Journal of Economic Entomology, 110(1), 2017, 80–86
doi: 10.1093/jee/tow273
Advance Access Publication Date: 28 December 2016
Research article

Effects of Low-Oxygen Environments on the Radiation Tolerance of the Cabbage Looper Moth (Lepidoptera: Noctuidae)

Catriona H. Condon,[1] Sabrina White,[1] Robert L. Meagher,[2] Laura A. Jeffers,[3] Woodward D. Bailey,[4] and Daniel A. Hahn[1,5]

[1]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611 (chcondon1@gmail.com; sabrinawhite407@gmail.com; dahahn@ufl.edu), [2]USDA-ARS, Center for Medical and Veterinary Entomology, Gainesville, FL 32608 (rob.meagher@ars.usda.gov), [3]USDA-APHIS-PPQ Center for Plant Health Science and Technology, Raleigh, NC 27606 (laura.a.jeffers@aphis.usda.gov), [4]USDA-APHIS-PPQ Center for Plant Health Science and Technology, Miami, FL 33158 (Woodward.D.Bailey@aphis.usda.gov), and [5]Corresponding author, e-mail: dahahn@ufl.edu

Subject Editor: Lisa Neven

Received 13 October 2016; Editorial decision 1 November 2016

Abstract

Ionizing radiation is used as a phytosanitary treatment to mitigate risks from invasive species associated with trade of fresh fruits and vegetables. Commodity producers prefer to irradiate fresh product stored in modified atmosphere packaging that increases shelf life and delays ripening. However, irradiating insects in low oxygen may increase radiation tolerance, and regulatory agencies are concerned modified atmosphere packaging will decrease efficacy of radiation doses. Here, we examined how irradiation in a series of oxygen conditions (0.1–20.9 kPa O_2) alters radiotolerance of larvae and pupae of a model lepidopteran *Trichoplusia ni* (Hubner) (Diptera: Noctuidae). Irradiating in severe hypoxia (0.1 kPa O_2) increased radiation tolerance of insects compared with irradiating in atmospheric oxygen (20.9 kPa O_2). Our data show irradiating pharate adult pupae at 600 Gy in moderately severe hypoxia (5 kPa O_2) increased adult emergence compared with irradiation in atmospheric oxygen (20.9 kPa O_2). Our data also show that in one of the three temporal replicates, irradiating *T. ni* larvae in moderately severe hypoxia (5 kPa O_2) can also increase radiotolerance at an intermediate radiation dose of 100 Gy compared with irradiating in atmospheric oxygen conditions, but not at higher or lower doses. We discuss implications of our results in this model insect for the current generic doses for phytosanitary irradiation, including the recently proposed 250 Gy generic dose for lepidioptera larvae, and temporary restriction on irradiating commodities in modified atmosphere packaging that reduces the atmosphere to < 18 kPa O_2.

Key words: phytosanitary irradiation, modified atmosphere, hypoxia

Ionizing radiation is used to treat fresh commodities after harvest to prevent the spread of pest species to new areas. Irradiation damages many components of insect cells and can lead to sterility or death through the accumulation of dominant lethal mutations in DNA (Robinson 2005). Due to this broad mode of action, phytosanitary irradiation treatments can be highly effective against a wide range of insects. Moreover, because phytosanitary irradiation is broadly effective against insect pests, generic doses developed to disinfest one commodity can often be used for the treatment of other fresh commodities (Hallman 2012).

Currently, the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) requires a generic absorbed dose of 400 Gy to control insect pests that are present on fresh commodities (APHIS 2015). An exception is that the 400 Gy generic dose is not recommended for controlling pupae or adults of Lepidoptera, although these life stages are very rarely found in fresh commodities (Hallman et al. 2013a,b). Where tephritid fruit flies are the only pests of concern for a commodity, a generic dose of 150 Gy has been approved for use by both the USDA APHIS and the International Plant Protection Convention (IPPC; APHIS 2015, Hallman et al. 2013). Both generic doses are well below the 1 kGy limit approved by the United States Food and Drug Administration for fresh fruit and vegetables (FDA 1986). Additional generic treatment doses are being developed, as growers are increasingly interested in expanding international market access of fresh commodities using phytosanitary irradiation (PI, hereafter; Follett 2009, Hallman et al. 2013a,b).

PI is an increasingly attractive treatment for commodity growers because irradiating produce has several advantages over heat, cold, and fumigation treatments, such as allowing riper and thus higher quality fruits to be imported (Hallman 2012). Fresh commodities

can also be irradiated after packing, allowing produce to be shipped and treated in a packaging that maintains an atmosphere optimal for product shelf life and quality (Wang 1990, Follett and Wall 2012). Modified atmosphere packaging (MAP, hereafter) that maintains a low-oxygen environment during transport slows the rate of plant respiration and delays ripening, providing a higher value product to the marketplace (Kader 1980, Wang 1990). MAP packaging also acts as an additional pest-proofing layer preventing pests from reinfesting a commodity after a phytosanitary treatment. Using MAP in combination with PI treatments is highly attractive to growers; however, this combination is of concern to regulators because severe hypoxia (<1 kPa O_2) is known to increase the radiation tolerance of insects (Follett and Neven 2006, Hallman et al. 2010).

