2011 International Nuclear Atlantic Conference - INAC 2011
Belo Horizonte,MG, Brazil, October 24-28, 2011
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-04-5**

PREPARING THE WAY FOR COMING AREA WIDE INTEGRATED PEST MANAGEMENT PROJECTS AGAINST THE NEW WORLD SCREWWORM, *Cochliomyia hominivorax*, IN MERCOSUR

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ABSTRACT

The New World Screwworm (NWS), *Cochliomyia hominivorax*, was eradicated from the USA, Central America to Panama, but in most tropical regions of Latin America, the NWS is still a serious threat to livestock, provoking estimated annual losses of US$ 1.8 billion in Brazil. Between January and May 2009, a pilot-project was performed at the Brazil-Uruguay border. As the results were positive, novel regional Area-Wide Integrated Pest Management projects are being planned. To set a mass-rearing center based in South America is strategic when considering long-term programs. In partnership with CENA/USP and the Biofactory MOSCAMED Brazil, a project to produce sterile NWS started on 2009. The project aimed to maintain a colony of a regional NWS strain, to develop a mass-rearing system and a sterilization protocol by X rays, and to study the sterility induction in regional strains. A colony was successfully established. The adults were kept in cages and fed on a diet (honey and spray dried egg). The larvae were reared in a medium made of spray dried blood, spray dried egg, milk, water, formalin and Ecogel®. Egg hatch has been of 80±10%. From F_1 to F_{22}, the total amount of pupae produced was about 38 L (≈ 315,400 pupae). The mean adult emergence and sex ratio were 86.7±3% and 0.59±0.08 respectively. The mean pupal weight was 47.1±1.7 mg. The estimated X ray doses to induce 99% sterility in males and females were 43.7 Gy and 47.5 Gy, respectively. To produce 1.5 L of pupae, the current cost is about US$ 15.00.

1. INTRODUCTION

The discovery that X or gamma rays could induce sufficient lethal dominant mutations in insect reproductive systems to provoke sterilization [1] was realized by the scientists of the US Department of Agriculture in 1946, which had searched for a method to sterilize insects massively for a few years. Those scientists had theorized that if a large amount of the target insect could be reared, sterilized and released in the field, the sterile insects would mate with the wild insects, but the resulting offspring would not be viable. Consequently, a decrease in the reproductive rate of the wild population would occur along the releases and even the eradication of the target population could be reached [2]. The first demonstration of the feasibility of such technique, the Sterile Insect Technique (SIT), was performed against the New World Screwworm, *Cochliomyia hominivorax* (Coquerel, 1858) (Diptera: Calliphoridae) [3, 4, 5].

The *C. hominivorax* fly is an obligate parasite of warm blooded animals and provokes primary myiasis in pre-existent wounds. The females have a high reproductive rate and can oviposit up to 450 eggs at 3-4 days interval at the periphery of wounds or body orifices. Larvae hatch after 12-20 h and craw immediately to the wound and start feeding superficially of the wound fluids. Larvae of 2nd and 3rd instars go deeper to feed of muscular tissues. When mature, the larvae abandon the animal and pupate in the soil [6]. The entire life cycle can be completed in only 14 days under ideal conditions and may lead easily to epidemic breaks. As an example, 1.3 million cases of myiasis were reported in 1934 on Southeastern USA. [7]. Larvae from a unique oviposition may kill small animals and multiple infections may kill adult cattle [8].

In 1957, an Area-Wide Integrated Pest Management (AW-IPM) program against the *C.hominivorax* started in Florida. After the success of this program and with the union among breeders, researchers and the federal government, eradication programs against the *C.hominivorax* were launch in other states from Southwestern and Southeastern USA. The NWS was declared eradicated from the USA in 1982, from Mexico in 2001 and, finally, from Central America in 2004 [9]. During the campaigns in the USA and Mexico, up to 500 million sterile flies per week were reared at the biofactory in Tuxtla Gutierrez, Mexico. A buffer zone of 300,000 km^2 was set at the Darien Gap, Panama, through the release of 25-50 million of sterile males per week to avoid reinfestation coming from South America [10]. The direct annual revenues from the eradication campaigns for the cattle industry were estimated in US$ 896 million, US$ 328 million and US$ 87.8 million in the USA, Mexico and Central America, respectively [9].

