EFFECTS OF GAMMA RADIATION ON THE STERILITY AND BEHAVIORAL QUALITY OF THE CARIBBEAN FRUIT FLY, *Anastrepha suspensa* (LOEW) (DIPTERA:TEPHRITIDAE).[1]

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SUMMARY: Pupae of *Anastrepha suspensa* (Loew) were irradiated 2 days before adult eclosion in an air atmosphere with 15, 20, 25, 30, 50 and 70 Gy of gamma radiation (Co-60). The radiation effects on sterility and other parameters of quality and behavior of males and females of caribfly were established. Males became fully sterile with a dose of 50 Gy and females laid no eggs when exposed to 25 Gy. Radiation had no significant effect on adult eclosion, sex ratio, flight ability and irritability, but female mortality was affected significantly by radiation, showing higher survival rates in low dosage treatments. The mating behavior of the males was reduced significantly by increasing the radiation doses.
Key Words: *Anastrepha suspensa*, gamma radiation, sterility, behavior

EFEITOS DA RADIAÇÃO GAMA NA ESTERILIZAÇÃO E COMPORTAMENTO DA MOSCA-DO-CARIBE, *Anastrepha suspensa* (LOEW) (DIPTERA:TEPHRITIDAE)

RESUMO: Pupas de *Anastrepha suspensa* (Loew) foram irradiadas dois dias antes da emergência dos adultos em atmosfera de ar com as doses de 15, 20, 25, 30, 50 e 70 Gy de radiação gama (Co-60). Foram avaliados os efeitos da radiação sobre a esterilidade e outros parâmetros de qualidade e comportamento de machos e fêmeas de mosca-do-caribe. Machos tornaram-se totalmente estéreis com uma dose de 50 Gy e as fêmeas não ovipositaram quando expostas a 25 Gy. A radiação não teve efeito significativo sobre a taxa de emergência de adultos, na razão sexual, na habilidade de vôo e na irritabilidade desses insetos. Somente a mortalidade das fêmeas foi afetada significativamente pela radiação, causando uma maior sobrevivência nas dosagens mais baixas. A atividade de acasalamento dos machos foi reduzida significativamente com o incremento da dosagem de radiação.
Descritores: *Anastrepha suspensa*, radiação gama, esterilização, comportamento.

INTRODUCTION

The Caribbean fruit fly, *Anastrepha suspensa* (Loew) (caribfly) (Diptera:Tephritidae), is a highly polyphagous pest in Florida, attacking more than 80 species of fruits in 23 families (SWANSON & BARANOWSKI, 1972). This fly was initially considered an urban pest rather than a commercial pest when it appeared in 1965 (WEEMS, 1966). It attacks and damages most varieties of dooryard fruits in South Florida. Later, in 1974, after an embargo by Japanese officials of a grapefruit shipment with caribfly larvae, *A. suspensa* became an important and economic quarantine pest (HORNYAK, 1976). This also caused a quarantine against fresh Florida citrus bound for Arizona, California, Texas and Hawaii (CLARK & WEEMS, 1989). Export problems arose again after the U.S. Environmental Protection Agency banned the use of ethylene dibromide (EDB) for fumigation of fresh fruit, particularly grapefruit (ETHYLENE, 1983).

With the demise of EDB, the citrus industry and several government agencies proposed

[1] Presented as a poster at the FAO/IAEA International Symposium on Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. Vienna, Austria, 19-23 October 1992.

and have established citrus growing areas that are considered free of the caribfly. One of the possible methods to be used to eliminate fly populations and to prevent re-infestation into these established areas is the sterile male release approach.

A sterile male release program, in which gamma irradiation was used to produce sterilized adults, was used to suppress caribflies in Key West, Florida, in 1970. The flies were 1 to 4 days old at the time of irradiation and the doses used ranged from 50 to 75 Gy. Irradiation studies showed the sterilizing dose for 10-day-old pupae was 40 Gy and for 12-day-old pupae was 80 Gy. The test was continued for a two-year period and ended June 1972. During the final months of the experiment, the fly population reached the lowest level. However, the population recovered rapidly and returned to normal by August 1972 because of migration of native flies from adjacent islands (BURDITT et al., 1975).