When insects are irradiated in severe hypoxia (<1 kPa O_2), higher doses of radiation are required to prevent adult emergence or induce sterility than in normal air, and the response occurs across insect taxa and stages of development (Balock et al. 1963; Fisher 1997; Hallman 2000, 2004, 2005, 2012). Before and during a phytosanitary irradiation treatment, USDA APHIS Plant Protection and Quarantine prohibits the use of modified atmosphere packaging that creates atmospheres of <18% O_2 surrounding fresh fruits, vegetables, and cut flowers (L.A.J., personal communication). Furthermore, the International Standards for Phytosanitary Measures number 28 (ISPM 28) Phytosanitary Treatments for Regulated pests states that phytosanitary irradiation treatments may not be performed on commodities stored in modified atmospheres (IPPC 2009). However, there is one exception to this general rule, phytosanitary irradiation of the Oriental fruit moth *Grapholita molesta* (Busck) in severely hypoxic environments (<1 kPa O_2) has been approved (ISPM 28, Annex 11, IPPC 2010). However, little data exist that directly examines the effect of irradiating insects in hypoxic environments that are used for commodity transport. Follett et al. (2013) used different MAP bags to test the effect of phytosanitary irradiation treatments in severe (1–4 kPa O_2), moderately severe (3–8 kPa O_2), and moderate (11–15 kPa O_2) hypoxia on late-instar melon fly (*Bactrocera cucurbitae* Coquillett) reared on artificial diet and inserted into the cavity of papayas. More adult flies emerged after irradiating larvae in severe hypoxia than in normal air, but this result was not statistically significant (Follett et al. 2013). Furthermore, this slight trend toward greater survival in severe hypoxia was only observed at a dose of 50 Gy, and substantial numbers of melon fly larvae (9,000) and medfly larvae (3,800) treated in severe hypoxia at the 150 Gy generic dose for fruit flies failed to emerge as adults (Follett et al. 2013). Additional work is required to investigate at what levels of hypoxia radioprotective effects are induced in insects and whether irradiating insects in hypoxia may reduce the efficacy of the radiation doses recommended for phytosanitary irradiation.

In this paper, we used the cabbage looper moth, *Trichoplusia ni* (Hubner) (Diptera: Noctuidae), as a model insect to study the effects of irradiation across a range of low-oxygen conditions to examine the degree to which hypoxia influences radiation-induced mortality and sterility. Specifically, we examined the effect of irradiation in five oxygen atmospheres between 20.9–0.1 kPa O_2 (all <0.01 kPa CO_2, balanced with N_2) on cabbage looper late fifth-instar cabbage looper larvae and late pharate adults ~48 h prior to emergence (late stage pupae). Previously, López-Martínez et al. (2016) showed that irradiating *T. ni* in severe hypoxia (<1 kPa O_2) increased the radiation tolerance of larvae, early pupae, and late pharate adults. Here, we conditioned larvae and late pharate adults to 0.1, 5, 10, 15, or 20.9 kPa of O_2 (<0.01 kPa CO_2, balanced with N_2 in all) for 1 h and then irradiated insects in these atmospheres. Because insect life stages further in development toward adulthood tend to be more radiation resistant (Hallman et al. 2010), we used two series of radiation doses to examine the effect of low-oxygen conditions on radiation-induced sterility. Larval *T. ni* were irradiated at 0, 50, 100, 150, or 200 Gy, whereas late pharate adults were irradiated at 0, 200, 400, 600, or 800 Gy. We predicted that with decreasing O_2 content, both larvae and pharate adults would have increasing tolerance to radiation treatments. We find that irradiating *T. ni* in 5 kPa O_2 at room temperature (~21–23 °C) can increase the tolerance of these insects to some irradiation treatments compared with insects irradiated at standard levels of atmospheric oxygen.

Materials and Methods

Cabbage looper larvae were reared at the United States Department of Agriculture's Agricultural Research Service's Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS-CMAVE) in Gainesville, FL. Larvae were reared on a pinto bean diet at low density with two to four larvae per well (4 by 4 by 2.5 cm) and maintained under a long-day photoperiod of 14:10 (L:D) h at 25 ± 1°C (Guy et al. 1985). We irradiated *T. ni* at two different stages of their development: late fifth-instar wandering larvae (11–12 d after egg hatch) and late pharate adults ~48 h before emergence (16–17 d after egg hatch), two stages that we expect to be among the most tolerant, even though pharate adults are not likely to be found in commodities. A late pharate adult is a nearly developmentally complete adult that has detached from the pupal cuticle, but has not emerged from the pupal cuticle (Klowden 2007). This stage is often referred to as the late pupal stage and is well known to differ physiologically from individuals early in pupal development (Chapman 2013). Only females were irradiated in this latter stage of development; these individuals were sexed during pupation (~14–15 d, Guy et al. 1985) and typically irradiated 24–48 h before adult emergence.

