Anoxia-conditioning hormesis alters the relationship between irradiation doses for survival and sterility in the cactus moth, *Cactoblastis cactorum* (Lepidoptera: Pyralidae)

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Abstract

One of the most important components of a program that has a sterile insect technique (SIT) component is an appropriate irradiation dose. Knowing the organismal dose-response enables the selection of a dose that induces the highest level of sterility while preserving the sexual competitiveness and other desired qualities of the sterile insect. Finding this balance in Lepidoptera is crucial because of the use of inherited (F_1) sterility, where the irradiated parent must be competitive enough to mate while its offspring must be sterile. Manipulations of atmospheric oxygen content have been shown to be an effective way of lowering post-irradiation somatic damage while preserving sterility and improving sterile insect performance, particularly in fruit flies. In this study we tested the irradiation dose response of adults of the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), and the effects of those doses on sterility, longevity, and F_1 performance, and whether a nitrogen conditioning-treatment (anoxia) prior to and during irradiation affected these metrics. We found that male and female fecundities were not impacted by dose or atmospheric treatment, but females were sterilized at lower doses than males. Eggs of irradiated parents took longer to hatch than those of unirradiated controls, and offspring of moths irradiated in anoxia lived longer in the absence of food and water. Anoxia conditioning rescued female fertility at intermediate doses but had no similar rescue effect on male fertility, which was always greater than female fertility at a given dose. Males generally lived longer than females and anoxia had a strong effect in lowering the male mortality rate and extending lifespan at a given dose. We show evidence that anoxia-conditioning prior to and during irradiation as part of a lepidopteran program with an SIT component could improve parental and larval performance and longevity.

Key Words: sterile insect technique; dose response; invasive species; inherited (F_1) sterility

Resumen

Uno de los componentes más importantes en la técnica del insecto estéril (TIE) es la dosis usada para irradiación. El conocimiento sobre la respuesta a la dosis es necesario y de suma importancia para ajustar la dosis optima que induzca el mayor grado de esterilidad manteniendo la calidad del insecto y su competitividad sexual. Encontrar este balance en lepidopteros es esencial dado la inducción de esterilidad heredada (esterilidad F_1), donde el macho irradiado tiene que ser competitivo para lograr el apareamiento efectivo dando descendencia estéril. Se ha demostrado un mayor grado de efectividad haciendo manipulaciones atmosféricas de oxigeno, el cual reduce el daño somático que resulta de la irradiación. Este tipo de tratamiento preserva la esterilidad de las palomillas y mejora el rendimiento de los insectos estériles, particularmente las moscas fruteras. Aquí ponemos a prueba la respuesta a la dosis de adultos de la palomilla del nopal, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), y el efecto de los dosis en la esterilidad, longevidad, y el rendimiento de la generación F_1. También estudiamos como el acondicionamiento con nitrógeno (anoxia) antes y durante la irradiación afecta estas métricas de rendimiento. Encontramos que la fecundidad de machos y hembras no fue impactada por la dosis usada o el tratamiento atmosférico, aunque las hembras son efectivamente esterilizadas con dosis más bajas que los machos. Los huevecillos de adultos irradiados tomaron más tiempo para eclosionar que los huevecillos de adultos no tratados, y la cría de insectos irradiados en anoxia sobrevivieron más en la ausencia de comida y agua. El acondicionamiento con anoxia rescato la fertilidad de las hembras en dosis intermedias pero no tuvo ningún efecto en la fertilidad de los machos, la cual siempre fue mejor que la fertilidad de las hembras en cualquiera de las dosis probadas. Los machos generalmente vivieron más que las hembras, y la anoxia redujo la mortalidad y extendió la longevidad de los machos. Aquí presentamos evidencias que demuestran que el acondicionamiento de anoxia antes y durante la irradiación como parte de un programa de TIE para lepidopteros puede mejorar el rendimiento de los adultos y las larvas al igual que su longevidad.

Palabras Clave: técnica de insecto estéril; respuesta de dosis; plagas invasivas; heredada (F_1) de esterilidad

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The sterile insect technique (SIT) is a powerful, environmentally safe, control tactic utilized in area-wide integrated pest management (AW-IPM) programs for the suppression, containment, prevention, or eradication of pest species (Hendrichs et al. 2005). The SIT typically involves the use of radiation (gamma, X-ray, or E-beam) to induce sterility, and sterile insects are released into infested areas. Sterile insects are expected to mate with wild fertile insects, therefore producing unviable eggs that will result in a reduction of the population of the pest. SIT was a major component of the program to control the spread of the invasive cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) from Florida through the Gulf Coast and into the Southwestern USA and Mexico until the termination of this program in 2011 (Hight et al. 2005; Goñalons et al. 2014).

All SIT programs must settle on a target dose that is high enough to induce sterility, but that is not so high that the insects' performance is degraded (Bloem et al. 2001). Somatic damage during the irradiation process leads to poor post-irradiation performance (Bakri et al. 2005; López-Martínez & Hahn 2012). This irradiation damage can be manifested as decreased dispersal ability (flight ability), lower mating success, and shorter lifespan (Hooper 1971; Ohinata et al. 1977; Robinson 2002; Nestel et al. 2007; López-Martínez & Hahn 2012, 2014). Thus, selecting an appropriate sterility dose represents a balance between desired genomic DNA double-stranded breaks that act as dominant-lethal mutations in offspring, and the unwanted secondary somatic damage that affects parental performance. A major source of irradiation-induced damage is the splitting of gaseous oxygen and water in insect tissues into free radicals that can bind to proteins, lipids, and DNA/RNA; disrupting physiological functions (von Sonntang 1987). Free radicals can continue to damage tissues long after irradiation is completed. Thus, oxidative stress is associated with loss of performance in several traits closely tied to successful sterile insect releases (Calkins & Parker 2005).

Historically, programs with a SIT component attempted to compensate for poor sterile insect quality by increasing the number of insects released assuming that quantity can win over quality (Calkins & Parker 2005). However, several models show that increasing the performance of sterile insects can lead to more effective control, despite maintaining low residual fertility (Rull et al. 2011). It has long been known that fruit flies irradiated in low-oxygen (hypoxic) or no oxygen (anoxic) environments performed better than flies irradiated in normal-oxygen (normoxic) environments. These fruit flies had higher treatment survival, were more sexually competitive, and lived longer without sacrificing sterility (Hooper 1971; Wakid 1973; Sharp et al. 1975; Zumreoglu et al. 1979). Some low-oxygen approaches are actively used in programs today. For example, the large Mediterranean fruit fly facility at El Pińo, Guatemala that provides the sterile male flies for the Moscamed Program, routinely seals pupae in airtight bags allowing pupae to deplete oxygen in those bags prior to irradiation (FAO/IAEA/USDA 2003).

Recently, our group has shown that exposure to anoxia prior to and during irradiation leads to greater post-irradiation organismal performance in both the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera; Tephritidae) and the cactus moth (López-Martínez & Hahn 2012, 2014; López-Martínez et al. 2014). Furthermore, we have shown that exposure to anoxia increases the antioxidant capacity of both Caribbean fruit flies and cactus moths and reduces post-irradiation oxidative damage. Thus, we propose that anoxic conditioning helps to reduce irradiation damage through a hormetic effect.

