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EFFECTS OF IRRADIATION ON THE COURTSHIP BEHAVIOR OF MEDFLY (DIPTERA, TEPHRITIDAE) MASS REARED FOR THE STERILE INSECT TECHNIQUE

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ABSTRACT

The effects of routine irradiation of the mass-reared males of the medfly, *Ceratitis capitata* on their mating performance were re-evaluated. Male courtship behavior was observed and quantified both in laboratory (video recording cages) and field cage conditions. For the experiments, samples of the strains routinely mass-reared for SIT operations at Seibersdorf, Austria; Mendoza, Argentina and Metapa, Mexico, were used. No major qualitative differences were found in the courtship pattern between irradiated and non-irradiated males. However, the results revealed that the process of routine irradiation as commonly used in the mass rearing facilities at the time of the experiments, reduces the mating performance of the sterilized males nearly two-fold. A whole range of quantitative differences between the irradiated and non-irradiated males were detected and described, and their implications for the efficiency of SIT operations are discussed. In contrast, partial sterilization with low doses of radiation did not affect the mating competitiveness of the treated males to a noticeable degree. In view of the results obtained, and due to the current wider use of "male-only" strains in SIT operations, a re-evaluation of the sterilization strategy and irradiation doses for males used in SIT is recommended.

Key Words: medfly, *Ceratitis capitata*, mating behavior, courtship, sterilization, irradiation, SIT

RESUMEN

Los efectos de irradiaciones rutinarias de machos criados en masa de la mosca del Mediterráneo, *Ceratitis capitata* sobre su capacidad de apareamiento fueron re-evaluados. El comportamiento del cortejo de los machos fue observado y cuantificado tanto en el laboratorio (grabaciones de videos en jaulas) y en condiciones de jaulas en campo. Para los experimentos, muestras de las razas que rutinariamente son criadas en masa para operaciones SIT en Seibersdorf, Austria; Mendoza, Argentina y Metapa, México fueron utilizados. No se encontraron diferencias cualitativas mayores en los patrones de cortejo entre machos irradiados y no irradiados. Sin embargo, los resultados revelaron que el proceso de irradiación rutinaria comúnmente utilizado en las instalaciones de cría en masa para el momento de los experimentos, reduce la capacidad de apareamiento de los machos estériles aproximadamente dos veces. Una gran variedad de diferencias cuantitativas entre los machos irradiados y los no irradiados fue detectado y descrito, y sus implicaciones en la eficiencia de las operaciones SIT son discutidas. En contraste, esterilización parcial con bajas dosis de radiación no afectó la competitividad de apareamiento de los machos tratados en un grado notable. En vista de los resultados obtenidos, y debido al uso más amplio y corriente de razas "solo-machos" en las operaciones SIT, una re-evaluación de la estrategia de esterilización y las dosis de irradiación utilizadas para los machos en SIT es recomendada.

The effectiveness of Sterile Insect Technique (SIT) programs is determined by the ability of released sterile males to successfully mate with and inseminate wild females. However, during the

process of establishing a strain (or its creation), along the rearing process, and during treatment before release, the insects are subject to highly artificial conditions, including extreme population

densities, a sterilization process, and sometimes, genetic manipulation. These factors adversely affect the biological fitness of the treated insects and their performance during SIT operations. In fact, a certain degree of reduction in insect quality seems to be an unavoidable cost in maintaining the economic efficiency in the mass rearing process.

In the practice of SIT operations, a moderate reduction in the quality of the mass-reared insects may be tolerated, because it can be compensated by higher release rates. This is especially true when the target insect pest has a simple mating system, closely resembling a stochastic process. In such cases, well represented by the tsetse, *Glossina morsitans morsitans* (Saini 1985), the courtship is usually reduced and females have a limited chance of rejecting males. Mating decisions are made by males and direct male-male competition is limited. Hence, the ability of the mass-reared tsetse males to copulate seems to be sufficient for SIT to be effective. However, the quality requirements for mass-reared insects may greatly increase when the SIT operation targets a pest having a more sophisticated mating system.

