Oxygen atmosphere potentiates radiation effects on *Brevipalpus yothersi* (Trombidiformes: Tenuipalpidae)

Andre Ricardo Machi[1,2], *and Valter Arthur*[2,1,*]

Abstract

The objective of the study was to compare the effect of pure oxygen to that of ambient air on gamma irradiation of *Brevipalpus yothersi* (Baker) (Trombidiformes: Tenuipalpidae). Flasks containing the mites were irradiated in a Gammacell-220 irradiator with Cobalt-60 emitting gamma radiation at a rate of 381 Gy/h. Seventy mites per flask replicated 4 times were irradiated in either pure oxygen or air with 0 (control), 200, 230, 270, or 300 Gy as the intended doses. All eggs, deutonymphs and adults were counted each day and the parameters of egg production, egg hatch, development and mortality were recorded. Data were analyzed with ANOVA and means were separated with Tukey's Honestly Significant Difference (HSD) test at 5% probability. Generally, irradiation of females with progressively larger doses—whether in oxygen or in air—resulted in progressively greater negative biological effects, and these effects were greater when females were irradiated in oxygen than in air. Non-irradiated gravid females exposed to pure oxygen deposited 79.3 ± 0.3 eggs per female compared to 73.0 ± 0.3 per female in ambient air. The numbers of eggs oviposited by females irradiated with the largest dose (300 Gy) were 29.1 ± 0.2 in air and 18.1 ± 0.3 in oxygen. In the ambient air + 270 Gy treatment egg hatch was 3.8 ± 0.1%, but in the oxygen + 270 Gy treatment it was 0%. When females were irradiated in air with 300 Gy, egg hatch was totally prevented. The number of F_1 deutonymphs per P generation female irradiated with 270 Gy in ambient air was 4.0 ± 0.1, but the corresponding number that descended from females irradiated in pure oxygen was significantly reduced to zero. Percentage survival of females at 22 d post treatment was 13.0 ± 0.1 d when females were irradiated in oxygen with 270 Gy compared to 16 ± 0.2 d when irradiated in air with 300 Gy. Therefore, 300 Gy is recommended as an appropriate candidate for phytosanitary irradiation of *B. yothersi* in air, and 270 Gy is recommended as an appropriate candidate for phytosanitary irradiation of *B. yothersi* in oxygen.

Key Words: deutonymph; egg viability; irradiation; leprosis mite; longevity; phytosanitary treatment

Resumen

El objetivo del estudio fue comparar la influencia de oxígeno puro a la de aire normal sobre el efecto de varias dosis de radiación gamma en *Brevipalpus yothersi* (Baker) (Trombidiformes: Tenuipalpidae). Se irradiaron los matraces que contenian los ácaros en un irradiador Gammacell-220 con Cobalto-60 que emite la radiación gamma a una velocidad de 381 Gy/h. Se irradiaron setenta ácaros por matraz replicados 4 veces con oxígeno puro o con aire con 0 (control), 200, 230, 270 y 300 Gy como las dosis previstas. Todos los huevos, deutoninfas y adultos se contaron cada día y se registraron los parámetros de la producción de huevos, eclosión de los huevos, el desarrollo y la mortalidad. Los datos fueron analizados con ANOVA y los medios se separaron con la prueba de la Diferencia Honestamente Significativa de Tukey (DHS) al 5% de probabilidad. En general, la irradiación de las hembras con dosis progresivamente más altas—ya sea en oxígeno o en aire —resultó en progresivamente mayores efectos biológicos negativos, y estos efectos fueron mayores cuando se irradiaron las hembras en oxígeno que en el aire. Las hembras grávidas-no irradiadas expuestas al oxígeno puro depositaron 79,3 ± 0,3 huevos por hembra en comparación con 73,0 ± 0,3 por hembra en el aire normal. El número de huevos depositados por las hembras irradiadas con la dosis más alta (300 Gy) fueron 29,1 ± 0,2 en el aire y el 18,1 ± 0,3 en oxígeno. En el aire + 270 escotilla normal de tratamiento fue de 3,8 Gy huevo ± 0,1%, pero en el 270 + tratamiento con oxígeno fue de 0,0 Gy es ± 0.0%. Cuando las hembras se irradiaron en el aire con 300 Gy, eclosión de los huevos se evito totalmente. El número de deutoninfas F_1 por hembra de la generación P irradiadas con 270 Gy en aire normal fue 4,0 ± 0,1, pero el número correspondiente que descendia de las hembras irradiadas en oxígeno puro se redujo significativamente a 0,0 ± 0,0. El porcentaje de sobrevivencia de las hembras a 22 días pos-tratamiento fue de 13,0 ± 0,1 d cuando las hembras fueron irradiadas en oxígeno con 270 Gy en comparación con 16 ± 0,2 d cuando fueron irradiadas en el aire con 300 Gy. Por lo tanto, la irradiación de los ácaros ya sea en el aire con 300 Gy o en oxígeno con 270 Gy impedió completamente la reproducción. Por lo tanto, se recomienda 300 Gy como un candidato adecuado para la irradiación fitosanitaria de *Brevipalpus yothersi* en el aire, y Gy se recomienda 270 como un candidato adecuado para la irradiación fitosanitaria de *Brevipalpus yothersi* en oxígeno.

