Mating Compatibility and Competitiveness between Wild and Laboratory Strains of Eldana saccharina (Lepidoptera: Pyralidae) after Radiation Treatment

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Mating compatibility and competitiveness between wild and laboratory strains of *Eldana saccharina* (Lepidoptera: Pyralidae) after radiation treatment

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Abstract

The efficacy of the sterile insect technique (SIT) applied as part of area-wide integrated pest management (AW-IPM) depends on efficient transfer of sperm carrying dominant lethal mutations from sterile males to wild females. Success or failure of this strategy is therefore critically dependent on quality and ability of sterile males to search for and copulate with wild females. The African sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is an economic pest of sugarcane targeted for control in South Africa using an AW-IPM approach with a SIT component. As part of further steps towards development of the technique, levels of mating competitiveness and compatibility were assessed by observing the extent to which individuals from different populations interbreed when confined together under both laboratory and semi-field conditions. Three types of pair-wise competition experiments were conducted: non-irradiated laboratory adults vs. non-irradiated wild adults, irradiated (200 Gy) laboratory adults vs. non-irradiated wild adults, and non-irradiated laboratory adults vs. irradiated (200 Gy) laboratory adults. Data from these tests were used to generate indices for mating performance and measuring sexual compatibility between strains. Irrespective of trial location, wild moths did not discriminate against irradiated or laboratory-reared moths, indicating no negative effects on acceptability for mating due to laboratory rearing or radiation treatment. In general, irradiated males mated significantly more than their wild counterparts regardless of the type of female, which indicated that they were still as competitive as their wild counterparts. The mating indices generated showed no evidence of incipient pre-mating isolation barriers or sexual incompatibility with the wild strain. Data presented in this paper therefore indicate that there is scope for further development of the SIT as an addition to the arsenal of tactics available for AW-IPM of this economic pest.

Key Words: African sugarcane stalk borer; sterile insect technique; mating behavior; mating indices; sexual selection; performance.

Resumen

La eficacia de la técnica del insecto estéril (TIE) aplicado como parte de la gestión de toda la zona integrada de plagas (AW-IPM) se requiere la transferencia eficiente de los espermatozoides portadores de mutaciones letales dominantes de machos estériles a las hembras salvajes. Por lo tanto, el éxito o el fracaso de esta estrategia depende críticamente de la calidad y capacidad de los machos estériles para buscar y copular con hembras salvajes. El barrenador de la caña de África *Eldana saccharina* (Lepidoptera: Pyralidae) es una plaga económica de la caña de azúcar específica para el control en Sudáfrica utilizando el enfoque de gestión integrada de plagas con un componente de la TIE. Como parte de nuevas medidas para el desarrollo de la técnica, los niveles de competitividad de apareamiento y la compatibilidad se evaluaron mediante la observación de la medida en que los individuos de diferentes poblaciones se cruzan cuando se confina juntos, tanto en condiciones de laboratorio y semi-campo. Se realizaron tres tipos de ensayos de competencia por parejas: adultos de laboratorio no irradiados vs. adultos salvajes no irradiados; irradiados (200 Gy) adultos de laboratorio frente a los adultos salvajes no irradiados: y adultos de laboratorio no irradiados vs. irradiados (200 Gy) adultos de laboratorio. Los datos de estas pruebas se usaron para generar índices simples para el seguimiento del rendimiento de apareamiento y la medición de la compatibilidad sexual entre las cepas. Independientemente de la ubicación del ensayo, polillas silvestres no discriminaban contra cualquiera de las polillas irradiadas o criados en el laboratorio, lo que indica sin efectos negativos debido a laboratorio de cría o tratamiento de radiación. En general, los machos irradiados aparearon significativamente más que sus contrapartes salvajes independientemente del tipo de sexo femenino que indica que eran todavía tan competitivo como sus contrapartes salvajes. El apareamiento índices generados no mostró evidencia de barreras de aislamiento pre-apareamiento incipientes o incompatibilidad sexual con la cepa salvaje. Los datos presentados en este trabajo, por lo tanto indican que hay un margen para un mayor desarrollo de la TIE como una adición al arsenal de tácticas disponibles para AW-IPM de esta plaga económica.

