DEVELOPMENT OF QUALITY CONTROL PROCEDURES FOR MASS PRODUCED AND RELEASED BACTROCERA PHILIPPINENSIS (DIPTERA: TEPHRITIDAE) FOR STERILE INSECT TECHNIQUE PROGRAMS

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DEVELOPMENT OF QUALITY CONTROL PROCEDURES FOR MASS PRODUCED AND RELEASED *BACTROCERA PHILIPPINENSIS* (DIPTERA: TEPHRITIDAE) FOR STERILE INSECT TECHNIQUE PROGRAMS

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ABSTRACT

Quality control procedures for *Bactrocera philippinensis* Drew & Hancock 1994 (Diptera: Tephritidae) used in sterile insect technique (SIT) programs were established in the mass rearing facility at the Philippine Nuclear Research Institute. Basic studies on pupal irradiation, holding/packaging systems, shipping procedures, longevity, sterility studies, and pupal eye color determination in relation to physiological development at different temperature regimes were investigated. These studies will provide baseline data for the development of quality control protocols for an expansion of *B. philippinensis* field programs with an SIT component in the future.

Key Words: *Bactrocera philippinensis*, quality control, Oriental fruit flies, pupal eye color, longevity, sterility

RESUMEN

Los procedimientos de control de calidad para *Bactrocera philippinensis* Drew & Hancock 1994 (Diptera: Tephritidae) usados en programas de la técnica de insecto estéril (TIE) fueron establecidos en la facilidad de cria en masa del Instituto Filipino de Investigación Nuclear. Estudios básicos sobre la irradiación de las pupas, sistemas de almacenaje/empaque, procedimientos del envio, longevidad, estudios de esterilidad y la determinación del color de ojo de la pupa en relación con el desarrollo fisiológico en regimenes diferentes de temperatura fueron investigados. Estos estudios proveerán una linea de información básica para el desarrollo de protocolos de control de calidad para una expansión de los programas de campo para *B. philippinensis* con un componente de TIS en el futuro.

Quality control is important for monitoring the performance of mass reared insects for use in the sterile insect technique (SIT) (Boller et al. 1981). To meet this requirement, routine quality control tests on egg hatchability, pupal weight and size, percent adult emergence, longevity, flight dispersal, and mating ability are used. The effect of pupal holding conditions, irradiation, and packaging procedures must also be assessed and threshold values for each quality control parameter need to be established.

After eradication of *Bactrocera philippinensis* Drew & Hancock 1994 (Diptera: Tephritidae) with the SIT in Naoway Islet, Philippines (Manoto et al. 1996), a feasibility study based on an integrated control program was initiated in Guimaras Island (Covacha et al. 2000). The Philippine Nuclear Research Institute upgraded the fruit fly mass rearing facility in order to produce 25 million pupae per week. The *B. philippinensis* colony has been mass reared in the laboratory for more than 100 generations (Rejesus et al. 1975). The quality control procedures being developed for this species were based on those developed for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann).

MATERIALS AND METHODS

Standard Specifications

Routine quality control includes measurements of pupal size, percent adult emergence, flight ability, sex ratio, response to stress, and mating propensity. Preparation of samples, observation, and gathering of data were done by following or modifying the procedures in the manual for "Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies" (FAO/IAEA/USDA 2003). Minimum specifications required for weekly and monthly routine quality control tests were established on pre-irradiation, post-irradiation, and post shipment pupae to serve as guides in monitoring fly quality and for inclusion into the manual.

Monitoring Fruit Fly Quality

Release of sterile flies in Guimaras Island by ground commenced in Apr 2001 and for every batch of released sterile flies routine quality control checks in the pre-irradiation, post-irradiation, and post-shipment were carried out. Data from quality control tests were tabulated and analyzed to assess variation in the quality specifications. A database of results for routine quality control tests was constructed for reference purposes.

Pupal Irradiation

Samples of *B. philippinensis* pupae obtained from the stock colony were marked with 1.5 g fluorescent dye (Dayglo®) and held in glass vials 2 d before emergence. The glass vials containing 25 mL of pupae were irradiated with ^{60}Co Gamma Cell 220 Irradiator facility with doses of 0, 25, 40, 50, 75, 100, 150, and 200 Gy. After irradiation, samples of pupae were prepared for the following tests.

Emergence and Flight Ability Tests

One hundred pupae, counted into a Petri dish, were placed at the base of a 10-cm black PVC pipe coated with talcum powder, inside a large cage. Percent adult emergence was based on the number of adults emerging from the pupal samples. Non-flying, fully emerged, partially-emerged, and deformed flies were counted and recorded. Flight ability (flies escaping from the black PVC pipe) was determined based on the number of unemerged pupae and residual flies remaining in the Petri dish.

