Locomotion activity meter for quality assessment of mass-reared sterile male moths (Lepidoptera)

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Abstract

Irradiation is used to provide sterile insects from mass-rearing facilities, but irradiation can degrade insect quality. A system is described that uses repeatable pheromone stimuli to activate male moths housed with clean airflow in a commercially available insect activity meter, for potential use in quality assessment of mass-reared moths in sterile insect programs. We tested sexually mature wild and sterile light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), males at the commencement of scotophase in a simple assay for increased mate-seeking activity after a 2 s stimulus with a 4-component synthetic pheromone source for 2 s, after a 5 s baseline of activity. Male activity at the upwind end of the glass tube was assessed by infrared beam. Next, we tested if a temperature shock at 30 °C in the pupal stage for various durations (0, 1, 2 or 4 h) would have an effect on male moth response to the sex pheromone. The results indicated similar baseline activity in clean airflow, but a significantly greater response after pheromone stimulation from non-irradiated males, compared with irradiated males. Responses from irradiated males averaged 78% of the control response (*n* = 320). The temperature shock did not appear to change the response of the non-irradiated moths (*n* = 64), but there was a slight decline in response by irradiated moths at the 2 and 4 h temperature shock durations. The system could be readily modified to be suitable for factory scale quality assurance.

Key Words: *Epiphyas postvittana*; sterile insect technique; competitiveness; fitness; irradiation; light brown apple moth

Resumen

Se utiliza la irradiación para proveer insectos estériles en las instalaciones de cría en masa, pero la irradiación puede degradar la calidad del insecto. Se describe un sistema que utiliza estímulos repetibles de feromonas para activar las polillas machos alojados con el flujo de aire limpio en un metro de actividad de insectos comercialmente disponibles, para su uso potencial en la evaluación de la calidad de las polillas criadas en masa en los programas de insectos estériles. Se probaron machos salvajes y sexualmente maduros y tambien polillas esteriles de la polilla de color marrón claro de la manzana (PMCM), *Epiphyas postvittana* (Walker) (Lepidoptera, Tortricidae), al inicio del escotofase en un ensayo simple para aumentar la actividad de búsqueda para las hembras después de 2 s de estímulo de una fuente hecha de una feromona sintética de 4 componentes durante 2 s, después de una línea de base de actividad de 5s. Se evaluó la actividad de los machos al apice del tubo de vidrio contra el viento por un rayo infrarrojo. A continuación, hemos probado si un choque de temperatura a 30 ° C en la fase de pupa para diversas duraciones (0, 1, 2 o 4 h) tendría un efecto sobre la respuesta de los machos a la feromona sexual. Los resultados indicaron actividad basal similar en el flujo de aire limpio, pero una respuesta significativamente mayor después de la estimulación de feromonas de los machos no irradiados, en comparación con los machos irradiados. El promedio de la respuesta de los machos irradiados fue el 78% de la respuesta del control (n = 320). El choque de temperatura no pareció cambiar la respuesta de las polillas no irradiados (*n* = 64), pero hubo una ligera disminución en la respuesta de las polillas irradiadas en las 2 y 4 horas de duración de choque de temperatura. El sistema podría ser modificado de manera adecuada para la asegurar de calidad de escala de fábrica.

Palabras Clave: *Epiphyas postvittana*; técnica del insecto estéril; competitividad; aptitud; irradiación; la palomilla marrón de la manzana

Pest management programs targeting Lepidoptera have increasingly looked to the sterile insect technique (SIT) for solutions because of its selectivity and proven track record against several species (Bloem et al. 2005; Bloem et al. 2007). The SIT relies on mass-rearing and release of sterilized but competitive males to locate and mate with wild female insects, to provide population suppression. Improvements in