Palabras Clave: la caña de azúcar de África tallo barrenador; la técnica del insecto estéril; la conducta de apareamiento; los índices de apareamiento; la selección sexual; el rendimiento.

During the last decades, the sterile insect technique (SIT) has gained recognition as an environment-friendly and effective control tactic for use in area-wide integrated pest management (AW-IPM) programs against many lepidopteran pests (Vreysen et al. 2007). The African sugarcane stalkborer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is a key pest of sugarcane in East, West and South Africa

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(Atkinson 1980; Chinheya et al. 2009) and is targeted for control using an AW-IPM approach with an SIT component. Examples of successful SIT initiatives targeting lepidopteran pests include the program in the USA and Mexico against the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (Carpenter et al. 2008), the Okanagan-Kootenay Sterile Insect Release (OKSIR) suppression program against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in British Columbia, Canada (Bloem et al. 2007), the containment program against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in the San Joaquin Valley, California, USA (Henneberry 1994) and the suppression program against the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa (Carpenter et al. 2007).

Although the concept of the SIT is simple, its implementation is more complex (Seawright 1988). This is partially due to higher mortality rates of the sterile insects and their tendency to lose their ability to perform behaviors that allow them to successfully mate and interrupt reproduction of wild females (Calkins & Parker 2005), as they go through a potentially behavior-altering chain of processes from mass-rearing and irradiation in an artificial environment to final release in the target area. Understanding courtship behavior and mating systems of species targeted for SIT and how they are influenced by mass-rearing and irradiation is one of several crucial steps that may lead to improvements in sterile male performance (Hendrichs et al. 2002). This would reduce the sterile to wild male over-flooding ratios routinely applied to compensate for the lower effectiveness of mass-produced sterile insects (Hendrichs et al. 2002). The mating performance of sterile mass-reared male insects is estimated to be between a third and half of that attained by wild males (FAO/IAEA/USDA, 2003). Should mating competitiveness be enhanced, it would significantly lower the costs of SIT application. The effectiveness of the SIT in AW-IPM strategies is based, in part, on the efficient transfer of sperm carrying dominant lethal mutations from sterile males to wild females (Bushland & Hopkins 1953; Knipling 1955). Lepidopteran species are amongst the most radiation-resistant insects (LaChance 1985), usually requiring such a high radiation dose to achieve full sterility that it reduces their competitiveness and performance in the field (Proverbs 1962; LaChance 1985). However, Walton (2011) demonstrated that F_1 (inherited) sterility is attainable in *E. saccharina* using sub-sterilizing radiation doses comparable with other lepidopterans such as the false codling moth (Bloem et al. 2003) and the cactus moth (Tate et al. 2007). The use of F_1 sterility is one way to circumvent the negative effects associated with the high radiation resistance (Suckling 2003; Soopaya et al. 2011). This method is a variation on the original SIT approach and uses male insects for release that are partially sterile, and thus have superior competitiveness because they have been exposed to a lower radiation dose. Use of F_1 sterility can be more effective at overall population suppression due to the more competitive mating of the released insects and the resulting heterozygote offspring that carry dominant lethal genotypes, which are passed into the wild population (LaChance 1985). Because success of the SIT is dependent on the quality and competitiveness of sterile males in the field, F_1 sterility is now the preferred approach for managing lepidopteran pests with SIT (LaChance 1985; Suckling 2003).