Recent studies on the medfly, *Ceratitidis capitata* (Wiedemann), mating system have shown that wild medfly males form leks to collectively attract females. This provides the females with an opportunity to compare, nearly at the same time, the qualities of several males present in a lek. To mate, the males have to engage in an elaborate courtship and wild females are known to be choosy in selecting a male for mating (Arita & Kaneshiro 1985, Calcagno et al. 1996, Calkins 1987, Harris et al. 1988, Hendrichs et al. 1991, Lux et al. 1996, Prokopy & Hendrichs 1979, Whittier & Kaneshiro 1991, Whittier & Kaneshiro 1995, Whittier et al. 1994). Consequently, the success of SIT for medfly control largely depends on the mating competitiveness of released irradiated males being directly challenged by their wild counterparts in competition for position in leks and the attention of wild females. Hence, simply the ability to copulate may not be sufficient to fully guarantee their efficiency. This implies that even minor deficiencies in the mass-reared sterilized males may have profound consequences for their mating success in the field, and consequently, on the costs and efficiency of medfly control operations.

Currently, irradiation is the only method available to effectively sterilize insects. The impact of an irradiation on the quality of the mass reared medfly males, in particular on their ability to mate, has been tested in the past. It has been concluded that the irradiation process causes no major mating deficiencies, if conducted according to the recommended standards (Hayashi & Koyama 1981, Holbrook & Fujimoto 1970, Hooper 1970, Hooper 1971a, Hooper 1971b, McInnis et al. 1985,

Wong et al. 1983, Wong et al. 1982). Hence, possible detrimental effects of irradiation, though reported by several authors (Favret et al. 1995, Haish 1969, Haish 1970, Hooper & Katiyar 1971, Lux et al. 1996, Lux et al. 1997), are generally considered to be of negligible importance for the effectiveness of SIT operations.

Indeed, the SIT has been used successfully in the USA and Latin America in numerous operations for large-scale medfly control or eradication (Hendrichs 1996, Penrose 1996). Though the technique is effective, efforts to improve its efficiency even further, continue. With increasing emphasis being placed on the quality of the produced and released insects, the present study was initiated to re-investigate the impact of irradiation on the performance of medfly males. To obtain more detailed insights into the subtleties of medfly courtship behavior, video recordings of courtship sequences and quantitative ethological analyses were made.

MATERIALS AND METHODS

The experiments were replicated in three locations: Seibersdorf (Austria), Mendoza (Argentina) and Metapa (Mexico), using three different medfly strains routinely mass-reared for SIT operations. To ensure unbiased data collection as well as comparability of the results, the video recording experiments were conducted by independent researchers from the three locations following a standard protocol, while most of the ethological analysis of the recorded material was conducted by the same team at ICIPE using a consistent methodological approach.

Biological Material

The following medfly strains were used:

- In 1994-1995, the G-47 temperature sensitive lethal (*ts/l*) strain, created and produced in the mass-rearing facility of FAO/IAEA Joint Division, Seibersdorf, Austria, was used. At the time of the experiments, the strain was mass-produced for a pilot SIT demonstration programme in Tunisia and, for this study, samples of routinely produced and irradiated flies were used. Later on, maintenance of the G-47 strain was discontinued. The pupae of the G-47 strain were gamma-irradiated (minimum absorbed dose: 14 Krad) in oxygen containing atmosphere, following the procedures used at IAEA at the time of experiments. Courtship behavior of both irradiated and non-irradiated medfly males was video-recorded for 90 min.
- In 1996, the wild type Mendoza strain adapted to mass-rearing conditions was used. This strain, produced in the mass-rearing facility located in Mendoza, Argentina, was used for

the large-scale medfly control/eradication program in Cuyo and Patagonia regions. For logistic reasons, a sample of flies from Mendoza facility was transferred to the INTA laboratory in Buenos Aires and reared for one generation following Teran's (1977) method as described by Calcagno et al. (1996). Pupae were irradiated 48 h before emergence with a Philips X ray emission device, under normal atmosphere. The applied doses were as follows: 14, 7 and 3.5 Krad, and an additional non-irradiated group was used as a control. Pupae were kept under controlled conditions (23-25°C, L:D 12:12). Every day, emerged adults were sexed, to insure virginity of both males and females. The effects of irradiation were tested under field cage conditions and video-recorded for 30 min in the laboratory in small chambers. For the field tests, pupae were X-irradiated with doses of 14, 7, 3.5 Krad and non-irradiated control. For the video recording experiment only 14 Krad and control (non-irradiated) males were used. The dose of 14 Krad is equivalent to the irradiation dose used in Seibersdorf in the above-described experiments.