The NWS fly still is a serious threat to animal and human health in Cuba, some Caribbean islands and most part of South America (except Chile). Since 1996, eradication efforts were made in Jamaica, but the program suffered several constraints and little progress was reached until 2005 [11].

In 2006, the *Comisión México-Americana para la Erradicación del Gusano Barrenador del Ganado* (COMEXA) presented a regional project to the Brazilian Ministry of Agriculture and a pilot-area was defined between the Brazil-Uruguay border, were the losses due to the NWS accounted US$ 150,000/year. The project was performed between January and May 2009, the sterile flies were imported from the biofactory in Tuxtla Gutierrez and released by the Uruguayan Air Force, with COMEXA as the agency in charge of the execution and the Banco Interamericano de Desenvolvimento (BID) as the investor. After 13 weeks of releases, the project revealed positive outcomes [12], and now new suppression or eradication programs against the NWS are being planned for the MERCOSUR countries.

Importing the sterile flies could be a constraint for these new programs due to issues related specially to the quality of the insects and costs [13]. As example, during the NWS eradication program at Libya in 1990, the shipment cost to send the pupae from Tuxtla Gutierrez to Tripoli was four times higher than the release costs [14]. Therefore, setting a rearing center of sterile flies in South America would be strategic for the new AW-IPM projects against the NWS in MERCOSUR.

2. MATERIAL AND METHODS

2.1. Insects and Environmental Conditions.

The experiments were performed at the Biofactory MOSCAMED Brazil, Juazeiro, Bahia, in controlled environment rooms (33±2ºC, 65% relative humidity, photoperiod of 12:12 (light: dark) h). The parental generation came from UNICAMP pupae (originally collected in Goias) in September 2009 and, since then, a colony has been reared for 20 generations at CENA/USP and, after August 2010, for 10 generations at the Biofactory MOSCAMED Brazil. The adults were kept in cages (77 x 154 x 74 cm) and fed on a diet (20% of honey and 80% of spray dried egg). The oviposition substrate was a mix of larval diet disposal and blood. The larvae were reared in a gelled medium made of spray dried blood, spray dried egg, milk, water, formalin and Ecogel® (to make 1 L: 60 g of spray dried blood, 40 g of spray dried egg, 40 g of milk powder, 30 g of Ecogel®, 1 mL of formaldehyde and 800 mL of water).

2.2. Irradiation Procedures.

The X rays used were generated by a self-contained low-energy irradiator, the RS-2400, which was acquired by the MOSCAMED Brazil facility in 2009 from the RadSource Technologies, Georgia, USA (Rad Source Inc., www.radsource.com). This unit operated at 150 keV and 45 mA, giving a dose rate of 10.8 Gy min^{-1} at the irradiation position. Pupae were positioned inside the canisters (178 mm diameter by 167 mm long), which were suspended by cradles that revolved around the horizontal X ray tube. For each exposure, dosimetry was performed following the Gafchromic® dosimetry system (Gafchromic HD-810 film; International Specialty Products, Wayne, NJ 07470, U.S.A.) [15].

2.3. Irradiation Effect on Fertility.

The *C. hominivorax* pupae 24 h before adult emergence were irradiated at 0 (control), 10, 25 and 60 Gy of X rays. After irradiation, the pupae were separated according to the treatments. To assess adult emergence and sex ratio, fifty irradiated pupae were separated for each replicate, with three replicates per dose, and placed in Petri dishes until emergence. After emergence, the number of males and females that emerged was assessed.

To evaluate male sterility, thirty irradiated males were placed with forty non-irradiated females in Styrofoam cages (10 x 30 x 30 cm) with mosquito netting curtains in the sides and kept together for a week. To assess the sterilization dose for females, forty irradiated females were exposed to thirty fertile males under the same conditions. The treatment with males and females, both irradiated, was also performed for each dose, exposing thirty males to forty females. Water and adult diet (rapadura) were offered *ad libitum*. All cages from the bioassays were distributed in a randomized design, with 4 replicates for each dose, and up to two egg collections per cage were made. Seven days old females laid eggs over an egging device, which consisted of a Petri dish fulfilled with a mixture of larval diet disposal and citrated bovine blood, covered partially with filter paper. Before being offered to the females, the egging devices were heated to 40 ºC in conventional oven and were exposed for 4 h inside the cages.