Mass-rearing, nevertheless, is necessary to produce the large number of males required for the SIT (Weldon 2005), and most rearing facilities often maintain the same strain for long periods of time (Rössler 1975a). This, consequently, may result in deterioration of insect quality after a certain number of generations (Partridge 1996). The biotic and physical conditions in mass-rearing facilities are very different from those in the field, and can greatly affect the phenotype of the sterile strain by selecting for traits required for efficient laboratory performance rather than field performance (Simmons et al. 2010). Changes in the gene pool due to artificial selection pressures imposed by the laboratory environment can be countered by regularly replacing the colony with field collected material (Calkins & Parker 2005). Often a production bottleneck is encountered when introducing field material, as only a fraction of the field collected individuals survive and reproduce during the initial phase of laboratory colonization (Leppla 1989). This may result in a delayed attainment of sufficient colony size to sustain SIT operations, as well as strain incompatibility and sexual isolation due to a reduction of the new strain's initial gene pool and variations in behavior of the mass-produced insects (Calkins & Parker 2005).

Preliminary laboratory and non-competitive trials have shown that adult male *E. saccharina* treated with an ionizing gamma radiation dose of 200 Gy, the optimum radiation dose for *E. saccharina* in an inherited sterility program (Walton 2011), called significantly earlier and achieved higher mating frequencies with wild females, than their wild counterparts (Mudavanhu et al. 2011). The primary question now is whether or not irradiated *E. saccharina* males reared in the laboratory are competitive with wild male *E. saccharina* when both occur in the same environment. It is tempting to simply release irradiated laboratory-reared adults into the target area to compete with wild adults. However, if the irradiated laboratory-reared moths are not competitive in this environment, several reasons could explain this inferiority. These include degradation of moth quality due to radiation (Calkins & Parker 2005), handling (Terblanche et al. 2008), laboratory rearing regime (which also disrupts synchrony) (Weldon 2005), the laboratory colony being lab-adapted in a way that promotes assortative mating (Calkins & Parker 2005), population size of *E. saccharina* in the wild being inaccurately estimated, or all of the above.

Levels of mating competitiveness and compatibility can be evaluated cost-effectively by observing the extent to which individuals from 2 populations interbreed when confined together in a field-cage setting (Taret et al. 2010). Therefore, the objectives of this study were to: (1) examine the effect of laboratory rearing and radiation (with gamma rays of 200 Gy) on mating competitiveness and compatibility; (2) examine the possibility of additive or synergistic effects due to laboratory rearing and irradiation on mating competiveness and compatibility compared with wild populations; and (3) investigate the overall performance of different adult treatments/strains in 2 different mating arenas. The data from these tests will be used in simple, reproducible and meaningful indices for tracking performance and making comparisons between strains (Cayol et al. 1999; Taret et al. 2010).

Material and Methods

STUDY POPULATIONS

Wild *E. saccharina* were collected from sugarcane plantations near Tinley Manor on the subtropical north coast of KwaZulu-Natal, South Africa (29.446 °S, 31.258 °E; 31 m asl), either as large instar larvae and reared to pupal stage on 8 mL of artificial *E. saccharina* diet (Walton 2011) in 30 mL plastic vials with screened lids or as pupae and stored individually in transparent multi-cell (n = 32) emergence trays sealed with perforated plastic wrap. These were placed in incubators (MRC® LE-509, Holon, Israel) at 26 ± 1 °C, 60 ± 10% RH and a 12:12 h L:D photoperiod.

Mass-reared *E. saccharina* were also obtained as pupae from the mass-rearing facility at the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe, Durban. The laboratory pop-

ulation was cultured for 4 generations since the previous infusion of wild stock. Mass-reared *E. saccharina* were marked by adding Calco Red to their larval diet to distinguish them from wild adults (Walton & Conlong 2008). The live insects were stored as pupae in containers as described above, packed into cardboard boxes that contained shredded paper and couriered by air to the Conservation Ecology and Entomology Department at Stellenbosch University, Western Cape, where all the experiments were conducted. No additional insulation from temperature variability was provided for the pupae during air transport. On arrival the pupae from the different populations were maintained under the same conditions as described. The pupae were checked at 08:00 h daily for emergence. Newly emerged adults were separated by sex and treatment type (in groups of 10 individuals) into cylindrical transparent 1 liter plastic containers (Plastics for Africa®) with screened lids. The highly active adults were provided with paper towel to perch on as well as to prevent damage to their wing scales.