Hormesis is a process by which low-level exposure to stress alters an organism's physiology—typically by inducing stress-resistance mechanisms—that provides an organism protection against subsequent stresses (Calabrese et al. 2007). Hormesis is a common topic of inquiry in biomedicine and aging, but the potential importance of hormetic-conditioning effects is increasingly being recognized in both ecology (Costantini et al. 2010) and applied pest management; including studies of insecticide toxicology (Morse 1998; Cutler 2013), phytosanitary treatments (Hallman 2010; Boardman et al. 2011), and SIT for both flies and moths (Chidawanyika and Terblanche 2011; López-Martínez & Hahn 2012, 2014; López-Martínez et al. 2014).

While common in fruit fly programs that have a SIT component, anoxia conditioning prior to and during irradiation is not regularly used in lepidopteran programs, but may be a beneficial hormetic treatment that could improve the field performance of sterile moths (Ashraf et al. 1975; Fisher 1997; Robinson 2005). The use of the SIT against Lepidoptera differs in many aspects from SIT against Diptera. Lepidoptera are typically irradiated as adults rather than as pupae, requiring different procedures for manipulating atmospheres during irradiation. Lepidopterans also require much higher doses of radiation to achieve sterility than Diptera, possibly because lepidopterans differ in having holocentric chromosomes (Bauer 1967; Carpenter et al. 2005). Complete sterility is not the goal for many lepidopteran SIT programs because the high doses required to induce the numerous dominant-lethal mutations needed for sterility often yield sterile insects with unacceptably low performance in dispersal and mating (Bloem et al. 2001; Carpenter et al. 2005). Instead, lepidopteran SIT programs typically use partial sterility wherein a portion of the offspring of the irradiated generation develop, but are themselves sterile (F_1 sterility) (North 1975). Partial sterility programs can effectively suppress pest insect populations despite some larval production as long as female fertility within the pest population declines substantially with time (Bloem et al. 2001; Carpenter et al. 2005). However, the irradiation doses required to induce adequate levels of partial sterility in most lepidopterans are still generally more than double the doses needed to induce complete sterility in fruit flies, and likely to have negative effects on the performance of released insects (Parker & Mehta 2007).

Treatments that reduce off-target damage from irradiation and preserve the performance of released insects while maintaining adequate levels of sterility, like anoxia conditioning, could have positive impacts on the efficacy and economy of lepidopteran programs with a SIT component. Robinson (1975) reported that direct male sterility (parental sterility) in the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) was only achieved in a nitrogen environment using 1.5 times the dose necessary in air (380 Gy in anoxia vs. 255 Gy in normoxia). Promisingly, the higher dosage delivered in anoxia did not reduce the competitiveness of the anoxia-irradiated moths, but did reduce the production of F_1 progeny, suggesting that irradiation in anoxia was accompanied by reduced somatic cellular damage without a potential reduction in inherited sterility. Unfortunately, the literature contains few such studies.

Thus we investigated the effects of anoxic conditioning on adult performance and F_1 sterility after irradiation using *C. cactorum* as a model system. We previously found that male moths treated with anoxia prior to and during irradiation with 200 Gy had a higher incidence of flight, better dispersal, mated more, and lived longer than irradiated moths not treated with anoxia (López-Martínez et al. 2014). This improvement in organismal performance was correlated with an increase in total antioxidant capacity triggered by the 1 h anoxia conditioning hormesis and a decrease in irradiation-induced free radical damage to lipids and proteins. However, when irradiated with 200 Gy, the F_1 progeny of anoxia-treated male moths had ~ 3.5% residual fertility whereas the F_1 progeny of male moths irradiated in normal-oxygen environments were completely sterile (López-Martínez et al. 2014). This small level of residual F_1 fertility in anoxia-treated moths is unlikely to represent a problem for effective population control by the SIT because it is

so close to full sterility that populations are still likely to decline, especially if the anoxia-treated males have greater mating performance and longevity after release. More research is needed to determine whether irradiation of lepidopterans in anoxic or hypoxic environments will generally necessitate greater irradiation doses to induce complete sterility than the doses needed in normal atmospheres, as suggested by Robinson (1975) and López-Martínez et al. (2014). Here we expand on our previous work studying the effects of anoxia conditioning on post-irradiation performance of the cactus moth by including additional doses below and above the 200 Gy target dose we previously used, up to a high dose of 400 Gy. We quantify the ability of groups of F_1 larvae to build feeding webs, an important function for these gregarious family groups of early instar larvae, and larval longevity under food and water stress, in addition to estimating the longevity, fecundity, and fertility of treated adults. We also test several methods for assessing longevity of adult moths, an important parameter in quality control for the SIT, showing that housing moths in groups in large containers gives very different results than housing moths individually in small containers.

Materials and Methods

INSECT REARING

Cactoblastis cactorum used in all experiments were collected as pupae from a colony reared at the USDA-ARS Crop Protection and Management Research Unit in Tifton, GA. Prior to adult emergence, unsexed moth pupae encased in their spun cocoons were individually transferred into small (30 mL), white, opaque plastic cups with clear plastic lids that had been perforated to allow ample air flow. As moths emerged, they were sexed and separated into treatment groups in a 4 °C room to limit flight and mobility during sorting. Both treated and non-treated control moths were kept in 2 L plastic-bucket cages with mesh tops at a density of 5 moths per cage. All cups and cages were kept in a temperature (24 °C) and humidity controlled (> 50% RH) room under a photoperiod of 14:10 h L:D.

RADIATION TREATMENTS

Male and female adult moths were irradiated within 24 h of emergence using a Gammacell Cs^{137} irradiator (GC45, Ottawa, Ontario, Canada) that had a dose rate of 8.16 Gy/min at the Florida Accelerator Services and Technology facility within the Division of Plant Industry of Florida Department of Agricultural and Consumer Services. Moths were inserted in the center of the cylinder used for irradiation to ensure dose uniformity from replicate to replicate. Moths were packed between small, custom-made, gel ice packs to minimize moth activity by maintaining them at a temperature range of 4 to 6 °C during irradiation. These ice packs maintained the temperature under 6 °C for up to 3 h, although irradiation time did not exceed 50 min in this series of experiments. We exposed moths to 1 of 4 irradiation doses (exposure times): 100 Gy (12 min and 15 s), 200 Gy (24 min and 31 s), 300 Gy (36 min and 46 s), and 400 Gy (49 min and 1 s). The control moths were not treated (0 Gy [0 min]). The target dose for male F_1 sterility in cactus moth is 200 Gy (Carpenter et al. 2001; Tate et al. 2007).

Moths were exposed to 2 atmospheric treatments: normoxia (normal atmospheric air; the presence of oxygen) and anoxia (nitrogen atmosphere; the absence of oxygen). Moths were kept in custom-sized polypropylene bags that were intact to maintain anoxia, or bags that had been thoroughly perforated using a 26-gauge needle to promote ample airflow (normoxia treatment). For each treatment, 25 male or female moths were placed in each bag. For anoxia treatments, moths were placed in polypropylene bags and flushed with nitrogen for 2 min, and then the bags were heat sealed as previously described (López-Martínez et al. 2014). Moths were kept without oxygen for 1 h prior to irradiation and were irradiated in the absence of oxygen. After irradiation, the bags were opened and oxygen reperfusion occurred. The time of anoxia exposure was adjusted in the untreated, 100, 200, and 300 Gy treatments so that the total duration of anoxia experienced by all moths in all treatments would be 1 h and 50 min (the length of the anoxia in the 400 Gy treatment prior to reperfusion). Preliminary studies showed no effect of slightly longer anoxia on treatment mortality; anoxia exposure had to exceed 6 h before survival differences were noted 72 h post-treatment. Moths in the normoxia treatments were placed in the perforated polypropylene bags for the same period of time as the anoxia-treated moths.