After collection, the egg masses were set on moist black filter paper following the methodology described by Berkebile & Skoda [16] and kept in BOD (33 ºC for 4 h and 25ºC for 48 h). Egg hatch was evaluated 72 h after egg mass collection.

2.4. Data Analysis.

For statistical analysis of sterility, the data corrected to the appropriate control value was Probit transformed and linear regression analysis against the log of the radiation dose performed [17]. The estimated D_{99} (dose that induces 99% sterility) for the X radiation was taken as the baseline for comparisons. For adult emergence and sex ratio, the same regression analysis was applied. The later analyses were performed by the statistical program SAS 9.1. [18].

3. RESULTS

The colony of *C. hominivorax* has been reared for 30 generations. Egg hatch had been of 80±10%. From F_1 to F_{22}, the total amount of pupae produced was about 38 L (≈ 315,400 pupae). The mean adult emergence and sex ratio were 86.7±3% and 0.59±0.08 respectively. The mean pupal weight was 47.1±1.7 mg.

In the sterilization tests, no dosimetry result differed significantly from the target dose. The target dose values were, therefore, used throughout. The linear regression lines of the Probit transform of sterility against the logarithm of dose for X radiation are presented at Figures 1 and 2. Estimated doses to give 99% sterility (D_{99}), with upper and lower confidence limits are listed in Table 1.

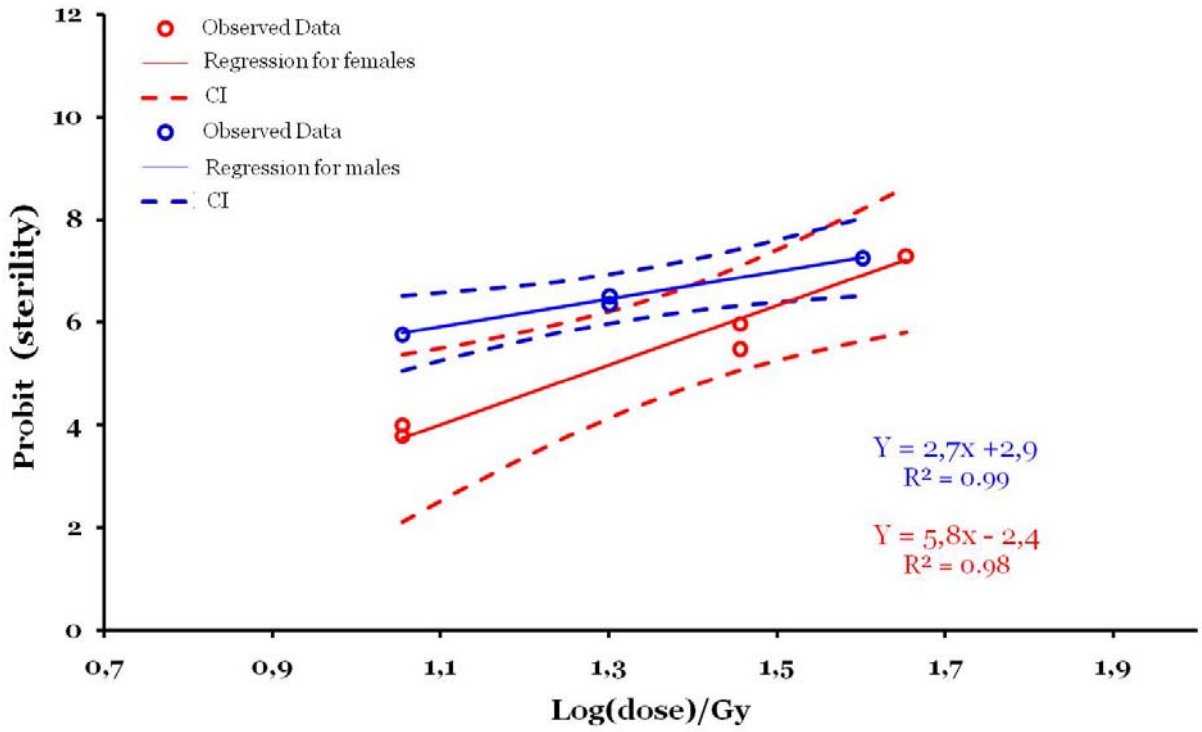

Figure 1. Linear regression of Probit transformed sterility on log dose for *C.hominivorax* males and females with the 95% confidence intervals.