Palabras Clave: deutoninfa; viabilidad de los huevos; irradiación; ácaros de la leprosis; longevidad; tratamiento fitosanitario

[1]University of São Paulo, Department of the Environment and Radiobiology, Institute of Nuclear Energy Research (IPEN), Lineu Prestes Avenue, 2242. University City, 05508-000 São Paulo, SP, Brazil

[2]University of São Paulo, Center for Nuclear Energy in Agriculture (CENA), Department of the Environment and Radiobiology. Piracicaba –SP 13400-970, Brazil

*Corresponding author; E-mail: armachi@cena.usp.br

Resumo

O objetivo foi comparar a influência de oxigênio puro e do ar normal sobre os efeitos das diferentes doses de radiação gama sobre *Brevipalpus yothersi* (Baker) (Trombidiformes: Tenuipalpidae). Para a irradiação, frascos contendo os ácaros foram irradiados em um irradiador gama de Cobalto 60, tipo Gammacell-220 sob uma taxa de dose de 381 Gy / h. Foram utilizados 70 ácaros por frasco, em um total de 4 repetições em oxigênio puro e ar normal nas doses de 0 (controle), 200, 230, 270 e 300 Gy. Todos os ovos, deutoninfas e adultos foram contados diariamente e os parâmetros de produção de ovos, eclosão de ovos, desenvolvimento e mortalidade foram avaliados. Os dados foram analizados com ANOVA e as médias com Tukey's Honestly Significant Difference (HSD) a 5% de probabilidade. Nas fêmeas irradiadas expostas ao oxigênio ou ar foi observado um progressivo aumento dos efeitos biológicos, estes efeitos foram mais evidenciados quando as fêmeas foram irradiadas em oxigênio. Fêmeas grávidas não irradiadas expostas ao oxigênio puro tiveram uma oviposição de 79,3 ± 0,3 ovos por fêmeas comparada a 73,0 ± 0,3 por fêmeas em ar normal. Nas fêmeas irradiadas com doses mais altas (300 Gy) foram 29,1 ± 0,2 em ar e 18,1 ± 0,3 em oxigênio. No tratamento com ar normal + 270 Gy a eclosão dos ovos foi de 3,8± 0,1%, mas no tratamento com oxigênio + 270 Gy foi 0,0 ± 0,0%. Quando as fêmeas foram irradiadas com 270 Gy em ar normal foi de 4,0 ± 0,1, mas o número de descendentes a partir das fêmeas irradiadas em oxigênio puro foi reduzido significantemente a 0,0 ± 0,0. A porcentagem de sobrevivência das fêmeas 22 dias após o tratamento foi de 13,0 ± 0,1 dias, quando as fêmeas foram irradiadas em oxigênio com 270 Gy comparado com 16,0 ± 0,2 dias quando irradiadas em ar com 300 Gy. Assim, 300 Gy em ar e 270 Gy em oxigênio são as doses recomendadas para prevenir a completa reprodução dos ácaros e podendo ser usada para a irradiação fitossanitária de *B. yothersi*.