Gafchromic HD-810 film (International Specialty Products, Wayne, New Jersey, USA) was used to quantify the accuracy of the doses delivered. Two dosimeters were taped to each bag (top and bottom), and were read 24 h after irradiation treatment. The average absorbed dose received by the moths never exceeded 10% above or below the target dose. Values of treatment doses were as follows: 100 Gy target, 108 Gy mean absorbed dose with dose-uniformity ratio (DUR) of 0.95; 200 Gy target, 214 Gy mean absorbed DUR 1.02; 300 Gy target, 301 Gy mean absorbed DUR 1.0; and 400 Gy target, 411 Gy mean absorbed DUR 1.0.

STERILITY AND F_1 LARVAL SURVIVAL

Groups of 5 treated males or females were placed in a 2 L plastic bucket with a mesh lid. Five untreated control males or females of the opposite sex were added to each cage. Each treatment was replicated over 5 consecutive weeks, using different cohorts of moths each week. Treatments were coded as follows: normoxia and no irradiation = NxNr; normoxia and the following 4 levels (Gy) of irradiation: Nx100, Nx200, Nx300 and Nx400; anoxia and no irradiation = AxNr; and anoxia and the following 4 levels of irradiation: Ax100, Ax200, Ax300 and Ax400. Eggsticks were collected daily for 7 d after treatment. No eggs were oviposited in any treatment beyond 7 d, and all moths were monitored daily until they had died. The total eggs oviposited, total eggsticks produced, eggs per eggstick, average d of egg-laying, d until oviposition, percentage egg hatch, time to hatch, longevity under stress, and percentage webs built by each gender were evaluated.

Cactoblastis cactorum females lay eggs one on top of the other in cactus spine-like sticks (eggsticks). The number of eggsticks laid, eggs per eggstick, and d of egg-laying were recorded for each treated individual to determine whether dose or atmospheric treatment affected multiple aspects of fecundity. For fertility, beyond recording the proportion of eggs hatched and the time taken for egg hatching, 2 behavioral aspects of neonate larvae were monitored as a metric of the ability of the F_1 larvae to survive the stress of the lack of food (either artificial diet or host plant). Individual eggsticks were placed in 30 mL cups and kept at 25 °C and 75% RH. Survival of the hatched larvae was recorded. Furthermore, newly hatched larvae feed as a group under the presence of a protective silk web that may serve to protect them from predators, parasitoids, and desiccation (Dickle 1991). We also recorded the ability of the hatched larvae to group together and build a communal web in the absence of food by scoring groups for either the presence or absence of a web.

ADULT LONGEVITY ASSAY DESIGN

Activity and mating can have strong effects on organismal life history traits, such as lifespan, but some investigators choose to perform

longevity tests on either solitary organisms or on single-sex groups. Our goal was to determine whether estimates of longevity would be effected by housing of moths in the following 3 ways: 1) individually in containers small enough to prevent flying, 2) in single gender groups in large containers that allowed flight but prevented mating, or 3) in mixed gender groups in large containers that allowed for the full range of activities, including flight and mating (50:50 male:female). The individual housing treatment consisted of 23 male moths or 23 female moths (treatment 1) individually placed in small (30 mL), white, opaque, plastic cups with clear perforated lids. These cups were large enough to confine individual moths but small enough to prevent running, flying, and extraneous mating activity; the moths were alone and inactive. The single gender cages were 2 L plastic buckets with mesh lids and were filled with 23 male moths or 23 female moths (treatment 2). The mixed gender cages contained 23 males and 23 females (treatment 3) in 2 L plastic buckets with mesh lids. These 2 L cages allowed for the full range of behavioral activities such as walking, flying, mating, and egg-laying. All cups and cages were checked daily and dead moths were removed and sexed. This experiment of 3 treatments assessing the effects of social environment on longevity was repeated twice using 2 different cohorts of moths for each treatment to inform as to the design of the quality control assay for longevity in this study.

ADULT LONGEVITY AFTER TREATMENT

In the experiment above with unirradiated individuals we found that moths housed in groups, as is common in SIT facilities, had shorter lifespans than moths housed individually. Thus for evaluating the effects of our treatments on longevity irradiated moths were housed in large (2 L) containers that included individuals of both sexes in equal proportions to assess whether irradiation dose and anoxia treatment affected longevity. Longevity was tracked for each treated and untreated control individual that was used in the dose-response experiment. Every 24 h after the initial treatment, mortality was assessed and dead individuals were removed from the 2 L plastic bucket cages and sexed. We present both the hazard mortality and median longevity for each treatment to allow comparisons with previous work (López-Martínez & Hahn 2014) and data in the literature.

STATISTICAL ANALYSES

The dose-response experiment, which included sterility and longevity assays, was repeated across 5 cohorts of moths. Total eggs oviposited, eggsticks produced, eggs per eggstick, oviposition d, percentage egg hatch, time to egg hatch, longevity under stress, support of web building, and d until oviposition were analyzed by gender using 3-way ANOVAs, using radiation dose, atmospheric treatments, gender, and their interactions as explanatory factors. Whenever the sexes differed in their response, we separated the genders for analysis using 3-way ANOVAs, with radiation dose, atmospheric treatments, gender, and their interactions as explanatory factors, followed by Tukey's HSD tests or linear contrasts to separate out treatment groups. Longevity data were analyzed using a proportional hazards model with atmospheric treatment (normoxia vs. anoxia), radiation dose (0, 100, 200, 300, or 400 Gy), and their interaction as factors. Whenever atmospheric treatment had an effect on the hazard mortality rate, we report the risk ratio for normoxic- vs. anoxia-treated individuals. The experiment to assess the effects of individual housing vs. group housing on longevity was repeated twice using different cohorts of moths. Cohort was initially fit as a random factor in all preliminary models. However, there was never a detectable effect of cohort in any analysis, and thus data were combined across cohorts for all analyses.

Results

FECUNDITY

There was no effect of anoxia during irradiation on the timing of the initiation of egg laying, but irradiation of males slowed the initiation of egg laying by their mates by 1 d compared to females mated to untreated males (F = 11.06; df = 1,94; P = 0.0013). Neither dose of irradiation delivered nor the atmosphere in which it was delivered affected the timing of egg laying. Females mated with males from all irradiated treatment groups began laying eggs 3 d after treatment compared with 2 d in untreated males (F = 0.80; df = 9,37; P = 0.6153). There were no differences in the timing of the onset of egg laying when treated or control females were mated to untreated males (F = 0.97; df = 9,39; P = 0.4769).

