

Irradiation of *Ecdytolopha aurantiana* (Lepidoptera: Tortricidae) pupae in oxygen requires a lower dose to strongly reduce adult emergence and prevent reproduction than irradiation in air

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Abstract

The purpose of this research was to determine if γ -irradiation of lepidopteran pupae in oxygen would have a greater effect in either preventing adult emergence or in the successful development of F_1 life stages than γ -irradiation of pupae in air. Pupae of the citrus fruit borer, *Ecdytolopha aurantiana* (Lima) (Lepidoptera: Tortricidae), were irradiated in either 100% oxygen or in normal air. In each experiment, thirty 10 d-old pupae of *E. aurantiana* in each of 4 replicates were treated with either 0, 100, 200 or 300 Gy of γ radiation. After irradiation the percent emergence of adults, and the percent viability of F_1 eggs and larvae were compared with the corresponding results of irradiation in air. The results showed that irradiation of the pupae in pure oxygen significantly reduced the adult emergence and egg hatch compared to irradiation of the pupae in air. Thus irradiation in oxygen of pupae with 300 Gy completely prevented emerged adults from producing eggs. Therefore, irradiation of *E. aurantiana* pupae with 300 Gy in oxygen is a viable candidate as a phytosanitary treatment. In contrast, irradiation in air of pupae with 300 Gy resulted in 5% egg hatch, although 100% of these larvae died as first or second instars. Therefore based on the criterion of 100% mortality of F_1 larvae, 300 Gy in air can also be considered to be a viable candidate for a phytosanitary treatment. These results indicate that the dose required for (PI) treatment may be reduced by increasing the oxygen gas content in fruit packages or containers.

Key Words: adult emergence; citrus fruit borer; F_1 egg hatch; γ radiation; phytosanitary irradiation

Resumen

El propósito de esta investigación fue determinar si la irradiación- γ de pupas de lepidópteros en oxígeno tendría un mayor efecto en la prevención de la emergencia de los adultos o en el desarrollo exitoso de los estadios de la F_1 que en la irradiación- γ de pupas en el aire. Las pupas del barrendador del fruto de cítrico, *Ecdytolopha aurantiana* (Lima) (Lepidoptera: Tortricidae), fueron irradiados en 100% de oxígeno o en aire normal. En cada experimento, treinta pupas de 10 días de edad de *E. aurantiana* cada una en 4 réplicas fueron tratadas con 0, 100, 200 o 300 Gy de radiación- γ . Después de la irradiación, se compararon los porcentajes de emergencia de los adultos y viabilidad de los huevos y larvas de la F_1 con los resultados correspondientes de la irradiación en aire. Los resultados mostraron que la irradiación de las pupas en oxígeno puro redujo significativamente la emergencia de adultos y eclosión de los huevos en comparación con la irradiación de las pupas en el aire. Por lo tanto, la irradiación en oxígeno de pupas con 300 Gy impidió completamente que los adultos emergidos produjera huevos. Por lo tanto, la irradiación en oxígeno de pupas de *E. aurantiana* con 300 Gy es una prerrogativa viable como tratamiento fitosanitario. Por el contrario, la irradiación en el aire de las pupas con 300 Gy dio lugar a 5% eclosión de los huevos, aunque el 100% de las larvas murieron como estadios de primera o segunda. Por lo tanto, basado en el criterio del 100% de mortalidad de larvas de F_1 , 300 Gy en el aire también puede ser considerado como un candidato viable para un tratamiento fitosanitario. Estos resultados indican que la dosis requerida para el tratamiento (IF) se puede reducir al aumentar el contenido de oxígeno en envases de frutas o contenedores.