Gamma radiation causes sterility in *A. suspensa* but it is important to know what other biological aspects are affected. Irradiation of pupae with 100 Gy of gamma radiation 2 days before eclosion decreases the distance flown, velocity and duration of flight, and affects negatively the mating behavior (SHARP, 1980; SHARP & WEBB, 1977).

Modifiers of radiosensitivity can be used to reduce undesirable effects and, the most frequent technique is to decrease the oxygen supply to the sample to be irradiated. Irradiation of caribfly pupae in nitrogen had fewer deleterious effects on the overall performance of the fly. Also, males from pupae irradiated with 50 Gy in nitrogen were 99.9% sterile (SHARP et al.,1975).

A new study indicated that irradiation of pupae at one or two days prior to eclosion resulted in sterility at lower doses than expected. A dose of only 30 Gy resulted in completely sterile flies with minimal effects on quality or effectiveness (CALKINS et al., 1988).

The Florida State Department of Agriculture and Consumer Services, Division of Plant Industry (FDASC,DPI) operates a caribfly rearing facility in Gainesville, FL, with a production level of about 21 million flies per week (MASS, 1991).

To assure total sterilization of the flies, the mass produced mature pupae have been irradiated with 70 Gy (SMITTLE & HOLLER, personal comunication) for use in field releases in South Florida.

The objective of this study was to determine the levels of sterility of male and female caribflies irradiated as pupae 2 days before eclosion, with different doses of radiation and to examine other parameters of quality and behavior (fecundity, mortality, adult emergence rate, flight ability, mating propensity and F1 sterility).

MATERIALS AND METHODS

Insects used in this study were from the USDA/ARS colony. Larvae were reared on a corncob grit-based diet (GREANY et al., 1976). Mature larvae emerging from the diet were collected for a 3 hour period and kept in fine vermiculite for pupation.

The pupae were maintained in moist vermiculite for 11 days at 26°C and 80% RH. On the 12th day, they were separated from the vermiculite by sifting and were irradiated in a Cs-137 source (Radiation Machinery Gammator M, Parsippany, N.J), with a dose rate of 1515 R/min. Lots of ca. 1500 pupae were held in a 20 ml ventilated plastic container during the exposures of 15, 20, 25, 30, 50 and 70 Gy in air.

After irradiation, the pupae were placed in small cages with sugar-protein and water sources. Within one day of emergence the flies were sexed and kept in separate rooms for 10 days.

Flies in the control were subjected to all of the same handling procedures, except for irradiation.

The mating and oviposition cages were modified plastic cups, with food and water supplied. The top cup (500 ml) consisted of a screened cap and a 5 cm diam. hole in the bottom that was covered by a dome of waxed cloth to serve as an oviposition site.

This was affixed to another cup (250 ml) with ca. 150 ml of water at the bottom to assure high humidity to prevent egg desiccation and to provide water to the flies through a dental wick.

To assess the sterility of males and females separately, irradiated males were mated with normal females and normal males were mated with irradiated females.

Control cages consisted of normal males mated with normal females. All treatments were replicated four times. Ten pairs were placed in each cage and all cages were held under a 14 hour photophase at 26.5°C and 65-70 % RH. Eggs were collected daily for 10 days and then twice a week until the 31st day.

During egg collections, adult mortality was recorded. All eggs collected were 0 to 24 hours old. Eggs adhered to the inside surface of the wax dome were washed with a jet spray of 0.03 % sodium benzoate solution onto black filter paper in 90 mm Petri dishes. They were incubated under moist conditions at 26.5°C for 4 days. Hatchability was determined under magnification. Some of the hatched larvae were transferred to a cup containing larval diet for development. The resulting adults were then crossed with non-irradiated flies of the opposite sex and their F1 fertility was evaluated.

Data from each radiation dose were collected on the following parameters: adult eclosion rate, percent effective flight, irritability (startle test) and mating propensity.

