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Authors: Allinghi, Armando, Calcagno, Graciela, Petit-Marty, Natalia, Gómez Cendra, Paula, Segura, Diego, et al.

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COMPATIBILITY AND COMPETITIVENESS OF A LABORATORY STRAIN OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) AFTER IRRADIATION TREATMENT

ARMANDO ALLINGHI[1], GRACIELA CALCAGNO[2], NATALIA PETIT-MARTY[3], PAULA GÓMEZ CENDRA[2], DIEGO SEGURA[3], TERESA VERA[4], JORGE CLADERA[3], CECILIA GRAMAJO[4], EDUARDO WILLINK[4] AND JUAN CÉSAR VILARDI[2]

[1]Comisión Nacional de Energía Atómica, CNEA, Argentina

[2]Depto. Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales Universidad Buenos Aires. (1428) Buenos Aires, Argentina

[3]Instituto de Genética, INTA, Castelar, Argentina

[4]Estación Experimental Agro-industrial Obispo Colombres, Tucumán, Argentina

ABSTRACT

We evaluated under semi-natural field cage conditions sexual compatibility and competitiveness of a laboratory strain (LAB) compared to a wild population (TUC) of *Anastrepha fraterculus* (Wiedemann). The LAB strain is produced under semi-mass rearing conditions at the Estación Experimental Agroindustrial Obispo Colombres facility (Tucumán, Argentina). Wild flies were obtained at Horco Molle (Tucumán, Argentina) from infested guava fruits. LAB pupae were irradiated (^{60}Co) 48 h before adult emergence. The tested doses were 0 (control), 40, 70, and 100 Gy. Twenty-five males and 25 females each of TUC and LAB were released into cages and mating pairs collected. Only 1 irradiation dose was considered at a time. Females were separated and allowed to lay eggs into artificial fruits to estimate induced sterility from the corresponding hatching rate. Copulation start time did not differ significantly between strains nor among irradiation treatments. Copulation duration showed highly significant differences among irradiation doses, but no differences between strains. The index of sexual isolation (*ISI*) and the relative sterility index (*RSI*) indices indicated that LAB and TUC are fully compatible, males from TUC and LAB did not differ in mating competitiveness, and irradiation within the range tested did not affect these indices. Non-irradiated LAB females exhibited higher mating propensity than TUC ones. However, a significant reduction in the female relative performance index (*FRPI*) index was observed with increasing irradiation dose. The analysis of induced sterility indicated that treatment with 40 Gy reduces male fertility from about 80% to 0.75%, and higher doses produce total sterility. In females, the 40 Gy dose reduces fertility to about 2% and higher doses prevent egg laying.

Key Words: mating compatibility, *Anastrepha fraterculus*, Irradiation, mating indices, fruit fly, Tephritidae

RESUMEN

Se evaluó bajo condiciones semi-naturales en jaulas de campo la compatibilidad y la competitividad sexual de una línea de laboratorio (LAB) con respecto a una población salvaje (TUC) de *Anastrepha fraterculus* (Wiedemann). La línea de laboratorio se produce en condiciones de cría semi-masiva en las instalaciones de la Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina). Las moscas salvajes se obtuvieron de frutas infestadas de guayabos en Horco Molle (Tucumán, Argentina). Las pupas de laboratorio fueron irradiadas (^{60}Co) 48 horas antes de la emergencia del adulto. Las dosis utilizadas fueron 0 (control), 40, 70, y 100 Gy. Se liberaron 25 machos y 25 hembras de TUC y LAB dentro de las jaulas y se recolectaron las parejas formadas. Sólo se considero 1 dosis de irradiación por vez. Las hembras apareadas fueron separadas y se les permitió poner huevos en frutas artificiales para estimar la esterilidad inducida a través del porcentaje de eclosión. La hora de inicio de la cópula no difirió significativamente entre poblaciones ni entre los tratamientos de irradiación. La duración de la cópula mostró grandes diferencias entre dosis de irradiación pero no entre cepas. Los índices *ISI* (aislamiento) y el *RSI* (esterilidad relativa) indican que LAB y TUC son totalmente compatibles, los machos de TUC y LAB no difieren en su competitividad y la irradiación dentro del rango de dosis utilizadas tampoco afectó este índice. Las hembras LAB no irradiadas muestran una mayor propensión para el apareamiento que las hembras de TUC. Sin embargo se observó una reducción significativa del índice *FRPI* (actuación relativa de hembras) a medida que se aumenta la dosis de irradiación. El análisis de la esterilidad inducida indica que con dosis de 40 Gy la fertilidad disminuye del 80% al

0.75%, y con dosis mayores la esterilidad fue total. Las hembras irradiadas con dosis de 40 Gy tienen una fertilidad de aproximadamente 2% y con dosis mayores no ponen huevos.