Fecundity and Sterility Tests

Five replicates of 100 pupae of each dose were counted and placed for emergence in screened cages (30 × 30 × 40 cm) and provided with food and water. The flies were allowed to lay eggs for 10 d after emergence in a small egging device containing a wet sponge. Samples of eggs were counted, held in Petri dishes on damp cloth, and observed for egg hatch 3 d later.

Holding/Packaging of Pupae for Irradiation and Shipment to Guimaras Island

Trials on horizontal and vertical holding/packaging of pupae for irradiation and shipment were conducted and compared with the standard packaging system currently used. Each holding/packaging method was evaluated to determine the effect of length, size, and position/arrangement of polyethylene plastic bags. Three soft ice packs measuring 11 × 7.5 inches were placed inside the cardboard box as coolant. A laboratory thermometer was inserted inside the box to check the temperature at 4 h intervals for 48 h. At the same time, samples of pupae were taken at random from different sausage bags to determine the effect of each treatment on adult emergence and flight ability. The packages used with their corresponding specifications are shown in Fig. 1. Percent adult emergence and fliers were tabulated and checked to see if they passed the minimum specifications (Obra & Resilva 2003).

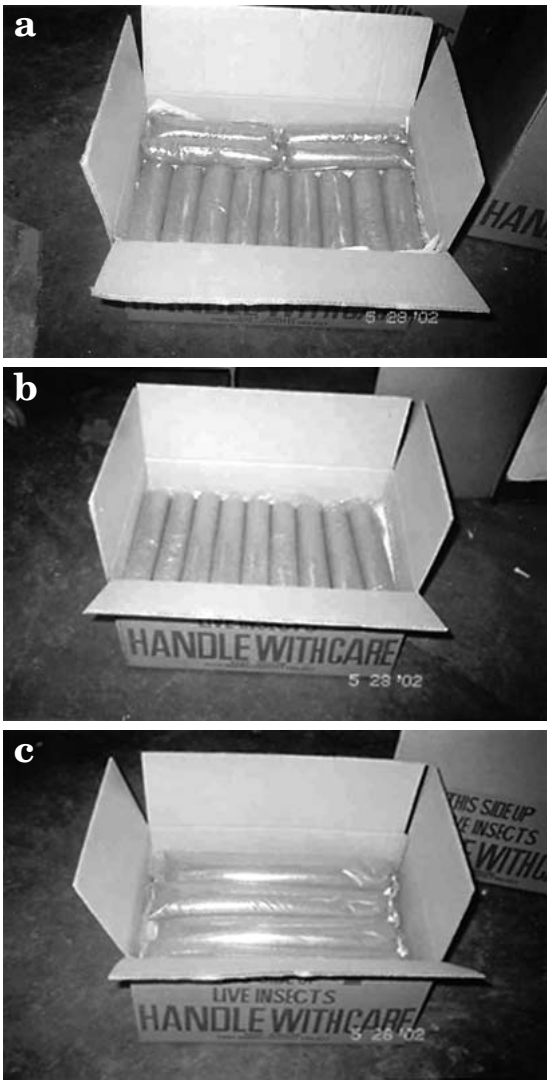


Fig. 1. Specifications and arrangement of 3 different packaging systems evaluated for irradiation and pupal shipment. a. Standard arrangement size of bags (cm) = 10.16 × 28, weight of pupae/sausage = 460 g, No. of sausage/box = 52, No. pupae/box = 1.9 million. b.Vertical arrangement size of bags (cm) = 10 × 40, weight. of pupae/sausage = 600 g. No. of sausage/box = 36, No. pupae/box = 1.7 million. c. Horizontal arrangement size of bags (cm) = 15 × 51, weight of pupae/sausage = 2000 g, No. of sausage/box = 12, No. pupae/box = 1.9 million.

Determination of Pupal Eye Color in Relation to Physiological Development

Development of pupae based on daily eye color changes (Ruhm & Calkins 1981) at different temperatures of 22-32°C (room temperature), 15, 19, and 28°C were determined. Pupal samples at 15, 19, and 28°C were placed in a controlled environment with an Echo Therm Chilling Incubator. Daily changes of eye color at each temperature were monitored by taking close up photographs with an Intel QX3 Computer Microscope at 60× magnification. Duration of pupal development and eye color changes of each pupal group were noted and matched with the color scale of the Soil Munsel Color Charts (Anonymous 2000).

