

EFFECTS OF PRE-IRRADIATION CONDITIONING OF MEDFLY PUPAE (DIPTERA: TEPHRITIDAE): HYPOXIA AND QUALITY OF STERILE MALES

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ABSTRACT

Irradiation of pupae in sterile insect technique (SIT) projects is usually undertaken in hypoxic atmospheres, which have been shown to lessen the deleterious effects of irradiation on the quality of adult sterile flies. Although this is the accepted technology in most mass-rearing and sterilization facilities, to date no information has been generated on the actual levels of oxygen (O₂) in pupae-packing containers during irradiation. The present study utilized recently-developed technology to investigate the O₂ level inside bags in which pupae of Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) are packed prior to irradiation, the ability of pupae to create hypoxic environments in these bags, and the effect of O₂ atmospheres on the quality of irradiated males. Pupae, 1 d before adult emergence, were shown to deplete the O₂ level in sealed bags in approximately 1 h. The rate of O₂ consumption was dependent upon pupal age and incubation temperature. Incubation temperature did not significantly affect the quality of pupae or mating capacity of resultant adult males if pupae were irradiated under maximal hypoxic conditions inside packing bags. In contrast, mating competitiveness drastically decreased when pupae were irradiated under ambient O₂ conditions, with the packing bag open. There was no difference in the mating capacity of males when pupae were irradiated in sealed bags under either 10% or 2% O₂ levels, or under maximal hypoxia. Normal doses of fluorescent dye, applied to pupae to mark sterile flies, did not affect the ability of pupae to create hypoxic conditions inside packing bags, nor the quality control parameters of either pupae or adults. Current practices in mass-rearing facilities are discussed in the light of these results.

Key Words: *Ceratitis capitata*, oxygen levels, pupal respiration, mating competitiveness, irradiation, sterile insect technique

RESUMEN

La irradiación de pupas en proyectos de mosca estéril usualmente se hace bajo condiciones de hipoxia. Esta condición ha demostrado ser menos detrimento a la calidad de las moscas que la irradiación en atmósferas con proporción normal de oxígeno. Aunque esta ha sido por mucho tiempo parte del protocolo de irradiación en plantas de producción de mosca estéril, hasta ahora no se ha medido el contenido de oxígeno dentro de los recipientes de empaque de pupa durante la irradiación. El presente estudio investigó los contenidos de O₂ en los contenedores de pupas de la mosca de las frutas del Mediterráneo (*Ceratitis capitata* Wiedemann), la habilidad de pupas de crear hipoxia dentro de los contenedores, y los efectos del contenido de O₂ durante la irradiación del contenedor en la calidad y capacidad de apareamiento de moscas estériles. Pupas de un día antes de emerger como adultos crearon atmósferas de máxima hipoxia dentro del empaque en aproximadamente una hora. La proporción de consumo de O₂ en contenedores sellados es dependiente de la edad de la pupa, y de la temperatura de incubación. La temperatura de incubación no afectó significativamente la calidad ni la capacidad de apareamiento de machos derivados de pupas irradiadas bajo condiciones de hipoxia. Sin embargo, la capacidad de apareamiento de machos irradiados como pupas en contenedores abiertos y en condiciones oxigenadas fue drásticamente afectada. En comparación a los resultados anteriores, atmósferas de 2% y 10% O₂ durante la irradiación no afectaron la capacidad de apareamiento de moscas estériles. Polvo fluorescente, aplicado a pupas para marcar las moscas estériles, no tuvo efectos sobre la capacidad de las pupas de crear hipoxia. Los resultados de este estudio se discuten en base a las prácticas actuales de producción e irradiación.

Translation provided by the authors.

Routine irradiation of fruit flies in control programs integrating the sterile insect technique (SIT) is usually undertaken when pupae are packed in containers that have been closed or her-

metically sealed for some period prior to their exposure to the radiation source (Schwarz et al. 1985). One variation of this method consists of the constant flushing of the pupae with nitrogen gas during irradiation (Fisher 1997). These techniques have been adopted to attain “reduced-oxygen atmospheres” during irradiation. These procedures have been shown to lessen the “oxygen effects” of “air irradiation” on mating performance and competitiveness of sterile males (Hooper 1971; Ohinata et al. 1977). While these practices are common in many fruit fly mass-rearing and irradiation facilities, and are recommended in the international fruit fly quality control manual (FAO/IAEA/USDA 2003), the actual levels of oxygen (O_2) inside sealed bags during irradiation have not, to our knowledge, been previously determined. The present study took advantage of recently developed technology for O_2 measurements in air environments to characterize O_2 atmospheres during packing and irradiation of pupae of the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann).