To determine the percentage of emerged adults and the percentage of emerged adults capable of flight, pupae were placed in a Petri dish lid with a black filter paper at the bottom. A black plastic tube (14 cm high x 8.25 cm i.d.) with the inside surface coated with unscented talcum powder was inserted into the lid (CALKINS & ASHLEY, 1987). Each treatment consisted of 100 pupae per tube, replicated 4 times. The test was repeated 3 times. The startle test was conducted for males and females separately when the adults were 3 to 4 days old, using a startle device developed by Boller (CALKINS et al., 1979). The insects were chilled for 7 minutes and placed into the bottom container of the startle unit.

After an adaptation period of 30 minutes, the partitions of the tubes were removed to allow bright light to enter from above and for flies to fly upward in the direction of the overhead light. After 3 minutes, the partitions of the system were closed and the flies in each level of each container were counted and the startle activity index (SAI) was calculated.

For the mating propensity test, the insects were sexed within 24 hours of eclosion, and placed in separate rooms for 10 days. Five special plastic cages (BOLLER et al., 1981) were used. On the day of the test, 25 males of a specified radiation dose were transferred into each cage and, after 15 minutes, 25 non-irradiated females were introduced into each cage.

Copulating pairs were removed during 10 minute intervals for 1 hour. The test was repeated 3 times for flies of each irradiation dose and each time the positions of the cages on the table were randomized to avoid any position effects on the mating results. Insects irradiated at 15 Gy and 25 Gy were not tested because of space limitations.

Analyses of variance (ANOVA) were calculated for the test results and the means were separated using the Waller-Duncan k ratio procedure, ($P \leq 0.05$, $k=100$) (SAS, 1985).

RESULTS AND DISCUSSION

Irradiation of caribfly pupae at two days before eclosion resulted in 100 % sterility in males at a dose of 50 Gy. At lower dosages, the sterility was not total but was very high.

For males irradiated at 15, 20, 25 Gy, the sterility were 98.1%, 99.4% and 99.9%, respectively. At a dose of 30 Gy, the mean male sterility was 99.9% after 7190 eggs examined during 31 days oviposition period (TABLE 1). These results are very close to those of CALKINS et al.(1988). They found 100% sterile males with 30 Gy in 1620 eggs examined during the first 6 days of oviposition. Also, their probit analyses indicating males 95% sterile at 13 Gy and 99% sterile at 16 Gy are close to our observed results. BURDITT et al.(1975) found that irradiation of 12-day-old pupae in air at 40, 60 and 80 Gy resulted in 99.8%, 99.97% and 100% male sterility, respectively. SHARP et al.(1975) reported that irradiation of pupae 2 days before adult eclosion in a nitrogen atmosphere with 20, 30, 40 and 50 Gy, produced males of 93.6%, 95.5%, 99.8% and 99.9% sterility, respectively.

Females were more sensitive to ionizing radiation than males. Females become fully sterile with a dose of 25 Gy. Hatchability was 47.3% at 15 Gy and dropped to 16.6% at 20 Gy. However, greater radiation effects were observed on fecundity which was drastically reduced with increasing radiation dose. Females exposed as mature pupae to 25 Gy or higher produced no eggs (TABLE 2). This is a normal radiobiological reaction because radiation interferes with cell division in the reproductive system of the females during its initial stage of development, causing complete atrophy of the ovaries (WALDER & CALKINS, 1992). Other authors also observed the lack of egg production in irradiated females, but radiation dosage was not the same for all of them, probably due to the variation of the dose rate used and/or different ages (stages) of pupae for irradiation (BURDITT et al., 1975; CALKINS et al., 1988; SHARP et al., 1975).

TABLE 1 - Effects of gamma radiation on sterility of Caribbean fruit fly males, *A. suspensa* (Loew), mated with non-irradiated females.