Palavras Chave: deutoninfa; viabilidade de ovos; irradiação; ácaro da leprose; longevidade; tratamento fitossanitário

Brevipalpus mites (Trombidiformes: Tenuipalpidae) are polyphagous and can be found infesting citrus (*Citrus* spp.; Sapindales: Rutaceae), grape (*Vitis* spp.; Vitales: Vitaceae), coffee (*Coffea* spp.; Gentianales: Rubiaceae) and many ornamental plants in various regions of the world. In Brazil *Brevipalpus* mites are vectors of citrus leprosis virus (CiLV), the cause of the main viral disease of the Brazilian citrus industry (Childers & Rodrigues 2005; Bastianel et al. 2010).

The identification of *Brevipalpus phoenicis* (Geijskes), cited as the only vector of leprosis in Brazil has been reviewed by Beard et al. (2015), and synonyms of *B. phoenicis* have been confirmed as separate species; specifically these are *Brevipalpus hondurani* Evans, *Brevipalpus papayensis* Baker, *Brevipalpus phoenicis* sensu lato (Geijskes) and *Brevipalpus yothersi* Baker.

Recent studies made by Mineiro et al. (2015) confirmed the occurrence *B. yothersi* in citrus in municipalities of Artur Nogueira, Barretos, Campinas, Caraguatatuba, Cordeirópolis, Descalvado, Guarujá, Holambra, Itirapina, Jaguariúna, Monte Alegre do Sul, Reginópolis and Santos. *Brevipalpus yothersi* has now been recognized as one of the main vectors of citrus leprosis in Brazil (Ochoa et al. 2011; Roy et al. 2015, Beard et al. 2015), while *Brevipalpus phoenicis* sensu lato is actually little found in the country (Beard et al. 2015).

The life cycle of *Brevipalpus* spp. consists of the following stages: egg, larva, protonymph, deutonymph and adult (Childers et al. 2001). Duration from neonate emergence from the egg to the adult of *B. phoenicis* ranges from 10.6 d at 86 °F (30 °C) to 27.3 d at 68 °F (20 °C) under laboratory conditions (Haramoto 1969), and prolonged periods of temperatures either above 86 °F or below 68 °F (20 ºC) are fatal to immature stages (Kessing & Mau 1992). Nevertheless *Brevipalpus* spp. persist in greenhouses located in areas that experience much harsher temperatures.

Postharvest phytosanitary irradiation is growing in commercial application and offers some advantages compared with other treatments for the control of quarantine pests on exported commodities. Irradiation takes less time than fumigation and leaves no undesirable residues, while being at least as effective as any other existing method insect and mite control. Also, while the development of resistance to insecticides and acaricides is a growing problem, resistance to irradiation has never arisen in arthropods (Tilton & Burditt 1983; Byrne 1996). The use of methyl bromide as a fumigant to protect commodities is being phased out; indeed the 1997 Montreal Protocol Agreement stipulated that methyl bromide usage would be completely phased out by 2005 in developed countries and by 2015 in developing countries, (UNEP 2009). Nevertheless, certain uses of methyl bromide are exempt from phase-out, and these include the strictly regulated quarantine and preshipment applications. The use of modified atmospheres, i.e., the modification of the composition of the gaseous environment, can be advantageous in the control of insects and mites, but when used during irradiation can reduce the efficacy of gamma radiation(Heather & Hallman 2008). The major effect of this reduction in efficacy is thought to be due to reduced oxygen; therefore, increased oxygen during irradiation might increase efficacy.

The influence of gases during the irradiation of biological material was observed for the first time in 1921 with regard 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), depended on the oxygen tension during irradiation, but the frequency of chromosome aberrations produced by α-rays was not affected by oxygen tension. Whiting (1954) showed that x-irradiation in the parasitic wasp, *Habrobracon* sp. (Hymenoptera: Braconidae), with 500 roentgens in air produced more mutations in the eggs than 1,100 roentgens 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). 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.

Santos et al. (1975) irradiated adults of the house fly, *Musca domestica* L. (Diptera: Muscidae) in atmospheres greatly enriched with 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 ambient air was 10 krad (100 Gy), while the dose required to do so in either carbon dioxide, helium, nitrogen, or a partial vacuum was 16 krad (160 Gy)—a 1.6-fold increase caused by the depletion of oxygen in the ambient atmosphere.