- In 1998, a bisexual strain was used, which was mass-produced in the MOSCAMED facility in Metapa, Mexico for medfly suppression operations along the Mexican—Guatemalan border. The insects were taken directly from the mass rearing facility. The pupae were gamma-irradiated (minimum absorbed dose: 14 Krad) in hypoxia (closed full container, at least 30 min before exposure), following the procedures used at MOSCAMED at the time of experiments. Courtship behavior of both irradiated and non-irradiated medfly males was video-recorded for 30 min.

In all the experiments, both the irradiated and non-irradiated males were paired with non-irradiated mass-reared females originating from the same strain. All insects were virgin and mature, 7-12 days old.

Field-cage Experiments

The field cage was built around a young citrus tree. Every day 30 virgin mature males from Mendoza strain representing each class of irradiation dose (14, 7, 3.5 Krad and non-irradiated control) were released into the cage at 08.00 AM (local time). After one hour, in which they had the opportunity to establish the territory and integrate into leks, 30 virgin non-irradiated females were released into the cage. Two days before the tests, the males were etherized and marked on the scutellum with a water-based paint, using color codes to identify the corresponding radiation dose.

Every hour from 10:00 AM to 14:00 PM, the cage was monitored and number of mating pairs was

recorded. The mating pairs were removed and the number of pairs along with the male category (radiation dose) and the position of the mating pair inside the cage were recorded. Every day after the test, all non-mated individuals were removed from the cage. The experiment was repeated 8 times between April 30 to May 9 1996.

Video Recording

The recordings were conducted using the methodology and set-up proposed by Lux (1994). A sound-proof room was maintained at approximately 23-26°C. The equipment consisted of a Sony Hi 8 video camera (Model CCD-TR805, Japan) with a Novoflex (Germany) macro lens, a color TV, a Hi 8 videocassette recorder, and a microphone (Sennheiser, Model K6P/MKE102, Germany).

Cylindrical mating cages (70 mm × 85 mm diam.) were used, made from acrylic pipe. The top of the cage was covered with a Petri dish and the open basis was placed on a 2 mm thick transparent glass plate. The video recording was carried out through the glass from below. To simulate natural field conditions, a fresh lemon or coffee leaf was placed inside the cage and fixed to its top cover and, as it usually happens in the field, the males tended to establish their territories on the underside of the leaves. A microphone for recording sound signals was inserted through a lateral hole in the cage. Another lateral hole permitted the release of flies into the cage.

In the experiments with the Seibersdorf strain, the males used for video recording were selected at random from the mass reared population. In the case of Mendoza and Moscamed strains the males were randomly pre-selected. Each morning, several mating cages were prepared. Approximately 30-60 min prior to the start of the recording, a male was gently placed into each mating cage and allowed to calm down and establish a territory. The first male that began to call (i.e., release pheromone from the abdomen) continuously for 5 min was chosen for the first recording.

Five min after releasing the male into the cage (Seibersdorf) or five min after the males started calling (Moscamed and Mendoza), a female was gently released into the same cage. Immediately following release of the female, behavior of the male and its interactions with the female were recorded for 30 min, except the Seibersdorf strain, where male behaviour was recorded for 90 min. The video recordings were carried out between 10:00 AM and 02:00 PM, which was found to be the period of the highest diurnal mating activity rate. About 30-40 recordings were conducted for each strain and treatment. A male was considered successful if copulation occurred within the observation period.

Data Analysis

Male behavior was categorized into the following activities/stages: 1. Stationary (the male remains still, cleaning), 2. Stationary Calling (the male remains still and calling, i.e. emitting pheromone), 3. Mobile Calling (calls as in the previous case but being mobile), 4. Fanning (continuous fanning-wing vibration upon detecting the presence of a female so that pheromone is directed towards her), 5. Wing Buzzing (intermittent buzzing that is produced by means of an intense wing vibration, performed alternately with short periods of head rocking, which starts when a female approaches and remains in front of the male), 6. Head Rocking (rapid movements of the head rotating toward both sides, which accompany the Buzzing), 7. Missed Jump (male jumps, trying to mount the female, but he fails to achieve it), 8. Violent Mounting Attempt (male jumps onto a female but she rejects him violently, only exceptionally this activity is followed by copulation), 9. Peaceful Mounting Attempt (male jumps onto a female and reorients parallel to her, the female is cooperative and does not object, usually this activity is followed by copulation), 10. Copulation (after jumping and reorienting on the female the male is accepted, which is followed by intromission of the aedeagus), 11. Fight (aggressive interaction initiated by the male, the female or both).

