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COMPARISON OF LONGEVITY BETWEEN A LABORATORY STRAIN AND A NATURAL POPULATION OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) UNDER FIELD CAGE CONDITIONS

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ABSTRACT

The South American fruit fly *Anastrepha fraterculus* (Wiedemann) is one of the most destructive fruit pests in this region, infesting major fruit crops. Implementation of the sterile insect technique (SIT) as part of an area-wide integrated approach against this species requires information on the survival of mass-reared and sterilized insects in the field and their ability to mate with wild females. The survival rates in field cages of both non-irradiated and irradiated laboratory flies were compared with that of wild flies. Both types of laboratory flies survived longer than their wild counterparts over the 8 days under the experimental conditions. The irradiation dose (70 Gy) did not affect survival of the laboratory reared flies. Our results improve the prospect of integrating the SIT into the control of *A. fraterculus* populations in Argentina.

Key Words: *Anastrepha fraterculus*, SIT, genetic control, longevity, survival

RESUMEN

Anastrepha fraterculus (Wiedemann), la mosca sudamericana de la fruta, es una de las plagas más destructivas en la región que infesta a los principales cultivos de frutas. La implementación de la Técnica del Insecto Estéril (TIE) como parte de un manejo integrado en áreas extensivas contra esta especie requiere ensayos que demuestren que los insectos producidos en forma masiva y esterilizados son capaces de sobrevivir en el campo y aparearse con las hembras silvestres. Se comparó la supervivencia de individuos de una línea de laboratorio, tanto irradiados como no irradiados con la de individuos de una población natural. Los dos tratamientos de moscas de laboratorio sobrevivieron más tiempo que las salvajes durante los 8 días y en las condiciones ensayadas. La dosis de radiación (70 Gy.) no afectó la supervivencia de las moscas criadas en laboratorio. Nuestros resultados mejoran las perspectivas de integrar la TIE en el control de las poblaciones argentinas de *A. fraterculus*.

Translation provided by the authors.

Anastrepha (Diptera: Tephritidae) is a genus of American origin, present in the subtropical and tropical regions (Hernández-Ortíz 1992). It includes nearly 180 species, several of which are regarded as pests of economic importance (Norrbom & Kim 1988; Steck 1991; Hernández-Ortíz 1992; Aluja 1994). A widely distributed species of this genus is *Anastrepha fraterculus* (Wiedemann) the South American fruit fly. It is one of the most destructive fruit pests in the neotropical region, infesting about 80 host plants including major fruit crops such as peach, plum, and mango (Norrbom & Kim 1988). It causes quarantine restriction for fruit trade and commercial losses in several countries (Steck 1998). In Argentina, it is abundant in the northwest and the northeast (Vergani 1956), which apparently represent the southern limits of the two unconnected distributions reported for their continental distribution (Salles 1995; Steck 1998). These regions are characterized by hot and wet subtropical climate and are separated by an extremely arid region (Cabrera & Willink 1980). Gene flow between populations of the northwest and northeast seems restricted only to what is allowed by fruit trade.

Traditional control methods for this pest species are based on the extensive use of chemical insecticides, but because they are associated with environmental harmful effects, the use of biological and genetic methods is now being encouraged. One of them is the sterile insect technique (SIT), based on the release of mass-reared and sterilized insects in order to reduce pest populations (Knipling 1959, 1968). It requires that the number of released sterile males should be higher than the number of wild males in the target pop-

ulation, increasing the probability for a wild female to copulate with a sterile male. This method has been very successful in eradication of the New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) from southern USA to Central America (Wyss 2000) and North Africa (Lindquist et al. 1992), the control of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in many Latin America regions (Hendrichs et al. 1995), and the suppression of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in Canada (IAEA 2001). The main advantages of SIT are that it is environmentally friendly, species-specific, compatible with other control methods, and its effectiveness increases as the size of the target population declines (Benedict & Robinson 2003).