Initially, we investigated how pupae of different ages, packed in sealed polyethylene bags commonly used in many mass-rearing facilities, modify the O_2 environment inside the bag. A second objective investigated the effect on fruit fly quality of several “pupae packing pre-irradiation protocols” commonly performed in different medfly mass-rearing facilities, on the O_2 environments in packing bags, and on the effect of irradiation upon resultant adult males. Specifically, we investigated the effect of incubating pupae for a certain period of time (usually 1 h) at low temperatures before sending the pupae to the irradiator, as reported by Schwarz et al. (1985). This type of manipulation, and the transfer of pupae from the “cold environment” into the room where the irradiation chamber is located, may expose the pupae to several changing temperature regimes (in tropical facilities temperatures in irradiation rooms may be above 25°C). We also investigated the effect of irradiating pupae at different periods after the bags were sealed, and determined O_2 level in the sealed bag environment at the start of irradiation. Finally, we investigated the effect of dusting pupae with fluorescent dye during packing and before irradiation upon the ability of pupae to modify the O_2 environments inside the sealed bags. Treating pupae with fluorescent dye prior to irradiation is a routine method commonly used to identify released sterile flies in traps in the field.

MATERIALS AND METHODS

Source of Insects

Male medfly pupae of the temperature sensitive lethal genetic sexing strain VIENNA 8 (Franz 2005) were obtained from the colony main-

tained in the research facility of the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, at Seibersdorf, Austria. Pupae were collected at the appropriate age, as specified in each of the experiments.

Measurement of Oxygen Levels

Between 400 and 500 mL of pupae were placed inside 4.5-L polyethylene bags (15 cm width × 45 cm height, 1.5 mm thick), commonly used to pack, irradiate, and ship medfly pupae in some mass-rearing facilities (e.g., El Pino, Guatemala). The bags were perforated in two places by screwing male and female luer connectors, which opened to the inner and outer sides of the bag (a hole in the plastic was punctured through the connectors). The connectors facing the outside were then attached to vinyl tubing. One of the connectors directed the air emerging from the sealed bag into an O_2 sensor (a lead- O_2 battery, Model No. S-102, Qubit Systems Inc., Kingston, Ontario, Canada). The bags were sealed, leaving an empty space of 3-5 cm above the pupae. An air pump (0.4 liters/min) pumped air through the O_2 sensor after which it was directed into the sealed polyethylene bag. Thus, air was circulated throughout the entire experimental period in a closed circuit. The depletion of O_2 over time, as affected by respiration of the pupae in the sealed system, was registered by a data logger (Vernier Software and Technology, Beaverton, Oregon, U. S. A.) that generated 1 measurement per min. Each experiment was discontinued when measurements showed a stable low O_2 level (close to 0%) for a period of 10 min (“maximal hypoxia”). Experiments were conducted at 24°C except when otherwise specified.

Irradiation and Quality Control Procedures

In experiments where pupae were irradiated, a ^{60}Co source in a Nordion Gamacell-220 (Nordion, Canada®) was used. Dose was calculated at 150 Gy. A Gafchromic dosimeter placed in the center of the bag, however, showed an average dose during the experiments of 157 Gy. The approximate time spent in the irradiation chamber for this dose was 8.5 min. After irradiation, samples of pupae were used to investigate the following quality control parameters: pupal weight, number of pupae in 5 mL, % adult emergence, and flight ability index (FAO/IAEA/USDA 2003). These quality control parameters were also determined for non-irradiated pupae of the same batch of the experiment (control).

An additional test investigated the mating competitiveness of flies in the different treatments in each of the experiments (including a non-irradiated control). For each treatment 25 sexually mature males (each marked with a dot of differently-colored paint on the prothorax to dif-

ferentiate treatments) were released into a field cage inside a greenhouse together with 25 non-irradiated and sexually mature virgin females (with an Egyptian genetic background that had been maintained in the laboratory since 1968). The greenhouse was kept at 25°C. A potted citrus tree, pruned to facilitate observations of mating pairs, was placed in the center of each cage. Mating pairs were counted once an hour for 12 continuous hours, giving 12 observations during the entire experiment. Each observation was considered a replicate. Differences in mating competitiveness between treatments was calculated from the average and variance of the number of mating pairs found during each 12-h observation period based on General Linear Models (Statgraphics 5 Plus 2000, Manugistics, Inc.).