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mass-rearing techniques, knowledge of genetics, trapping systems and other factors may account for increased interest in the SIT (Klassen & Curtis 2005; Vreysen et al. 2013). In moths, inherited sterility (IS) offers considerably greater suppressive potential from the release of partially sterile insects with a lower quality deficit due to the lower irradiation dose. Any F_1 offspring resulting from a sterile male: wild female cross produces completely sterile F_2 progeny, which increases the cost effectiveness of the IS approach over fully sterile releases (Carpenter et al. 2005). Lower radiation doses required to induce IS enable the release of moths that live longer, are stronger fliers, and mate more frequently than fully sterile insects (Carpenter 2000; Bloem et al. 2006; Stringer et al. 2013). Suppression or eradication was achieved using the SIT within area-wide programs for the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Bloem et al. 2005; Bloem et al. 2007) and the Australian painted apple moth, *Teia anartoides* Walker (Lepidoptera: Lymantriidae) (Suckling et al. 2007b). The SIT has been investigated with this goal for the European grape vine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) (Bloem et al. 2005), the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Carpenter et al. 2007) and the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Kean et al. 2011; Soopaya et al. 2011; Jang et al. 2012).

LBAM is an invasive tortricid from southeastern Australia, and its current known distribution outside of its natural range includes New Zealand, the USA, and some European countries (Suckling & Brockerhoff 2010). Sterility has been examined in the context of the SIT and modeled with competitiveness parameters to estimate the over-flooding ratio required for population suppression (Kean et al. 2011).

The SIT requires assessment of the effects of irradiation and other factors on insect quality (Vreysen 2005), and considerable effort has been expended on this for fruit flies (Cáceres et al. 2007), and more recently moths (Simmons et al. 2010). Many authors have used simple activity measures for flies, such as flight ability out of a cylinder, but this approach may have limitations for moths. Carpenter et al. (2012) described a very cost-effective and simple bioassay for assessing the quality of sterilized codling moths for both field and laboratory by counting the number of released moths that have flown from a cylinder over a period of around 3 days. While this assay is effective and affordable, it does come at the expense of time.

Wind tunnel assessment has been used to determine the effect of irradiation and other factors on male Australian painted apple moth arrival at a female (Suckling et al. 2004; Stephens et al. 2006) with quality assessment performed weekly during the painted apple moth eradication program in New Zealand (Suckling et al. 2004; Simmons et al. 2010). The evaluation proved to be valuable at detecting and improving insect quality by altering the handling process (Stephens et al. 2006). The reduction in quality caused by irradiation was used in a model designed to estimate the over-flooding ratio (Kean et al. 2007) during the male-only release program. Digital tracking of LBAM flight behavior in a wind tunnel also found some effect of irradiation on male quality of individuals, which was evident in the field with recaptures in hedgerows and vineyards (Suckling et al. 2011). The need for specialized facilities or release into the environment (which adds variables) reduces the practicality of this approach for routine assessment of quality.

Other assays of male behaviors in response to pheromone stimulus have been conducted, including a wing fanning assay in glassware (Bartell & Shorey 1969). We have previously assessed several methods for their suitability at detecting the effects of irradiation on male moth competitiveness (Suckling et al. 2011; Stringer et al. 2013). The close correlation between proclivity of an individual for activation and wing fanning and then arrival after a sustained zig-zag flight (Suckling et al. 2011) led us to hypothesize that direct assessment of wing fanning after pheromone stimulus might be a suitable measure of insect quality.

We tested the suitability of a commercially available locomotor activity meter (LAM), originally designed for vinegar flies. This project followed earlier use of locomotor activity meters for assessing Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) quality and competitiveness after irradiation (Dominiak et al. 2014). Here, we sought to understand whether impacts of irradiation on laboratory-reared male LBAM could be detected using observations of behavior obtained during pheromone stimulus. The focus was on upwind walking and wing fanning, because these behaviors were correlated with arrival and they effectively showed a dose response with irradiation (Suckling et al. 2011). We also tested whether heat shocks of various durations would have an effect on the quality of male moths in conjunction with irradiation.

Materials and Methods

INSECTS

Male LBAM pupae were obtained from Plant & Food Research in Auckland, reared on a modified Singh diet (Singh 1983) at 18 °C, 60% RH, and a photoperiod of 16:8 h L:D. After the pupae were exposed to the treatments, a 10% sucrose solution was provided for newly emerged adults to feed on. Only adult males aged ≥ 24 h were used in the experiments. Colonies of adult moths were relocated to the room where the experiments took place and were kept at 18 ± 1 °C with reverse phase (16:8 h L:D) lighting until needed.