Prior to irradiation, 10 larvae or 10–14 pharate adults were placed in open petri dishes inside 1,180-ml plastic chambers (18.5 by 11.9 by 7.3 cm, Rubbermaid, Atlanta, GA). Each chamber had a three-way, leur-lock valve (Cole-Parmer, Vernon Hills, IL) glued into holes at both ends, which enabled the air inside to be flushed when the lid was sealed. Insects were placed in a chamber and the lid was sealed with vacuum grease and fastened with a zip tie for additional tension. Then, chambers were flushed at a flow rate of 500 ml/min for 5–10 min with one of five oxygen concentrations: 0.1, 5, 10, 15 kPa of O_2, or ambient O_2 atmosphere (~20.9 kPa O_2). Hypoxic gases of 5.02, 10.04, and 15.04 kPa of O_2 in a balance of N_2 (±2%, <0.01 kPa CO_2) were mixed in K-type pressurized cylinders and certified by Airgas, Jacksonville, FL. Oxygen concentrations were verified on a FOXBOX field gas analysis system (Sable Systems International, Las Vegas, Nevada, NV). For the most hypoxic treatment, chambers were flushed with industrial-grade nitrogen (~0.1 kPa O_2), also prepared in a K-type pressurized cylinder by Airgas (Jacksonville, FL). Ambient O_2 in the control treatments was first dried and scrubbed with a Drierite and Ascarite II column to remove CO_2 and then pumped into the chamber at a flow rate of 100 ml/min. Chambers were flushed with treatment gases from the gas cylinders (0.1–15 kPa O_2) for 5–10 min at ~500 ml/min so that each chamber would be completely flushed three to six times. After flushing, larvae and pharate adults were acclimated to the oxygen atmosphere for 1 h before irradiation. All insects were irradiated after 1 h and remained in the atmospheric treatment for a total of 2 h, all at room temperature (22–24 °C), to ensure that atmospheric treatment conditioning was of the same length for all radiation treatments.

To verify that oxygen consumption by insects inside the chamber did not greatly alter the target oxygen concentration of the hypoxia treatments, 10 fifth-instar wandering *T. ni* were exposed to our two most hypoxic treatments (5 and 10 kPa O_2) without irradiation at room temperature (21–23 °C) and full light. The chambers were filled with the target atmosphere and sealed as described above, and 10 ml of air was extracted with a syringe at the time the chambers were closed and again after insects were sealed inside the chambers for 2 h. We measured the volume of O_2 in the 10-ml samples using the FOXBOX field gas analysis system and compared the relative percent change in O_2 between the initial sample and after 2 h. Larvae were found to decrease the target oxygen concentration by <2 kPa O_2 during the 2-h atmospheric conditioning at room temperature (21–23 °C).

All atmospheric treatments were applied and insects were irradiated at the Florida Department of Agriculture and Consumer Services (FDACS) in Gainesville, FL, at room temperature (21–23°C). Pharate adults were irradiated at 0, 200, 400, 600, and 800 Gy using a Varian L-1000A electron-beam irradiator (5.2 MeV, 1.5 kW). Controls that did not receive radiation (0 Gy group) were also transferred to the FDACS facility, exposed to modified atmospheres, and transferred back afterwards to mimic the spectrum of manipulations performed on irradiated individuals. E-beam irradiation was delivered in passes of 200 Gy, requiring chambers in the higher irradiation treatments to pass multiple times through the irradiator on an automated conveyer belt, each pass took 12 min. To irradiate larvae, we used the same electron beam accelerator with a copper plate to convert electron beam radiation into X-rays. Larvae were X-ray irradiated at 0, 50, 100, 150, and 200 Gy. X-ray radiation doses were delivered based on time during a single pass, with higher doses requiring longer exposure. For both X-ray and E-beam irradiation, dose accuracy was confirmed using Gafchromic HD-810 film (International Specialty Products, Wayne, NJ; uncertainty 5.6% at 95% confidence level). For E-beam doses above 400 Gy, new dosimeters were attached to the chambers after the first two passes of 200 Gy. Absorbed doses of radiation were within 10% of the target radiation dose for all treatments (Dose uniformity ratio <1.1). In total, 708 larvae and 964 pharate adults were irradiated across five irradiation doses and five oxygen treatments. For each atmosphere × radiation treatment, three to five independent replicates of 8–12 individuals each were performed with insects from temporally distinct cohorts (i.e., temporal replicates) taken from the USDA-ARS CMAVE colony across a period of 10 wk.

To determine the effect of atmospheric oxygen content on irradiation treatments, we scored the adult emergence of *T. ni* irradiated at both larvae and pharate adult stages. After irradiation, larvae and pharate adults were carefully moved to individual 30-ml plastic cups with a perforated lid for observation and maintained under a long-day photoperiod in a chamber at 14:10 (L:D) h and 25 ± 1 °C. For both life stages that were irradiated, adult emergence was scored as either a success or failure: a success was scored when an adult emerged without any visible defect and had the ability to fly when prodded. Pharate adults typically emerged with 24–48 h after the irradiation treatment. Irradiated larvae emerged as adults 5–6 d after the treatment. Larvae that died within 24 h of the treatment failed to pupate and were presumed to have died from handling injury. These larvae could not be identified as male or female and were excluded from the analysis.