In Argentina, the SIT has been successfully implemented against the most common tephritid pest, *C. capitata* (Aruani et al. 1996; De Longo et al. 2000). The use of this technique to control *A. fraterculus* requires political support and grower organization to allow an area-wide approach, and a more detailed knowledge of its biology. One requirement for SIT to be successful is that released insects must survive in the field and mate with wild insects. Mating success is probably related to interactions with other males and to female choice (Partridge & Halliday 1984). Also, at least in related tephritids, such as *C. capitata*, morphometric traits appear relevant in mate choice (Norry et al. 1999; Kotiaho et al. 2001; Rodriguero et al. 2002a, b).

Previous studies by our group verified that *A. fraterculus* populations of different regions in Argentina are fully compatible among themselves (Petit-Marty et al. 2004). Nevertheless, as the mass-rearing process and sterilization may cause a loss of fitness (Shelly et al. 1994; Lance et al. 2000; Alphey 2002; Benedict & Robinson 2003), survival of laboratory reared sterile insects should be evaluated under field conditions in order to predict their performance in control programs that integrate the SIT. Field cages with host trees provide a suitable model to simulate some aspects of field conditions.

The main objective of this study was to compare the survival of a laboratory-reared strain of *A. fraterculus* (irradiated and non-irradiated) with that of a wild population in order to develop a non-expensive, easy to follow method that can be applied as a routine quality control test for sterilized, mass-reared insects in SIT based control programs.

Materials and Methods

Insects and Methods of Handling

Two populations were tested. The first one was collected from the wild and the second was a laboratory strain, reared since 1997 under semi mass-rearing conditions (Jaldo et al. 2001) at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán Province, in the northwest of Argentina. Cages were held in a rearing room with 25 ± 1°C, 80 ± 10% R.H. and a photoperiod of 12:12 (L:D) (Vera et al. 2007). Wild flies were collected at Horco Molle, (26°48'S, 65°20'W), in the same province, from wild guava fruits, *Psidium guajava* L. (Myrtaceae).

Infested guava fruits were put on a sand layer to allow pupation. Emerging pupae were sieved and transferred to glass flasks. Both wild and laboratory reared pupae were sent to the Instituto Nacional de Tecnología Agropecuaria (Castelar, Buenos Aires, 58°40'W, 34°40'S), where they were kept in glass flasks (3 liters) and maintained under controlled conditions (23 ± 2°C, 70 ± 5% R.H. and a photoperiod of 12:12 (L:D) until adult emergence. Previously, half of the laboratory reared pupae were irradiated at the Centro Atómico Ezeiza (Comisión Nacional de Energía Atómica, Argentina) with a sterilizing median dose of 70 Gy in a Gammacell 220 ^{60}Co irradiator. Irradiation was performed 2 d before adult emergence, at room temperature, air atmosphere and 1 atmosphere of pressure with a dose rate between 1.0904 and 1.0785 Gy/min.

Sex Separation

Every day, emerging adults from all treatments were removed from the flasks and transferred to new 3-L glass containers. The following day they were sorted by sex and supplied with water and adult food composed of brown sugar and hydrolyzed corn protein. This diet promoted normal sexual development in laboratory strains (Manso 1998). Adults were kept under laboratory conditions (20-27°C, 60 ± 20% R.H. and a photoperiod of 12:12 (L:D) until the moment of release into the field cages.

Fly Marking

Two d after emergence flies were marked to identify their origin. They were placed in a netting bag (1 mm mesh), carefully immobilized and labeled with a dot of water-based paint (Témpera Alba, Alba, Inc., Argentina) on the mesonotum. Five colors (green, red, white, blue, and yellow) were interchanged sequentially each labeling day. After painting, groups of 25 flies were placed in 1-L containers, covered with a mesh, and provided with food and water and held under laboratory conditions. They were released into the field cages 2 d after marking.

Field Test

The experiments took place at Instituto Nacional de Tecnología Agropecuaria, between 25 Mar

and 8 Apr 2004. Eight outdoor nylon screened cages (2.5 m high × 3 m diameter) were placed over rooted young (about 1.5 m high) tangerine trees (one per cage). Two flasks (50 cm^3) filled with water were placed in each cage. The flasks had a perforated lid with a gauze wick (partially outside the flask). As a result, flies could take water from the gauze without polluting the water inside the flask. Two pieces of dry peach suspended from a wire fixed to the roof of the cage were used as food source; this was shown to be a suitable food source in preliminary tests (data not shown).