Sterile adults were obtained by irradiating freshly emerged laboratory-reared adult *E. saccharina* at the radiation facility of SIT Africa Pty (LTD), ARC-Infruitec/Nietvoorbij, Helshoogte Road, Private Bag X5026, Stellenbosch, 7599. Treatment samples were exposed to 200 Gy gamma radiation delivered at a rate of 3.75 Gy/min from a panoramic ^{60}Co point source centrally located on a rotating turntable 1 m in dia. The samples were placed on one or more of 8 smaller counter-rotating turntables, each 200 mm in diameter and situated equidistant on the periphery of the main turntable. The resulting dual rotation of the adults facilitated uniform dose distribution throughout the sample. The dose was verified by Sterin® or RadTag® indicators in each container with adults. All irradiation treatments commenced at 08:00 h. Adults were transported to the radiation facility (3 km distance) in the same containers in which they were irradiated. Experiments were conducted approximately 7 h after exposure to radiation. In all experiments, only freshly emerged (zero-day old) virgin male and female adults were assayed, and discarded after use.

EXPERIMENTAL DESIGN

The mixed population mating tests were conducted under both controlled laboratory and field cage conditions. The laboratory and field cage trials were implemented concurrently in order to examine for possible interactions between adult strain performance and type of cage (for example a particular adult strain may be competitive with another in the small bench-top cage but less competitive in the large walk-in cage with sugarcane or vice versa). Tests were conducted over a 12 h scotophase starting at dusk in both the laboratory and field mating arenas.

Laboratory Setup

For the laboratory trials, a transparent Perspex® bench-top cage (300 mm × 300 mm × 300 mm) was installed in a climate and photoperiod controlled room (26 ± 1 °C, 60 ± 10% RH, 12:12 h L:D photoperiod). Access to the bench-top cage was by means of a transparent Perspex® lid, while ventilation was by means of white cotton mesh cloth on the side walls. To enable a comparison of the onset times of mating between laboratory and outdoor cage tests, laboratory lights were synchronized with daylight.

Field setup

A large cylindrical outdoor walk-in field cage (3 m diam, 2 m height) fabricated of Lumite® (poly-monofilament) screen of mesh (thread count size 32 × 32) was used for the field experiments. The field cage was installed on an open area in a cleared apple orchard surrounded by a pine tree wind break at Welgevallen Farm in Stellenbosch (33.947 °S, 18.872 °E) (Chambers et al. 1983). Screening of the cage was light brown in color to allow good light penetration. The field cage was provided with a single 22 month-old potted sugarcane plant (variety 'NCo 376'). The night temperature at the field site between 18:00 to 06:00 h ranged from 13.6 ± 1.4 °C to 20.5 ± 1.5 °C while the average relative humidity was 73.7 ± 3.5%. The photoperiod of 12:12 h L:D of the field site during Oct–Nov (duration of this study) matched the average natural light: dark cycles in the areas of collection. Although wind speed and direction may have a bearing on the results, they were not recorded. For both the field and laboratory tests the proportion of mating (FAO/IAEA/USDA 2003) was used to determine the suitability of the test environment and treatment adults for mating, as well as the suitability of the data for inclusion in the analysis.

Release into Experimental Cages

To distinguish strain types during the choice tests, the insects were lightly marked on the dorsal part of the thorax using black and red Pentel® permanent markers prior to cage observations. Males were released into the experimental cages 1 h prior to testing to allow them to disperse, acclimate to the cage environment and establish territories. Females were released 1 h later (at the start of each test).