We also examined whether irradiation in low-oxygen conditions affected the fertility of pharate adults. After irradiation, female *T. ni* that successfully emerged as adults were moved to a clear 180-ml perforated plastic cup with access to 2.25% honey and 2% sugar solution and maintained under a long-day photoperiod of 14:10 (L:D) h at 25 ± 1°C. Twenty-four hours later, we placed a single nonirradiated male *T. ni* from the same cohort with the irradiated female for 48 h. After this time, a paper coffee filter was added as an oviposition site. Female *T. ni* were left to lay eggs on the paper coffee filter for four nights (4–8 d after adult emergence), during the time when females have the highest fecundity (Shorey 1963, Landolt 1997). The filter was then removed and stored in an individual plastic bag with a moist cotton ball to maintain a humid environment (~100% RH) for egg development. Viable eggs hatched within 4 d, and fertility was scored by the presence or absence of newly hatched larvae on the filters.

Statistical Analysis

Emergence and fertility data were binomially distributed and all data were analyzed using logistic regression. We used a bias-reduced generalized linear model (Firth 1993) because many of our treatment levels produced data where all insects responded the same way (i.e. all 1 or all 0). Fitting the bias reduction GLM (brglm) improves the maximum likelihood estimation of the model coefficients and standard errors (Kosmidis and Firth 2010). All data were analyzed using R and the brglm library (Kosmidis and Firth 2010). Irradiation treatment and oxygen atmosphere were modeled as categorical fixed effects. Because random effects are currently not implemented in the brglm package, we also fit temporal cohort as a fixed effect. Likelihood ratio tests were performed to determine the significance of the fixed effects and their interactions. Post hoc multiple comparisons testing was performed to investigate emergence and fertility at the different treatment levels, with *P*-values corrected using the Holm method (Holm 1979). For all analyses, significance was taken at $P < 0.05$. Data presented in the figures are based on the coefficient estimates and their standard errors from the best fitting model for each dataset. The best fitting model was determined via likelihood ratio tests of competing models.

Results

Larvae

Irradiating *T. ni* in 0.1 kPa O_2, our severe hypoxic treatment, increased the radiation tolerance of both male and female larvae (between 100–200 Gy) compared with larvae irradiated in all other oxygen concentration treatments (Fig. 1A and B). Female *T. ni* larvae were more susceptible to radiation than males, but this effect did not vary across oxygen or radiation treatments (sex: $\chi^2_1 = 7.7$, $P < 0.01$). Larvae treated at 0.1 kPa O_2 had the greatest proportion of adult emergence, with the greatest effect of 0.1 kPa O_2 occurring between 100–200 Gy (oxygen × radiation dose interaction: $\chi^2_{16} = 65.2$, $P < 0.001$). This effect of 0.1 kPa O_2 was most apparent at 100 Gy, where ~90% of larvae that were irradiated in severe hypoxia emerged as adults, while the emergence rate of moths from the hypoxic (5, 10, and 15 kPa O_2) and normoxia (20.9 kPa O_2) treatments fell to 30% or less.

At the radiation treatment of 100 Gy, larvae that were treated in 5 kPa O_2 also had a greater rate of emergence than those irradiated in normoxic air (20.9 kPa O_2; Fig. 1A and B). However, this increase in adult emergence after 5 kPa O_2 conditioning occurred in only one of the three larvae cohorts that we performed this experiment with (radiation × cohort interaction: $\chi^2_8 = 40.44$, $P < 0.001$). In one of the experimental replicates, 7 out of 10 larvae emerged as adults after 100 Gy irradiation in 5 kPa O_2, whereas in the two remaining replicates, no adults emerged despite the fact that radiation doses

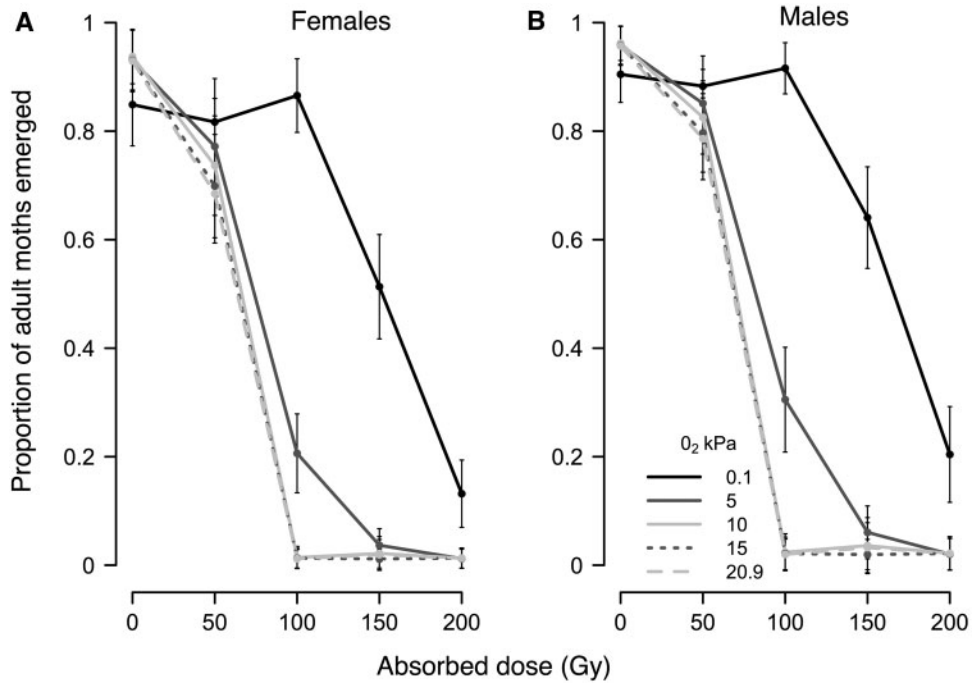

Fig. 1. Proportion of (**A**) female and (**B**) male adult *T. ni* moths that emerged after irradiation as wandering fifth instars in oxygen environments 0.1–20.9 kPa O_2. Data presented are based on the coefficients from the best-fit statistical model and their standard errors.

and gas concentrations for this unusual replicate did not stand out as different from the other two replicates.