Experiment 1: Effect of Pupal Age on O₂ Depletion in Sealed Bags

Non-irradiated pupae 3, 2, and 1 d before adult emergence were used; 500 mL of pupae of each age were placed in separate polyethylene bags equipped with O₂ sensors as described above. For each pupal age, we used 2-3 replicates. A hermetically sealed bag with no pupae inside was used as a control. The real pupal ages were confirmed by using "pupal emergence grids", which included a random sample of 100 pupae (one pupa per grid space), and by following the emergence from pupae over time. Oxygen levels were measured as described above at a constant temperature of 24°C. Airflow in the closed system was started when the bags were sealed (= time 0). The rate of O₂ depletion was determined by measuring the O₂ levels every min. Differences in O₂ depletion rates (at 10 min after sealing), and time until the attainment of maximal hypoxia inside the bags, were investigated with a one-way ANOVA; means were separated by LSD (Statgraphics 5 Plus 2000, Manugistics, Inc.).

Experiment 2: Influence of Incubation Temperature on Attainment of Maximal Hypoxia and Quality of Irradiated Males

Polyethylene bags equipped with O₂ sensors as described above were filled with 450 mL of non-irradiated pupae at an estimated age of 1 d before adult emergence. The bags were laid flat to reduce the accumulation of metabolic heat and the pupae were then incubated for 1 h with the bags open ("preconditioning incubation"). Two preconditioning incubation temperatures were used, 16°C and 24°C; 16°C was selected because preconditioning incubations at 16°C are performed in some facilities to halt pupal development before irradiation (FAO/IAEA/USDA 2003). An incubator was used to attain 16°C while 24°C was the temperature in the experimental room. After incubation, bags

were hermetically sealed and immediately connected to the closed airflow system to measure O₂ consumption as specified above. Oxygen consumption was investigated under two constant temperature regimes ("post-sealing incubation temperatures"): 16°C and 24°C.

Measurements at 16°C were performed with the bag and the air line inside the incubator. For this, the door of the incubator was closed with the vinyl tubes emerging through the rubber door seal to conduct the air of the closed system to the O₂ sensor. We ensured a free flow of air through the system without affecting the temperature inside the incubator. In the case of incubation at 24°C, the entire system was in a room maintained at this constant temperature. Thus, we had four treatments which combined the two preconditioning incubation temperatures (PC) and the two post-sealing-incubation temperature (PS): (1) 24°C PC and 24°C PS; (2) 24°C PC and 16°C PS; (3) 16°C PC and 24°C PS; and (4) 16°C PC and 16°C PS.

After reaching a constant level of maximal hypoxia in the sealed bag system, O₂ measurements were discontinued, pupae were irradiated inside the sealed bags, bags were opened, and samples of pupae were subjected to quality control tests as specified above. We repeated the experiment twice with different batches of pupae.

Experiment 3: Oxygen levels in Packing Bags during Irradiation and Effect upon Male Fly Quality

As in Experiment 2 we used pupae at an estimated age of 1 d before adult emergence, placing 450 mL of non-irradiated pupae in four polyethylene bags. Three out of the 4 bags were sealed simultaneously but only 1 of the bags was used to monitor the O₂ level. Since bags were sealed simultaneously with the same type and quantity of pupae, we made the assumption that the reading in the monitored bag would be representative of those in the other bags. The 4th bag was left unsealed. The sealed bags were then individually irradiated 15, 30, and 60 min after sealing, this being when the O₂ level inside the monitored bag reached approximately 10%, 2%, and maximal hypoxia, respectively. In addition, we irradiated the unsealed bag that was presumed to have an ambient O₂ environment during irradiation. Total irradiation time lasted approximately 8.5 min, during which interval the O₂ level inside the sealed bags are presumed to have decreased further. Thus, the O₂ levels mentioned above are those expected at the onset of irradiation. The experiment was replicated twice with different batches of pupae of the same age. After irradiation the bags were opened and samples of pupae from each bag were separately subjected to the quality control tests. Non-irradiated pupae which were not exposed to hypoxia were used as a control.