The first d (Day 0), at noon, 18 males and 25 females of wild origin were released into each of the 8 cages. Additionally, the same number of male and female irradiated laboratory flies were release into 4 cages (#1, #2, #3, and #4) and non-irradiated laboratory flies into the remaining 4 (#1', #2', #3', and #4'). All the cages had the same number of flies, half from wild and half from laboratory origin. Two d later (Day 2) all surviving flies were recovered from 2 cages (#1 and #1'). In each case the number of flies of each strain (wild or laboratory) was recorded. Both cages were refilled with flies of the same age as that of Day 0. The same procedure was followed on Day 4 with cages #2 and #2', on Day 6 with cages #3 and #3', and on Day 8 with cages #4 and #4'. All flies recovered by aspiration from Day 2 to Day 8 had been released on Day 0 and represent, respectively, the survivors after 2, 4, 6, and 8 d. On d 10 all survivors were aspirated from all cages. At this moment the flies in cages #1, #2, #3, and #4 had survived respectively 8, 6, 4, and 2 d (they correspond to the second release into each cage).

With this protocol we obtained for each set of laboratory flies (non-irradiated and irradiated) 2 replicates of relative survival at 2, 4, 6, and 8 d with respect to wild flies. An additional release was performed on d 11 in two cages, one with non-irradiated laboratory flies and the other with irradiated laboratory flies, in both cases co-released with the wild flies (in proportion 1:1). On Day 13 both cages were emptied, providing a third replicate of the survival after 2 d. These extra replicates allowed us to compensate for the fact that (due to lack of material), one of the earlier replicates for 2 d had wild insects 3-4 d older than laboratory insects.

In 1 replicate (corresponding to survivors at 4 d) 22 (instead of 25) females from the non-irradiated laboratory strain and 16 (instead of 18) wild males were released. These numbers were taken into account to estimate expected values when performing the statistical analysis. It was not possible to have more replicates because of the increasingly cold weather. Meteorological data (temperature, relative humidity, wind speed, and sunshine) were recorded every h at the meteorological station of the Instituto de Clima y Agua, located 2 km away from the experimental site.

Data Analysis

Homogeneity among replicates was analyzed by means of homogeneity χ^2 tests. The statistical significance of any departure from equal performance of populations was tested by means of a χ^2 test of goodness of fit comparing the number of wild and laboratory reared flies recovered alive with the expected 1:1 ratio (i.e., the null hypothesis is that the probability of survival is the same for both groups.) An alternative method of analyzing relative survival through time was based on evaluating the regression of the proportion of laboratory flies (number of laboratory flies/total number of flies) recovered on the number of days spent into the field cages.

In order to compare performance between populations, a new variable was defined as the proportion between laboratory-reared and total recovered flies. Arithmetic means over replicates were calculated for each period (2, 4, 6 and 8 d) and used to perform the regression analysis. The average proportion of laboratory flies recovered at each age (2, 4, 6, and 8 d) was compared between the cages with irradiated and non-irradiated laboratory flies by means of a Wilcoxon pair wise test. All statistical analyses were performed with Statistica (5.1) for Windows (Stat Soft, Inc. 2000).

Results

Temperature and humidity during the field tests (Table 1) varied within ranges that are considered favorable for *A. fraterculus*. Sunshine ranged between 0.3 and 10 h of direct light (effective heliophany, 3 to 88% of relative heliophany). The weather was benign, except for a single rainy day with strong winds.

Wild flies were compared with both irradiated and non-irradiated laboratory flies, and the total number of wild flies released in all was twice each of the 2 other groups. Total numbers of male and female flies recovered alive at each time period are presented in Tables 2 and 3, respectively. Results for males (Table 2) indicate that the numbers of flies recovered for both laboratory classes (irradiated and non-irradiated) were similar to wild flies for the shorter periods, but significantly higher for the longest period of 8 d. Results for females (Table 3) are similar for non-irradiated females, however wild females caged with irradiated females had an unusually high survival rate at 8 d (14% vs. 5.6-6.0% in equivalent cells for wild males and females of Tables 2 and 3), meaning that in this case no significant differences were found between wild females and irradiated laboratory females.