There were no effects of either atmosphere or irradiation dose on any fecundity parameter when either males or females were treated. When treated females were mated with untreated control males, there was no effect of either irradiation dose or atmospheric treatment on the total number of eggs oviposited (62 eggs for lifetime fecundity; F = 0.83; df = 9,39; P = 0.5905), the number of eggs oviposited per eggstick (35 eggs/eggstick; F = 0.70; df = 9,38; P = 7058), the number of eggsticks produced per female (1.8 eggsticks; F = 1.26; df = 9,39; P = 0.2903), or the number of oviposition d (2.8 d; F = 1.10; df = 9,39; P = 0.3868). Similarly, when treated males were mated with untreated control females, there was no effect of either irradiation dose or atmospheric treatment on the total number of eggs oviposited per female (68 egg for lifetime fecundity; F = 0.68; df = 9,37; P = 0.7237), the number of eggs oviposited per eggstick (~38 eggs/eggstick; F = 0.39; df = 9,37; P = 0.9316), the number of eggsticks produced (~1.9 eggsticks; F = 0.84; df = 9,37; P = 0.5832), or the number of d during which untreated control females mated with treated males oviposited eggs (~3.4 d; F = 0.80; df = 9,37; P = 0.6153).

FERTILITY

Irradiation of female moths in anoxia strongly improved their fertility (Fig. 1A; $F_{\_model}$ = 132.10, df = 9,39; P < 0.0001; $F_{\_atm\ treatment}$ = 7.26; df = 1,39; P = 0.0104; $F_{\_dose}$ = 284.30; df = 4, 39; P < 0.0001; $F_{\_atm\ treatment\ x\ dose}$ = 2417.41; df = 4,39; P < 0.0001). When females were irradiated in normoxia and mated to untreated control males, female fertility (egg hatch) dropped steadily with increasing irradiation dose from 98% for the untreated control (0 Gy) to less than 1% for females treated with 100 Gy. None of the eggs that were oviposited by females that had been treated with 200 Gy or more hatched. Anoxia conditioning had a strong effect on fertility, substantially rescuing fertility in the 100 Gy (33%), and the 200 Gy (6%) treatment group, and even a few females produced viable eggs when treated with 300 Gy (~1%) compared with normoxia-irradiated females.

The time taken for eggs from treated females to hatch was also strongly influenced by both irradiation dose and atmospheric treatment (Fig. 1B; $F_{\_model}$ = 4249.06, df = 9,29; P < 0.0001; $F_{\_atm\ treatment}$ = 4620.62; df = 1,29; P < 0.0001; $F_{\_dose}$ = 5056.13, df = 4,29; P < 0.0001; $F_{\_atm\ treatment\ x\ dose}$ = 2001.28, df = 4,29; P < 0.0001). When females were treated with 100 Gy, eggs from females irradiated in anoxia hatched 1 d earlier on average than eggs from females treated in normoxia (linear contrast, P < 0.001). When considering eggs from anoxia-treated females only, higher-doses of radiation progressively slowed the time taken for eggs to hatch.

Anoxia conditioning did not affect the fertility of treated males regardless of the radiation dose applied, but there was a strong decrease in fertility with increasing doses of radiation (Fig. 1C; $F_{\_model}$ = 28.24; df

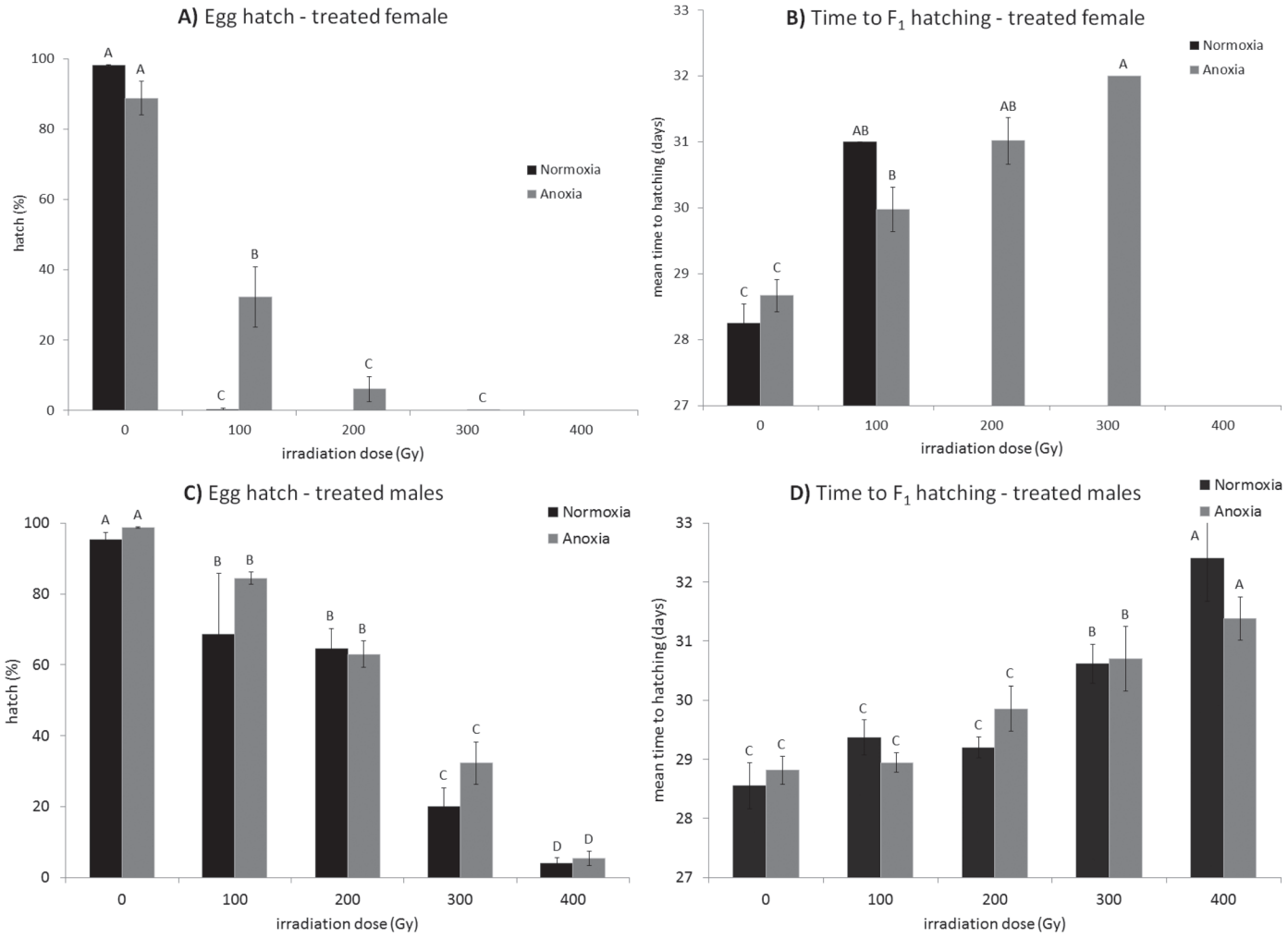

Fig. 1. Fertility (as measured by egg hatch success and time to hatch) of *Cactoblastis cactorum* treated as adults at 5 levels of irradiation and 2 levels of atmospheric conditions. Means and standard errors are plotted. Letter designations refer to Tukey's post hoc analysis.