PHEROMONE

Samples of (*E*)-11-tetradecenyl acetate (E11-14Ac, 99.7% purity), (*E,E*)-9,11-tetradecadienyl acetate (E9E11-14Ac, > 99% purity), (*E*)-11-tetradecen-1-ol (E11-14OH, > 99% purity), and (*E*)-11-hexadecenyl acetate (E11-16Ac, > 99% purity) were purchased from Plant Research International, Wageningen, The Netherlands and prepared at a 95:5:1:0.5 ratio (El-Sayed et al. 2011) in hexane at 100 µg loading on rubber septa (Thomas Scientific Inc., Philadelphia, Pennsylvania, USA).

EXPERIMENT 1

Pupae were placed on tissue paper and were irradiated in Petri dishes (Soopaya et al. 2011). Irradiations were conducted at the Institute for Environmental Science and Research in Christchurch using an external beam Cobalt^{60} Theratron unit (Atomic Energy of Canada Ltd.), which is a single radiation source that irradiates from above the target. Dose rate was 0.31 Gy/min, 55 cm from the source. A 3 mm thick layer of Perspex® was placed on top of the Petri dishes during irradiation to ensure electronic equilibrium, and full dose deposition at the top surface of the pupae. To ensure that the dose was uniform across the pupae, the dishes were inverted when half the required dose had been administered, and were irradiated inverted for the second half of the irradiation. Given the relatively large source-sample distance, and the sample flipping precautions, it was estimated that the maximum variability in the dose to any point in the sample was no more than ± 1% with a 95% level of confidence. There were 2 treatments: 0 Gy (non-treated) and 300 Gy. Each run consisted of 16 replicates of each treatment in randomly assigned positions. Twenty runs were performed for a total of 320 replicates to determine if there was a detectable difference between the irradiated and non-irradiated LBAM male activity after exposure to the sex pheromone blend.

EXPERIMENT 2

Half of the pupae were irradiated as described above while the other half were left non-irradiated. Both irradiated and non-irradiated pupae were then exposed to 1 of 4 levels of heat shock within 12 h of the irradiation treatment. The heat shock exposure of pupae was 0, 1, 2 or 4 h at 30 °C in a controlled atmosphere chamber. There were 8 treatments in total (2 × 4 factorial). Each run consisted of 4 replicates of each treatment randomly placed in a commercially-available electronic actinography LAM, with 32 glass cylinders and recording channels (LAM10, Trikinetics, USA; Fig. 1). Sixteen runs were performed to give a total of 64 replicates of each treatment. This set of treatment combinations was performed to simulate accidental heat exposure during travel to emergence and release sites of an SIT program, and to determine if temperature and radiation exposure may interact, affecting the response of males to the female-produced sex pheromone.

LOCOMOTOR ACTIVITY METER

The LAM was slightly modified by removing the back plate so that air could flow freely through each tube. The LAM was housed in a ventilated box (H × W × D: 34 cm, 46 cm, 25 cm; flow = 1.2 m / s) to pull the pheromone through the tubes and remove it to a waste airstream. Moths from each treatment were selected at random and placed 1 per tube. Each tube (L × D: 125 mm, 25 mm) had a 25 mm aluminium mesh insert at each end, which left 75 mm of space in the tube for the moths to move around. After trialling different configurations, we positioned the tubes so that there was 25 mm between the infrared sensors and the mesh inserts on the up-wind side; otherwise it was too difficult to detect the wing fanning of the moths near the mesh. Moths were stimulated with a 'puff' of the sex pheromone for 2 s presented at the upwind end of each tube (Fig. 1), and activity was recorded with the tripping of 1 or more of the 3 infrared beams when the moth was within 1 cm of the upwind opening near the stimulus. Activity counts were made every 30 s to establish a 2 min pre-pheromone exposure baseline (4 counts), followed by 2 min period post-exposure to the sex pheromone. The total before and total after counts were calculated for each replicate and these counts were used for analysis in both experiments.