Palabras Clave: emergencia de adultos; barrendador del fruto de cítrico; eclosión de los huevos de F_1 ; radiación- γ ; irradiación fitosanitaria

Resumo

O objetivo desta pesquisa foi determinar se a irradiação γ em pupas de lepidópteros em oxigênio teria um efeito maior para prevenir a emergência de adultos e o desenvolvimento da fase de vida de F_1 do que a irradiação γ em pupas no ar. Pupas da broca citrúcula, *Ecdytolopha aurantiana* (Lima) (Lepidoptera: Tortricidae), foram irradiadas nos gases: 100% de oxigênio e ar normal. Em cada experimento, foram utilizadas 30 pupas de *E. aurantiana* com 10 dias de idade, com 4 repetições por tratamento e foram tratados com: 0 (controle), 100, 200 e 300 Gy de radiação γ . Após a irradiação, a percentagem de emergência de adultos, e a percentagem de viabilidade de ovos e larvas F_1 foram comparados com os resultados correspondentes das pupas irradiadas no ar. Os resultados mostraram que a irradiação de pupas em oxigênio puro reduziu significativamente a emergência dos adultos e eclosão de ovos em comparação com a irradiação de pupas no ar. Assim, a irradiação de pupas em oxigênio com 300 Gy evitou completamente a emergência de adultos e produção de ovos. Portanto, a irradiação de pupas de *E. aurantiana* com 300 Gy em oxigênio é um candidato viável como

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tratamento fitossanitário. Em contraste, a irradiação no ar de pupas com 300 Gy resultou em 5% de eclosão dos ovos, embora 100 % destas larvas morreram como primeiro ou segundo estádios. Portanto, com base no critério de porcentagem de mortalidade de larvas F_1 , 300 Gy em ar, também pode ser considerado um candidato viável para um tratamento fitossanitário. Estes resultados indicam que a dose necessária para o tratamento (PI) pode ser reduzida pelo aumento do teor do gás oxigênio em embalagens de frutas ou em outros recipientes.

Palavras Chave: emergência de adultos; broca do citrus; F_1 eclosão dos ovos; radiação γ ; irradiação fitossanitário

Increased levels of oxygen in the ambient environment are known to enhance the effects of γ rays and x-rays in biological tissues. The influence of gases during the irradiation of biological material was observed for the first time in 1921 with respect to cellular effects in irradiated ascarid (Ascaridida: Ascarididae) roundworms (Holthusen 1921). Thoday & Read (1947) found that the frequency of chromosome aberrations produced by x-rays in root tip cells of the broad bean, *Vicia faba* L. (Fabales: Fabaceae), was dependent on oxygen tension during irradiation, while the frequency of chromosome aberrations produced by α -rays is not affected by oxygen tension. Whiting (1954) showed that x-irradiation of the parasitic wasp, *Habrobracon* sp. (Hymenoptera: Braconidae), with 500 roentgens in air produced more mutations in the eggs than 1,100 roentgen applied in nitrogen. In addition, Baumhover (1963) observed that a rapid buildup of anoxia greatly protected pupae of the screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), packed into a gas-tight canister against the induction of sexual sterility by γ -irradiation. More specifically, Baumhover found that screwworm pupae packed tightly into an air-tight canister quickly depleted their oxygen supply and built-up the concentration of carbon dioxide, so that if the pupae were held in this way for about 30 min at room temperature the γ -ray dose needed to induce complete sterility had to be increased ~2-fold compared to irradiation in ambient air. There are many published reports that the application of oxygen during the irradiation of insects with γ -rays or X-rays enhances radiation damage, whereas the depletion of the oxygen content with either carbon dioxide or nitrogen diminishes the effect of γ - or X-irradiation (Smittle 1967).

Santos et al. (1975) irradiated adults of the house fly, *Musca domestica* L. (Diptera: Muscidae), in atmospheres greatly enriched with either hydrogen, nitrogen or oxygen and concluded that hydrogen reduced the negative effect that irradiation has on the lifespan of the house fly but oxygen increased the negative effect on the lifespan. Ohinata et al. (1977), investigated the effects of several gases used as the atmosphere while irradiating Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), pupae and they reported that the dose required to induce almost complete sterility in normal air was 10 krad (100 Gy), while the dose required to do so in either carbon dioxide, or helium, or nitrogen or in a partial vacuum was 16 krad (160 Gy)—a 1.6-fold increase caused by the depletion of oxygen in the ambient atmosphere.