Evaluation of Different Shipping Coolants for Sterile Pupae

Different shipping coolants such as soft ice, ice packs, and plastic ice trays were evaluated to determine suitable cooling materials for pupal shipment. Three boxes of pupal samples, arranged in the standard packaging system, were prepared and irradiated with the multi-purpose gamma irradiation facility. After irradiation, three sets of each coolant wrapped in newspaper were placed on the top of each cardboard box. The lids of the boxes were closed and secured with packaging tape. Data collection procedures were similar to those used for packing/holding.

Determination of Optimal Irradiation Doses Range

Samples of pupae were prepared and irradiated with doses inside of the irradiation chamber ranging from 52-56 Gy, 63-67 Gy, and 67-74 Gy. Sterility was checked by mating 50 irradiated males or females with 50 non-irradiated males or females in cages $30 \times 30 \times 40$ cm. Samples of eggs were collected weekly in moistened plastic vials over a 3-week period to determine egg hatch for each treatment. As many as 500 eggs were collected and counted during each egging.

Statistical Analysis

All data obtained in all experiments were tested in a randomized complete block design with 5 replications, evaluated, and subjected to an analysis of variance (ANOVA), with the honestly significant difference value calculated as Tukey's statistic at $\alpha = 0.05$ (SAS Institute 1990).

Results and Discussion

Standard Specifications for *B. philippinensis*

Table 1 shows the standard specifications for the essential weekly and monthly quality control tests of mass-reared *B. philippinensis*. These values were based on the minimum mean data obtained in a year's production in the rearing facility. The minimum weight set for pupae was 11.13 mg with a diameter of 1.75 mm. Minimum emergence rate and fliers with a 10-cm flight tube in pre-, post-irradiation, and post-shipment were 90.3, 85.2, and 80.4% for emergence, respectively, and 77.3, 73.2, and 70.1% for fliers, respectively. Minimum values of 50.2, 45.3, and 40.2% survival after 28-32 h was acceptable when newly emerged flies were subjected to stress tests in pre- and post-irradiation and post shipment, respectively. Mating propensity indicates an acceptable mean mating index of 50.2% (pre-irradiation), 45.1% (post-irradiation), and 40.3% (post-shipment) for 10 day-old flies.

Routine quality control checks were done on sterile flies sent to Guimaras for release. Released sterile flies passed the minimum specifications set for pupal size, adult emergence, adult fliers, and other quality control parameters tested.

Irradiation Studies

Table 2 shows data on the effects of different doses of gamma radiation on adult emergence, flight ability, fertility, and longevity. Statistical analysis of the adult emergence data showed no significant difference for all doses tested, compared to the control group. Flight ability data indicate that a high proportion of flies, 93.3-97.7%, escaped from the flight tube after doses of 25-100 Gy. At higher doses, the number of fliers progressively decreased from 73.9 to 69.0%. Females irradiated as pupae with 25-40 Gy were not sterile with egg hatch of 25.0 and 3.2%, respectively. When pupae were irradiated at 50 Gy and above, 100% sterility was achieved in all adult females. With regard to longevity tests, no significant difference was observed following irradiation with 25-75 Gy. However, increasing the dose beyond 100 Gy progressively affected the survival, resulting in a decrease in adult longevity after 5 weeks.

Holding/Packaging of Pupae for Irradiation and Shipment to Guimaras

Mean percent emergence and adult fliers in all packaging arrangements showed satisfactory results on pupae randomly-sampled every 4 h from 0-48 h. Similar results were observed in flight ability tests in which a high proportion of adults capable of flight (75-99%) escaped from a 10-cm flight tube. These findings indicate that horizontal and vertical arrangement of pupae were acceptable holding/packaging methods comparable to the standard packing system currently used for sterile fly shipment in Guimaras. Packing of pupae in cardboard boxes with 3 ice packs eliminates overheating of the pupae inside the box.

TABLE 1. SPECIFICATIONS FOR REQUIRED WEEKLY OR MONTHLY QUALITY CONTROL PARAMETERS OF MASS-REARED *B. PHILIPPINENSIS* FOR USE IN SIT PROGRAMS IN THE PHILIPPINES.