Quantitative ethological analysis was performed using QuantEtho software (Lux 1989). The program selected a specified part of each observation and calculated:

- mean duration of each activity (its variability and standard error),
- number of occurrences and total time spent for each activity,
- probability of passing from other activities to the given one (input chances),
- probability of passing from the given activity to others (output chances), and
- ratio of time spent for each activity.

For each analyzed part of an observation, a chart of time-budget was prepared showing the percentage of time spent on each activity during the observation.

Each time when the two observed individuals (a male and a female) were closer to each other than 3 cm, responses of both, the male and female, as well as the interactions between them, were quantified. When necessary, the frame-by-frame function of the time-lapse video recorder was used, which allowed a 1/30 second reviewing resolution and analysis of very short-lasting responses, such as touching a male by the front legs of a female.

The analysis of all recordings produced a large body of numerical data and charts and only a

small part of it is presented in this paper. The complete data set was recorded on CD-ROM (Lux et al. 1999) and deposited at FAO/IAEA at Seibersdorf, Austria and at ICIPE, Nairobi, Kenya.

RESULTS AND DISCUSSION

A typical sequence of a successful male courtship was described by Feron (1962) and is comprised of the following main steps: calling, fanning (wing vibration), wing buzzing, mounting attempt and copulation. Several other behavioral elements were described by Lux & Gaggli (1995), which indicate that medfly courtship is a kind of a “dialogue” with intensive exchange of signals between sexes. Females display a rich repertoire of responses to courting males, such as touching a male with her head or front legs, brief jumping towards the male, short wing vibrations and stretching wings just after mounting. Most of these activities are very short-lasting, within a range of 1/30-1/10 second, and thus were not noticed in the earlier studies.

For all the strains tested, no major qualitative differences were found in the courtship pattern between irradiated and non-irradiated males. In both cases, the courtship sequences were composed of the same major behavioral steps and each activity, if it occurred, was performed in a similar manner. The irradiated males were generally able to display courtship and mate. However, several consistent quantitative differences between irradiated and non-irradiated males were noticed.

The most important difference was that the average mating performance of the irradiated males was reduced nearly two-fold as compared to the non-irradiated males. However, during 30 min of the video-recording experiment, the mating success of males from the Seibersdorf strain was about 8 times lower compared to that of the Moscamed and Mendoza strains observed over the same period. With such an extremely low numbers of mating males, the difference between the irradiated and non-irradiated Seibersdorf males was difficult to interpret and was not significant (Table 1). The low performance of this strain is most likely to have been caused by the fact that the males used for recording were selected at random from the entire mass-reared population. In contrast, the males from the other two strains were pre-selected before recording, and only sexually motivated and calling males were used. Indeed, in the case of the Moscamed and Mendoza strains, the number of matings was much higher, and in both strains, the reduction in mating performance caused by the irradiation process reached 40%. However, only 30 pairs were observed from the Moscamed strain, and the difference between the irradiated and non-irradiated males, though close to the borderline of

TABLE 1. EFFECT OF IRRADIATION ON MATING SUCCESS (DURING 30 MIN OBSERVATION).

Strain origin	Irradiated males			Non-irradiated males			Reduction in performance	
	Mating success	S	F	Mating success	S	F	percent	P*
Seibersdorf G-47	3.6%	1	27	7.1%	2	26	50.0%	0.553
Moscamed	30.0%	9	21	50.0%	15	15	40.0%	0.114
Mendoza	37.5%	15	25	62.5%	25	15	40.0%	0.025

*Significance level in one-sided chi² contingency test.
S—successful males which started copulation within 30 min.
F—failures, number of unsuccessful males which failed to copulate within 30 min.

statistical significance ($P = 0.114$), was not significant. In the case of the Mendoza stain, more pairs (40) were observed, and the same level of reduction was highly significant ($P = 0.025$) (Table 1). It was also noticed that the irradiated males engaged in mating later than the non-irradiated ones, a trend well represented in the time budgets of the Moscamed strain (Fig. 1). Remarkably, the differences between irradiated and non-irradiated males were noticed even though the video-recordings were conducted in extremely limited space and each female was forced to repeatedly interact with a given single male, having only a “NO-choice and NO-retreat” option.