Hallman (2004) irradiated 5th instars of the oriental fruit moth, *Grapholitha molesta* Busck (Lepidoptera: Tortricidae) present in fruit with a target dose of 200 Gy (195–232 Gy measured). In ambient atmospheres, no adults emerged from 58,779 fifth instars; however, in atmospheres flushed with nitrogen, 5.3% of adults emerged from 44,050 fifth instars, but these adults died at a faster rate than control adults and without laying eggs. Thus Hallman (2004) recommended a dose of

232 Gy—the maximum recorded when 200 Gy was targeted—to disinfest fruit of the oriental fruit moth under both ambient and hypoxic atmospheres.

Data regarding irradiation of mites are scarce. For the Eriophyidae, Tarsonemidae, Tenuipalpidae and Tetranychidae families of mites in the order Trombidiformes—which are quarantine pests for which postharvest phytosanitary treatments are needed—the necessary dose in air might be at least 350 Gy (Heather & Hallman 2008).

The objective this research was to evaluate and compare the effect on *B. yothersi* of various doses of gamma radiation and to determine how these doses are affected by irradiation in either pure oxygen or ambient air.

Materials and Methods

MITE REARING

The experiments were performed in the Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture (CENA / USP), Piracicaba city, Sao Paulo, Brazil. The mite colony was obtained from cultures that had been maintained in the laboratory for more than 3 yr in the Department of Acarology, College of Agriculture "Luiz de Queiroz" (ESALQ / USP), Piracicaba city, Sao Paulo, Brazil. The taxonomic identifications of *B. yothersi* was made with assistance of the Prof. Dr. Gilberto José de Moraes. The mite colony was reared continuously on Brazilian broad bean (*Canavalia ensiformis* L. DC; Fabales: Fabaceae) plants under laboratory conditions of 25 ± 1 °C, 70 ± 5% RH and a 14:10 h L:D photoperiod. Thus the colony was maintained in 40 × 27 × 11 cm plastic trays provided with *C. ensiformis* leaves and surrounded by hydrophobic cotton and Stickem® entomological glue (PROMIP, Limeira –SP, Brazil) to prevent possible escape of mites. Thereafter, the mites were placed in separate 1.5 × 1.5 × 1.5 m wooden cages covered with organdy fabric and maintained in a greenhouse.

Canavalia ensiformis plants infested with mites were used to obtain gravid adult females. The mites were identified under a stereomicroscope and gravid females were individually transferred with a fine-tip brush to a Petri plate containing a Brazilian broad bean leaf for determining the number of eggs oviposited per female, and the number that reached the deutonymph stage.

IRRADIATION PROCEDURES

The gravid adult female mites were transferred on Brazilian broad bean leaves into 250 mL flasks for irradiation in a Gammacell-220 irradiator (Atomic Energy of Canada, Ottawa, Ontario, Canada) located in CENA / USP with a source of Cobalt- 60 emitting gamma radiation at a rate of 381 Gy/h. The intended doses for the irradiated samples were 200 and 300 Gy. Gammachrome dosimeters with range dose of 0.1–3 kGy were used, and they were read with a Genesy 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 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.

The mites were irradiated at doses of 0 (control), 200, 230, 270, or 300 Gy, with a total of 4 replications per treatment with 70 mites per each flask for a total of 280 mites per each treatment in either air or pure oxygen (Santos et al. 1975; Arthur et al. 2016). To assess the effects of irradiation on F_1 eggs laid at 22 d after the female had been irradiated, a sample of 100 eggs in each of 4 replicates per treatment was taken.

In preparation for irradiation, the gravid adult females were conditioned in 250 mL flasks. Pure oxygen from a 10 m^3 cylinder (White Martins Ltda, Sao Paulo, Brazil) was introduced into each flask with the mites (Fig. 1). 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 the 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. In pre-conditioning the samples before irradiation, the flasks were immediately closed after they had been filled with oxygen and in the same moment they placed into the irradiator. The duration of irradiation was 31.4 min for 200 Gy and 47.2 min for 300 Gy. Samples irradiated in ambient air (78% nitrogen, 21% oxygen and 1% miscellaneous gases) were treated in essentially the same manner as those irradiated in oxygen, except they were exposed only to air at the ambient atmospheric pressure. In the oxygen plus 0 Gy treatment, the mites were exposed to oxygen for 47.2 min.