Translation provided by the authors.

Anastrepha fraterculus (Wiedemann) the South American fruit fly (Stone 1942) is an important pest of fruit production in Argentina and the species is abundant in the northwestern and northeastern regions (Vergani 1956). The range of *A. fraterculus* overlaps at least partially with that of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). The programs of suppression or eradication of the latter species, integrating the Sterile Insect Technique (SIT) in this country, have shown a remarkable success (SENASA 1997, http://www.senasa.gov.ar/vegetal/mosca1.php), and point to the necessity of developing and applying similar control strategies for *A. fraterculus* (Guillén & Sanchez 2007).

We analyzed under laboratory conditions the optimal irradiation dose and pupal age at the moment of irradiation to induce sterility in *A. fraterculus* (Allinghi et al. 2007). Irradiated males were able to transfer sperm and exhibited apparently minimal effects, if any, of the irradiation on their performance in comparison with non-irradiated males. However, the *sine qua non* condition for the SIT is sexual compatibility between sterilized and released laboratory reared flies and wild flies. Therefore, it is necessary to evaluate under quasi-natural conditions the mating performance of laboratory males when competing with wild males for wild females.

In the present work we evaluated, on field-caged host trees the sexual compatibility and competitiveness of a laboratory strain (LAB) in relation to a wild population from Tucumán (Argentina) (TUC). We also analyzed the effects of different radiation doses on mating competitiveness, strain compatibility, fertility, copulation duration, and copulation start time.

Materials and Methods

The LAB strain used in this study was produced under semi-mass rearing conditions (Jaldo et al. 2001) since 1997 at the Estación Experimental Agroindustrial Obispo Colombres facility (Tucumán, Argentina). Wild flies were obtained from fruiting guava trees *Psidium guajava* L. (Myrtaceae) at Horco Molle (26°48'S, 65°20'W) from Tucumán, Argentina. The LAB strain and the collected fruits were sent to the Laboratory of Insects, Instituto Nacional de Tecnología Agropecuaria (INTA), in Castelar, Argentina. The collected fruits were placed on plastic trays over a layer of sand to allow pupation. The sand was periodically sifted to obtain pupae, which were then placed in plastic 1-L flasks. The LAB and the TUC pupae were maintained under controlled conditions (25 ± 1°C, 80 ± 5% rh and a photoperiod of 12:12 L:D) until adult emergence.

LAB pupae were irradiated 48 h before adult emergence (Allinghi et al. 2007) at the Centro Atómico Ezeiza facility (Comisión Nacional de Energía Atómica, Argentina) in a Gammacell 220 (MDS Nordion, Canada) irradiator (^{60}Co source) with a dose rate of 1.4 Gy/min. Lots of 500 pupae were held in 20-mL ventilated glass containers during the exposures of 40, 70, and 100 Gy in normal atmosphere. After irradiation, the pupae were placed in 3-L glass containers. Flies of the control group were subjected to all of the same handling procedures except irradiation. Emerging adults were removed from the flasks every 24 h. To facilitate sorting by sex, flies were anaesthetized by exposure to a temperature of 0°C for 10 min. Fifty individuals of each sex were placed in separate 3-L glass containers and supplied with water and adult food. The food consisted of a 2:1 dry mixture of brown sugar: hydrolyzed corn protein (R. M. SAIC). Manso (1998) showed that laboratory strain adults fed this diet developed to sexual maturity. Adults were kept under laboratory conditions (25 ± 2°C, 60 ± 20% r.h.), and a photoperiod of 12:12 (L:D) until sexually mature. De Lima et al. (1994) reported flies under such conditions reach sexual maturity in 16 d. In a pilot field cage test, we found an increasing proportion of mating with fly age; however, mortality also increased with age. The age of 20 ± 1 d after emergence was found to be the best compromise between maturity and viability.