The regression analyses showed the same trend in all cases (Fig. 1). The association between the ratio of laboratory:wild in the recovered flies and the number of days in the cage was

TABLE 1. WEATHER CONDITIONS DURING THE TESTING PERIOD. WIND SPEED WAS MEASURED 2 M ABOVE SOIL SURFACE. (ALL DATA CAME FROM THE INSTITUTO DE CLIMA Y AGUA, INTA-CASTELAR, METEOROLOGICAL STATION.)

Day	Max temp (°C)	Mean temp (°C)	Min temp (°C)	Wind speed (km/h)	Precipitation (mm)	Humidity (%)
25 Mar	33.0	25.2	17.4	4.0	0.0	63
26 Mar	32.6	24.5	16.4	4.1	0.0	61
27 Mar	31.0	23.0	15.0	4.3	0.0	59
28 Mar	30.6	23.8	17.0	7.3	0.0	65
29 Mar	32.0	26.0	20.0	6.0	0.0	56
30 Mar	30.6	26.0	21.5	11.9	0.0	69
31 Mar	27.4	24.2	21.0	6.2	0.5	79
01 Apr	28.2	22.5	16.8	5.1	0.0	67
02 Apr	32.0	25.8	19.6	3.3	0.0	76
03 Apr	34.0	28.0	22.0	3.7	0.0	68
04 Apr	34.8	28.8	22.8	10.9	0.0	59
05 Apr	30.7	25.9	21.0	9.4	26.3	70
06 Apr	25.8	22.3	18.8	3.4	0.0	89
07 Apr	28.2	22.6	17.0	2.6	0.0	74
08 Apr	28.0	22.0	16.0	9.2	5.0	87

positive and highly significant ($P < 0.01$), indicating that on average laboratory flies survive longer under protected field cage conditions than wild flies. Wilcoxon matched pairs tests indicated that the differences were not significant ($P = 0.29$ and $P = 0.72$ for male and female regressions, respectively). In order to avoid any bias due to the different age in 1 of the replicates (2 d), the complete analysis was repeated after removing this case (i.e., 2 replicates for 2 d in cage, as well as for the other periods). Significance levels remained unchanged.

DISCUSSION

Even though weather conditions affect absolute survival, the present analysis was based on relative viability of laboratory flies with respect to wild flies. As the weather was similar most of the days we consider that it was not a factor that could have modified the results in different cages.

Field cages, as a semi-natural environment, represent a compromise between the laboratory and the open field. Within closed field cages flies are much more protected from abiotic and biotic factors such as predators than under open field conditions (Hendrichs et al. 1993; Hernández et al. 2007). Nevertheless, the uncontrolled conditions in an outdoor field cage test significantly reduced survival of flies with respect to laboratory tests. Only 6-35% of flies could be recovered after 8 d inside the cages, whereas flies survived longer periods of time under laboratory conditions (data not shown). This result suggests that closed field cages still represent a challenging environment, where weather variation could impose extra mortality and where it is not possible to avoid completely the presence of predators such as spiders.

TABLE 2. *ANASTREPHA FRATERCULUS* MALES RECOVERED FOR EACH PERIOD (DAYS IN THE FIELD CAGE) AND CHI SQUARE TESTS FOR GOODNESS OF FIT TO COMPARE RELATIVE SURVIVAL OF LABORATORY FLIES WITH RESPECT TO WILD FLIES. DATA ARE PRESENTED AS AN ABSOLUTE NUMBER (n) OF INSECTS RECOVERED IN ALL THE REPLICATES FOR THE SAME PERIOD AND PERCENT SURVIVAL (%) (100 × RECOVERED/RELEASED).[1]