After irradiation, the leaves containing the mites were removed from the flasks and placed on new leaves in dishes for counting of individuals. The different phases of *B. yothersi* (eggs, protonymphs, deutonymph and adults) in the F_1 generation on leaves were observed daily to record viability, fertility and mortality.

STATISTICAL ANALYSIS

The experiments were completely randomized, and the data were evaluated with ANOVA (analysis of variance) using the Statistical Analysis System (SAS) version 9.0® (SAS Institute 2002). The Tukey HSD test at 5% probability was used to separate the means.

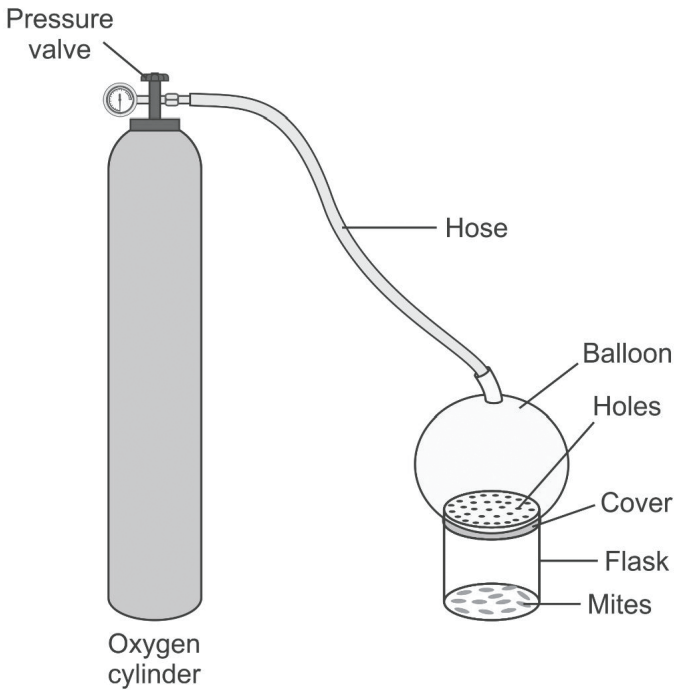

Fig. 1. Experimental set-up for replacing ambient air surrounding *Brevipalpus yothersi* adult females with oxygen prior to irradiation.

Results

EFFECT OF IRRADIATING GRAVID FEMALES IN EITHER AIR OR OXYGEN ON NUMBER OF EGGS LAID

Table 1 shows that the number of eggs laid by non-irradiated females in oxygen was slightly greater than those laid in ambient air. The number of eggs laid by irradiated females decreased significantly as the dose increased both in ambient air and in pure oxygen. Moreover, Table 1 shows that there were substantial and significant differences in the number of eggs laid by irradiated female mites when the same dose was applied in either ambient air or oxygen. Thus when the females were irradiated in air with 200 Gy, the number of eggs laid per female was 63.4 ± 0.2 versus 46.3 ± 0.5 in oxygen. Likewise, when the females were irradiated with 300 Gy in air the number of eggs laid per female was 29.3 ± 0.2 versus 18.1 ± 0.3 in oxygen.

EFFECT OF IRRADIATING GRAVID FEMALES IN EITHER AIR OR OXYGEN ON NUMBER OF F_1 DEUTONYMPHS

Table 2 shows that the number of F_1 deutonymph progeny of non-irradiated females exposed only to air (56.3 ± 0.2) was slightly greater than the number of deutonymph progeny of non-irradiated females (52.3 ± 0.2) exposed to oxygen for 47.2 min—the duration needed to irradiate females with 300 Gy. Irradiation of females with progressively larger doses—whether in oxygen or in air— resulted in the production of progressively fewer deutonymphs, and this effect was greater when females were irradiated in oxygen than in air. In addition Table 2 shows that the number of F_1 deutonymphs per P generation female irradiated with 270 Gy in ambient air was 4.0 ± 0.1, but the corresponding number that descended from females irradiated in pure oxygen with 270 Gy was significantly reduced to 0. However to achieve this latter result by irradiating in air the dose had to be increased to 300 Gy.