Hallman & Hellmich (2009) reported that the radiotolerance of European corn borer, *Ostrinia nubilalis* (Hübner), (Lepidoptera: Crambidae) pupae—the most tolerant stage that infests commodities in trade—was neither affected by having been reared on an artificial or a meridic diet versus on the corn ear nor by temperature in the range 1–13 °C, but was positively affected by low oxygen content. Using F_1 egg hatch as their criterion of efficacy of irradiation, Hallman & Hellmich (2009) found that the minimum absorbed dose for phytosanitary irradiation of *O. nubilalis* increased from 233 Gy in normal air to 343 Gy in air with reduced oxygen levels—a 1.5-fold increase.

Modified atmospheres are used to prolong the shelf life of stored commodities, and these usually involve storage in an atmosphere with low oxygen content. These low oxygen concentrations have been found to reduce efficacy of phytosanitary irradiation (Hallman 2004a, b, 2005). Also, cold temperatures (above freezing) have been hypoth-

esized to reduce the efficacy of irradiation (Hallman 2001), although the only thorough study of this subject focused on the differences in efficacy between apple maggot, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), third instars irradiated in apples (*Malus domestica* Borkh; Rosales: Rosaceae) held at temperatures in the range 1–24 °C (Hallman 2004b).

Ionizing radiation treatments are increasingly being used as phytosanitary treatments against quarantine pests. A generic treatment of 400 Gy against all insect species has been approved for commodities entering the United States except commodities potentially infested with pupae and/or adults of Lepidoptera. The literature indicates that irradiation with 400 Gy of some adult Lepidoptera, namely, the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelichiidae), and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), fail to provide complete control (Hallman & Phillips 2008).

The radiotolerance of an insect species reaches its greatest level in the late pupae (pharate adults) and adult stages, and therefore the minimum absorbed dose to prevent F_1 egg hatch for the above 2 species when irradiated as adults was examined. Also, because hypoxia is known to increase radiotolerance in insects, the radiotolerance of the Angoumois grain moth was tested in a hypoxic atmosphere. A dose range of 336–388 Gy was sufficient to prevent F_1 egg hatch from a total of 22,083 irradiated adults of the Indian meal moth. Dose ranges of 443–505 and 590–674 Gy were sufficient to prevent F_1 egg hatch from a total of 15,264 and 13,677 irradiated adults of the Angoumois grain moth in ambient and hypoxic atmospheres, respectively (Hallman & Phillips 2008). A generic dose of 600 Gy for all insects in ambient atmospheres might be efficacious, although many fresh commodities may not tolerate it when applied on a commercial scale (Hallman & Phillips 2008).

The citrus fruit borer *Ecdytolopha aurantiana* (Lima) (Lepidoptera: Tortricidae) is an economically important pest of tree fruits in Latin America. Bento et al. (2001) summarized the distribution and biology of this pest as follows. *Ecdytolopha aurantiana* occurs in most Neotropical areas, including Brazil, Argentina, Costa Rica and Trinidad-Tobago. The larvae of *E. aurantiana* feed on and destroy citrus, (*Citrus* L.; Sapindales: Rutaceae) fruits, guava, *Psidium guajava* L. (Myrtales: Myrtaceae), banana, *Musa acuminata* Colla (Zingiberales: Musaceae), coconut, *Cocos nucifera* L. (Arecales: Arecaeae), cherimola, *Annona cherimola* Mill. (Magnoliales: Annonaceae), lychee, *Litchi chinensis* Sonn. (Sapindales: Sapindaceae), macadamia, *Macadamia integrifolia* Maiden & Betche (Proteales: Proteaceae) and sugar apple (frutadoconde), *Annona squamosa* L. (Magnoliales: Annonaceae).

Ecdytolopha aurantiana causes damage to various citrus fruits in the State of Sao Paulo and in 10 other Brazilian States, with consequent reduced citrus production in these regions (Prates & Pinto 1988, 1991). Yield losses of up to 50% in infested areas were estimated to occur in the State of Sao Paulo (Garcia et al. 1998), and crop losses throughout Brazil were estimated at US\$ 50 million per yr (Anonymous 2000).