When given more time, the males from the Seibersdorf strain finally reached a similar mating frequency as the two other strains. However, when the females were forced to interact with the males for so long in the restricted space, over 40 meetings might occur during the 90 min of observation. Under such conditions, the consistent (albeit not significant) differences in performance between the irradiated and non-irradiated males gradually became relatively less apparent (50%, 15% and 12% in reduction in mating success during 30, 60 and 90 min of observation, respectively). The results appear to indicate that the method for selection of males from the mass-reared population, and the duration of the quality control test, are important factors determining the sensitivity of the test and its ability to detect deficiencies in male quality. More research, however, will be required to confirm and quantify such effects and to optimize the quality control tests.

Analysis of the recordings further revealed that the irradiated males were more passive and less vigorous. They spent relatively more time passive (“resting”), and less time walking or flying (Table 2). Activity indices, ratio of the time spent active (walking or flying) to the time spent passive, as measured for the whole sample of males from each strain, not only represented the overall vigor of the strain, but were also found to be indicative of the strain’s mating success (Fig. 2).

It appears that the irradiated males were less motivated sexually; more frequently they did not

call (did not emit pheromone) just before meeting with a female, even though they sometimes mated during the same meeting (Table 3). These non-calling males did not attract females, but rather met them by chance. As a consequence, the frequency of meetings with females was lower for the irradiated males as compared to the non-irradiated males (Table 3). When very close to the approaching female, the irradiated males tended to move towards her, which often provoked a fight and resulted in more frequent aggressive interactions during meetings (Table 3).

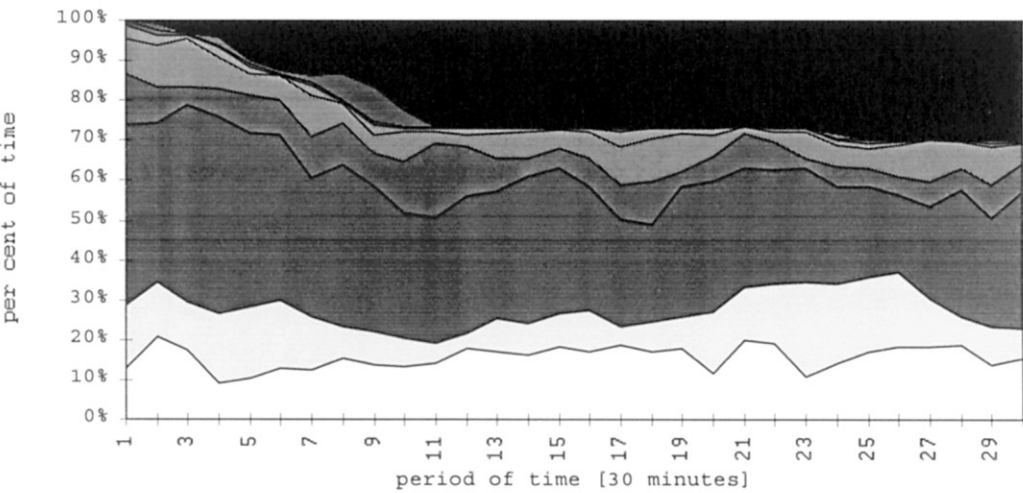
During courtship, females interacted with courting males by touching a male with her head or front legs, brief jumping towards the male, short wing vibrations, lowering wings and stretching wings just after mounting. Several differences were detected in the frequency of female responses to courtship between irradiated and non-irradiated males, both during wing vibration and buzzing (Table 4). Though it appears that the frequency and structure of female responses might be indicative of the quality of male courtship, the exact role of these behaviors and their relation to the probability of mate acceptance and successful mating remain unclear.

In general, non-irradiated males were more “patient” and allowed females to approach very closely and interact. During buzzing, they more frequently remained still and were less likely to approach or “push” the courted female (Table 5). Such a steady courtship has been reported to be a general trait of successful males, as opposed to the “impatient” courtship of those males which were usually later rejected by females and failed to mate (Lux & Gaggl 1995, Lux et al. 1996). Interestingly, in those cases where the courtship successfully progressed until a mounting attempt, no differences were noticed between irradiated and non-irradiated males.