Experiment 4: Effect of Fluorescent Dye on O₂ Consumption Patterns and Irradiation Effects

Two polyethylene bags were each loaded with 500 mL of pupae at an expected age of 1 d before adult emergence. The pupae in 1 bag were thoroughly mixed with 0.75 g of Day-Glo® fluorescent powder. The second bag was untreated and used as a control. The bags were hermetically sealed and the consumption of O₂ over time measured as described above. After reaching maximal hypoxia the bags were irradiated, opened, and separate samples of pupae subjected to quality control tests. In this experiment we did not investigate mating competitiveness.

RESULTS

All samples of pupae placed in adult emergence grids confirmed that the expected ages of pupae used for these experiments were true ages (results not shown).

Experiment 1: Effect of Pupal Age on O₂ Depletion in Sealed Bags

Fig. 1 shows the effect of pupal age upon the consumption of O₂ by pupae in the closed air system. These data show that older pupae consume O₂ more rapidly than younger pupae. The rate of O₂ consumption 10 min after the bag was sealed was significantly faster in pupae 1 d before adult emergence than in pupae 2 or 3 d before emergence (Fig. 2; $F = 20.9$; $df = 2,6$; $P < 0.01$). Simi-

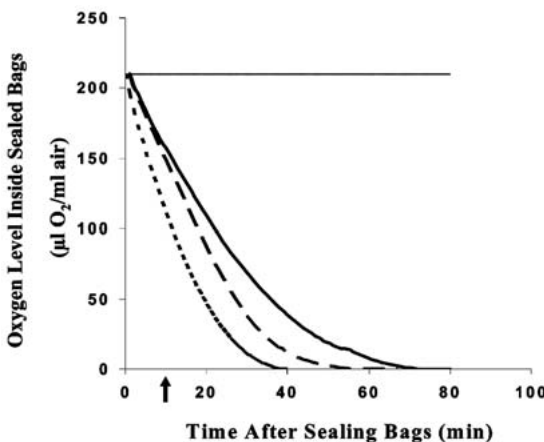


Fig. 1. Oxygen consumption curves for medfly pupae (VIENNA 8) sealed in bags as a function of pupal age: 1 d (-----), 2 d (-----) and 3 d (—) before adult emergence. The horizontal line at approximately 210 µL O₂/mL air (21% O₂) is the line obtained for the sealed bag when no pupae were present inside the bag. The arrow on the x-axis shows the time at which the rate of O₂ consumption by pupae was determined (data shown in Fig. 2).

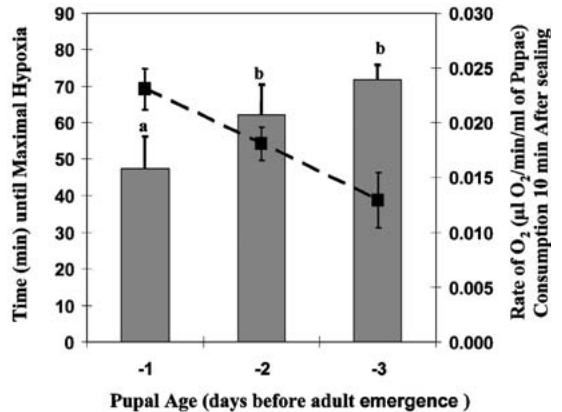


Fig. 2. Time needed by medfly pupae (VIENNA 8) to attain maximal hypoxia (bars and left y-axis), and average and standard deviation rate of O₂ consumption (broken line and right y-axis) as affected by pupal age (data from Fig. 1, 10 min after sealing bags). Different letters above bars stand for significant differences ($P < 0.05$) in time needed to attain maximal hypoxia (Kruskal-Wallis). Rate of oxygen consumption was significantly different for the 3 treatments (Kruskal-Wallis).

larly, the consumption rate was significantly faster in pupae 2 d before adult emergence than 3 d before emergence. The time needed by pupae to reach maximal hypoxia in the bag was significantly longer in pupae 3 d before adult emergence than in pupae 2 or 1 d before emergence (Fig. 2; $F = 9.3$; $df = 2,6$; $P < 0.05$).