= 9,37; $P < 0.0001$, $F_{\text{atm treatment}} = 2.13$; df = 1,37; $P = 0.1529$; $F_{\text{dose}} = 61.80$; df = 4,37; $P < 0.0001$; $F_{\text{atm treatment x dose}} = 0.60$; df = 4,37; $P = 0.6622$). Similarly, the time to egg hatch from treated males was not affected by anoxia conditioning, but there was a clear delay in egg hatching that was proportional to irradiation dose (Fig. 1D; $F_{\text{model}} = 10.86$; df = 9,33; $P < 0.0001$; $F_{\text{atm treatment}} = 0.13$; df = 1,33; $P = 0.7205$; $F_{\text{dose}} = 22.50$; df = 4,33; $P < 0.0001$; $F_{\text{atm treatment x dose}} = 1.44$; df = 4,33; $P = 0.2434$). Eggs of males treated with 400 Gy hatched on average 3 d after eggs of untreated control males (~12% longer) in both anoxia and normoxia treatments.

LONGEVITY OF HATCHED F_1 LARVAE UNDER STRESS

Longevity of newly hatched F_1 larvae when not provided any food or water (i.e., survivorship under stress) was strongly affected by the gender of the treated parent (Fig. 2; $F_{\text{model}} = 18.32$; df = 19,72; $P < 0.0001$; $F_{\text{atm treatment}} = 12.34$; df = 1,72; $P = 0.0008$; $F_{\text{dose}} = 20.74$; df = 4,72; $P < 0.0001$; $F_{\text{sex}} = 141.51$; df = 1,72; $P < 0.0001$; $F_{\text{atm treatment x dose}} = 3.61$; df = 4,72; $P = 0.0098$; $F_{\text{sex x dose}} = 10.29$; df = 4,72; $P < 0.0001$; $F_{\text{atm treatment x sex}} = 17.81$; df = 1,72; $P < 0.0001$; $F_{\text{atm treatment x dose x sex}} = 5.85$; df = 4,72; $P = 0.0004$). When considering the progeny of treated females mated to untreated males, larvae hatching from the eggs of females treated in anoxia with 100 Gy lived twice as long as larvae from females irradiated with 100 Gy in normoxia (Fig. 2A; $F_{\text{model}} = 18.10$; df = 9,39; $P < 0.0001$; $F_{\text{atm treatment}} = 27.47$; df = 1,39; $P < 0.0001$; $F_{\text{dose}} = 24.90$; df = 4,39; $P < 0.0001$; $F_{\text{atm treatment x dose}} = 8.51$; df = 4,39; $P < 0.0001$). Anoxia conditioning had no effect on the longevity of newly hatched larvae derived from mating irradiated males with unirradiated control females, and the longevity of these newly hatched larvae was not as strongly affected by radiation dose (Fig. 2B). Only those offspring from males irradiated with 400 Gy had a shorter lifespan than untreated controls (Fig. 2B; $F_{\text{model}} = 2.15$; df = 9,33; $P = 0.0531$; $F_{\text{atm treatment}} = 0.29$; df = 1,33; $P = 0.5923$; $F_{\text{dose}} = 4.56$; df = 4,33; $P = 0.0047$; $F_{\text{atm treatment x dose}} = 0.23$; df = 4,33; $P = 0.9205$).

SUPPORT WEB BUILDING IN F_1 LARVAE

The ability of a group of newly hatched sibling larvae to build a communal support web was strongly impacted by the gender of the treated parent, so the sexes were analyzed separately (Fig. 3; $F_{\text{model}} = 52.91$; df = 19,67; $P < 0.0001$; $F_{\text{atm treatment}} = 4.19$; df = 1,67; $P = 0.0445$; $F_{\text{dose}} = 133.75$; df = 4,67; $P < 0.0001$; $F_{\text{sex}} = 160.63$; df = 1,67; $P < 0.0001$; $F_{\text{atm treatment x dose}} = 2.85$; df = 4,67; $P = 0.0305$; $F_{\text{sex x dose}} = 35.87$; df = 4,67; $P < 0.0001$; $F_{\text{atm treatment x sex}} = 1.08$, df = 1,67; $P = 0.302$; $F_{\text{atm treatment x dose x sex}} = 6.46$; df = 4,67; $P = 0.0002$). When females were irradiated with 100 Gy there was no effect of anoxia conditioning on larval web building, but with regard to the larvae of anoxia-conditioned females, fewer groups of larvae built webs in the 200 Gy treatment than in the untreated and 100 Gy treatment groups (Fig. 3A; $F_{\text{model}} = 71.33$; df = 9,34; $P < 0.0001$; $F_{\text{atm treatment}} = 4.93$; df = 1,34; $P = 0.0332$; $F_{\text{dose}} = 134.43$; df = 4,34; $P <$

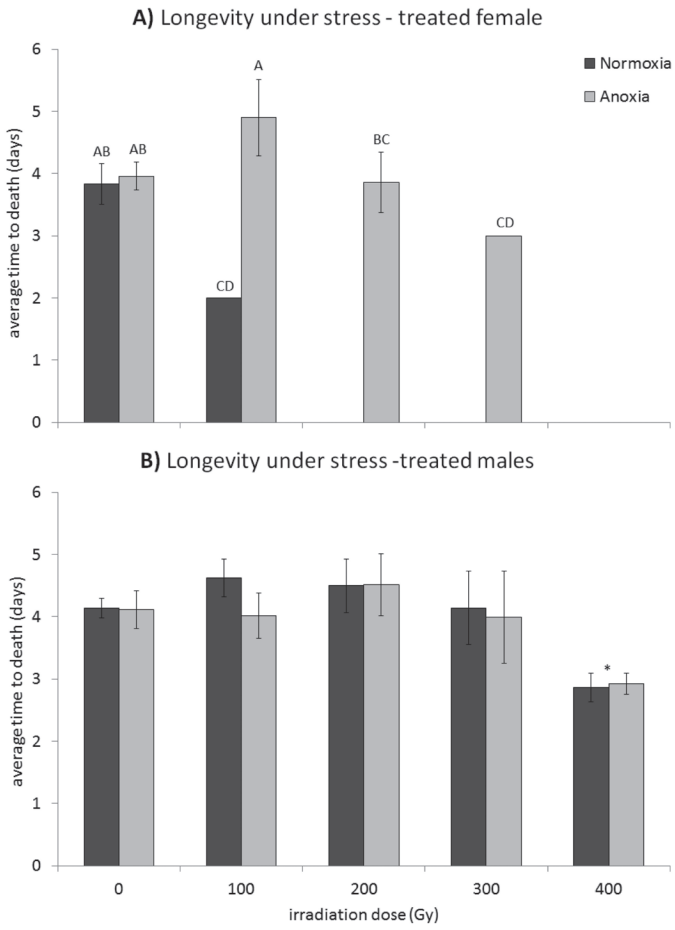

Fig. 2. Longevity (mean ± SE) of neonate *Cactoblastis cactorum* in the absence of food and water whose parents had been treated at 5 levels of irradiation and 2 levels of atmospheric conditions. Letter designations refer to Tukey's post hoc analysis.