Under the described experimental conditions with such drastically restricted space, frequent and random interactions may sometimes result in a successful mating, even in the case of males which are less sexually motivated or less competitive. In the field, however, a non-calling male

A. IRRADIATED MALES

Time budget



B. NON-IRRADIATED MALES

Time budget

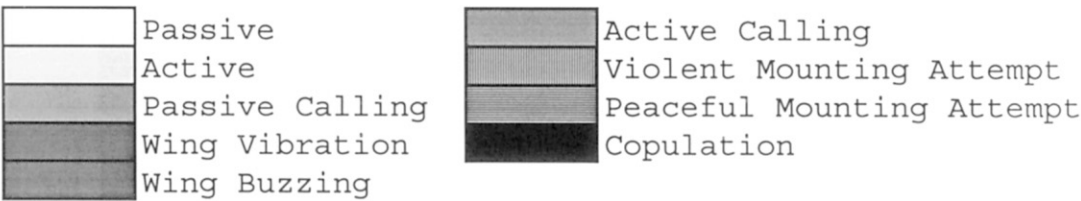
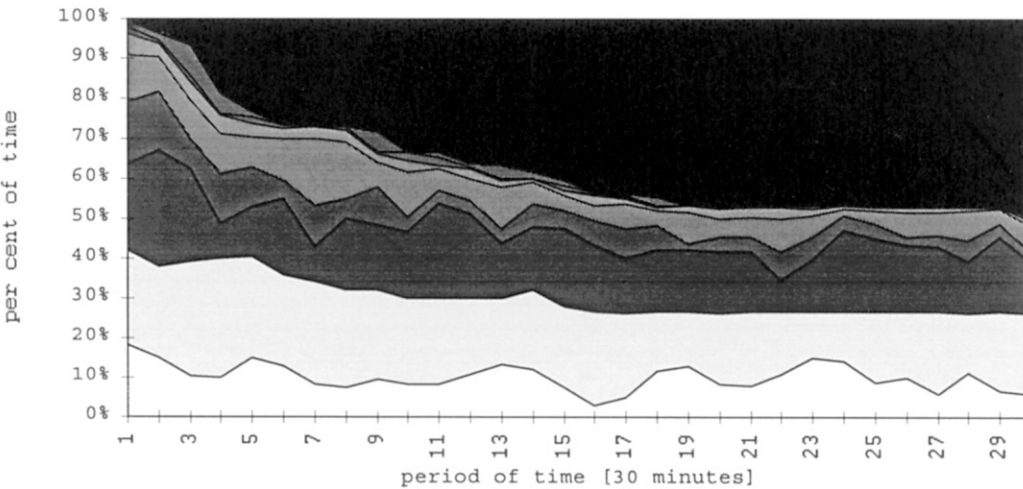


Fig. 1. Effect of irradiation on performance of medfly males (Moscamed strain).

TABLE 2. AVERAGE TIME IN SECONDS SPENT ON VARIOUS ACTIVITIES DURING 30 MIN OBSERVATION.

Activity	Seibersdorf G-47		Mendoza		Moscamed	
	IR	Non-IR	IR	Non-IR	IR	Non-IR
Passive	1306.4	1281.6	90.0	12.6	294.5	187.2
Active	432.1	501.6	158.6	43.7	215.9	372.6
Average activity index: (time Active/time Passive)	0.33	0.39	1.76	3.46	0.73	1.99
Significance level	n.s.		*		*	

*Significant difference ($P < 0.05$) in the average activity index between irradiated and non-irradiated males from the same strain, according to t-test conducted using activity indexes calculated for individual males from each group.
IR = irradiated, Non-IR = non-irradiated.

would have a negligible chance of meeting with a female and mating. Such males would most likely not appear or establish themselves in lek arenas or participate in calling activities, or attract females. Hence, females would be unlikely to find them and mate. Furthermore, those poorly performing males which, although suffering rejection several times, finally managed to mate, would be unlikely to have so many opportunities when released into the field. Therefore, the described differences between irradiated and non-irradiated males should be expected to be far more apparent under field conditions. Indeed, the detrimental effects of irradiation on mating performance were found to be more explicit in the field cage test (Table 6), where much more space was available and the females were given a chance to choose among males irradiated at various doses.

The results also revealed that with increasing dose of radiation, a male's ability to participate in lek formation was reduced. The males which received a high radiation dose (7 or 14 Krad) were recorded on the tree less frequently than the males which received the lowest dose (3.5 Krad) or no ra-

diation at all (Table 7). Although the integration of males into leks was not quantified in relation to the corresponding radiation dose, it was noticed that the males irradiated with 14 Krad tended to stay on the floor and only rarely joined leks on the tree. In addition, these males tended to keep calling on the tree in the afternoon when most of the other males had already stopped calling.