Parameter	Frequency	Pre-irradiation	Post-irradiation	Post-Shipment
A. Established minimum specifications[1]				
Pupal size	weekly			
min. pupal weight (mg)		11.00	nr	nr
min. pupal diameter (mm)		1.75	nr	nr
Sex ratio: min.% males	weekly	50.00	nr	nr
Emergence & flight ability	weekly			
min.% emergence		90.00	85.0	80.0
min.% fliers (10-cm tube)		77.00	73.0	70.0
Stress test: min.% survival, 28-32 h	weekly	50.00	45.0	45.0
Mating propensity	monthly	50.00	45.0	40.0
Boller's index (min), 10-d-old flies				
B. Mean QC test results[2]				
Pupal size	weekly			
min. pupal weight (mg)		12.52	nr	nr
min. pupal diameter (mm)		1.75	nr	nr
Sex ratio: min.% males	weekly	50.30	nr	nr
Emergence & flight ability	weekly			
min.% emergence		94.50	92.7	91.6
min.% fliers (10-cm tube)		86.10	81.5	71.7
Stress test: min.% survival, 28-32 h	weekly	57.60	56.3	53.4
Mating propensity	monthly	46.00	39.0	48.0
Boller's index (min), 10-d-old flies				

[1]Minimum standard specification established for one year period.
[2]Mean quality control test results of flies used in SIT release program. All data were collected in replicates. nr =not required.

Temperature was maintained between 15 to 28°C for 48 h. In addition, lining with plastic bubble wrap between the layers of "sausage bags" protects the pupae from mechanical injury by serving as a cushion while in transit.

Determination of Pupal Eye Color in Relation to Physiological Development

Daily changes in eye color during pupal development at 22-32°C (room temperature), 15, 19, and 28°C are shown in Table 3. The method for estimation of the pupal age is based on color changes compared with the color scale in the Munsel Soil Color Charts (Anonymous 2000).

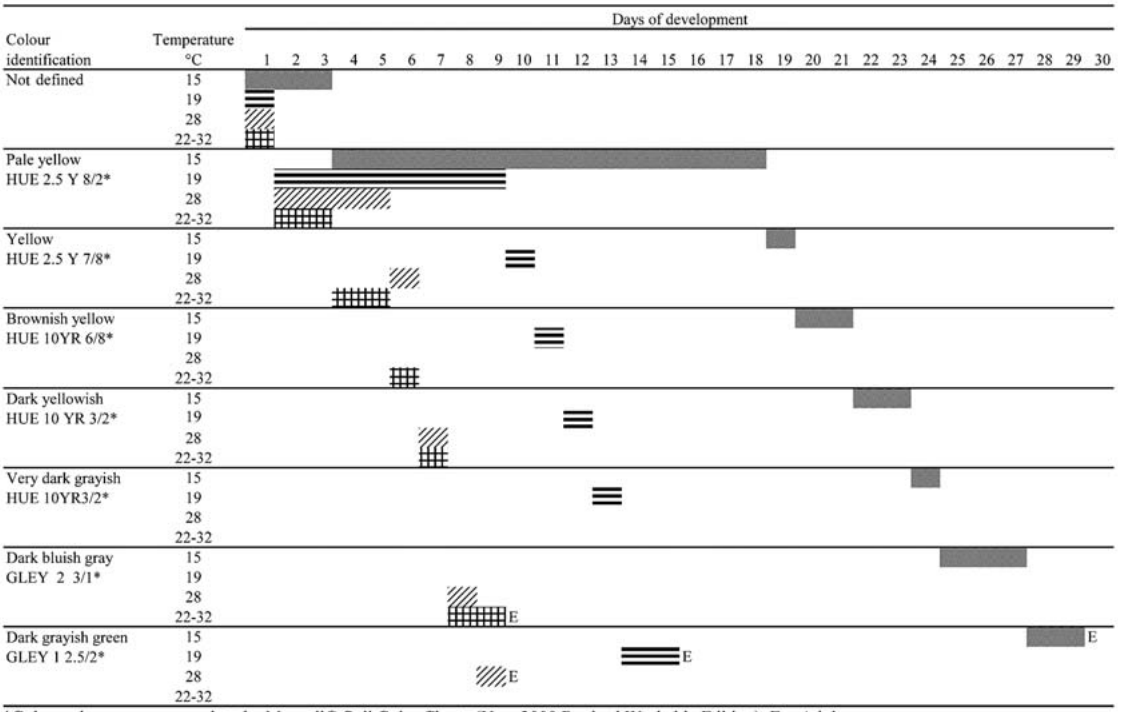
At 22-32°C (room temperature), pupal development is approximately 9 d. Dissection of the anterior part of the puparium is possible on the second day. On d 7 when pupae are irradiated, eye color is dark yellowish brown (HUE 10 YR 3/6). Adult flies start to emerge at 9 d and emergence is complete at 10 d.

TABLE 2. EFFECTS OF DIFFERENT DOSES OF GAMMA RADIATION (X ± SEM) ON THE ORIENTAL FRUIT FLY *BACTROCERA PHILIPPINENSIS* IRRADIATED 2 DAYS BEFORE EMERGENCE.* WITHIN A ROW, MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ($\alpha = 0.05$; ANOVA TEST).