Control of *E. aurantiana* is very difficult. Females usually deposit only 1 egg per fruit, and lay 150–200 eggs during their life. After hatching, larvae penetrate the peel and burrow into the fruit where they feed. Once the larva has entered the fruit, its control is impossible and the fruit no longer marketable. Although the pest prefers to infest ma-

ture fruits, it also infests immature fruits when the pest population is dense. The infested fruit abscises and rots on the soil surface. Pupation occurs in the soil (Cabrera-Asencio et al. 2012), as well as in the fruit (Biocontrol 2016). In Brazil the greatest levels of infestation of citrus fruits occur between Nov and Mar each yr.

It is important to determine if γ -irradiation of lepidopteran pupae in oxygen would have a greater effect on mortality of either the adults or their F_1 eggs and first instars than γ -irradiation in air. Thus the specific objective of this study was to determine the doses of γ -irradiation of *E. aurantiana* pupae in either oxygen or normal air required to either cause complete mortality of adults or of their F_1 progeny.

Material and Methods

SOURCE OF CITRUS FRUIT BORER PUPAE AND REARING PROCEDURES

A laboratory colony of *E. aurantiana* was established from locally field-collected specimens and reared in the laboratory for somewhat more than 20 generations on an artificial diet. The diet consisted of white bean (*Phaseolus vulgaris* L.; Fabales: Fabaceae) meal (750 g), wheat germ (60 g), soy (30 g), casein (30 g), yeast (30 g), ascorbic acid (3.6 g), sorbic acid (1.8 g), nipagin (1.5 g), tetracycline (113 mg), formaldehyde (3.6 mL), vitamin mixture (9 mL), agar powder (2.3 g), and water (1,200 mL). The vitamin mixture consisted of niacinamide (1 mg), calcium pantothenate (1 mg), thiamine (1 mg), riboflavin (0.25 mg), pyridoxine (0.25 mg), folic acid (0.25 mg), biotin (0.02 mg), vitamin B₁₂ (0.002 mg), and inositol (20 mg).

The freshly prepared hot diet was poured into 2.5 × 8.5 cm test tubes—to fill ¾ of the tube. After the diet had solidified and cooled to room temperature, 1 newly hatched larva was placed on the diet in each test tube. Each larva developed within the diet and pupated in the test tube. The pupae were removed from the test tubes and up to 200 were placed for adult emergence in each mesh-covered cage (25 × 25 × 25 cm). The adults were provided with a 10% honey solution in a 3 × 5 cm plastic vial with a perforated lid through which a dental roll was inserted for making the solution available through capillary action. The interior surfaces of the cage were covered with polyethylene sheets, which served as the substrate for oviposition. This use of polyethylene sheets greatly facilitated the counting of the eggs and the observation of hatching. The laboratory was maintained at 27 ± 2 °C, 70 ± 5% RH, and a photophase of 14 h. Under these conditions, the egg to egg generation time was 30 days.

EXPERIMENTAL SETUP AND PROCEDURES

Gammachrome dosimeters with range dose of 0.1–3 kGy were used and they were read using a Genesys 20 spectrophotometer. Dose certifications were made by the Institute for Energy and Nuclear Research – IPEN. The traceability of dose measurements was maintained by comparison with the international service assurance dose offered by the International Atomic Energy Agency, Vienna, Austria (Khoury et al. 2016).

The 250 mL flasks were centralized inside of the irradiator in order not to disrupt the uniformity of the radiation. Six dosimeters were positioned as follows: 1 on top of the flask, 1 at the bottom, and 4 equally-spaced at lateral positions. The uncertainty in each flask was ± 1.6%. The variation of measured doses was of ± 1.5% in the Gammacell-220 source.

Ten d-old pupae of *E. aurantiana* were used in this study. Each treatment had 4 repetitions. In each repetition 30 pupae were irradi-

ated with either 0, 100, 200 or 300 Gy; hence a total of 120 pupae were included in each treatment. In preparation for irradiation, the pupae were conditioned in 250 mL flasks. Pure oxygen from a 10 m³ cylinder (White Martins Ltda, Sao Paulo) was introduced into each flask with pupae. This was done by cutting a slit in the bottom of a rubber balloon and stretching it around the mouth of a 250 mL flask to make a gas-tight seal (Fig. 1). A flow of pure oxygen was introduced through the balloon to purge the flask and remove air. The flask was purged for several min and then sealed fully with the balloon partially inflated with oxygen to ensure that the flask contents were at a slightly greater pressure than atmospheric pressure. Control samples were treated in exactly the same manner as the irradiated samples except that they were irradiated in normal air (78% nitrogen, 21% oxygen and 1% miscellaneous gases) at the ambient atmospheric pressure.