Some of the progeny that descended from females irradiated with various doses both in air and in oxygen displayed morphological abnormalities—most notably in their legs, and some developed abnormally, while others died a few h after hatching.

EFFECT OF IRRADIATION OF GRAVID FEMALES IN EITHER AIR OR OXYGEN ON THEIR SURVIVAL AT 22 D POST TREATMENT

The results in Table 3 show that in the non-irradiated control treatments, percentage survival of the non-irradiated adult females at 22 d post irradiation in ambient air (21 ± 0.1) vs in pure oxygen (22 ± 0.1) were very similar. In the ambient air + 200 Gy treatment, percentage survival of the females at 22 d post treatment was 20 ± 0.1, which was almost the same as in the corresponding control. However, the percentage of survival at 22 d in the oxygen + 200 Gy treatment was 17 ± 0.2, which was not significantly different than in the air + 270 Gy treatment; and the percentage of survival in the oxygen + 230 Gy treatment was not significantly different than in the air + 300 Gy treatment. Yet even in the oxygen + 300 Gy treatment, 10.0% survived 22 d post irradiation.

Table 1. Average number of eggs (± SE) oviposited per of *Brevipalpus yothersi* female that had been irradiated in either ambient air or pure oxygen with 0 (control), 200, 230, 270, or 300 Gy of gamma radiation.

	Number of eggs (± SE)/female*	
Dose (Gy)	Pure oxygen	Ambient air
0	79.3 ± 0.2a	73.0 ± 0.2b
200	46.3 ± 0.5e	63.4 ± 0.2c
230	40.7 ± 0.1f	58.6 ± 0.2d
270	24.7 ± 0.1i	36.5 ± 0.2g
300	18.1 ± 0.3j	29.3 ± 0.2h
F	68.7	
P value	< 0.001	

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

Table 2. Average number (± SE) of deutonymphs per female that developed from the eggs oviposited by *Brevipalpus yothersi* females irradiated in either ambient air or pure oxygen with 0 (control), 200, 230, 270, or 300 Gy of gamma radiation.

	Number of deutonymphs (± SE)/female*	
Dose (Gy)	Pure oxygen	Ambient air
0	52.3 ± 0.2b	56.3 ± 0.2a
200	7.7 ± 0.3d	9.7 ± 0.3c
230	3.9 ± 0.1f	7.4 ± 0.2e
270	0.0 ± 0.0g	4.0 ± 0.1f
300	0.0 ± 0.0g	0.0 ± 0.0g
F	67.8	
P value	< 0.001	

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

EFFECT OF IRRADIATION OF GRAVID FEMALES IN EITHER AIR OR PURE OXYGEN ON PERCENTAGE HATCH OF THEIR EGGS

Table 4 shows the average percent viability (hatch) of eggs oviposited 22 d after *B. yothersi* females had been irradiated of in either ambient air or pure oxygen with 0 (control), 200, 230, 270, or 300 Gy of gamma radiation. The percentage hatch of eggs oviposited by non-irradiated females in the 2 treatments were fairly similar, being 88.6% ± 0.2 in ambient air vs. 85.6% ± 0.2 in pure oxygen. In the ambient air + 230 Gy treatment, the percentage of egg hatch was reduced to 9.9 ± 0.2% and compared to 2.95 ± 0.1% in the oxygen + 230 Gy treatment. However, in the oxygen + 270 Gy treatment, egg hatch was 0%, while 3.8 ± 0.1% hatched in the ambient air + 270 Gy treatment. When females were irradiated in air with 300 Gy, egg hatch was totally prevented.

Discussion

The modest reduction in percentage survival and the longevity of female adults irradiated in air with 200 Gy—which was only slightly less than in the non-irradiated control—may be attributed to the capacity

Table 3. Mean percentage survival (% ± SE) of *Brevipalpus yothersi* adult females at 22 d after irradiation in either ambient air or pure oxygen with either 0 (control), 200, 230, 270, or 300 Gy of gamma irradiation.