The success of an SIT program relies principally on reduction of the reproductive potential of wild females by induced egg sterility caused by the release of sterile males. This in turn depends on a balance among several factors, such as the degree of sperm sterility induced by irradiation; male mating competitiveness; and a male's ability to transfer sperm to female spermathecae in order to switch off her receptiveness, thus preventing re-mating and inducing egg laying behavior. Interestingly, males irradiated with 3.5 Krad mated in the same proportion as non-irradiated ones, indicating a similar mating competitiveness (Table 6). Therefore, the use of low doses of radiation allows good mating performance, although not ensuring total male sterility. In fact, it has been

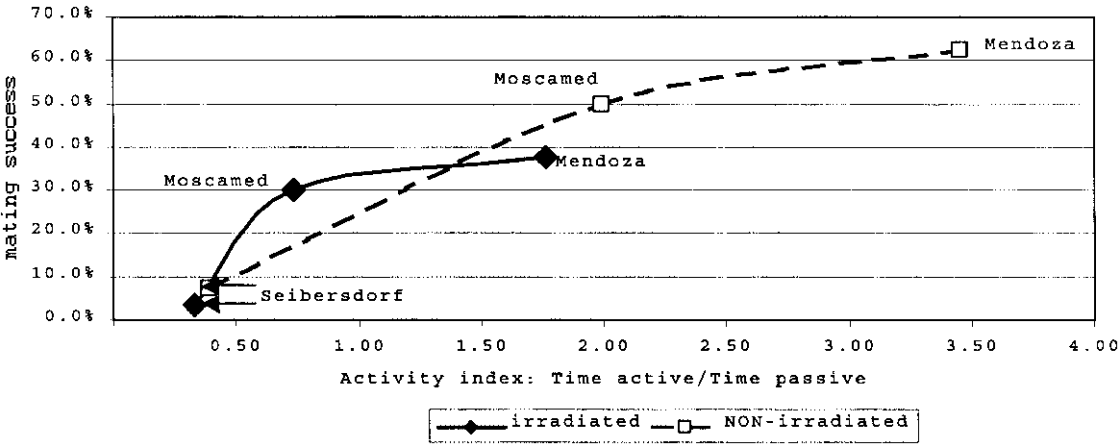


Fig. 2. Relation between activity index and mating success for irradiated and NON-irradiated males.

TABLE 3. THE EFFECT OF IRRADIATION ON MALE-FEMALE INTERACTIONS DURING MEETING.

Meeting (male & female are closer than 3 cm)	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	<i>P</i> ^{a,b}	IR	Non-IR	<i>P</i> ^{a,b}
Average number of meetings/pair	5.87	7.00	n.s.	4.68	8.92	*
Male calling just before the meeting	57%	73%	0.003	85%	90%	0.201
Male approaching female during meeting	22%	16%	0.154	6%	1%	0.013
Fight during meeting	18%	4%	0.000	10%	6%	0.211

^aSignificance level (presented in as the exact probability) in one-sided chi² contingency test, conducted using the actual frequencies of events.
^bSignificance level (presented as: n.s. if *P* >= 0.05, or * if *P* < 0.05) in t-test, conducted using numbers of events performed by each male.
IR = irradiated, Non-IR = non-irradiated.

demonstrated that partially sterile males irradiated with doses close to 3.5 Krad may actually be more effective in reducing overall reproductive potential of females (measured by number of non-hatched eggs) than totally sterile males irradiated with higher doses (Favret et al. 1995).
In the past, when only bisexual medfly strains were available and used, applying a high radiation dose which would guarantee total sterility of both sexes was necessary to ensure the efficiency of SIT treatment, as well for the regulatory reasons. Our results revealed, however, that such an irradiation strategy partially incapacitates the mass-reared males, which is substantially reducing their performance and increasing overall costs of the SIT operations. Since “male-only” strains are now available, the irradiation strategy should now be re-considered. Partially sterile but highly competitive males could be released

and may prove more effective than the totally sterile, but less competitive males produced at the moment.
Several improvements in the irradiation treatments have been suggested in the past (Hooper 1971a, Ohinata et al. 1977, Zumreoglu et al. 1979) which might reduce the damage caused by the sterilization process. However, since the irradiation process was widely considered to cause only insignificant damage to the sterilized males, in most mass rearing facilities, such improvements, though well known, have not been applied, and presentations of our early results (Lux et al. 1996b, Lux et al. 1997) were received with skepticism. To corroborate our earlier results, several additional independent experiments (reported in this paper) were conducted in Argentina and Mexico. The consistent results obtained not only confirm the significance of the negative effects of

TABLE 4. THE EFFECT OF IRRADIATION ON FEMALE RESPONSES DURING COURTSHIP.