Period (days)	W		Lab				W*		Irr			
	n	(%)	n	(%)	χ^2	P	n	(%)	n	(%)	χ^2	P
2	27	50.00	31	57.41	0.28	0.60	31	57.41	44	81.48	2.25	0.13
4	15	41.67	12	33.33	0.33	0.56	9	26.47	9	25.00	0.01	0.90
6	6	16.67	14	38.89	3.20	0.07	2	5.56	5	13.89	1.29	0.26
8	2	5.56	9	25.00	4.45	0.03	2	5.56	10	27.78	5.33	0.02
Total	50	30.86	66	40.74			44	27.5	68	41.98		

[1]W stands for wild flies released into the same cages that the laboratory reared stain (Lab), and W* for wild flies released together with laboratory reared and gamma irradiated flies (Irr).

TABLE 3. *ANASTREPHA FRATERCULUS* FEMALES RECOVERED FOR EACH PERIOD (DAYS IN THE FIELD CAGE) AND CHI SQUARE TESTS FOR GOODNESS OF FIT TO COMPARE RELATIVE SURVIVAL OF LABORATORY FLIES WITH RESPECT TO WILD FLIES. DATA ARE PRESENTED AS AN ABSOLUTE NUMBER (n) OF INSECTS RECOVERED IN ALL THE REPLICATES FOR THE SAME PERIOD AND PERCENT SURVIVAL (%) (100 × RECOVERED/RELEASED).[1]

Period (days)	W		Lab				W*		Irr			
	n	(%)	n	(%)	χ^2	P	n	(%)	n	(%)	χ^2	P
2	49	65.33	45	60.00	0.17	0.68	54	72.00	48	64.00	0.35	0.55
4	16	32.00	13	27.66	0.27	0.60	9	18.00	19	38.00	3.57	0.06
6	9	18.00	18	36.00	3.00	0.08	10	20.00	18	36.00	2.29	0.13
8	3	6.00	17	34.00	9.80	<0.01	7	14.00	9	18.00	0.25	0.62
Total	77	34.22	93	41.89			80	35.56	94	41.78		

[1]W stands for wild flies released into the same cages that the laboratory reared stain (Lab), and W* for wild flies released together with laboratory reared and gamma irradiated flies (Irr).

In addition to that, food and water were supplied in a way that flies had to actively forage for them.

The ability of a laboratory reared and sterilized fly to survive to sexual maturity under open field conditions is very important for the application of the SIT. Usually, in fruit fly suppression or and eradication programs, releases of sterilized flies occur once or twice a week (Dyck et al. 2005). It would be desirable that a significant proportion of the released flies survive this period. Thus, if flies are released in an immature state, as in our experiments, and as is common in most opera-