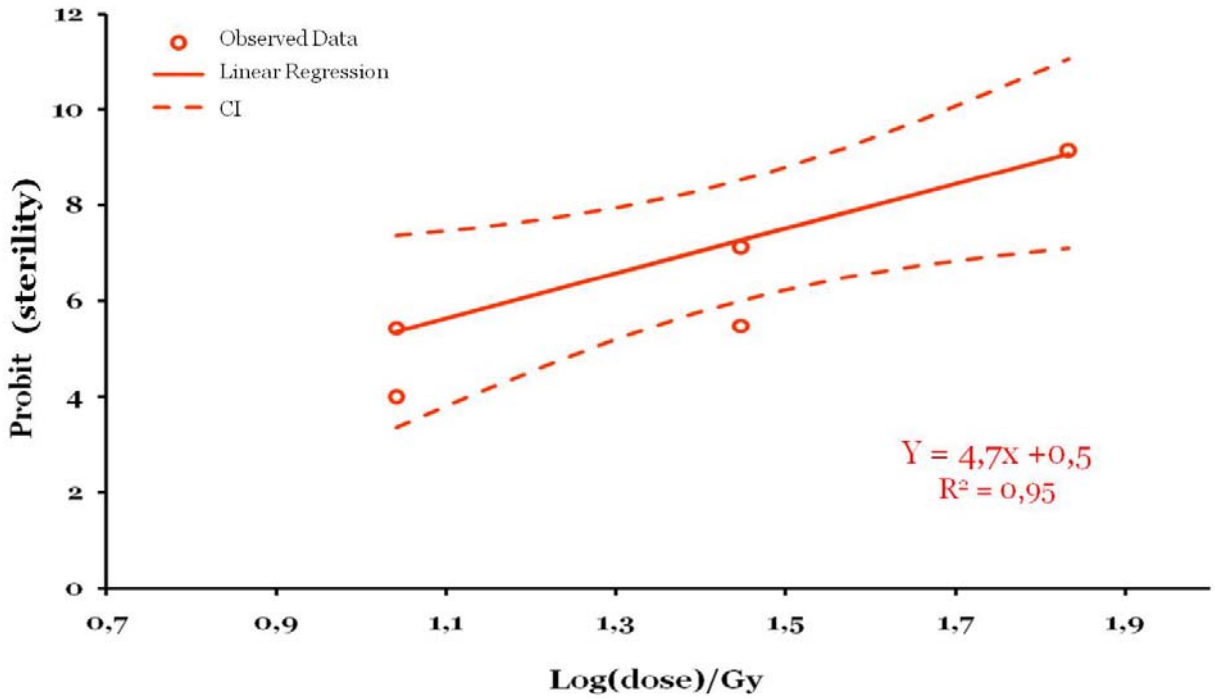

Figure 2. Linear regression of Probit transformed sterility on log dose for irradiated *C.hominivorax* males and females with the 95% confidence intervals.

Table 1. Radiation doses (Gy) calculated from the linear regression equations of Probit transformed sterility on log dose.

Treatments	Angular coefficient ± SE	*P*	Equation	D_{99} (Gy)
C. hominivorax irradiated males	2.7 ± 0.17	0.038	y = 2.9+2.7 log(x)[a]	43.7[b] (22.2; 85.5)[c]
C. hominivorax irradiated females	5.8 ± 0.31	0.033	y = -2.4+5.8 log(x)	47.5 (27.2; 83.2)
Irradiated male and female	4.7 ± 0.31	0.041	y = 0.5+4.7 log(x)	28.9 (16.9; 49.5)

[a] y = Probit and x = dose (Gy).
[b] D = Dose (Gy) that induces 99% sterility.
[c] Confidence Interval stated at 95% confidence level.

The *C. hominivorax* mean fertility in control groups was 91.9±1.6%. Other studies on methods for separating eggs from the masses and on larval diet revealed egg hatching in control groups ranging from 60% to 96% [16, 19].