At 150 Gy, ~50% of larvae treated in 0.1 kPa O_2 emerged as adults, while the proportion of moths that emerged after treatment in hypoxia (5, 10, and 15 kPa O_2) and normoxia (20.9 kPa O_2) was close to zero. At 200 Gy, the only adult *T. ni* which emerged had been irradiated in 0.1 kPa O_2.

Pharate Adults

Pharate adults also had greater adult emergence when treated with 0.1 kPa O_2 prior to and during irradiation, but only at the highest doses of irradiation at 600–800 Gy (oxygen × radiation treatment: $\chi^2_{16} = 38.2$, $P < 0.01$, Fig. 2). Oxygen environment did not affect the emergence of pharate adults in irradiation treatments between 0–400 Gy, but treatment in 0.1 kPa O_2 did increase the adult emergence of pharate adults at 600 Gy compared with those irradiated in hypoxia (5, 10, and 15 kPa O_2) and normoxia (20.9 kPa O_2; $P < 0.05$ for all). At 600 Gy, pharate adults irradiated in 0.1 kPa O_2 were more than four times more likely to emerge than those irradiated in normoxic air (Fig. 2). Importantly, irradiation in 5 kPa of oxygen at 600 Gy also increased the proportion of adult moths emerging compared with irradiation in the standard normoxic atmosphere of 20.9 kPa O_2 ($z = 2.2$, $P = 0.05$). At the very high irradiation dose of 800 Gy, 36% of pharate adults irradiated in 0.1 kPa O_2 still successfully emerged as adult moths, whereas emergence was <10% for pharate adults irradiated in all other oxygen treatments. As above for larvae, in these experiments with pharate adults, we detected a significant effect of temporal replicate on adult emergence (cohort: $\chi^2_3 = 8.8$, $P = 0.03$). Some cohorts had higher adult emergence than others in both treatment and control groups (20.9 kPa O_2 with no radiation).

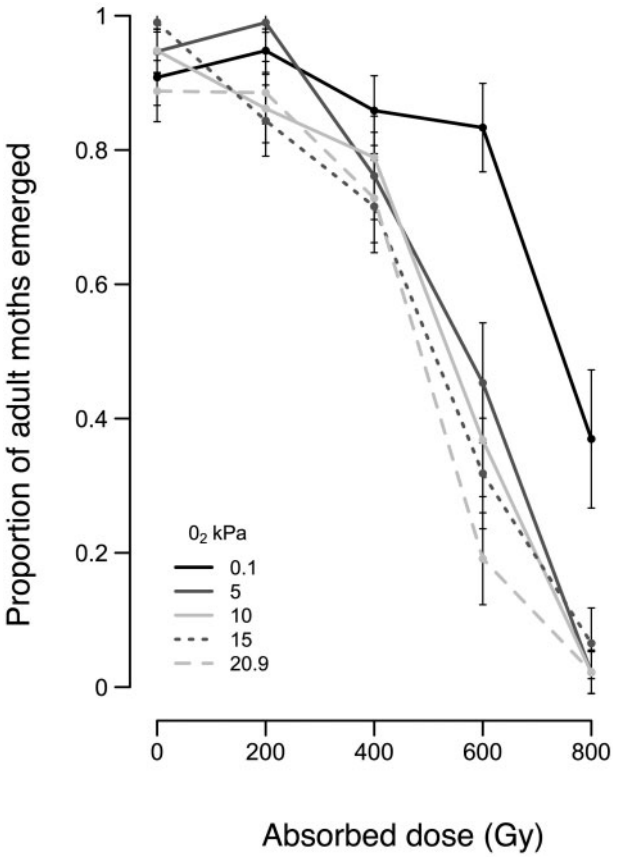

Fig. 2. Proportion of adult female *T. ni* moths that successfully emerged after irradiation as pharate adults (late pupae) in oxygen environments 0.1–20.9 kPa O_2. Data are based on the coefficients from the best-fit statistical model and their standard errors.