Dose (Gy)	Emergence[1] (%)	Fliers[1] (%)	Mortality[1] (%)	Egg hatch[1] (%)
0	98.7 ± 0.4 ab	97.9 ± 0.6 a	14.8 ± 3.8 a	83.8 ± 4.6 a
25	98.4 ± 1.0 ab	94.4 ± 1.7 ab	15.9 ± 2.9 ab	25.1 ± 5.2 b
40	97.4 ± 1.9 ab	94.9 ± 2.1 b	14.9 ± 3.8 a	3.2 ± 2.8 c
50	98.8 ± 0.8 a	94.2 ± 0.9 b	12.4 ± 2.4 a	0
75	98.1 ± 0.6 ab	93.2 ± 0.8 b	13.5 ± 3.3 a	0
100	97.9 ± 0.6 ab	93.3 ± 0.9 b	26.3 ± 5.45 c	0
150	97.5 ± 0.8 b	73.9 ± 4.6 c	19.4 ± 3.8 b	0
200	95.3 ± 2.7 c	69.0 ± 5.0 d	30.7 ± 5.2 d	0

*Data are means of 5 replicates. [1]Analysis of variance results for % emergence were ($F = 2.06$; $df = 7,16$; $P > 0.11030$, % fliers ($F = 49.99$; $df = 7,16$; $P < 0.0001$), % mortality ($F = 8.43$; $df = 7,16$; $P < 0.00002$), % egg hatch ($F = 371.31$; $df = 7,16$; $P < 0.0001$).

TABLE 3. CHANGES IN EYE-COLOR OF THE ORIENTAL FRUIT FLY, *B. PHILIPPINENSIS* AT DIFFERENT TEMPERATURE OF PUPAL DEVELOPMENT.



*Color codes were compared to the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition). E = Adult emergence

At 15°C, pupal development takes about 29 d. Dissection of the puparium is possible 4 d after pupation, and at d 22-23 the eyes are dark yellowish brown (HUE 10 YR 3/6 and 3/4, respectively) and the pupae can be irradiated. Adult flies begin emerging on d 30.

Pupal development is approximately 15 d at 19°C. Radiation can be applied at d 12 with eye color of dark yellowish brown (HUE 10 YR 3/8). Adult flies start to emerge after 16 d.

At 28°C, pupal development requires 9 d. Eye color is dark yellowish brown (HUE 10 YR 3/6)

TABLE 4. STERILITY OF ORIENTAL FRUIT FLY, *B. PHILIPPINENSIS* IRRADIATED WITH DIFFERENT RANGE DOSES OF GAMMA IRRADIATION.

Dose range (Gy)	Crosses[1] (50 Males × 50 Females)	Number of eggs sampled	% Egg hatched
A. 52-56 Gy	U × U	8,000	86.76
	U × IR	1,055	8.93
	IR × U	8,000	0.66
	IR × IR	360	3.10
B. 63-67 Gy	U × U	8,000	90.40
	U × IR	687	7.13
	IR × U	7,900	0.33
	IR × IR	176	2.50
C. 67-74 Gy	U × U	8,000	87.71
	U × IR	—	—
	IR × U	7,956	—
	IR × IR	—	—

[1]U = non-irradiated flies; IR = irradiated flies. Eggs were collected starting 10 d after emergence for 3 consecutive weeks.

and is noticeable on d 7 when the pupae can be irradiated. Adult flies started emerging after 9 d and emergence was complete in 10 d.

Evaluation of Different Shipping Coolants

Mean adult emergence and flight ability data were obtained from pupae packed in boxes with soft ice, ice packs, and plastic ice trays in a standard arrangement/packaging system. The use of ice packs and soft ice were equivalent and met the minimum specifications set for emergence and adult fliers. Similar results were also observed for plastic ice trays; however, a decrease in adult fliers was noted when pupae were stored more than 44 h. A possible explanation for low fliers in plastic ice trays appeared to be due to an increase in temperature up to 32°C that begins after 44 h.

Determination of Optimal Irradiation Doses Range

Table 4 shows the effects of irradiation on the sterility of irradiated with 4 different dose ranges. Pupal irradiation with doses lower than 67 Gy did not prevent egg hatch. When pupae were irradiated with doses ranging from 63-67 Gy, egg hatch was between 0.3-0.7% when irradiated males were paired with non-irradiated females, or from 7.1-8.9% when non-irradiated males were paired with irradiated females. However, when the dose range was increased to 67-74 Gy, egg hatch was completely suppressed. These results suggested that the best irradiation range to achieve complete sterility with a Gamma-cell 220 should be between 67 and 74 Gy.

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