Experiment 2: Influence of Incubation Temperature on Attainment of Maximal Hypoxia and Quality of Irradiated Males

The rate at which O₂ was consumed by pupae in hermetically sealed bags when incubated at different preconditioning (PC) and post-sealing-bag (PS) temperatures is shown in Fig. 3. Pupae incubated at 24°C during both PC and PS consumed O₂ faster than pupae incubated first at 24°C during PC and then transferred after 1 h to 16°C for PS incubation (Fig. 3A). Similarly, pupae incubated during PC at 16°C and then transferred to 24°C consumed O₂ faster than pupae incubated at 16°C for both PC and PS (Fig. 3B). The average durations (2 replicates) needed for pupae to reach maximal hypoxic conditions during these different schedules were 57.5 min for 24°C PC + 24°C PS; 87.0 min for 24°C PC + 16°C PS; 98.0 min for 16°C PC + 24°C PS; and 148.5 min for 16°C PC + 16°C PS.

Table 1 shows results of the quality control tests after pupal irradiation under the 4 temperature combination protocols and in the control. Average and standard deviation are for 2 replicate experiments. Mating competitiveness test was performed only with 1 of the replicates. Pupal weight, number of pupae in 5 mL, % adult emer-

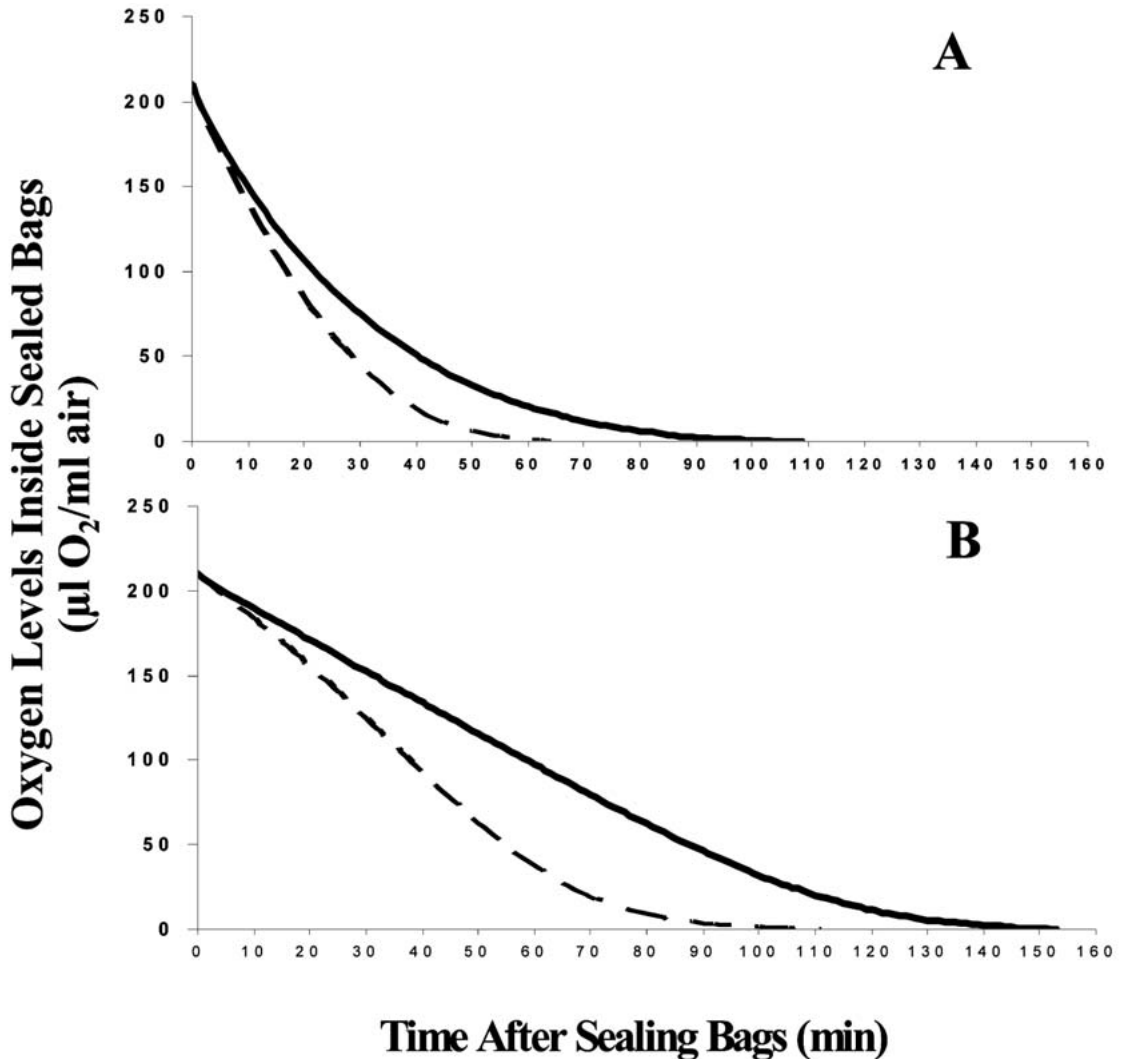


Fig. 3. Oxygen consumption curves for medfly pupae (VIENNA 8) 1 d before emergence packed in sealed bags as a function of 2 pre-irradiation temperature incubation protocols. PC = pre-conditioning incubation protocol; PS = post-sealing incubation protocol. (A) PC of 24°C; (B) PC of 16°C; (-----) O₂ curves for PS of 24°C; (—) O₂ curves for PS of 16°C.

gence and flight ability did not differ significantly between treatments and control. Similarly, incubation temperature protocols did not have any significant effect upon the mating competitiveness of irradiated males (Fig. 4). However, as expected the mating competitiveness of non-irradiated males (control) was significantly greater than that of irradiated males (Fig. 4).

Experiment 3: Oxygen levels in Packing Bags during Irradiation and Effect upon Male Fly Quality