STATISTICAL ANALYSIS

Counts were analysed using a hierarchical generalized linear modelling approach (Lee et al. 2006). Treatments (radiation, temperature

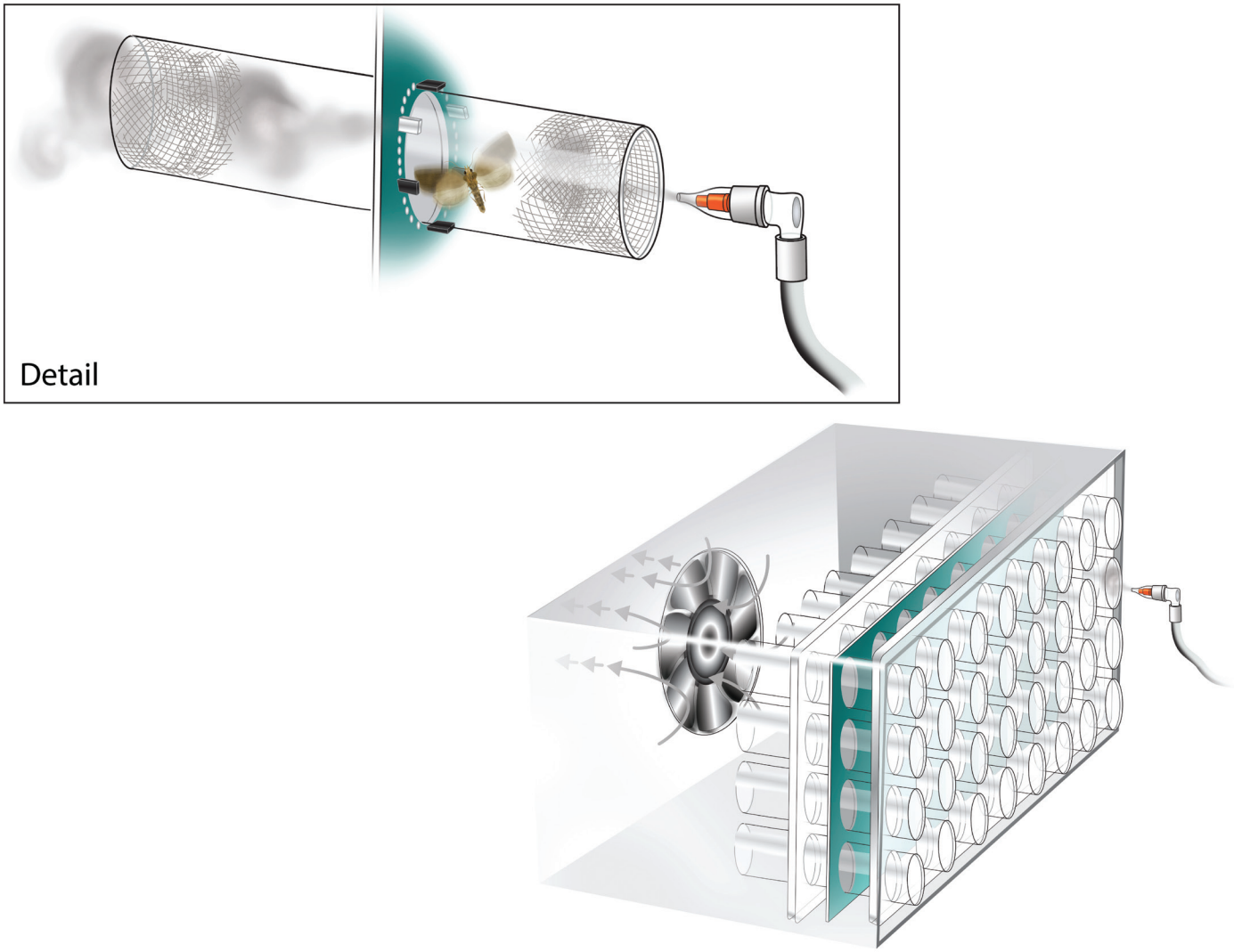

Fig. 1. An illustration of the modified Locomotion Activity Monitor set-up used for the bioassays in this study. Synthetic pheromone was puffed (2 s) from upwind to stimulate LBAM males into wing fanning. Each time a male moved through the beam array it was counted as 1 observation. Comparisons were made between pre-pheromone exposure activity and post-pheromone exposure activity for each treatment.

shock exposure time, before/after pheromone and the interactions between these; and also factors to make selected contrasts) were included as fixed effects with a Poisson distribution, and random effects (effects that increase variability, such as runs, and moths within runs) were included with a gamma distribution, with logarithmic links for both types of effect. The importance of a random effect was assessed with a χ^2 test of the change in deviance on dropping the term, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2013a), and fixed effects similarly, using GenStat's HGFTEST procedure. For both experiments, the random factors between runs and between moths within runs for the same treatment were both found to be important, and thus were included in the final analysis, to adjust results for them.