Fertility

We examined how the fertility of pharate adults was affected by different oxygen and radiation treatments by recording whether larvae

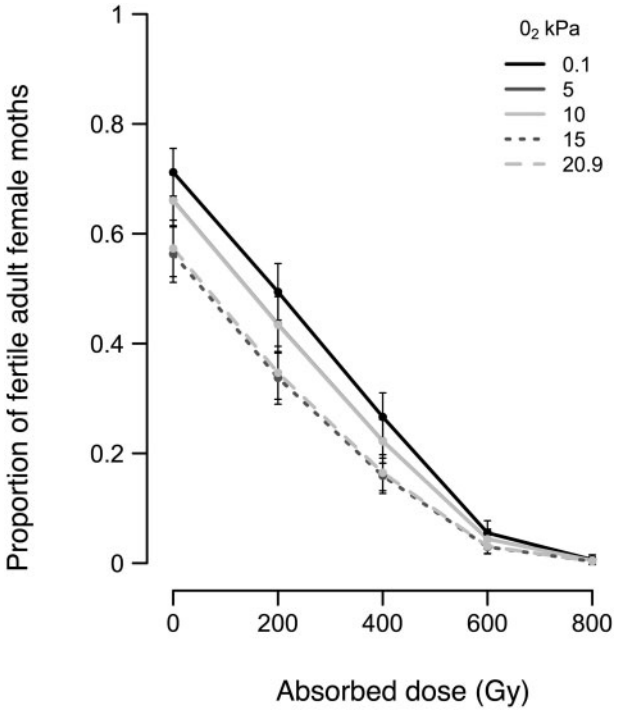

Fig. 3. Proportion of fertile female *T. ni* moths after irradiation as pharate adults (late pupae) in oxygen environments 0.1–20.9 kPa O_2. Data are based on the coefficients from the best-fit statistical model and their standard errors.

hatched from eggs laid by females that were irradiated in the previous experiment. Increasing radiation decreased the proportion of fertile females across all oxygen treatments ($\chi^2_4 = 267$, $P < 0.001$), while oxygen atmosphere also affected fertility ($\chi^2_4 = 9.55$, $P = 0.05$), but no interaction was detected between these treatments (Fig. 3). We used multiple comparisons to test for differences in atmosphere (pooled across radiation treatments) and found that females irradiated in 0.1 kPa O_2 had a marginally significantly greater probability of being fertile than female moths conditioned and irradiated at 15 kPa O_2 ($\chi^2_3 = 2.5$, $P = 0.08$).

There was a marginally significant effect of temporal replicate (cohort: $\chi^2_3 = 6.98$, $P = 0.07$), but there was no interaction between cohort and atmosphere or radiation treatment. Multiple comparison tests between cohorts of pharate adults revealed only a marginally significant difference between two of the cohorts, with one cohort having higher fertility across all treatment and control groups ($Z = 2.3$, $P = 0.08$, all other comparisons $P > 0.1$, data pooled for radiation and atmosphere treatments).

Discussion

Commodity producers have expressed a growing interest in using phytosanitary irradiation with modified atmosphere packaging to disinfest insects from their product and keep produce fresh while in transport. Atmospheres that increase the shelf life of fresh fruit and vegetables vary with the product, but some can be very hypoxic (1–5 kPa O_2). For example, apples, peaches, and pears have a recommended storage atmosphere of <5 kPa O_2 (Kader 1980, Yahia 2009). Currently, USDA APHIS PPQ prohibits packaging that creates an environment of <18 kPa O_2 before or during the irradiation treatment to ensure that irradiation remains an effective phytosanitary treatment, and the International Plant Protection Committee (IPPC) also discourages the use of phytosanitary irradiation for commodities in modified atmospheres (see ISPM 28, Annex 11 for irradiation of Oriental fruit moth under hypoxia for the single exception). The outcome of research examining the effect of irradiating insects in a range of hypoxic environments from 0.1–10 kPa O_2 will be needed to determine whether these regulations are likely to be altered in the future.

Generally, we found that insects that were treated with 0.1 kPa O_2 prior to and during irradiation had greater radiation tolerance than those irradiated in atmospheric oxygen. Our data show that irradiating *T. ni* larvae at 5 kPa O_2 can increase tolerance to a X-ray treatment at 100 Gy compared with irradiating in normal air. However, this effect was present in only one of the three cohorts we treated. The tolerance of pharate adults exposed to a 600 Gy radiation treatment was increased at 0.1 kPa O_2 and 5 kPa O_2 compared with insects irradiated in normal atmospheric conditions (20.9 kPa O_2), and the effect was apparent across all cohorts. However, when pharate adults were treated with the very high dose of 800 Gy, only the individuals treated in 0.1 kPa O_2 had greater tolerance. Follett et al. (2013) also showed a trend toward an increased radiation tolerance in melon fly larvae irradiated in MAP bags that produce severe hypoxia (~1–4 kPa O_2) compared with fly larvae irradiated in normoxia. Overall, our data suggest that irradiating in hypoxic environments from 0.1–5 kPa O_2 can affect the radiation tolerance of *T. ni*, but these responses need to be further investigated at oxygen levels between 0 and 10 kPa O_2 to determine at what concentration of oxygen the protective effect of irradiating in hypoxia emerges.

The radiation dose applied and the developmental age of an insect will determine whether irradiation treatments are successful in low-oxygen conditions. The radiation tolerance of *T. ni* increases with developmental stage and the deleterious effects of radiation are buffered in 0.1 kPa O_2 (López-Martínez et al. 2016). Lepidopteran larvae are the most likely life stage to be found in commodities, thus we tested wandering larvae because they are the most radio tolerant larval stage (Hallman et al. 2013a,b; López-Martínez et al. 2016). When larvae were treated with 200 Gy in atmospheres between 5–20.9 kPa O_2, none emerged as adults, but ~15–20% of larvae did later successfully emerge as adults when treated at 200 Gy in 0.1 kPa O_2. A recent review of the literature has suggested a generic dose of 250 Gy to prevent lepidopteran larvae from successfully emerging as adults (Hallman et al. 2013a,b), but more work will be needed to determine whether that generic dose will be uniformly effective for larvae held in hypoxic or hypercapnic (high CO_2) environments.