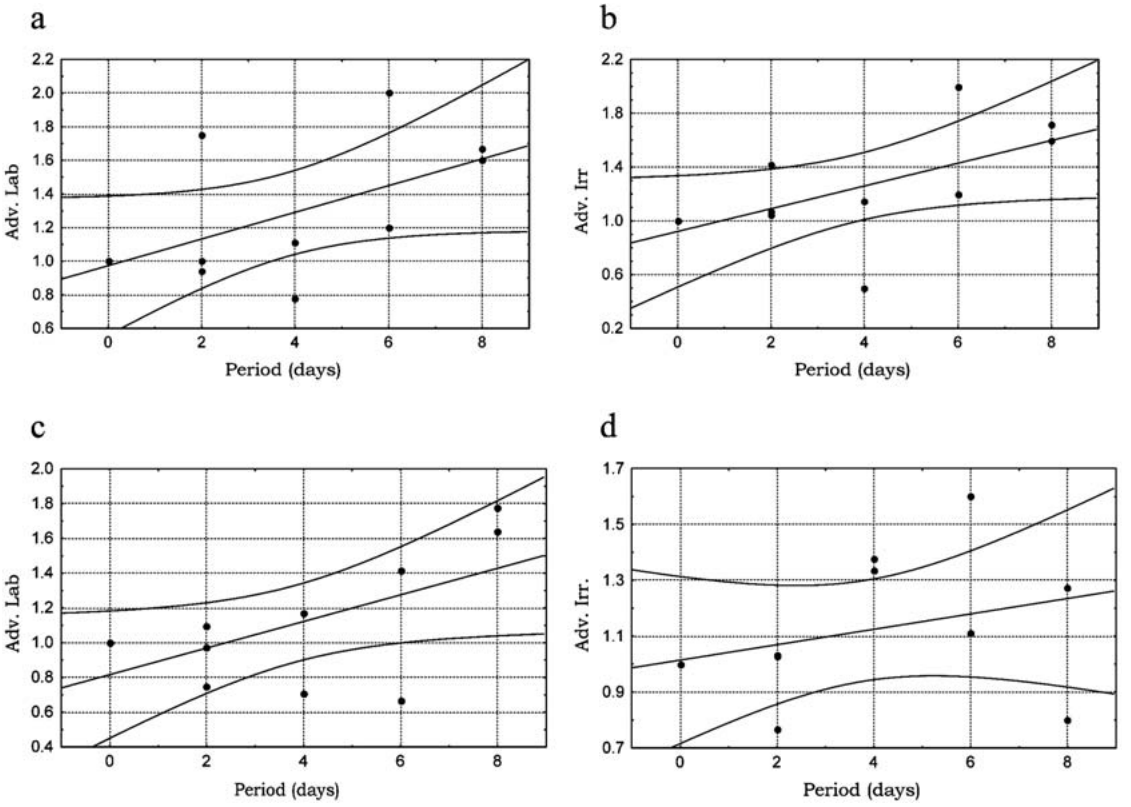

Fig. 1. Regression (95% confidence) of relative advantage of laboratory non-irradiated or irradiated flies with respect to wild flies on the number of days survived after release into field cages. Relative advantage was calculated as the proportion of non-irradiated or irradiated laboratory flies in the total number of recovered flies at each period: (a) non-irradiated males (r = 0.569, P = 0.0004); (b) irradiated males (r = 0.593, P = 0.0006); (c) non-irradiated females (r = 0.602, P = 0.0006); and (d): irradiated females (r = 0.316, P = 0.0000).

tional programs, only a proportion of males reaches sexual maturity, because on average they require at least one week to achieve maturation (Segura et al. 2005). Results of the current test suggest that roughly only 25% of sterilized flies are able to survive 8 d in field cages.

The relation between the number of released flies and the survivors after a week should be considered when estimating the number of flies to be released and the frequency of releases. Most flies survived the first 2 d in field cages. Therefore, if mature sterilized males were released, significantly more males could participate in sexual activities, and a considerable proportion of males would have the ability to continue mating during the following days. This assumption is strongly supported by the fact that survival of the laboratory-reared flies was higher than that of wild flies even after irradiation.

Mass rearing can affect fly fitness (Cayol 2000). Additionally, radiation can damage some physiological processes, leading to reduction of survival (Spates & Hightower 1970; Crystal & Whitten 1976) and/or mating competitiveness (Calcagno 2001; Allinghi et al. 2002; Calcagno et al. 2002; Lux et al. 2002). It is not unreasonable to suppose that the advantage in survival that laboratory-reared flies showed in our experiments is due to good rearing conditions in the laboratory facility. It is impossible to predict exactly how this fitness would change if a major *A. fraterculus* mass rearing system was used, but our results based on a small-scale rearing suggest that manipulation and irradiation did not reduce survival, which would be good for the application of the SIT to control this insect. Nevertheless, there are a number of factors that could modify these results as laboratory and wild flies have undergone entirely different selection pressures. Characteristics that are relevant for flies to survive and mate in the laboratory may not be the same as those needed to survive and mate in field (Mayer et al. 1998).

Obviously, all these considerations are to be added to the effect of handling and transportation of the flies to be released in field that could cause damage and possibly stress the insects. It is possible that released flies could have reduced dispersal compared to wild flies (Mayer et al. 1998). As in every laboratory strain, lack of diversity could affect the ability to survive, reach sexual maturity, find a potential mate, and copulate in the field. Implementation of SIT requires continuous surveillance to evaluate survival of sterile flies, probably through the implementation of regular field cage and open field tests.

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