Three d prior to each experiment, flies were labeled to identify their origin. This was done by placing approximately 10 flies in a mesh bag (1 mm mesh diameter) where, one at a time, they were gently immobilized and painted on the thorax with a dot of water-based paint (Tempera Alba, Alba, Inc., Argentina). Colors green, red, white, and yellow were interchanged sequentially each day. After labeling, 25 flies were placed in 1-L containers with food and water and held under laboratory conditions until required. Outdoor nylon screened cages (2.9 m tall × 3 m diameter) were erected over rooted 1.5 m tall, 4-year-old tangerine trees, *Citrus reticulata* Blanco (Rutaceae). Field cages were identified by number, and each day treatments and observers were randomly assigned to them. In field cages, 25 males and 25 females each of TUC and LAB strains were released. For each radiation dose, 6 replicates were made. Only 1 irradiation dose was considered at a time.

Because mating occurs mainly in the morning (Malavasi et al. 1983; Morgante et al. 1983; De Lima et al. 1994; Petit-Marty et al. 2004), the observation period was from 08.00 h to 13.00 h. Males were released 15 min before females to allow establishment in the cage. Only healthy marked flies were released, while non-active or dead flies were replaced. For each mating pair, the following data were recorded: copulation start time, copulation location (fruit, net, ground, stem, abaxial-adaxial side of a leaf, height in the tree), and male and female colors. The pairs were gently induced to walk into 20-mL plastic vials and placed in the shade until the mating couple disengaged. This moment was recorded as the copulation end-time. These field cage tests were performed at INTA Castelar (Buenos Aires Province) between April 4 and 16, 2002. Temperatures, relative humidity, and sunshine records during this period were favorable for fly requirements. Copulation start time and copulation duration were compared among laboratory irradiated flies by one-way analysis of variance.

Sexual compatibility was estimated by means of the index of sexual isolation (*ISI*) (Cayol et al. 1999) and the relative sterile index (*RSI*) (McInnis et al. 1996). Male and female competitiveness was evaluated respectively through male (*MRPI*) and female relative performance (*FRPI*) indices (Cayol et al. 1999). The statistical significance of any departure from random mating or equal performance of each sex was tested, following Petit-Marty et al. (2004), by means of an independence chi squared test taking into account the total number of each mating combination (*ISI*), the total number of mated and unmated males (*MRPI*) or females (*FRPI*), of each population. Compatibility and relative performance analyses were based only on those trials where the percentage of mating was sufficiently high (>20% of mated females). Matings occurring on the cage screen or on the floor were not included, following the international fruit fly quality control manual (FAO/IAEA/USDA 2003).

For each treatment, induced sterility was evaluated from the percent of egg hatching. At the end of the experiments in the field cages, females were separated according to radiation treatment and male origin and transferred to 3-L flasks. They were allowed to lay eggs into artificial fruits (Manso 1998). Eggs were collected and incubated in Petri dishes, and the hatching rate was recorded.

Results

For both LAB and TUC flies, most matings occurred on the lower side of peripheral leaves at an intermediate canopy height. Copulation start time (Table 1) did not differ significantly between strains and the irradiation treatment did not show any effect on this variable ($F = 0.23$, $P = 0.63$ and $F = 3.16$, $P = 0.08$ for males and females, respectively). Copulation duration (Table 1) showed highly significant differences among treatments ($F = 4.97$, $P < 10^{-3}$ and $F = 10.08$, $P < 10^{-7}$ for males and females, respectively). These differences are totally attributable to the irradiation treatment. Indeed, TUC and non-irradiated LAB flies did not differ significantly in copulation duration ($P = 0.88$ and $P = 0.41$ for males and females, respectively), but if these two classes are grouped and compared with irradiated flies the differences are highly significant ($F = 18.71$, $P < 10^{-4}$ and $F = 40.08$, $P < 10^{-9}$ for males and females, respectively).

The analysis of mating compatibility by means of the *ISI* indicated that LAB and TUC are fully compatible. The estimated values did not depart significantly from that expected for random mating, and no effect of irradiation was observed (Table 2). Males from TUC and LAB did not differ in mating competitiveness, and irradiation did not affect this index. Non-irradiated LAB females exhibited higher mating propensity (*FRPI* signifi-

Table 1. Copulation start times and mating duration (hrs:min) of TUC[1] and LAB[2] flies with different irradiation doses.