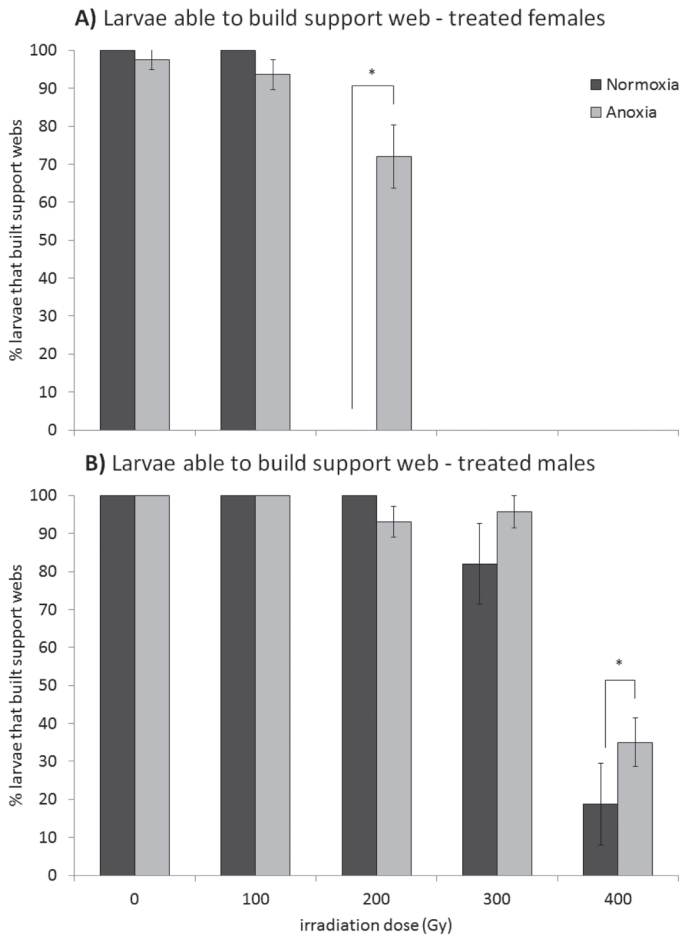

Fig. 3. Success rate (mean ± SE) of neonate *Cactoblastis cactorum* at building communal webs in the absence of food and water whose parents had been treated at 5 levels of irradiation and 2 levels of atmospheric conditions.

0.0001; $F_{\_atm\ treatment\ x\ dose}$ = 9.41; df = 4,34; P < 0.0001). Anoxia conditioning had no effect on communal web building by the progeny of treated males, and the effect of radiation dose was also less pronounced on the progeny of treated males than the progeny of treated females with the only detectable drop in communal web building occurring at the highest dose of 400 Gy (Fig. 3B; $F_{\_model}$ = 19.82; df = 9,33; P < 0.0001; $F_{\_atm\ treatment}$ = 0.51; df = 1,33; P = 0.4834; $F_{\_dose}$ = 43.39; df = 4,33; P < 0.0001; $F_{\_atm\ treatment\ x\ dose}$ = 0.85; df = 4,33; P = 0.5056).

ADULT LONGEVITY ASSAY DESIGN

Individually-housed males and females lived longer than their counterparts that were housed together in either single-sex or mixed-sex groups (Fig. 4; $F_{\_model}$ = 45.06; df = 5,6; P = 0.0001; $F_{\_sex}$ = 155.57; df = 1,6; P < 0.0001; $F_{\_grouping}$ = 31.86; df = 2,6; P = 0.0006, $F_{\_sex\ x\ grouping}$ = 3.50, df = 2,6; P = 0.1250), and overall males were longer-lived than females (P < 0.0001). Thus, in the subsequent experiment to evaluate effects of irradiation doses and anoxia on longevity, moths were housed in large cages that included individuals of both sexes in equal proportions.

ADULT LONGEVITY AFTER TREATMENT

The mortality rates of treated individuals were affected by atmospheric treatment, dose, gender, and their interactions (Fig. 5; $\chi^2_{\_model}$ = 102, df = 19, P < 0.0001, $\chi^2_{\_atm\ treatment}$ = 10.8, df = 1, P = 0.001, $\chi^2_{\_dose}$ = 34.7, df = 4, P < 0.0001, $\chi^2_{\_sex}$ = 25.1, df = 1, P < 0.0001, $\chi^2_{\_atm\ treatment\ x\ dose}$ = 20.3, df = 4, P = 0.0004, $\chi^2_{\_atm\ treatment\ x\ sex}$ = 9.65, df = 1, P = 0.0019, $\chi^2_{\_dose\ x\ sex}$ = 4, df = 19, P = 0.0296, $\chi^2_{\_atm\ treatment\ x\ dose\ x\ sex}$ = 11.6, df = 4, P = 0.0207). Anoxia conditioning reduced mortality risk in males irradiated at all doses, but anoxia had no effect on mortality risk in untreated males. Across all radiation doses, males irradiated in normoxia had ~45% greater probability of dying than males irradiated in anoxia (risk ratio = 1.45, 1.13–1.86 95% CI). In agreement with the reduced risk when combining anoxia conditioning with irradiation, males irradiated in anoxia lived longer than males irradiated in normoxia across all radiation doses (Fig. 6). In contrast to the pattern in males, there was no consistent effect of anoxia conditioning mortality risk in females. Anoxia-conditioned females irradiated with 300 Gy had a shorter median longevity than females irradiated with 300 Gy in normoxia (Fig. 6), but there was no effect of anoxia at any other level of irradiation. There was no effect of anoxia conditioning on either the mortality risk or median longevity when males or females were not irradiated.

Discussion

The efficacy of SIT relies on releasing insects that are both sterile and sexually competitive with wild insects in the field. This can be a challenge in use of sterility to suppress lepidopteras because the high doses of radiation needed to induce complete sterility have off-target effects on organisms that affect the performance of sterilized insects (Bloem et al. 2001). Thus, we suggest that hormetic-conditioning ap-

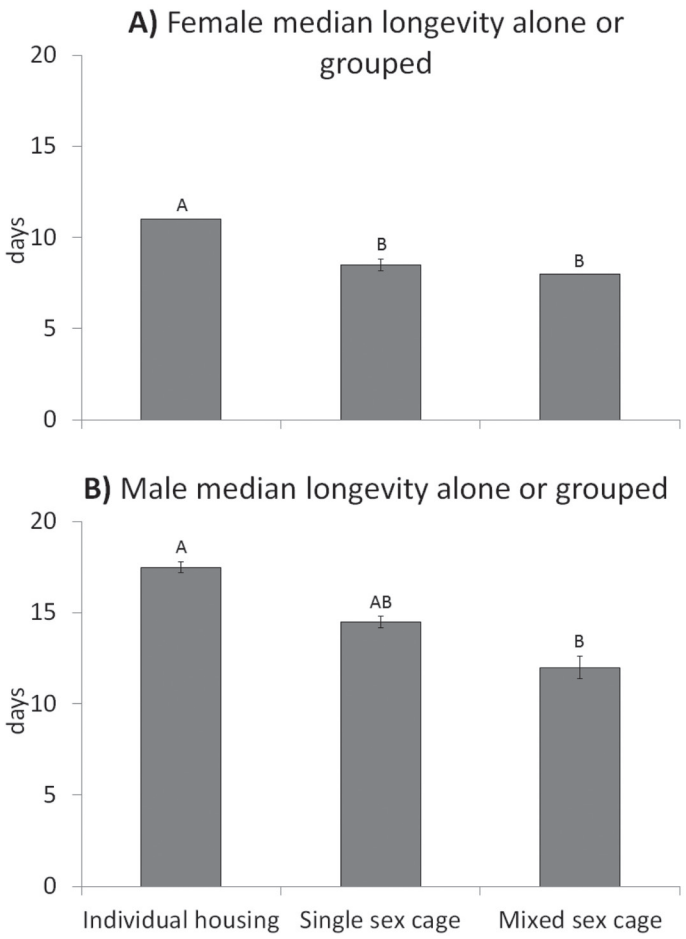

Fig. 4. Median longevity (median ± SE) of untreated control (no irradiation or atmospheric treatment) female and male *Cactoblastis cactorum* adults housed in 3 different conditions; individually in restrictive cups, together in single gender cages that allowed movement, and together in mixed gender cages that allowed movement and mating. Letter designations refer to Tukey's post hoc analysis.