Fig. 5 shows the approximate O₂ levels: I, in an open bag (ambient O₂ environment); II, 15 min after sealing ($\pm 10\%$ O₂ level); III, 30 min after

sealing ($\pm 2\%$ O₂ level); IV, maximal hypoxia (close to 0% O₂ level). The rate of O₂ consumption and the time needed to reach maximal hypoxia were very similar in the 2 replicates: at 10 min after bag-sealing O₂ consumption was 9.6 and 9.2 $\mu\text{L O}_2/\text{mL air}/\text{min}$, and maximal hypoxia was reached after 52 and 58 min, in replicates 1 and 2, respectively.

Table 2 shows the effects of irradiation under several O₂ environments in packing bags upon pupal weight, number of pupae in 5 mL, % adult emergence, and flight ability. Average and variance are for 2 replicate experiments. The mating competitiveness test was performed only with one of the replicates. None of the treatments with reduced O₂ levels had a significant effect upon the

TABLE 1. EFFECT OF DIFFERENT PRE-IRRADIATION TEMPERATURE REGIMES ON QUALITY CONTROL PARAMETERS OF IRRADIATED MEDFLIES.

Treatment PC→PS*	Avg. pupal weight (mg/pupae) ± SD	Avg. no. pupae in 5 mL ± SD	Avg. adult emergence (%) ± SD	Flight ability index (%) ± SD
24°C→24°C	8.65 ± 0.07	285 ± 1	87.7 ± 9.1	84.8 ± 8.6
24°C→16°C	8.50 ± 0.28	270 ± 25	83.1 ± 16.5	85.7 ± 10.4
16°C→24°C	8.70 ± 0.14	285 ± 3	86.7 ± 12.3	86.2 ± 9.7
16°C→16°C	8.55 ± 0.07	291 ± 13	84.2 ± 13.3	86.0 ± 7.5
Control**	8.75 ± 0.07	298 ± 12	92.7 ± 2.6	91.1 ± 7.5
<i>H</i> ***	4.1	3.7	1.0	1.5
<i>P</i>	>0.05	>0.05	>0.05	>0.05

*Preconditioning Temperature → Post-Sealing Temperature.

**Control consisted of non-irradiated pupae, and pupae that did not undergo hypoxia treatment and preconditioning and post-sealing-bag temperature incubations. Control pupae were maintained at 24°C until processing.

****H*—Kruskal-Wallis Non-Parametric one way ANOVA.

quality control parameters (Table 2). In contrast, irradiation under ambient O₂ level, although not statistically significant, reduced adult emergence and flight ability, and significantly affected the mating competitiveness of irradiated males (Table 2, Fig. 6). As expected, non-irradiated males (control) performed significantly better than sterile males in the mating competitiveness test (Fig. 6).