Estimated mean counts and associated 95% confidence limits for before and after activity levels, and for the ratio between them were obtained on the transformed (log) scale, and back-transformed for presentation. Analyses were carried out with GenStat (GenStat Committee 2013b).

Results

EXPERIMENT 1

Between 40 and 60% of moths for each treatment showed no activity either before or after pheromone stimulation (Table 1) with about 36% of moths over both treatments showing no activity in either period. However, there was no evidence that a lack of activity was associated with the radiation treatment, since the percentage of inactive moths was similar for irradiated and un-irradiated moths. The raw activity data shows that moth activity increased after moths were exposed to the sex pheromone (Fig. 2).

Table 2 and Fig. 2 summarize activity levels for irradiated and un-irradiated moths. Prior to pheromone stimulation, activity levels were similar for both treated and untreated moths, at about 2.7 activity counts. Activity levels for both rose after stimulation, but the increase was noticeably greater for the un-irradiated moths: There was a significant interaction between radiation and after: before (χ^2 = 12.76; df = 1; $P < 0.001$), indicating that the after: before pheromone ratio was modified in irradiated moths in comparison to un-irradiated moths. The after: before activity ratio was about 40% greater for un-irradiated moths compared to irradiated moths.

EXPERIMENT 2

Around half of moths showed no activity before or after pheromone exposure (Table 3). A third of moths showed no activity at all (both zero); these inactivity levels were therefore quite similar for the 2 experiments. Table 4 and Fig. 4 summarize the activity measured for experiment 2. Changes in activity with respect to increasing temperature shock exposure varied both between before and after pheromone exposure, and between irradiated and un-irradiated moths (χ^2 = 11.52; df = 3; P = 0.009 for the time by radiation by before/after interaction).

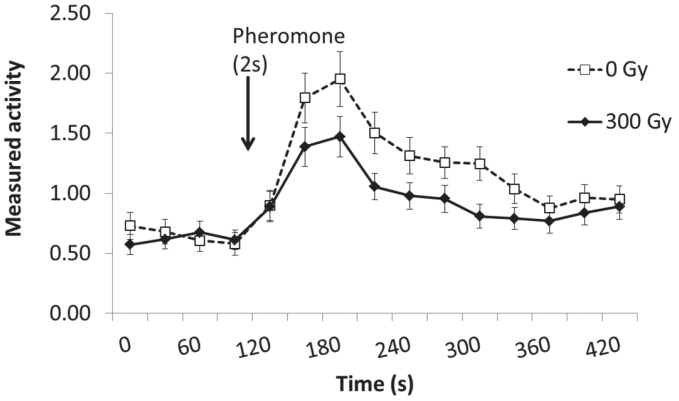

Fig. 2. Measured activity of male *Epiphyas postvittana* as summed counts at 30 s intervals in a locomotor activity monitor, before and after pheromone stimulus of irradiated and non-irradiated moths.

Thus, the after:before activity ratio temperature exposure response varied between irradiated an unirradiated moths. The change in activity with increasing exposure was inconsistent, and activity did not vary significantly with temperature shock duration for either treatment, either before or after stimulation (χ^2 = 4.50, 1.71, 1.70, 1.36; df = 3; P = 0.212, 0.635, 0.637, 0.715 for the temperature effect, for before, without / with radiation, and after without / with radiation respectively).

The after:before ratio also did not vary significantly with temperature for irradiated (χ^2= 6.729; df = 3; P = 0.081) or un-irradiated (χ^2 = 1.993; df = 3; P = 0.574) moths, but the response pattern did vary noticeably. For 0 and 1 h shock, the ratio was fairly similar for irradiated and un-irradiated moths, but it increased between 1 and 2 h exposure for un-irradiated moths, but decreased for irradiated moths (thus, the significant 3-way interaction).