proaches may be particularly useful for improving the organismal performance of sterile moths. Here we explore the effects of anoxia-conditioning before and during irradiation across a range of radiation doses from 100–400 Gy on the post-irradiation performance of the cactus moth.

We found no effect of anoxia-conditioning or of irradiation up to a dose of 400 Gy on any aspect of fecundity for either gender of *C. cactorum*. This is probably because *C. cactorum* were irradiated as adults, usually within 24 h of emergence, and by the time females emerged they had already matured their full complement of eggs. Three groups of 15 unmated females each were dissected and an average complement of 145 eggs/female was present within 12 h of emergence and prior to irradiation. Both untreated and irradiated females lived up to 8 d on average, but only oviposited eggs for a maximum of 6 d. At the time they died, all females retained at least about a third of the complement of eggs they had at emergence—whether they were irradiated or not. The fecundity of untreated females was also not affected by mating with irradiated males, regardless of the radiation dose received by males or whether they were anoxia-conditioned or not.

Anoxia-conditioning prior to and during irradiation had a dramatic effect on fertility in irradiated females mated with untreated males, but there was no effect of anoxia-conditioning on fertility of irradiated males when mated with untreated females. Increasing radiation doses led to lower fertility in both males and females, but this effect was substantially greater in females as expected for other Lepidoptera (Bloem et al. 2001). Previously we found that a 1 h pre-treatment of anoxia followed by irradiation with 200 Gy in anoxia strongly improved cactus moth performance in the laboratory, while preserving male inherited sterility at 96% (López-Martínez et al. 2014). A similar pattern was found in this study with 100 and 200 Gy wherein female fertility was partially rescued by anoxia conditioning (Fig. 1A), but there were no such effects on males at any radiation dose.

There is growing interest in the transgenerational effects of stress and how the experiences of parents may affect the development of the offspring (Ho & Burggren 2010; Ayyanath et al. 2013); thus newly hatched cactus moth larvae were observed until they succumbed to starvation/dehydration. Offspring from irradiated parents took longer to hatch once the eggs were oviposited, the developmental delay increased with increasing radiation dose, and there was no detectable effect of anoxia-conditioning on the radiation-induced developmental delay in egg hatching. When females were irradiated it is possible that this delay in embryonic development was due to a combination of genomic DNA damage and somatic damage done to the matured eggs when females were irradiated that must be repaired during embryonic development. However, because the effects of radiation dose on the delay in hatching were most conspicuous in eggs fertilized by irradiated males, we expect that the developmental delay we observed was due to fragmentation of paternal genomic DNA.

Anoxia conditioning substantially enhanced larval survival when females were irradiated. Very few larvae were able to hatch from females treated with 100 Gy in normoxia, but those that did hatch had 50% shorter lifespans than larvae from untreated controls (Fig. 2A). Larvae from mothers that were anoxia conditioned and irradiated with 100 Gy lived ~2.5 times longer than those irradiated in oxygen with 100 Gy. Anoxia treatment had even more dramatic effects on larvae from female moths treated in anoxia with 200 Gy, which not only were able to hatch while their normoxic 200 Gy counterparts did not, but they also lived up to 4 d under stress, similar to larvae of untreated females. Likewise, when considering females treated with 300 Gy, only those larvae whose mother was treated with anoxia were able to hatch, suggesting that the protective effects of anoxia treatment before and during irradiation extended beyond rescuing performance in the parental generation and trans-generationally improved the performance of the offspring (López-Martínez et al. 2014), even in the absence of food and water.

In nature, egg hatch from eggsticks is synchronized and sibling larvae group together to build a communal feeding web on the surface of cactus pads that may protect them from natural enemies and desiccation (Dickle 1991). To further quantify the effects of irradiation of parents on their offspring, sibling groups of larvae were observed for their ability to build communal webs. Not all larvae that were able to hatch survived for more than just a few hours under stress, and similarly not all those that survived were able to build support webs. When males were irradiated up to a dose of 300 Gy, there was no effect of anoxia treatment on the ability of larvae to build support webs. Nonetheless, there was a clear benefit of anoxia treatment with 400 Gy with ~35% of larval offspring from anoxia-treated males building webs compared with only ~20% of larval offspring successfully building webs when males were irradiated in normoxia with 400 Gy. Seventy percent of larvae from females treated with anoxia and 200 Gy were observed building support webs, compared with almost 100% of larvae from females treated with 100 Gy or untreated females regardless of atmospheric treatment. However, none of the larvae in the anoxia-300 Gy group