For both males and females of *C. hominivorax*, the fertility decreased significantly with the increase of radiation doses. For irradiated males, 99% sterility was reached at 43.7 Gy of X rays. Regarding the irradiated females, the estimated D_{99} was a little higher, 47.5 Gy (Table1).

Adult emergence and sex ratio were not significantly affected by the X ray dose range applied ($P > 0.05$) (Table 2). The emergence and sex ratio values were all higher than 70% and 0.5, respectively.

Table 2. Linear regression for adult emergence and sex ratio of *C. hominivorax* pupae irradiated at different X ray doses.

Quality control parameter	Dose (Gy)				Significance test for the linear regression[b]
	0	10	25	60	
Adult emergence	80 ± 5.8[a]	76.7 ± 12.0	93.1 ± 6.6	93.3 ± 6.7	$F_{1,11} = 3.34^{ns}$ $P = 0.21$
Sex ratio[c]	0.6 ± 0.07	0.6 ± 0.03	0.7 ± 0.03	0.6 ± 0.1	$F_{1,11} = 0.12^{ns}$ $P = 0.76$

[a] Mean ± standard error.
[b] Analyses of variance with F-test indicates if a significant linear regression can be fitted to the data or not (*ns* = not significant, $P > 0.05$).
[c] Sex ratio = nº females/(nº females + nº males).

Bushland & Hopkins [20] reported that the adult emergence and sex ratio of younger pupae (16 to 48 h old) irradiated at 2,500 R (24.3 Gy) and 5,000 R (48.7 Gy) were only 34.4% and 0.7, and 0.6% and 1, respectively. Bushland & Hopkins [4] irradiated 6 days old pupae with 48.7 Gy of X and gamma rays and did not find any significant difference between the values from the control and both types of radiation (all values of adult emergence and sex ratio were about 65% and 0.5, respectively).

When irradiated males were exposed to irradiated females, a significant reduction in fertility (approximately 99%) was observed at very low doses as 28 Gy of X rays, in comparison to the control group (Figure 2). Females irradiated at 60 Gy did not lay eggs. Similar results were found in literature.

The first bioassays for sterilization of *C. hominivorax* were performed during the 1950's. Bushland & Hopkins [20] reported that male and females from 5 days old pupae exposed to 2,500 and 5,000 R (24.3 and 48.7 Gy) of X rays were sterile, respectively, and the competitiveness of these sterile males was the same of fertile males in mating tests under laboratory conditions. After the development of gamma sources by the US Atomic Energy Commission, Bushland & Hopkins [4] performed tests comparing X and gamma rays and

concluded that no dose lower than 5,000 R (48.7 Gy) would be able to induce full sterility (batches with male and females together) and that it was more suitable to irradiate pupae at 5 days old or older. Two decades later, Crystal [21] re-examined the sterilization doses trying to find an optimal combination between dose and insect age. When pupae 72 h before the adult emergence were irradiated, the sterility of males irradiated at 2 krad (20 Gy) or more was higher than 95%, whilst the females were full sterile at 4 krad (40 Gy). In Brazil, Guevara [22] concluded that the ideal dose of gamma rays to sterilize two samples of *C.hominivorax* (one from SP and another from MG) would be 75 Gy (but the dosimetry results were not presented nor analyzed).

Under modified atmosphere, as with excess of CO_2, the sterilization doses range from 45 to 110 Gy [23, 24]. *C. hominivorax* adults may also be chimiosterilized, with males getting sterile at lower doses [25].

4. CONCLUSIONS

A colony of the New World Screwworm, *Cochliomyia hominivorax*, to produce sterile flies was successfully established in Brazil. The estimated X ray doses to induce 99% sterility in males and females were 43.7 Gy and 47.5 Gy, respectively. To produce 1.5 L of pupae (which serves to treat approximately 6,300 ha), the cost was about US$ 15.00.

ACKNOWLEDGMENTS

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for funding the project and the Biofactory MOSCAMED Brazil for the provision of facilities at Juazeiro, Bahia. Mention of trade names or commercial products in this article is sorely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors. This work was part of the thesis by Thiago Mastrangelo at the Universidade de São Paulo, Brazil.

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