	Percent survival at 22 d post-irradiation*	
Doses (Gy)	Irradiation in pure oxygen	Irradiation in ambient air
0	22.3 ± 0.1a	21.1± 0.1b
200	17.0 ± 0.2e	20.0 ± 0.1c
230	15.9 ± 0.1f	19.0 ± 0.2d
270	13.0 ± 0.1g	17.5 ± 0.2e
300	10.0 ± 0.2h	16.0 ± 0.2f
F	60.6	
P value	< 0.001	

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

Table 4. Mean percentage viability of eggs oviposited 22 d after *Brevipalpus yothersi* females had been irradiated in either ambient air or pure oxygen with 0 (control), 200, 230, 270, or 300 Gy of gamma radiation.

	Percentage of viable eggs laid on d 22	
Dose (Gy)	Pure oxygen	Ambient air
0	85.6 ± 0.2b	88.6 ± 0.2a
200	5.0 ± 0.1e	14.1 ± 0.1c
230	2.9 ± 0.1g	9.9 ± 0.2d
270	0.0 ± 0.0h	3.8 ± 0.1f
300	0.0 ± 0.0h	0.0 ± 0.0h
F	45.9	
P value	< 0.001	

To assess the effects of irradiation on F_1 eggs laid at 22 d after the females had been irradiated, a sample of 100 eggs per treatment was taken in each of 4 replicates.

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

of *B. yothersi* to resist stressful environmental changes and to maintain its longevity in order to reproduce (Force 1975; Sakurai 2000).

The results of this study are in agreement 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 concluded that oxygen potentiated the effect of irradiation in shortening the lifespan of the house fly compared to irradiating it in air. 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 the creation of intermediate and transient chemical products, i.e., free radicals, that are highly reactive and some of which have enhanced stabilities and relatively longer lifetimes (Quintiliani 1986).

The radiotolerance of insects and mites increases as development progresses through successive life stages, and differs in various taxonomic groups. Lee & Ducoff (1989) noted that irradiated organisms have the ability to repair some of the damage done to DNA by irradiation. Thus gamma radiation effects can be more harmful to cells and the consequences can range from cellular inhibition to death. However, as explained by Muller (1950), LaChance (1967) and Robinson (2005), the main cause of cell death and the induction of sexual sterility by ionizing radiation is the induction of dominant lethal mutations—most of which are chromosome breaks, chromosomal rearrangements and genetic imbalance created by the loss of chromosome fragments. Chromosomes are by far the most vulnerable to the breakage, genetically imbalanced recombination and loss in cells that are dividing. The proportion of cells that undergo division is very great in immature insects, but in most taxa only the germ cells—cells involved in gametogenesis—actively divide in adult insects. Most taxa of adult insects are very radioresistant because their somatic cells do not divide. However, in some species of the Curculionidae, such as *Anthonomus grandis* Boheman, the digestive enzymes of the gut are produced in apocrine cells that slough off the midgut epithelium and must be continuously replaced by cell division (Riemann & Flint 1967).

The modest reduction in percent survival and the longevity of female adults irradiated in air with 200 Gy—which was only slightly less than in the non-irradiated control—may be attributed to the capacity of *B. yothersi* to resist stressful environmental changes and to maintain its longevity in order to reproduce (Force 1975; Sakurai 2000). This great capacity of the adult to tolerate radiation damage strongly suggests that no vitally important somatic cells in *B. yothersi* adults undergo cell division, and that the genes in chromosomes damaged by the ionizing radiation continue to serve as templates for vitally important enzymes and other proteins and peptides. In any case, Hallman (2000) noted that in some taxa, including mites, arthropods irradiated with doses that completely prevent reproduction result in equal or greater longevity compared with non-irradiated adults.

According to our results, irradiation of gravid *B. yothersi* adult females with 300 Gy completely prevented hatch of F_1 eggs. Based on criterion of 100% of mortality F_1 eggs and F_1 deutonymphs, the dose of 300 Gy can also be considered a viable candidate for phytosanitary irradiation of *B. yothersi* in air, but this can be reduced to 270 Gy by irradiating in oxygen.

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