Experiment 4: Effect of Fluorescent Dye on O₂ Consumption Patterns and Irradiation Effects

Oxygen consumption curves of dyed pupae and undyed pupae 10 min after bag sealing were very

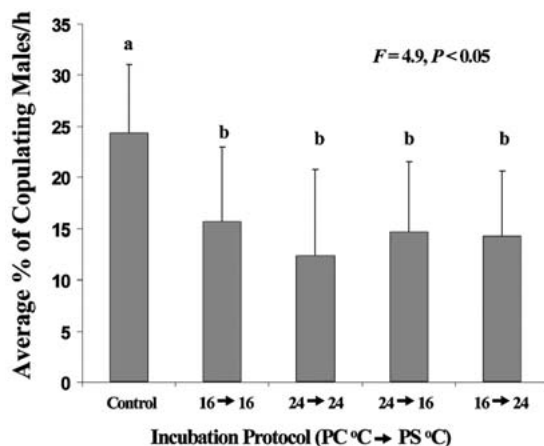


Fig. 4. Mating competitiveness (ability to form copulating pairs) of irradiated and non-irradiated (control) male medflies (VIENNA 8). Irradiated males were subjected to different incubation protocols (preconditioning, PC, and post-sealing-bag, PS, incubation temperatures protocols) before irradiation. Irradiation was performed after reaching maximal hypoxia in sealed bags during PS incubation. The figure includes the resultant *F* and *P* (General Linear Models) and step-wise separation of means (lower case letters).

similar: 8.7 and 8.6 μL O₂/mL air/min, respectively. Time to reach maximal hypoxia was 58 min for dyed pupae and 60 min for undyed pupae. Likewise, pupal weight, number of pupae in 5 mL, % emergence and flight ability was very similar between the 2 treatments, and comparable to the unirradiated control (data not shown).

DISCUSSION

With no renewal of O₂, pupae sealed in bags were expected to totally deplete the O₂ levels of the air through their metabolic activity (FAO/IAEA/USDA 2003). This study clearly showed that the O₂ level inside sealed bags loaded with pupae steadily declines over time. Time needed for the attainment of maximal hypoxia was dependent not only on the age of the pupae, but also

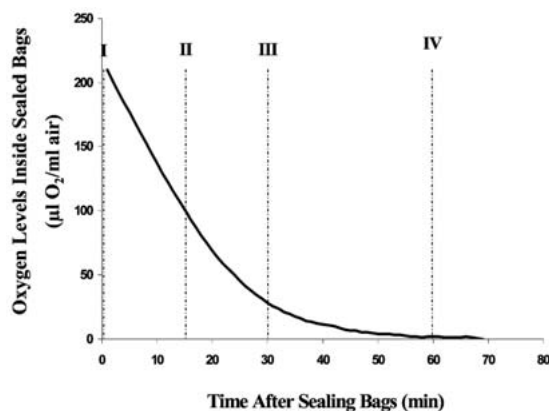


Fig. 5. Oxygen consumption curve and level of hypoxia attained for medfly pupae (VIENNA 8) 1 d before emergence packed in sealed bags: I. ambient O₂ level during irradiation; II. 10% O₂ level at the onset of irradiation; III. 2% O₂ level at the onset of irradiation; IV. Maximal hypoxia at the onset of irradiation.

TABLE 2. EFFECT OF DIFFERENT PRE-IRRADIATION OXYGEN LEVELS ON QUALITY CONTROL PARAMETERS OF THE IRRADIATED MEDFLY.

Treatment	Avg. pupal weight (mg/pupae) \pm SD	Avg. no. pupae in 5 mL \pm SD	Avg. adult emergence (%) \pm SD	Flight ability index (%) \pm SD
Maximum hypoxia	8.40 \pm 0.30	298 \pm 8	89.2 \pm 0.9	82.6 \pm 1.8
2% O ₂	8.50 \pm 0.30	303 \pm 1	90.9 \pm 2.9	84.2 \pm 1.9
10% O ₂	8.45 \pm 0.20	302 \pm 4	89.6 \pm 1.8	81.6 \pm 4.7
Ambient O ₂	8.55 \pm 0.20	303 \pm 11	85.3 \pm 6.8	67.4 \pm 13.4
Control*	8.45 \pm 0.40	301 \pm 8	89.4 \pm 2.3	86.3 \pm 0.7
<i>H</i> **	1.2	0.9	1.5	7.3
<i>P</i>	>0.05	>0.05	>0.05	>0.05

*Control consisted on non-irradiated pupae that did not undergo hypoxia treatment. Control pupae were maintained at 24°C until processing.

***H*—Kruskal-Wallis Non-Parametric One Way ANOVA.

on the temperature at which pupae are maintained. These results support the findings of Langley (1970) and Seo et al. (1990), that the rate of O₂ consumption is strongly dependent upon the rate of metabolic activity. Metabolic activity and respiration rate increased significantly in pupae close to adult emergence, and in pupae kept at high temperatures (Keister & Buck 1973; Seo et al. 1990).