When considering lepidopteran pests, pharate adults (a.k.a., late pupae) are much less likely to be found in commodities being treated than eggs or larvae, but we tested pharate adults because they are the most tolerant life stage and thus represent an upper threshold for radiotolerance in our model system approach (Hallman et al. 2013a,b; López-Martínez et al. 2016). Some adult moths also emerged when pharate adults were treated at the highest dose (800 Gy) in 0.1 kPa O_2, but these moths were unlikely to produce live offspring. Irradiating commodities surrounded in low-oxygen conditions at very high doses of 800 Gy and above will likely ensure that emergence as adults is not possible for treated larvae and that even though the more tolerant pupa and adult life stages may successful emerge as adults, complete or partial sterility will mitigate risk associated with these emerging adults. However, even a small increase above the current generic dose of 400 Gy may not be suitable for some commodities that are less tolerant to radiation (Wall 2015). Furthermore, because many commercial facilities have dose uniformity ratios >2, commodities at the edge of the load may receive more than double the target dose delivered to commodities in

the middle of the load. These high dose uniformity ratios within some commercial irradiation facilities make doses targeted above 400 Gy not practical within the current 1 kGy radiation limit for fresh commodities (Wall 2015). When treated at 400 Gy in ambient air, 70–90% of all irradiated female pharate adults emerged as adult moths, and some of these females produced live offspring (10–36%). Live offspring were recorded from female pharate adults that were irradiated at 400 Gy across all oxygen treatments. A large body of literature on irradiation in the context of lepidopteran sterile insect technique programs suggests that many nonlethal irradiation treatments (e.g., 100–400 Gy) applied to late pupae and adults result in F1 inherited sterility (reviewed in Carpenter et al. 2005, Hallman and Hellmich 2009). More research measuring F1 inherited sterility is required, but the generic dose of 400 Gy may be effective for lepidopteran pupae and adults if F1 inherited sterility is an acceptable outcome for phytosanitary irradiation treatments, perhaps even for those treatments at 400 Gy delivered in hypoxia.

In our experiments, larvae and pupae were held in low-oxygen conditions for 1 h prior to irradiation and for an additional hour during and after irradiation. Currently, phytosanitary irradiation treatments can occur in the country of origin or at a port of entry in the importing country. As a result, the commodity may be in the MAP bag for hours or weeks depending on the location of the treatment and transit time. In addition, insects may experience significant changes in atmosphere and temperature during transport because the atmosphere inside a MAP bag is designed to change as the commodity respires, and respiration is proportional to temperature for both fresh commodities and insects. As well as low-oxygen conditions, MAP bags can also maintain high CO_2 environments that create an atmosphere of high CO_2 with low O_2. Low O_2 and high CO_2 environments have a detrimental effect on insects, particularly when temperatures are high (Neven 2003, Neven and Hansen 2010, Yahia 2009), but how a combination of these treatments might alter the radiation tolerance of insects is uncertain. More research is required to investigate how these variables may influence the efficacy of phytosanitary irradiation.

Overall, our results suggest that exposure in severely hypoxic environments, specifically 0.1 and 5 kPa O_2, may increase the radiation tolerance of irradiated *T. ni*, while the effect of more mild hypoxic environments (10 and 15 kPa O_2) appears to be similar to irradiation in normal air (20.9 kPa O_2). More research is required to identify what oxygen concentrations and radiation doses may compromise the efficacy of irradiation as a phytosanitary treatment.

Acknowledgments

We thank Carl Gillis from the Florida Department of Agriculture and Consumer Services (FDACS) for X-ray and E-beam irradiation services. We thank Amy Rowley for maintaining the insect colony, and Genevieve Comeau and Mary Karcher for assistance with fecundity trials. Guy Hallman and Peter Follett both kindly provided important advice on research design and interpretation. Lisa Neven and two reviewers provided substantial advice in revising this manuscript for clarity. This research was supported by a grant (14-8130-0471-CA to D.A.H.) from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) and the IAEA/FAO Coordinated Research Project in The Development of Generic Irradiation Doses for Quarantine Treatments (#CRP D62008).

References Cited

(APHIS) U.S. Department of Agriculture. 2015. Treatment manual. (http://www.aphis. usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf)

Balock, J. W., A. K. Burditt, and L. D. Christenson. 1963. Effects of gamma radiation on various stages of three fruit fly species. J. Econ. Entomol. 56: 43–46.

Carpenter, J. E., S. Bloem, and F. Marec. 2005. Inherited sterility in insects, pp. 115–146. *In* A. V. Dyck, J. Hendrichs and A. S. Robinson (eds.), Sterile insect technique. Springer, Berlin/Heidelberg.

Chapman, R. F. 2013. The insects: Structure and function, 5th ed. Cambridge University Press, New York, NY.