Irradiated adults that died within 24 h after emergence were considered to be non-viable. Most non-viable adults had either deformed legs or abdomens or both. In each treatment the emerged males and females were allowed to mate. From each replicate in each treatment 100 eggs were collected and the percent hatch was determined.

STATISTICAL ANALYSIS

For analysis of variance with a completely randomized design, we used the Statistical Analysis System (SAS) version 9.0[®] (SAS Institute 2002). For the separation means, we used the Tukey HSD test at 5% probability.

Results

The numbers of adults that emerged after irradiation with various doses either in pure oxygen or normal air and the percent hatch of the F_1 eggs are shown in Table 1 (oxygen) and Table 2 (air). The percent adult emergence and the percent egg hatch in the irradiation in oxygen treatments were significantly different from either the control or the irradiation in air treatments.

EFFECT ON ADULT EMERGENCE AND EGG HATCH OF IRRADIATION OF PUPAE IN OXYGEN OR IN AIR

When the 10 d-old pupae were irradiated in oxygen (Table 1) with either 200 or 300 Gy the percent adult emergence was reduced from 93 ± 1.7 in the non-irradiated control to 50 ± 1.5 in the 200 Gy treatment and 13 ± 1.0 in the 300 Gy treatment, respectively. The corresponding percentages of F_1 egg hatch were 85 ± 1.5, 14 ± 1.3 and 0.0 ± 0.0, respectively. Thus irradiation of pupae in oxygen with 300 Gy prevented 100% of the irradiated population from reproducing. Therefore,

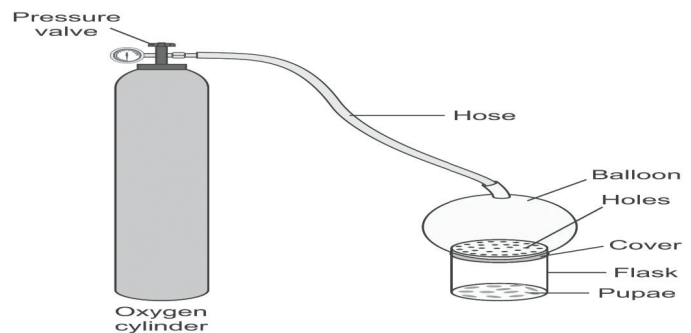


Fig. 1. Experimental set-up for replacing normal air surrounding *Ectodytolopha aurantiana* pupae with oxygen just prior to irradiation.

Table 1. Effects of irradiation in oxygen of 10 d-old *Ecdytoplopha aurantiana* pupae with either 100, 200 or 300 Gy of γ -radiation. The number of pupae irradiated in each treatment and the mean number ($\pm$ SD) of adults that emerged from them were recorded, and the percent adult emergence and the percent hatch of the eggs oviposited after emerged females and males had mated were calculated. Each treatment was replicated 4 times.

Dose (Gy)	No. of irradiated pupae/replicate	No. of emerged adults/replicate	Percent adult emergence	Percent hatch of F_1 eggs**
0	30	28 $\pm$ 1.2a	93 $\pm$ 1.7a	85 $\pm$ 1.5a
100	30	20 $\pm$ 1.3b	66 $\pm$ 1.5b	60 $\pm$ 1.5b
200	30	15 $\pm$ 1.1c	50 $\pm$ 1.5b	14 $\pm$ 1.3c
300	30	04 $\pm$ 0.9d	13 $\pm$ 1.0c ¹	No eggs
F	—	77.2	74.7	70.4
P values	—	< 0.001	< 0.001	< 0.001

*Means followed by the same letter do not differ by Tukey test at 5%.

**Percent hatch was based on samples of 100 eggs per treatment.

¹All of these adults were deformed and all of them died within 24 h.