	Males					Females				
	Start time		Duration			Start time		Duration		
Strain/dose	Avg	SE	Avg	SE	*n*	Avg	SE	Avg	SE	*n*
TUC	9:11	0:42	1:14	0:37	554	9:13	0:42	1:15	0:36	523
LAB/0	9:16	0:46	1:19	0:41	136	9:14	0:46	1:21	0:43	154
LAB/40	9:11	0:41	1:07	0:35	134	9:07	0:42	1:04	0:34	144
LAB/70	9:11	0:43	1:02	0:30	135	9:12	0:40	1:03	0:33	144
LAB/100	9:19	0:45	1:05	0:33	128	9:13	0:44	1:02	0:32	122

[1]TUC = wild flies from Tucumán obtained from guava fruits; non irradiated controls.
[2]LAB = laboratory strain reared at the Estación Experimental Agroindustrial Obispo Colombres.

TABLE 2. COEFFICIENTS OF SEXUAL ISOLATION (*ISI*), MALE (*MRPI*) AND FEMALE (*FRPI*) REPRODUCTIVE PERFORMANCE (CAYOL ET AL. 1999), AND RELATIVE STERILITY (*RSI*) (MCINNIS ET AL. 1996). *P*: SIGNIFICANCE WITH RESPECT TO RANDOM EXPECTED VALUES.

Dose	*ISI*	*P*[1]	*MRPI*	*P*	*FRPI*	*P*	*RSI*
0	-0.029	0.238	0.046	0.477	0.121	0.061	0.543
40	0.008	0.909	-0.023	0.709	0.034	0.534	0.484
70	0.067	0.585	-0.052	0.392	0	1	0.440
100	-0.004	0.751	0.004	0.949	-0.050	0.273	0.504

[1]*P* = probability of obtaining the observed results assuming random mating.

cantly higher than 0) than TUC females. However, a significant reduction in the *FRPI* was observed as irradiation dose was increased (r = -0.98; P = 0.014). The mating competitiveness of irradiated LAB males with TUC males for TUC female mates was measured by the *RSI*. The estimated *RSI* values approached 0.5, showing that at all radiation doses LAB males competed efficiently with TUC males for mating with TUC females (Table 2).

The analysis of induced sterility as a function of irradiation dose was based on the proportion of eggs hatching for the TUC strain. Egg hatch rate was estimated from all reciprocal crosses in all tests with the exception of the 70 Gy TUC-LAB test, in which the data collection was missed (Table 3). In each case 4 egg collections were obtained during a 12 d period. The results indicated that a treatment with 40 Gy reduces male fertility from about 80% to 0.75% and higher doses produce total sterility. In females, the 40 Gy dose reduces fertility to about 2% and higher doses prevent egg laying. No differences were observed among egg collection dates, indicating that fertility is not recovered after the irradiation.

DISCUSSION

The adaptation of insects to laboratory conditions, mass rearing, and sterilizing by irradiation is known to produce genetic and physiological effects in strains (Shelly et al. 1994; Lance et al. 2000; Alphey 2002; Benedict & Robinson 2003). These factors can influence the efficiency of mass reared and sterilized flies once they are released into the field in support of control programs integrating the sterile insect technique. Males of the Mexican fruit fly *Anastrepha ludens* (Loew) produced in bio-factories, for example, start their sexual activity well before wild ones. This may pose a problem in conventional strains involving the release of both sterile males and females, as these may mate among

TABLE 3. NUMBER OF EGGS SCORED, NUMBER OF EGGS HATCHED AND PERCENTAGE EGG HATCH IN ALL RECIPROCAL CROSSES AT VARIOUS RADIATION DOSES.

Treatment	Mating (male-female)	No. pairs	Hatched eggs	Total eggs	% hatch
0 Gy	LAB[1]-TUC[2]	57	351	437	80.32
	TUC-LAB	66	506	547	92.50
	TUC-TUC	48	336	422	79.62
	LAB-LAB	68	349	459	76.03
40 Gy	LAB-TUC	62	4	536	0.75
	TUC-LAB	68	1	46	2.17
	TUC-TUC	64	425	460	92.39
	LAB-LAB	66	0	0	0.00
70 Gy	LAB-TUC	59	0	464	0.00
	TUC-LAB	66	*	*	*
	TUC-TUC	75	291	336	86.61
	LAB-LAB	68	0	0	0.00
100 Gy	LAB-TUC	65	0	625	0.00
	TUC-TUC	64	316	398	79.40
	TUC-LAB	56	0	0	0.00
	LAB-LAB	56	0	0	0.00