(FDA) Food and Drug Administration. 1986. Irradiaton in the production, processing, and handling of food. Rules Regul. Fed. Regist. 51: 13376.

Firth, D. 1993. Bias reduction of maximum-likelihood estimates. Biometrika 80: 27–38.

Fisher, K. 1997. Irradiation effects in air and in nitrogen on Mediterranean fruit fly (Diptera: Tephritidae) pupae in Western Australia. J. Econ. Entomol. 90: 1609–1614.

Follett, P. A. 2009. Generic radiation quarantine treatments: The next steps. J. Econ. Entomol. 102: 1399–1406.

Follett, P. A., and L. G. Neven. 2006. Current trends in quarantine entomology. Annu. Rev. Entomol. 51: 359–385.

Follett, P. A., and M. M. Wall. 2012. Phytosanitary irradiation for export of fresh produce: Commercial adoption in Hawaii and current issues. J. Radioanal. Nucl. Chem. 296: 517–522.

Follett, P. A., M. Wall, and W. Bailey. 2013. Influence of modified atmosphere packaging on radiation tolerance in the phytosanitary pest melon fly (Diptera: Tephritidae). J. Econ. Entomol. 106: 2020–2026.

Guy, R. N., L. N. C, J. R. Rye, C. W. Green, S. L. Barette, and K. A. Hollien.1985. *Trichoplusia ni*, pp. 487–494. *In* P. Sing and R. F. Moore (eds.), Handbook of insect rearing. Elsevier, Amsterdam.

Hallman, G. J. 2000. Expanding radiation quarantine treatments beyond fruit flies. Agric. For. Entomol. 2: 85–95.

Hallman, G. J. 2004. Ionizing irradiation quarantine treatment against oriental fruit moth (Lepidoptera: Tortricidae) in ambient and hypoxic atmospheres. J. Econ. Entomol. 97: 824–827.

Hallman, G. J. 2005. Hypoxia reduces reproductive susceptibility of plum curculio (Coleoptera: Curculionidae) to ionizing radiation. Fla. Entomol. 88: 208–210.

Hallman, G. J. 2012. Generic phytosanitary irradiation treatments. Radiat. Phys. Chem. 81: 861–866.

Hallman, G. J., and R. L. Hellmich. 2009. Ionizing radiation as a phytosanitary treatment against European corn borer (Lepidoptera: Crambidae) in ambient, low oxygen, and cold conditions. J. Econ. Entomol. 102: 64–68.

Hallman, G. J., A. G. Parker, and C. M. Blackburn. 2013a. The case for a generic phytosanitary irradiation dose of 400 Gy for Lepidoptera that infest shipped commodities as pupae. J. Econ. Entomol. 106: 525–532.

Hallman, G. J., N. M. Levang-Brilz, J. L. Zettler, and I. C. Winborne. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. J. Econ. Entomol. 103: 1950–1963.

Hallman, G. J., V. Arthur, C. M. Blackburn, and A. G. Parker. 2013b. The case for a generic phytosanitary irradiation dose of 250 Gy for Lepidoptera eggs and larvae. J. Econ. Entomol. 106: 525–532.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6: 65–70.

Kader, A. A. 1980. Prevention of ripening in fruits by use of controlled atmospheres. Food Technol. 34: 51–54.

Klowden, M. J. 2007. Physiological systems in insects, 2nd ed. Elsevier, Burlington, MA.

Kosmidis, I., and D. Firth. 2010. A generic algorithm for reducing bias in parametric estimation. Elect. J. Stat. 4: 1097–1112.

Landolt, P. J. 1997. Cabbage looper (Lepidoptera: Noctuidae) fecundity maximized by a combination of access to water and food, and remating. Ann. Entomol. Soc. Am. 90: 783–789.

López-Martínez, G., R. Meagher, L. A. Jeffers, W. D. Bailey, and D. A. Hahn. 2016. Low oxygen atmosphere enhances post-irradiation survival of *Trichoplusia ni* (Lepidoptera: Noctuidae). Fla. Entomol. (in press).

Neven, L. G. 2003. Physiological effects of physical postharvest treatments on insects. HortTechnology 13: 272–275.

Neven, L. G., and L. D. Hansen. 2010. Effects of temperature and controlled atmospheres of coddling moth metabolism. Ann. Entomol. Soc. Am. 103: 418–423.

Robinson, A. S. 2005. Genetic basis of the sterile insect technique, pp. 95–114. *In* A. V. Dyck, J. Hendrichs and A. S. Robinson (eds.), Sterile insect technique. Springer, Berlin/Heidelberg.

Shorey, H. H. 1963. The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). II. Factors affecting adult fecundity and longevity. Ann. Entomol. Soc. Am. 56: 476–480.

Wall, M. M. 2015. Phytosanitary irradiation and fresh fruit quality: Cultivar and maturity effects. Stewart Postharvest Rev. 3: 6.

Wang, C. Y. 1990. Physiological and biochemical effects of controlled atmosphere on fruits and vegetables, pp. 197–223. *In* M. Calderon and R. Barakai-Golan (eds.), Food preservation by modified atmospheres. CRC Press.

Yahia, E. M. 2009. Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities. CRC Press, Boca Raton, Florida.