300 Gy in oxygen is a viable candidate as a phytosanitary treatment of *E. aurantiana* pupae.

However when the 10 d-old pupae were irradiated in air (Table 2) with either 200 or 300 Gy the percent adult emergences was reduced from 90 $\pm$ 1.7 in the non-irradiated control to 66 $\pm$ 1.5 in the 200 Gy treatment and 33 $\pm$ 1.2 in the 300 Gy treatment, respectively. The corresponding percentages of F_1 egg hatch were 83 $\pm$ 1.6, 22 $\pm$ 1.4 and 5.0 $\pm$ 1.3, respectively. Thus irradiation of pupae in air with 300 Gy did not prevent 100% of the irradiated population from reproducing. Nevertheless irradiation of the pupae with 300 Gy in air resulted in 100% mortality of the F_1 larvae. Therefore based on the criterion of 100% mortality of F_1 eggs, 300 Gy in air can also be considered to be a viable candidate for a phytosanitary treatment.

Also, if we compare the effect of irradiation with the various doses on adult emergence in oxygen with those in air, we can observe that 5 fewer adults per 30 in the 100 Gy and the 200 Gy treatments emerged, and 6 fewer adults per 30 emerged in the 300 Gy treatments. Therefore the effect of γ radiation in oxygen was 16.7% greater than in air in the 2 intermediate dose treatment and 46.7% greater in the 300 Gy treatment in oxygen versus in air. Possibly these few data points indicate that the effect of irradiation increases exponentially with increasing doses.

Discussion

Arthur (2004) reared *E. aurantiana* by artificially infesting orange (*Citrus × sinensis* (L.) Osbeck; Sapindales: Rutaceae) fruits. Using 1,000 pupae per treatment in normal air, Arthur irradiated 12 d-old pupae in artificially infested orange fruits and found that in the 400 Gy treatment 1.8% of the adults emerged, but in the treatment with 500 Gy—the next higher dose—no adults emerged, and thus, 100% of the pupae

or pharate adults were killed. These results indicated that the prevention of adult emergence by the irradiation of *E. aurantiana* pupae in air requires a dose greater than 400 Gy. Irradiation in air of eggs or larvae of all instars with 300 Gy applied was sufficient to prevent both the development to the adult stage and reproduction. Arthur (2004) also reported that in non-irradiated samples 901 adults emerged from 1,000 pupae (90.1% emergence), which is in close agreement with the emergence rate from non-irradiated pupae in this study, i.e., 93% of those held in oxygen and 90% of those held in air.

The results presented in Tables 1 and 2 illustrate that oxygen increases the ability of ionizing radiation to prevent the development of *E. aurantiana* pupae. Thus with respect to adult emergence there was an increase in radiosensitivity of approximately 16.7% in the 100 and 200 Gy treatments and 46.7% in the 300 Gy treatment. When observing the viability of the emerged adults, the difference between air and pure oxygen was less well pronounced between 0 and 100 Gy, but there was an observed enhancement to radiation effects in oxygenated samples irradiated at 200 and 300 Gy, which suggests that the effect of irradiation in oxygen increases strongly with increasing doses.

The results are also in accordance with other studies, for example, Santos et al. (1975) irradiated *Musca domestica* L. (Diptera: Muscidae) in either hydrogen, nitrogen or oxygen using air as the control; and they concluded that oxygen potentiated the effect of irradiation in shortening the lifespan of the house fly compared to that observed when irradiating it in air. More fundamentally, Wiendl et al. (1976) concluded that gamma irradiation in oxygen of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae) can accelerate insect metabolism, and that the effects of γ radiation can be very harmful at the cellular level with consequences that can vary from inhibition of cell division to cell death. Also, in radiation biology and chemistry, it is well known that oxygen can modify and enhance radiation induced reactions by enabling

Table 2. Effects of irradiation in air of 10 d-old *Ecdytoplopha aurantiana* pupae with either 100, 200 or 300 Gy of γ -radiation plus a non-irradiated control. The number of pupae irradiated in each treatment and the mean number ($\pm$ SD) of adults that emerged from them were recorded, and the percent adult emergence and the percent hatch of the eggs oviposited after emerged females and males had mated were calculated.. Each treatment was replicated 4 times.