Each cage was stocked with an equal sex ratio of 6 females and 6 males for each treatment. Three types of pair-wise comparison/competition experiments were conducted: (1) non-irradiated laboratory and wild adults ("L-W"), (2) non-irradiated laboratory and irradiated laboratory adults ("L-S"); (3) irradiated laboratory and non-irradiated wild adults ("S-W").

Experiment 1 examined the effect of laboratory rearing on mating competitiveness and compatibility between non-irradiated laboratory-reared and non-irradiated wild males. Experiment 2 examined the effects of irradiation on mating competitiveness and compatibility between non-irradiated laboratory-reared males and irradiated laboratory-reared males. Experiment 3 examined the possibility that there were additive or synergistic effects due to laboratory rearing and irradiation on mating competitiveness and compatibility by comparing irradiated laboratory-reared males with non-irradiated wild males. Each experiment compared the performance of different moth treatments/strains in the 2 different mating arenas, and 5 replicates were carried out for each experiment. Due to limitations in adult emergence and availability of field cages, replication was done by night where each trial consisted of one cage per location for each of 5 nights. All mating pairs were collected in perforated and lidded disposable foam cups at regular intervals (i.e., every h) during each observation period. Because experiments were conducted during the night, a Bushnell® Night Vision 2 × 24 mm Night Watch Monocular was used to locate adults in the cages.

Observations and Measurements

In each cage, the type of adults engaged in mating was recorded (e.g., in experiment 1, "L-W", adults were expected to pair as follows: wild ♀ × wild ♂; lab ♀ × lab ♂; lab ♀ × wild ♂; or wild ♀ × lab ♂). Please note the convention of listing the type of female first in any mating pair combination, because this system is adopted in the rest of the paper unless otherwise stated. The following parameters were recorded for each mating pair collected: type of female, type of male, mating frequency for each mating pair type, time of mating and cage type. The mated adults were neither replaced nor released back into the cages after mating and collection (Chambers et al. 1983).

STATISTICAL ANALYSIS

A factorial ANOVA was performed on the data from each experiment (1, 2, 3) with number of mating pairs as the dependent variable and with cage type (i.e., location), time of night mating occurred, and type of mating pair as the independent variables, using Statistica 10; Statsoft Inc., Tulsa, Oklahoma, USA. The suitability of the adults and the cage environmental conditions for mating was determined by calculating the participation in mating (PM) using the formula:

$$PM = \frac{\textit{No. of pairs collected}}{\textit{No. of females released}}$$

According to FAO/IAEA/USDA (2003) a PM value of 0.2 is regarded as the minimum proportion of mating for inclusion of data in compatibility tests. Mating indices (Cayol et al. 1999, 2002; Taret et al. 2010) were used to quantify sexual compatibility, performance and isolation between the adult strains in each trial. The index of sexual isolation (ISI) accounts for the number of pairs obtained for each possible mating combination and is calculated using the following formula:

$$ISI = \frac{(AA + BB) - (AB + BA)}{\textit{Total no. of matings}}$$

"AB" is the number of matings of "A" females with "B" males. This convention is followed in the other capital letter pairs in the above equation. The values of the ISI range from -1 ("complete negative assortative mating", i.e., adults only mate with partners from the opposite strain or population), through an equilibrium at 0 ("random mating", i.e., uniform sexual compatibility and therefore no mating preferences), to +1 ("complete positive assortative mating", i.e., adults only mate with partners from the same strain or population resulting in complete mating isolation).

Two other indices that account for variations in mating vigor (propensity to mate) were calculated. The male relative performance index (MRPI) and the female relative performance index (FRPI) are a measure of male and female mating propensity, regardless of their mating partners (Cayol et al. 1999). The formulae for the respective indices are:

$$MRPI = \frac{(AA + AB) - (BB + BA)}{\textit{Total no. of matings}}$$

$$FRPI = \frac{(AA + BA) - (BB + AB)}{\textit{Total no. of matings}}$$

The values of these 2 indices also range from +1 (i.e., all matings done by males (MRPI) or females (FRPI) of one type [the first to be listed (A)]), through an equilibrium at 0 (i.e., equal participation in mating by males or females of both types), to -1 (i.e., all matings achieved by males (MRPI) or females (FRPI) of the other type [(B)]). The MRPI and FRPI explain the role of the males and females of the 2 strains compared in each experiment, and thus complement the ISI very well (Taret et al. 2010).