		Radiation doses - Gray (Gy)						
Days		Control	15	20	25	30	50	70
1	nº eggs	431	463	609	395	646	571	600
	% hatch	92.9	2.6	0.4	0.4	0.0	0.0	0.0
2	nº eggs	586	628	637	524	687	569	688
	% hatch	95.3	2.1	0.9	0.0	0.0	0.0	0.0
3	nº eggs	657	480	730	566	596	718	594
	% hatch	95.0	2.8	0.7	0.0	0.3	0.0	0.0
4	nº eggs	701	710	723	646	658	727	825
	% hatch	97.0	2.3	0.8	0.1	0.0	0.0	0.0
5	nº eggs	543	625	625	590	658	597	744
	% hatch	94.9	1.4	0.6	0.1	0.0	0.0	0.0
6	nº eggs	492	567	612	523	566	660	611
	% hatch	95.8	1.8	0.3	0.3	0.0	0.0	0.0
7	nº eggs	449	529	579	465	504	556	599
	% hatch	93.9	1.4	0.7	0.0	0.0	0.0	0.0
8	nº eggs	393	499	439	396	428	407	498
	% hatch	91.1	1.8	0.2	0.3	0.0	0.0	0.0
9	nº eggs	426	449	439	396	428	407	498
	% hatch	93.9	1.8	0.3	0.3	0.5	0.0	0.0
10	nº eggs	402	390	521	388	451	446	528
	% hatch	87.4	2.0	1.2	0.0	0.4	0.0	0.0
14	nº eggs	365	272	382	316	330	431	476
	% hatch	83.4	3.0	0.0	0.0	0.0	0.0	0.0
17	nº eggs	342	381	402	310	352	424	476
	% hatch	85.1	1.3	0.8	0.0	0.0	0.0	0.0
21	nº eggs	275	318	351	253	292	357	429
	% hatch	83.5	1.3	0.8	0.0	0.0	0.0	0.0
24	nº eggs	247	257	174	248	235	302	362
	% hatch	89.8	2.2	0.0	0.0	0.3	0.0	0.0
28	nº eggs	170	254	258	202	237	287	346
	% hatch	87.1	2.2	0.0	0.0	0.3	0.0	0.0
31	nº eggs	128	186	158	109	121	200	256
	% hatch	84.7	1.5	0.0	0.0	0.0	0.0	0.0
TOTAL EGGS		6607	7005	7793	6278	7190	7721	8586
MEAN % HATCH		90.7	1.9	0.6	0.1	0.1	0.0	0.0

TABLE 2 - Effects of gamma radiation on sterility and fecundity of Caribbean fruit fly females, *A. suspensa* (Loew), mated with non-irradiated males.

		Radiation doses - Gray (Gy)						
Days		Control	15	20	25	30	50	70
1	nº eggs	457	60	5	0	0	0	0
	% hatch	94.3	39.8	20.0	0.0	0.0	0.0	0.0
2	nº eggs	486	52	0	0	0	0	0
	% hatch	93.0	55.4	0.0	0.0	0.0	0.0	0.0
3	nº eggs	623	45	0	0	0	0	0
	% hatch	96.4	52.3	0.0	0.0	0.0	0.0	0.0
4	nº eggs	623	12	0	0	0	0	0
	% hatch	94.7	48.5	0.0	0.0	0.0	0.0	0.0
5	nº eggs	469	8	0	0	0	0	0
	% hatch	95.0	33.3	0.0	0.0	0.0	0.0	0.0
6	nº eggs	427	5	0	0	0	0	0
	% hatch	92.2	40.0	0.0	0.0	0.0	0.0	0.0
7	nº eggs	463	6	0	0	0	0	0
	% hatch	96.6	12.5	0.0	0.0	0.0	0.0	0.0
8	nº eggs	375	6	0	0	0	0	0
	% hatch	87.4	16.7	0.0	0.0	0.0	0.0	0.0
9	nº eggs	395	6	0	0	0	0	0
	% hatch	86.9	44.4	0.0	0.0	0.0	0.0	0.0
10	nº eggs	376	2	0	0	0	0	0
	% hatch	85.4	0.0	0.0	0.0	0.0	0.0	0.0
14	nº eggs	345	2	0	0	0	0	0
	% hatch	83.8	50.0	0.0	0.0	0.0	0.0	0.0
17	nº eggs	307	1	0	0	0	0	0
	% hatch	87.5	0.0	0.0	0.0	0.0	0.0	0.0
21	nº eggs	258	2	1	0	0	0	0
	% hatch	89.6	0.0	0.0	0.0	0.0	0.0	0.0
24	nº eggs	191	1	0	0	0	0	0
	% hatch	87.6	0.0	0.0	0.0	0.0	0.0	0.0
28	nº eggs	123	0	0	0	0	0	0
	% hatch	84.6	0.0	0.0	0.0	0.0	0.0	0.0
31	nº eggs	121	0	0	0	0	0	0
	% hatch	90.6	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL EGGS		5978	207	6	0	0	0	8586
MEAN % HATCH		90.3	47.3	16.6	0.0	0.0	0.0	0.0