Dose (Gy)	No. of irradiated pupae/replicate	No. of emerged adults/replicate	Percent adult emergence	Percent hatch of F_1 eggs
0	30	27 $\pm$ 1.6a	90 $\pm$ 1.7a	83 $\pm$ 1.6a
100	30	25 $\pm$ 1.5a	83 $\pm$ 1.6a	59 $\pm$ 1.4b
200	30	20 $\pm$ 1.5b	66 $\pm$ 1.5b	22 $\pm$ 1.4c
300	30	10 $\pm$ 1.1c	33 $\pm$ 1.2b	5.0 $\pm$ 1.3d ¹
F	—	72.2	69.7	70.3
P values	—	< 0.001	< 0.001	< 0.001

*Means followed by the same letter do not differ by Tukey's HSD test at 5%.

**Percent hatch was based on samples of 100 eggs per treatment in a replicate.

¹The neonates either died or developed into second instars, but none of the latter survived.

the creation of intermediate and transient chemical products that are highly reactive and have greater stability and relatively longer lifetimes (Quintiliani 1986).

Spiracular function in pupae of the cecropia moth (*Hyalophora cecropia* [L.]; Lepidoptera: Saturniidae) is strongly affected by carbon dioxide and oxygen (Burkett & Schneiderman 1974). These authors found that when the intratracheal concentration of carbon dioxide is high or that of oxygen is low, the spiracular valves open; but when the intratracheal concentration of oxygen is high, they close. Moreover, they found that the carbon dioxide concentration needed to cause prolonged spiracular valve opening increased as the intratracheal oxygen concentration increased. These findings suggest that the oxygen concentration at the cellular level in *E. aurantiana* pupae might be increased by exposing the pupae to mixtures of carbon dioxide and oxygen shortly before and during irradiation to achieve phytosanitary control. In this way even lower gamma radiation doses than were found by irradiating pupae in pure oxygen may provide phytosanitary control of this and other insect pests.

Based on the present research and that of others, we concluded that placing infested produce in oxygen gas during irradiation can enhance the desired lethality of the use of ionizing radiation in phytosanitary irradiation. Further work would be necessary to determine if it would be practical and profitable to irradiate infested produce in oxygen gas as a commercial operation.