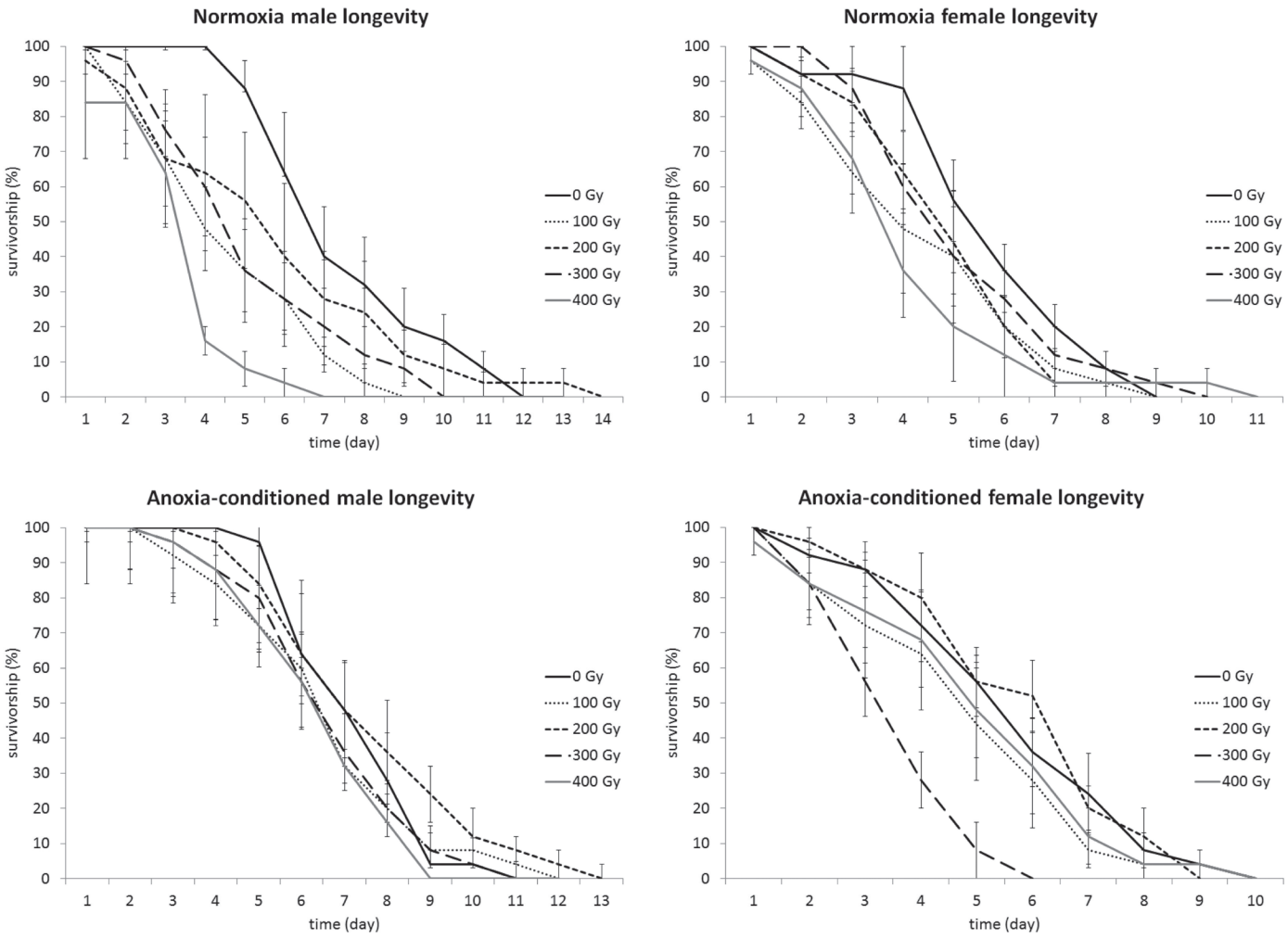

Fig. 5. Survivorship and longevity (mean ± SE) of female and male *Cactoblastis cactorum* adults treated at 5 levels of irradiation and 2 levels of atmospheric conditions.

were able to construct webs, even though they survived nearly as long as those in the anoxia-200 Gy group (Fig. 2A). Thus, survival time alone did not adequately describe larval performance.

Anoxia conditioning increased the lifespan of males irradiated at all doses, but did not extend the longevity of irradiated females. Long-lived sterile individuals may have a greater number of opportunities to mate with wild insects, and previous work has shown that Caribbean fruit flies sterilized in anoxia had increased lifespan and healthspan into old age, unlike individuals irradiated in normoxia (López-Martínez & Hahn 2014). In contrast to lifespan, healthspan is the duration of an organisms' lifespan in which the animal maintains adequate performance (Minor et al. 2010). Lifespan is routinely monitored in efforts to improve the performance of biological control agents, including sterile insects for release. However, longevity extension alone is not an improvement if performance is not maintained into old age. For example, López-Martínez & Hahn (2014) showed that anoxia-conditioning not only increased the lifespan of irradiated sterile male Caribbean fruit flies, but that anoxia-conditioned sterile males also maintained their sexual competitiveness better into old age than males irradiated in normoxia. Thus, we encourage research on whether the healthspan of important traits, like dispersal and mating competitiveness, is also maintained in interventions that extend longevity. Whether healthspan is increased by anoxia conditioning in irradiated cactus moths or other lepidopterans remains to be investigated.

It is noteworthy that anoxic conditioning clearly increased longevity in irradiated male cactus moths, but did not do so in irradiated female cactus moths. In fact, the only detectable effect of anoxia conditioning on mortality rates was that anoxically conditioned females irradiated with 300 Gy died earlier than females irradiated with 300 Gy in normoxia. Because this same trend towards greater hazard mortality in anoxia-conditioned females was not present in either higher or lower irradiation doses, anoxia-conditioning does not appear to generally decrease female performance, but neither is it helpful as it is in males. Why anoxia conditioning improved male longevity and not female longevity after irradiation is unclear, but female lepidopterans tend to have greater radiation sensitivity than males in general (Bloem et al. 2001; Carpenter et al. 2005) and more work is needed to determine the basis of this difference in hormetic responses between the sexes.

In the absence of any type of atmospheric treatment or irradiation, solitary males and females held in small cups were longer-lived than insects housed together in groups in larger cages where they could interact with each other and have more room for activity (walking, flying, and mating), confirming the cost of activity, interaction, and mating on lifespan. Because insects for programs with a SIT component will ultimately be expected to disperse and interact with other insects by mating—costly activities that are expected to decrease lifespan (Partridge et al. 2005)—it is important to consider the potential costs of

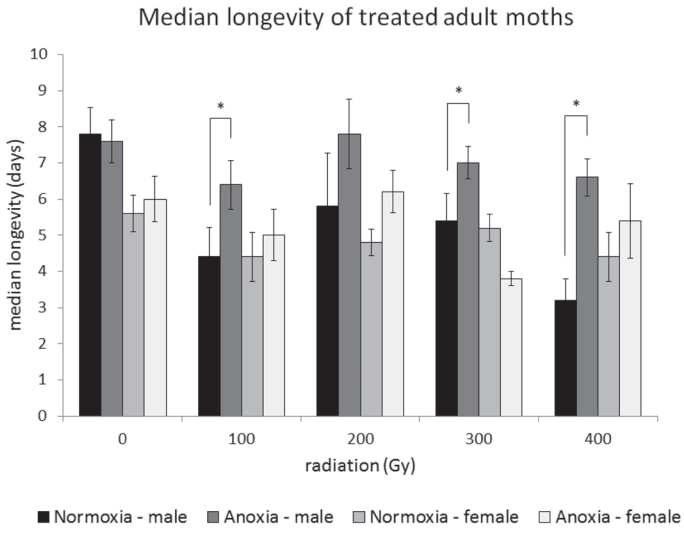

Fig. 6. Median longevity (median ± SE) of female and male *Cactoblastis cactorum* adults treated at 5 levels of irradiation and 2 levels of atmospheric conditions. Asterisks refer to significant differences at the 0.0001 level.

activity and mating when designing assays for longevity as part of any SIT quality control program or in any study of interventions that may improve the SIT.

This study showed that a conditioning treatment of anoxia 1 h prior to and during sterilizing irradiation had a protective effect that rescued parental sterility as well as larval survival after hatching in both irradiated males and irradiated females when they were mated to untreated individuals, as well as longevity in irradiated males but not in irradiated females. The ramifications of this work for lepidopteran SIT are that longer-lived irradiated males could be produced that have F_1 offspring with lower post-irradiation damage, and able to be more competitive than offspring of males irradiated in air. Because many lepidopteran programs that have a SIT component rely on inherited (F_1) sterility, our data suggest that the offspring of individuals treated with hormetic interventions, such as anoxia conditioning, can perform better and potentially improve the efficacy and economy of SIT programs by producing higher-quality sterile insects.

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