Discussion

When undertaking an area-wide program that includes the release of sterile males, it is important to determine the competitiveness of the insects being released in order to have the highest chance at success (Simmons et al. 2010; Kean et al. 2011). There are many quality assays that measure the physical capabilities of the released insect, such as the ability to fly in a wind tunnel or be recaptured after release (Suckling et al. 2011; Stringer et al. 2013). But possibly the most important quality to measure is the mass-reared insects drive and ability to mate (Simmons et al. 2010; Kean et al. 2011). By slightly modifying a commercially available locomotion activity monitor so that air could flow through the tubes, we were able to test the response of laboratory-reared LBAM males to the 4-component sex pheromone. We were able to see change in the pattern of activity, i.e., from low activity at the base level to higher activity in the post pheromone phase, finally settling down to near base levels after ~6 min (Fig. 2), which is similar to the recovery rates found by Bartell

Table 1. Number of *Epiphyas postvittana* males with zero activity count before, after and both before and after pheromone exposure in the locomotor activity monitor. The number of moths used for each radiation treatment was 320.

Radiation dose	Pheromone exposure		No. with 0 activity both before and after pheromone exposure
	Before	After	
0 Gy	190	137	112
300 Gy	172	154	117

Table 2. Mean activity counts for un-irradiated (0 Gy) and irradiated (300 Gy) *Epiphyas postvittana* males before and after pheromone exposure, and the mean after/before activity ratio (95% confidence limits). n = 320.

Radiation dose	Pheromone exposure		Activity ratio: After/Before
	Before	After	
0 Gy	2.6 (1.9, 3.6)	6.3 (4.7, 8.5)	2.4 (2.0, 2.9)
300 Gy	2.8 (2.0, 3.8)	4.9 (3.6, 6.7)	1.8 (1.5, 2.1)

Table 3. Numbers of *Epiphyas postvittana* males with zero activity count before, after and both before and after pheromone exposure. Treatments were radiation (0 Gy or 300 Gy) plus temperature shock (0, 1, 2, 4 h at 30 °C). The number of moths used for each treatment was 64.

Radiation dose	Temperature shock (h)	Pheromone exposure		No. with 0 activity both before and after pheromone exposure
		Before	After	
0 Gy	0	34	30	23
	1	28	29	19
	2	37	30	24
	4	39	25	24
300 Gy	0	41	31	28
	1	36	31	26
	2	29	34	24
	4	29	31	21

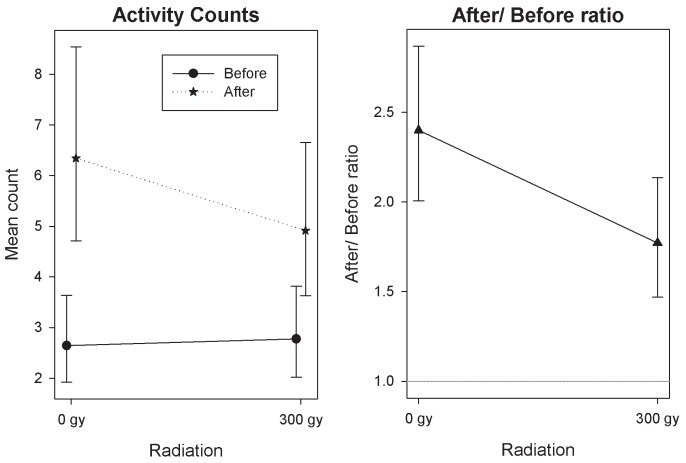

Fig. 3. Mean activity counts for before and after pheromone exposure (left), and the mean after/before activity ratio (right), for un-irradiated (0 Gy = −) and irradiated (300 Gy = +) *Epiphyas postvittana* males. Error bars are 95% confidence limits for each mean. An after/before ratio of 1 (marked) indicates an equal level of activity before and after pheromone exposure.

(1985). For this experiment we used 30 s reading intervals, but the interval can be adjusted down to every 1 s or up to 60 min, depending on how fine or coarse a resolution is needed in the behavior of the insects.

