%)
19.1 - 37.9 (40%)
38 - 56.9 (60%)
57 - 75.9 (80%)
76 - 95 (100%)
Traps
Males 70 Gy
0 - 14.6 (20%)
14.7 - 29.1 (40%)
29.2 - 43.7 (60%)
43.8 - 58.3 (80%)
58.4 - 73 (100%)
Traps
Females 70 Gy
2 - 26.5 (20%)
26.6 - 53.1 (40%)
53.2 - 79.6 (60%)
79.7 - 106 (80%)
107 - 133 (100%)
N
0 20 40
Meters

III. Conclusiones

Los resultados de la prueba de dosimetría biológica indicaron que los machos tratados con dosis de 60, 70 y 80 Gy, poseen una esterilidad >98%. Estos resultados soportan el hecho de reducir la dosis de irradiación sin comprometer la esterilidad de los machos liberados en campo. En las cruzas de machos estériles con hembras fértiles, hubo un mejor desempeño sexual en machos irradiados a 60 Gy, comparado con los que fueron irradiados a 70 y 80 Gy. Sin embargo, se registró un menor número de apareamientos comparado con machos silvestres. No se observó diferencia significativa en el número de apareamientos entre los machos irradiados a 50 Gy y los machos de cría fértiles (no irradiados). Este hecho, sugiere que la disminución del desempeño sexual también puede estar relacionada con otro factor como es el proceso de cría, ya que los machos fértiles de cría (no irradiados) igualmente registraron un bajo desempeño sexual.

La latencia a la cópula (tiempo transcurrido entre la liberación de los machos en jaula de campo y la cópula) fue mayor en los machos de cría no irradiados e irradiados con las diferentes dosis, y menor en los machos silvestres, las diferencias entre los valores fueron significativos. Este comportamiento puede afectar la eficiencia de la TIE ya que implica que las hembras silvestres podrían requerir de un mayor tiempo para evaluar a los machos de cría (irradiados o no), caso contario sucedió con los machos silvestres que fueron más efectivos en su cortejo para poder copular de forma rápida con las hembras silvestres. Se ha reportado en otras especies de moscas de la fruta como *Bactrocera tryoni* y *Anastrepha ludens*, que los machos estériles registran menor latencia en comparación con los machos silvestres (Radhakrishnan et al. 2009, Abraham et al. 2016). Con fundamentos en estos resultados, se considera que es necesario mejorar los procesos de cría para incrementar la calidad de los machos estériles que serán liberados (Quintero-Fong et al. 2016, Sánchez-Rosario et al. 2017).

No hubieron diferencias significativas en la probabilidad de que una hembra silvestre se re-aparee después de haberse apareado con un macho irradiado. Esto indica que la dosis de irradiación no tuvo un efecto sobre la habilidad del macho para inhibir la receptividad sexual de las hembras silvestres, lo cual favorece la eficacia de la TIE. El porcentaje de re-apareamientos de hembras copuladas con machos irradiados fluctuó entre 10-15%, siendo menor a lo reportado con machos silvestres (Aluja et al. 2009, Pérez-Staples y Aluja 2006). De acuerdo con los resultados del RSI, los machos irradiados con 60 Gy fueron más competitivos que los machos tratados a 80 Gy, dando como resultado índices de esterilidad de 0.37 y 0.25, respectivamente. Por lo tanto, irradiar a los machos a 60 Gy da mejores resultados en el desempeño sexual del insecto liberado. Los siguientes coeficientes de competitividad que se estimaron con la prueba de Fried fueron: de 0.57 en machos irradiados a 80 Gy y de 0.69 en machos irradiados a 70 Gy; considerando que los valores que se aproximan a 0 indican dominio por parte de los machos silvestres y cuando es hacia 1 indican dominio de los machos de cría. Este hecho mostró que los machos estériles son capaces de competir con éxito con los machos silvestres y que los machos irradiados a una menor dosis serán más competitivos.

En la dispersión de las moscas, en lo general, hubo mayor captura de hembras que de machos. Dector et al. (2016) sugieren que este resultado se debe a que las hembras de *A. obliqua* son más atraídas a los componentes sintéticos del Biolure que los machos (Diaz-Fleischer et al. 2009, Arredondo et al. 2014). La dispersión de machos irradiados a 70 y 80 Gy fue similar, no hubo diferencias significativas entre dichas dosis y hubo una mayor captura (~80%) durante los dos primeros días de haberse liberados. Por lo tanto, si la dosis de irradiación se disminuye 10 Gy no se afectará la dispersión de los machos, por lo que es pertinente analizar la dispersión de machos tratados a 60 Gy. El porcentaje de recaptura (4.04%) fue similar a los reportados en estudios previos con moscas irradiadas a 80 Gy (Hernández et al. 2007, Dector et al. 2016).

En conclusión, de acuerdo con los resultados obtenidos se sugiere reducir la dosis de irradiación a 60 Gy, con la cual se puede ocasionar una esterilidad aceptable en los machos estériles sin afectar su desempeño sexual, el cual incluye su latencia a obtener cópulas y su habilidad para inhibir los reapareamientos en las hembras.

Debido a que la cría masiva tiene mayor impacto sobre el desempeño sexual de los machos que la irradiación, se sugiere enfocar los esfuerzos a otros tratamientos como la aromaterapia y aplicación de hormonas para incrementar el desempeño sexual de los machos (Pereira et al. 2013), también es necesario mejorar el manejo de colonias en los sistemas de cría (Rull y Barrera-Landa 2007). Quintero-Fong et al. (2016) y Sánchez-Rosario et al. (2017) encontraron que mediante la selección es posible mejorar el desempeño sexual de los machos de cría masiva, y por lo tanto, incrementar la eficacia de la TIE.

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