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References Cited

- Anonymous. 2000. Tecnologia contra o bicho-furão. Revista da Fundecitrus 96: 8-10.
- Arthur V. 2004. Use of gamma radiation to control three lepidopteran pests in Brazil, pp. 45-50 *In* Irradiation as a Phytosanitary Treatment of Food and Agricultural Commodities. International Atomic Energy Agency, IAEA-TEC-DOC-1427, Vienna.
- Baumhover AH. 1963. Influence of aeration during gamma irradiation of screw-worm pupae. Journal of Economic Entomology 56(5): 628-631.
- Bento JMS, Parra JERP, Vilela EF, Walder JM, Leal WS. 2001. Sexual behavior and diel activity of citrus fruit borer *Ecdytoplopha aurantiana*. Journal of Chemical Ecology 27(10): 2053-2065.
- Biocontrole. 2016. *Ecdytoplopha aurantiana*. www.biocontrole.com.br. Accessed IV-11-2016.
- Burkett BN, Schneiderman HA. 1974. Roles of oxygen and carbon dioxide in the control of spiracular function in cecropia pupae. Biological Bulletin 147: 274-293.
- Cabrera-Asencio I, Vélez AL, Henríquez SA, and Santiago-Blay JA. 2012. *Melicoccus bijugatus* jacquin (sapindaceae), quenepa: a new host plant record for the citrus fruit borer, *Gymnandrosoma aurantianum* Lima, 1927 (Lepidoptera: Tortricidae) and the genus *Gymnandrosoma* in Puerto Rico. Life: The Excitement of Biology 1(1): 3-16.
- Colabone MAP, Aguiar SMN, Wiendl FM. 1981. Mortalidade e reprodução de *Zabrotes subfasciatus* (Boh., 1833) exposto a gases atmosféricos concentrados e duas temperaturas. Ciência e cultura 33(3): 416-418.
- Garcia MS. 1998. Bioecologia e potencial de controle de *Ecdytoplopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae), o bicho-furão-dos citros, através de *Trichogramma pretiosum* Riley, 1879. Tese de doutorado. Piracicaba, Escola Superior de Agricultura "Luiz de Queiroz", USP, 118 pp.
- Hallman GJ. 2001. Irradiation as a quarantine treatment, pp. 113-130 *In* Molins R. [ed.], Food Irradiation: Principles and Applications. Wiley, New York.
- Hallman GJ. 2004a. Ionizing irradiation quarantine treatment against oriental fruit moth (Lepidoptera: Tortricidae) in ambient and low oxygen atmospheres. Journal of Economic Entomology 97: 824-827.
- Hallman GJ. 2004b. Irradiation disinfestation of apple maggot (Diptera: Tephritidae) in low oxygen and temperature storage. Journal of Economic Entomology 97: 1245-1248.
- Hallman GJ. 2005. Low oxygen reduces reproductive susceptibility of *Plum curculio* (Coleoptera: Curculionidae) to ionizing radiation. Florida Entomologist 88: 208-210.
- Hallman GJ, Phillips TW. 2008. Ionizing irradiation of adults of Angoumois grain moth Lepidoptera: Gelechiidae and Indian meal moth (Lepidoptera: Pyralidae) to prevent reproduction, and implications for a generic irradiation treatment for insects. Journal of Economic Entomology 101(4): 1051-1056.
- Hallman GJ, Hellmich RL. 2009. Ionizing radiation as a phytosanitary treatment against European corn borer (Lepidoptera: Crambidae) in ambient, low oxygen, and cold conditions. Journal of Economic Entomology 102(1): 64-68.
- Holthusen, H. 1921. Beiträge zur Biologie der Strahlenwirkung, Untersuchungen an Askariden. Archiv der gesamten Physiologie Pflugers 187: 1-24.
- Khoury HJ, Mehta K, de Barros VS, Guzzo PL, Parker AG. 2016. Dose assurance service for low energy x ray irradiators using an alanine-EPR transfer dosimetry system. Florida Entomologist 99(special issue #2): 14-17.
- Ohinata K, Ashra FM, Harris EJ. 1977. Mediterranean fruit flies: Sterility and sexual competitiveness in the laboratory after treatment with gamma irradiation in air, carbon dioxide, helium, nitrogen and vacuum. Journal of Economic Entomology 70: 165-168.
- Prates HS, De S. Pinto WB. 1988b. Ocorrência da mariposa das laranjas (*Gymnandrosoma aurantianum* Lima, 1927) na citricultura paulista. Laranja 9: 117-124.
- Prates HS, De S. Pinto WB. 1991. Controle do "bicho-furão" na citricultura. Informativo Coopercitrus 60: 18-24.
- Quintiliani M. 1986. The oxygen effect in radiation inactivation of DNA and enzymes. International Journal of Radiation Biology 50(4): 573-594.
- Santos JH, Wiendl FM, Arthur V. 1975. Uso de hidrogênio durante a irradiação para aumentar a esperança de vida de adultos de *Musca domestica* (L.). Revista de Ciência Agronômica 5(1-2): 73-77.
- SAS Institute. 2002. The SAS system for windows, release 9.0. SAS Institute, Cary, N.C.
- Smittle BJ. 1967. Effect of an aeration on gamma irradiation of house fly pupae. Journal of Economic Entomology 60: 1594-1596.
- Thoday JM, Read J. 1947. Effect of oxygen on frequency of chromosome aberrations produced by X-rays. Nature 160: 608.
- Wiendl FM, Tornisiello VL, Walder JMM, Sgrillo RB. 1976. Efeito do oxigênio, nitrogênio e dióxido de carbono durante a irradiação gama Co⁶⁰ de *Sitophilus zeamais* Mots e *Zabrotes subfasciatus* Boh., Boletim Científico, Piracicaba, CENA.
- Whiting AR. 1954. The effects of oxygen on the frequency of X-ray induced mutation in *Habrobracon* eggs. Genetics 39: 851.