TABLE 3 - Progeny of Caribbean fruit fly parents, *A. suspensa* (Loew), irradiated at sub-sterilizing doses.

Parents	Larvae	Pupae	N° adults [1]		Eclosion
(♂ x ♀)	(N°)	(N°)	♂	♀	(%)
15 Gy x N	79	37	15 (A)	14 (B)	78.4
N x 15 Gy	13	6	1 (C)	2 (D)	50.0
20 Gy x N	20	5	2 (E)	2 (F)	80.0
25 Gy x N	9	4	2 (G)	0	50.0
30 Gy x N	5	3	1 (H)	1 (I)	66.7

[1]/ The letters in parenthesis are used for identification lines.

TABLE 4 - Fertility of F1 progeny mated with fertile (normal-N) Caribbean fruit flies, *A. suspensa* (Loew).

(LINE) x n pairs	Eggs	Egg hatch	% Fertility
(A) x N-females	916	634	69.2
(B) x N-males	545	429	78.7
(C) x N-females	322	221	68.6
(D) x N-males	201	189	04.0
(E) x N-females	575	489	85.0
(F) - d b m *	-	-	-
(G) x N-females	697	440	63.0
(H) - d b m *	-	-	-
(I) x N-male	11	0	0.0
Control	319	302	94.7

* d b m = died before mating

Some larvae, from sub-sterile irradiated males and females, completed development and pupae were obtained (TABLE 3). Emerged adults were crossed to non-irradiated adults to determine the F1 fertility. On the average, males from irradiated (15, 20 and 25 Gy) parents were less fertile than females, suggesting that some genetic damage occurred because of radiation.

The only live progeny from a male parent irradiated at 30 Gy was a fully sterile female that laid very few eggs (TABLE 4).

Only female mortality was affected by radiation (TABLE 5). Significant differences were observed after the 31 day observation period. Females treated with low radiation doses (15, 20 and 25 Gy) had higher or similar survival rates to the control whereas the higher radiation doses (30, 50 and 70 Gy) caused significantly more mortality. Similar results were reported for *A. suspensa* irradiated in nitrogen (SHARP et al., 1975).

TABLE 5 - Percentage of adult Caribbean fruit fly, *A. suspensa* (Loew), survival at the end of the sterility test (31 days).[1/]

TREATMENTS	IRR. ♂ x N-IRR. ♀		N-IRR. ♂ x IRR. ♀	
CONTROL	55.0 a	72.5 a	67.5 a	65.0 bc
15 Gy	72.5 a	80.0 a	82.5 a	62.5 bcd
20 Gy	65.0 a	67.5 a	60.0 a	75.0 ab
25 Gy	60.0 a	65.0 a	65.0 a	85.0 a
30 Gy	55.0 a	62.5 a	72.5 a	55.0 cd
50 Gy	50.0 a	62.5 a	62.5 a	45.0 d
70 Gy	52.5 a	80.0 a	62.5 a	52.5 cd

[1/] Insects were 10-days-old at the beginning of the test.
Means within a column followed by the same letter are not significantly different (Waller-Duncan means separation, $P < 0.05$, SAS Institute, 1985).