Pre-conditioning (PC) and post-sealing (PS) incubation temperatures had a marked effect upon the consumption of O₂ by pupae. As expected for a poikilotherm organism, incubation at 24°C accel-

erated the consumption of O₂ and the metabolism of pupae, while 16°C had a depressing effect upon both metabolism and O₂ consumption. The transfer of pupae from a PC temperature of 24°C to a PS temperature of 16°C reduced O₂ consumption to a lesser extent than the opposite situation, suggesting that it takes more time to warm up pupae and accelerate metabolic rate than to slow down metabolism with cooler temperatures. While temperature manipulation prior to attainment of hypoxia and irradiation affected the rate of O₂ consumption, these incubation protocols did not have any noticeable effect upon pupal quality and mating performance. In contrast, ambient O₂ levels inside bags during irradiation had, as previously demonstrated (Ohinata et al. 1977), an important negative effect upon mating activity and pupal quality.

The mechanism by which different O₂ environments during irradiation affect the quality of the fly has still not been fully investigated. However, the most acceptable hypotheses suggest that low O₂ tension in pupal tissue reduces the formation of toxic free radicals and peroxides during irradiation (Ohinata et al. 1977). Regardless of the mechanism by which the O₂ atmosphere influences the quality of the irradiated fly, the present study confirmed the fact that a hypoxic environment during irradiation enhances the mating performance and quality of sterile pupae (Bakri et al. 2005).

It is interesting to note that irradiation under 10% and 2% O₂ environments resulted in flies with similar mating competitiveness and quality to those irradiated under maximal hypoxia. This phenomenon could be the result of ongoing pupal metabolic activity, and O₂ consumption, during the time spent inside the irradiation chamber (8.5 min), which may have reduced further the O₂ levels inside the sealed bags. A further possibility is that below certain O₂ level inside the bags, the "Oxygen effect" (Hooper 1971; Ohinata et al. 1977) is not manifested. Our study was not able to detect this "threshold" O₂ level, which may be of theoretical, but not practical, interest.

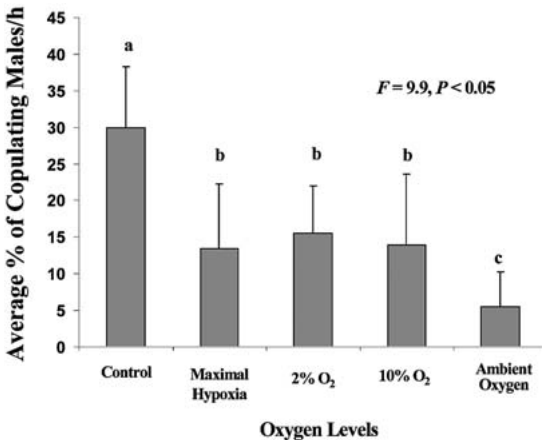


Fig 6. Mating competitiveness (ability to form copulating pairs) of irradiated and non-irradiated (control) male medflies (VIENNA 8) as affected by irradiation under different O₂ environments. Hypoxia levels as shown in Fig. 5: 60 min = maximal hypoxia inside sealed packing bags; 30 min = 2% O₂ level at the onset of irradiation; 15 min = 10% O₂ level at the onset of irradiation; 0 min = open bags with ambient O₂ levels. Control males were neither irradiated nor underwent hypoxia. The figure includes the resultant *F* and *P* (General Linear Models) and step-wise separation of means (lower case letters).

In practical terms, this study demonstrated that hermetically sealing bags containing pupae for irradiation, and keeping these pupae for approximately 1 h at temperatures of 24°C, is sufficient to create an optimal hypoxic environment inside these bags. It also demonstrated that routine treatment of pupae with fluorescent dye (1.5 g/kg of pupae) did not appear to affect the ability of pupae to consume O₂ inside the bags. The study also provided data on the effect of different incubation temperatures upon the ability of pupae to create hypoxic atmospheres inside packing bags, and upon the effects of some pre-irradiation incubation protocols currently carried out in several medfly rearing facilities. Specifically, our data suggests that keeping pupae in open bags at 16°C until irradiation may affect their later ability to create an optimal hypoxic environment inside sealed bags before and during irradiation. These last results, and the cost/benefit evaluations of incubating procedures, need to be re-assessed before final recommendations are made.

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