Mating competitiveness is significantly reduced by irradiation in Lepidoptera, and adverse effects have been shown on males of *C. pomonella* (Carpenter et al. 2012; Carpenter et al. 2013) and both male and female LBAM (Suckling et al. 2011; Stringer et al. 2013). Wind tunnel assessment of changes in moth flight ability after irradiation has been done on species in 2 families of Lepidoptera (Suckling et al. 2005; Suckling et al. 2007a). The wind tunnel system, which has been widely used in pheromone research (Baker & Vickers 1997), looks promising for measuring insect quality in irradiated Lepidoptera, but is not suitable for routine use. A need has also been expressed for a more automated system suitable for factory scale use. An automated system of testing single moths in behavioral assays would be desirable to advance automation of moth quality assessment. While simple and affordable bioassays have been developed to assess the quality of sterilized insects (Carpenter et al. 2012), the bioassay presented here using the LAM system demonstrates that 32 individual insects can be assessed (or many more as more modules are added) in a matter of min for each run.

The locomotion activity meter indicated that a significant reduction of around 23% occurred in activation response to pheromone (Table 2) for insects irradiated at 300 Gy compared with untreated controls. The estimate of a reduction to 77% of control values is similar to recapture rates with male LBAM irradiated at 300 Gy and released in hedgerows (75% of control values) and vineyards (78% of control values) (Suckling et al. 2011).

Abiotic stressors, such as changes in temperature, are known to have a deleterious effect on various life stages of insects. This is of particular interest when insects are reared and released for SIT programs. Unfortunately, temperature changes can happen when insects are transported from the rearing site to the release site. Even a short duration temperature shock has been known to affect the quality of sterile insects (Dominiak et al. 2007; Dominiak et al. 2014). Carpenter et al. (2012) found that irradiated codling moths were more likely to be of lower quality than non-irradiated after handling during transport. Gutierrez et al. (2010) demonstrated that the lower and upper threshold for all LBAM development stages was between 6.8 and 31.3 °C. In the same study, Gutierrez et al. (2010) refined the calculated optimum temperature of 19.15 °C by re-analyzing data from Danthanarayana's earlier study (Danthanarayana 1975). By using the upper limit of 30 °C, we stayed within the upper/lower temperature range of LBAM during our temperature shock experiment. While the various temperature shock durations did not appear to lower the competitiveness of the non-irradiated males, there was a slight negative effect on the irradiated males at the 2 and 4 h durations. This suggests limited tolerance for higher temperatures in this laboratory strain, which had been cultured for 134 generations.

Recent developments support the use of locomotion activity meters for assessing quality in irradiated fruit flies (Dominiak et al. 2014). Even before the pheromone was identified, LBAM were observed and characterized for response to sex pheromone (extracted

Table 4. Mean activity counts before and after pheromone exposure, and the mean after/before activity ratio, for un-irradiated (0 Gy) and irradiated (300 Gy) *Epiphyas postvittana* males exposed to 1 of 4 levels of temperature shock (0, 1, 2, 4 h at 30 °C) (95% confidence limits).

Radiation dose	Temperature shock (h)	Pheromone exposure		No. with 0 activity both before and after pheromone exposure
		Before	After	
		4.4 (2.8, 6.8)	5.6 (3.7, 8.4)	1.3 (0.9, 1.8)
	1	3.1 (2.0, 5.0)	4.1 (2.6, 6.4)	1.3 (0.8, 2.0)
	2	3.2 (2.0, 5.1)	5.7 (3.8, 8.6)	1.8 (1.2, 2.7)
	4	2.9 (1.8, 4.6)	5.0 (3.3, 7.6)	1.7 (1.2, 2.6)
300 Gy	0	3.4 (2.1, 5.3)	4.4 (2.9, 6.8)	1.3 (0.9, 2.0)
	1	3.2 (2.0, 5.1)	5.2 (3.4, 7.9)	1.6 (1.1, 2.4)
	2	4.5 (2.9, 6.9)	4.1 (2.7, 6.4)	0.9 (0.6, 1.4)
	4	3.8 (2.5, 6.0)	4.1 (2.7, 6.4)	1.1 (0.7, 1.6)