*Missing data.
[1]TUC = wild flies from Tucumán obtained from guava fruits; non-irradiated controls.
[2]LAB = laboratory strain reared at the Estación Experimental Agroindustrial Obispo Colombres.

themselves before having the opportunity to mate with wild counterparts (Moreno et al. 1991; Hernández et al. 2003). Liedo et al. (2002) observed that laboratory-reared females of *C. capitata* have greater mating propensity than wild females, and their age of maximum mating activity is earlier. Furthermore, Cayol (2000) reported that the high densities of flies in breeding cages may affect courtship, and matings tend to be faster.

During the strain colonization process for SIT application, the insects are faced with artificial conditions very different from nature and may experience genetic changes due to genetic drift and particular selective forces. These factors sometimes affect the efficiency of the SIT (Cayol 2000). The irradiation treatment to induce sterility was claimed to affect courtship behavior (Lux et al. 2002). Thus, the strain of *A. fraterculus* that is reared under semi-mass rearing conditions at the Obispo Colombres facility was evaluated under conditions that imitate those in nature as a prerequisite to being used in control programs with an SIT component. Outdoor field cages are an acceptable compromise between natural conditions and a controlled laboratory experimental system for monitoring strains (Robinson et al. 2002; FAO/IAEA/USDA 2003).

The present results show that the behavior of this laboratory strain is not substantially modified with respect to the natural population for Horco Molle. Average copulation start time was not statistically different between LAB and TUC. The preferred position in the tree for mating was conserved. Copulation duration was similar in non-irradiated LAB and TUC, but irradiation treatment significantly reduced this time. A similar trend was observed in *C. capitata* (Cayol et al. 1999), but the importance of this effect on the efficiency of the SIT is not clear. This is because there is not a direct relationship between copulation duration and the ability of males to transfer sperm. However, matings that are too short might increase the probability of female remating (FAO/IAEA/USDA; 2003).

The estimated *ISI* (-0.03 to 0.07) and *RSI* (0.44 to 0.54) values suggest total compatibility between the laboratory strain and the natural population of *A. fraterculus* analyzed here. *MRPI* (-0.05 to 0.05) did not differ from the expected, indicating similar male mating competitiveness of LAB and TUC. An important result linked to the possibility of applying the SIT to control *A. fraterculus* is that compatibility and mating performance of male LAB flies are not affected by irradiation for all tested doses. This results contrasts with those of Cayol et al (1999), who observed that under similar conditions LAB flies of *C. capitata* had reduced competitiveness ($MRPI \cong 0.09$; $RSI \cong 0.33$) and compatibility $ISI \cong 0.31$).

According to our *FRPI* estimates, LAB females have higher mating propensity than TUC females. A similar result was observed in other tephritids (Cayol 2000; Liedo et al. 2002), which suggests that LAB females are sexually more active and less selective. However, this higher mating propensity of LAB females was reduced as the applied irradiation dose increased.

Some authors observed that irradiated females do not lay eggs depending on the radiation dose and the developmental stage at the time of the irradiation treatment (Burditt et al. 1975; Velasco & Enkerlin 1982; Calkins et al. 1988). According to the present analysis of egg laying and hatching, *A. fraterculus* females treated with 40 Gy oviposited a reduced number of eggs compared to control females. Higher doses prevented all egg laying. Moreover, the treatment with 70 Gy of gamma irradiation applied 48 h before adult emergence ensured 100% sterility both in males and females. Furthermore, during the evaluation period (12 d) there was no evidence of recovery of fertility in females or males.

Recent results by Vera et al. (2006) indicate that some *A. fraterculus* populations from different regions in South America might be sexually incompatible and reproductively isolated, while Petit-Marty et al. (2004) observed complete compatibility between TUC and several geographically isolated populations from within Argentina. Alberti et al. (2002) also concluded that TUC and other populations from Argentina and southern Brazil (Pelotas) are not differentiated genetically. Therefore, it is expected that the LAB population from Obispo Colombres facility will behave similarly when facing natural populations from Argentina and southern Brazil. The high compatibility of LAB and TUC flies and the good competitiveness of irradiated LAB males observed in the present work encourage the application of the SIT at least at a sub-regional level to control *A. fraterculus* populations from Argentina and southern Brazil.

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