TABLE 6 - Effects of increasing doses of radiation on adult eclosion, sex ratio, flight ability, irritability and mating propensity of the Caribbean fruit fly, *A. suspensa* (Loew).[1/2/]

	Eclosion	SR	PEF	SAI		MMI
TREATMENTS	(%)			♂	♀	
Control	81.6 a	0.56 a	77.1 a	1.01 a	1.22 a	48.97 a
15 Gy	80.9 a	0.64 a	77.7 a	0.94 a	1.14 a	-
20 Gy	82.1 a	0.62 a	72.6 a	0.91 a	1.25 a	42.87 ab
25 Gy	82.6 a	0.58 a	77.3 a	0.98 a	0.69 a	-
30 Gy	81.9 a	0.61 a	72.3 a	0.79 a	1.13 a	37.23 bc
50 Gy	80.6 a	0.61 a	75.4 a	0.98 a	1.01 a	33.23 bc
70 Gy	79.8 a	0.63 a	70.5 a	0.80 a	0.68 a	31.67 c

[1/] SR - sex ratio; PEF - percent effective flight; SAI - startle activity index; MMI - mean mating index.
[2/] Means within a column followed by the same letter are not significantly different (Waller-Duncan means separation, $P < 0.05$, SAS Institute, 1985).

The percentage of eclosion and the sex ratio (SR) of caribfly adults were not affected by radiation at any dosage used. Apparently the dose of 70 Gy was more harmful and fewer flies flew out of the cylinders but, no significant differences were found among the averages. The irritability of the insects also was not significantly affected by the increasing radiation doses used, but they were found to be detrimental to mating behavior (TABLE 6).

CALKINS et al.(1988) reported similar results on the effects of radiation on adult eclosion, sex ratio and percent fliers, for the doses from 10 Gy to 70 Gy. In mating competitiveness tests, these authors also found deleterious effects of radiation on mating success. However, for lower doses tested (30 Gy), the males exhibited equal competitiveness with normal males. SHARP & WEBB (1977) and SHARP (1980) also reported deleterious effects of gamma radiation on some behavioral parameters of the caribfly, but they used a higher dose (100 Gy).

Technically, a sterile insect technique (SIT) program depends on good quality mass-reared insects for success. The assurance of sterility of the released insects is a critical factor for a SIT success.

The sterilizing process of a sterile insect release program must receive the same attention as rearing by program managers because good quality reared insects could become a problem in a control program if the radiation dose for sterilization is inappropriate. Usually the radiation dose recommended is determined under laboratory conditions where most of the insect development and environmental parameters are controlled and/or known and the treated sample is small. Sometimes, therefore, the ideal radiation dose does not work as expected in a massive sterilization program because some areas do not receive equal doses and some areas receive doses lower than expected in the irradiation chamber. The effectiveness of mass-reared, sterilized caribflies in an eradication program could be low or fail if the minimum radiation dose received by the pupae was not appropriate for complete female sterilization. Usually in a mass rearing facility, mature larvae are collected once a day and the mean pupation time is 4 days (LEPPLA, 1977). So, at the time of irradiation, despite the eye color record (RUHM & CALKINS, 1981), there are always some variations in physiological development of the pupae. The reproductive system in this insect phase is characterized by intensive cell proliferation, cell differentiation and cell migration. Short spans of time are critical to radiosensitivity.

Also, there are errors of dosimetry due to the geometrical variation of the irradiation chamber. Therefore, to compensate for these variations, the radiation dose should be a little higher than threshold to assure the desired sterility to all the insects.

The results of our study indicate that 50 Gy would be the appropriate dose of gamma radiation for a massive Caribbean fruit fly sterilization program, using homogeneous aged pupal samples and isodose radiation chambers.

ACKNOWLEDGEMENTS

This work was performed while Julio M. Walder was a Visiting Scientist at the USDA/ARS Insect Attractants, Behavior and Basic Biology Research Laboratory and was supported by the University of São Paulo (USP) and by a Post Doctoral Fellowship from the Comissão Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, Brazil. We thank Patrick Greany, Tim Holler and Burrell Smittle for reviews and constructive comments on the manuscript.

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Received December 10, 1992
Accepted March 22, 1993

Trabalho enviado para publicação em 10.12.92
Trabalho aceito para publicação em 22.03.93