Stage of courtship and female response	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	<i>P</i> ^a	IR	Non-IR	<i>P</i> ^a
Wing fanning (vibration)						
a. touching male with front leg/legs	0.62	0.93	*	0.11	0.11	n.s.
b. touching male him with head	0.21	0.14	n.s.	0.06	0.08	n.s.
c. sudden jump towards male	0.20	0.14	n.s.	0.01	0.08	*
d. vibrating wings (very short)	0.03	0.22	*	0.00	0.00	n.s.
e. lowering wings	0.00	0.00	n.s.	0.00	0.00	n.s.
Wing buzzing						
a. touching male with front leg/legs	3.94	3.53	n.s.	3.38	3.00	n.s.
b. touching male him with head	0.96	0.69	n.s.	0.71	0.96	n.s.
c. sudden jump towards male	0.38	0.45	n.s.	0.52	0.82	n.s.
d. vibrating wings	0.25	0.83	*	0.00	0.03	n.s.
e. lowering wings	0.10	0.00	*	0.00	0.00	n.s.

^aSignificance level (presented as: n.s. if *P* >= 0.05 or * if *P* < 0.05) in t-test, conducted using numbers of events performed by each female.
IR = irradiated, Non-IR = non-irradiated.

TABLE 5. THE EFFECT OF IRRADIATION ON MALE BEHAVIOUR DURING ADVANCED STAGE OF COURTSHIP (BUZZING).

Buzzing	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	P ^a	IR	Non-IR	P ^a
Male “patience” during buzzing:						
a. remains still while buzzing	56%	64%	0.28	53%	68%	0.026
b. approaches and/ or pushes female	44%	36%		47%	32%	

^aSignificance level (presented in as the exact probability) in one-sided chi² contingency test, conducted using the actual frequencies of events.
IR = irradiated, Non-IR = non-irradiated.

TABLE 6. FIELD CAGE TEST: EFFECT OF IRRADIATION DOSE ON MALE MATING PERFORMANCE (MENDOZA STRAIN).

Radiation dose (rad)	No. of matings	Mating success (%)	Results of Chi ² test
0	71	41 a	Chi ² = 0.04, P = 0.841 Chi ² = 26, P < 0.0001 Chi ² = 87, P < 0.0001
3500	69	40 a	
7000	29	16 b	
14000	5	3 c	

Numbers followed by the same letter are not significantly different.

TABLE 7. EFFECT OF IRRADIATION DOSE ON SELECTION OF MATING SITES WITHIN THE FIELD CAGE (MENDOZA STRAIN).

Radiation dose [rad]	Screen	Tree	Results of Chi ² test
0	18	53 a	Chi ² = 3.64, P = 0.16 Chi ² = 8.78, P = 0.032
3500	22	47 a	
7000	13	16 a	
14000	4	1 b	

Numbers followed by the same letter are not significantly different.

the irradiation process and re-emphasize the critical need for using hypoxia or a nitrogen atmosphere during irradiation, but have also triggered the process of re-investigating the sterilization strategy and methods.

The most recent findings (Rendon 1999) indicate that the detrimental effects of the same dose of irradiation were much more severe in the case of mass-reared males as compared to wild males. This suggests that reduced male tolerance to irradiation and the consequent increased severity of the damage caused by the irradiation process, may depend on the number of generations of a strain under mass rearing. If confirmed, this would add one more reason for periodically changing the mass-reared strains and maintaining strict quality control regimes in the rearing process.

It is being recognized that ensuring a high level of mating competitiveness of mass-reared irradiated males and, at the same time, maintaining economic efficiency in the mass rearing process, presents an enormous challenge. Ultimately, however, the increased rearing or processing costs are likely to be offset by benefits from

the increased efficiency of SIT operations, if the same results can be achieved by releasing smaller numbers of more competitive insects.

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