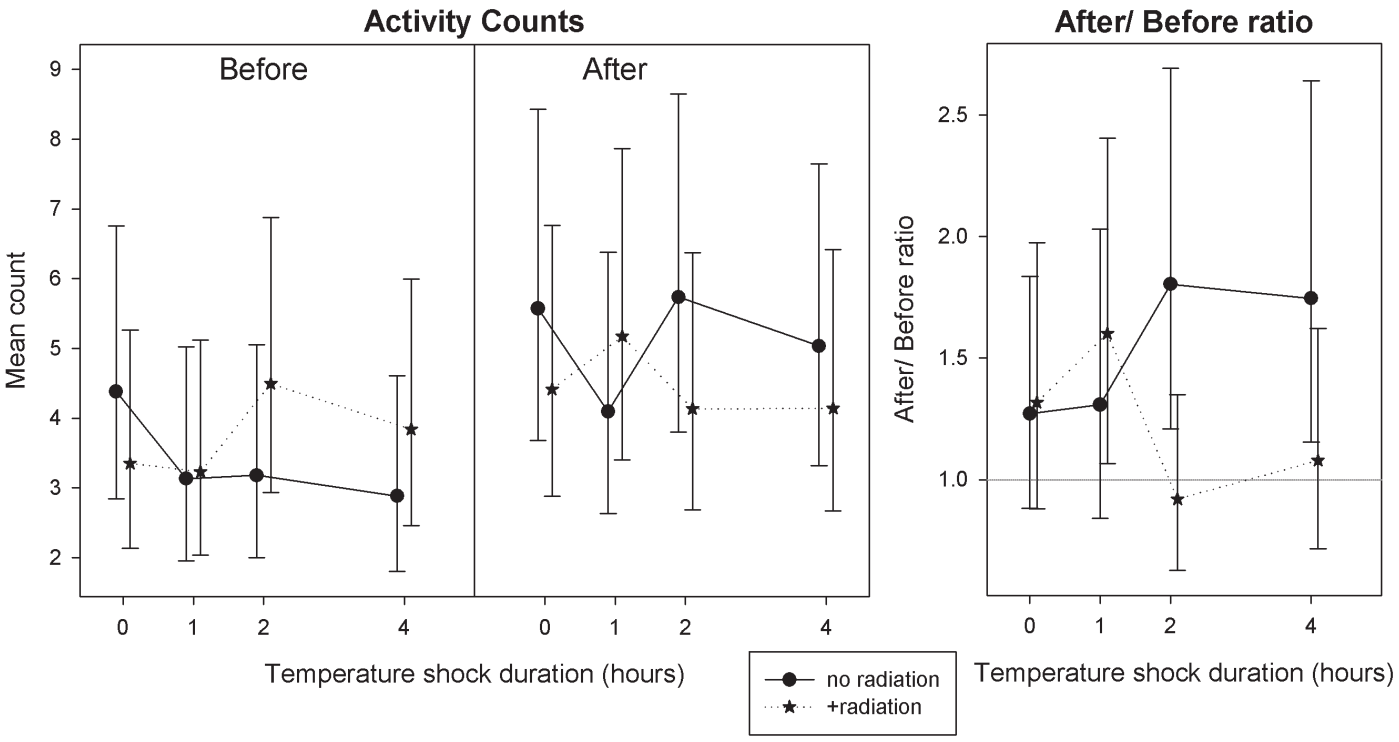

Fig. 4. Mean activity counts for before and after pheromone exposure (left), and the mean after/before activity ratio (right), un-irradiated (0 Gy) and irradiated (300 Gy) *Epiphyas postvittana* males exposed to 1 of 4 levels of temperature shock (0, 1, 2, 4 h at 30 °C). Error bars are 95% confidence limits for each mean. An after/before ratio of 1 (marked) indicates an equal level of activity before and after pheromone exposure.

from female moths), with multiple individuals caged in a rotating glass device to ensure clean air and a low background of activity (Bartell & Lawrence 1976). Modern variations to this could include motion recording using machine vision techniques after pheromone stimulation, but after initial experiments and after considering the advantages demonstrated in Dominiak et al. (2014), we pursued the use of the LAM. Pre-courtship behavior in LBAM involves male wing fanning and movement towards the female, which the assay described in the present study captures. Stimulation with pheromone and analysis of the response offers a number of benefits, because this behavior is correlated with flight and probability of mating, but can be measured far more easily.

The LAM was successful at measuring activity following pheromone stimulation including increased walking and wing fanning. It has potential for wider use in measuring insect quality and could prove to be amenable to automation. The commercially-available LAM would appear to have significant advantages including cost and ready availability along with a relatively fast turnover of replicates. This system could help with the assessment of sterile insects so that programs can better model/predict the effect of each release.

Acknowledgments

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