In order to give a reliable illustration of mating performance of the different adult treatments, all 3 mating indices were considered together. A chi-square test of independence was used to test for significant departures of the indices from 0. The number of homotypic (e.g., "AA") and heterotypic (e.g., "AB") couples within each cage and between both cage types were also compared using ANOVA, followed by Tukey's HSD post hoc tests to identify statistically homogenous groups at $P = 0.05$ in Statistica 10.

Results

PARTICIPATION IN MATING

The mean PM values obtained in all mating trials regardless of location (laboratory and field cage) confirmed that the cage environmental conditions were suitable for mating since 0.5 was the minimum proportion of matings recorded in the experiments (Table 1). In all tests the PM was above 0.2; therefore none of the data were rejected. In all tests there was no significant interaction ($F = 0.239$; df = 2,30; $P = 0.788$) between location and treatment with respect to participation in mating (Table 1). However, the PM value for experiment 2 in the laboratory location ("L-S") differed significantly ($F = 5.199$; df = 5,30; $P = 0.001$) from those for experiment 1 in both locations ("L-W") as well as that for experiment 3 in the field location ("S-W") (Table 1). These results show that significantly more matings took place in the small bench-top cage between non-irradiated laboratory and irradiated laboratory adults compared to tests involving other treatment types.

EFFECT OF LABORATORY REARING

There was a highly significant 3-way interaction ($F = 4.595$; df = 18, 280, $P = 0.001$) (Fig. 1) across time of night, mating type and location of trials. In the laboratory test the highest number of matings occurred during the first h of the scotophase (18:00 h–19:00 h; Fig. 1). These were mainly homotypic combinations of non-irradiated laboratory females with non-irradiated laboratory males ("L♀ × L♂") and mating frequency was significantly greater than the frequencies of all other mating combinations that occurred in this test. In the same locality the other mating types that occurred included non-irradiated laboratory females with non-irradiated wild males ("L♀ × W♂") and non-irradiated wild females with non-irradiated laboratory males ("L♀ × L♂"). No homotypic matings were observed between non-irradiated wild females and non-irradiated wild males ("W♀ × W♂"). In general, most matings occurred during the period 18:00 h–20:00 h with an additional mating peak between 22:00 h and 23:00 h.

In the field cage all matings occurred between 20:00 h and 22:00 h (Fig. 1). The mating combinations of non-irradiated wild females with non-irradiated laboratory males ("W♀ × L♂") and the non-irradiated laboratory females with non-irradiated laboratory males ("L♀ × L♂") scored the greatest mating frequencies. There was also some mating by non-irradiated laboratory females with non-irradiated wild males ("L♀ × W♂") at 20:00 h and some mating by non-irradiated wild females with non-irradiated wild males ("W♀ × W♂") at 22:00 h (Fig. 1). These data indicate that wild females were generally more responsive to non-irradiated laboratory males than they were to the non-irradiated wild males indicating no ill effect due to laboratory rearing.

In both the laboratory and the field cages, non-irradiated laboratory males mated more than non-irradiated wild males irrespective of the female involved. With respect to timing, mating occurred significantly earlier in the laboratory than in the field. In the outdoor walk-in field cage mating only commenced at 20:00 h and peaked until 22:00 h, whereas in the laboratory, peak mating time was between 18.00 h and 20.00 h (Fig. 1).

EFFECT OF IRRADIATION

There was no significant 3-way interaction across time of night, mating type and location on mating propensity between non-irradiat-

Table 1. Mating compatibility and performance of laboratory-reared, wild and irradiated (200 Gy) *Eldana saccharina* adults in pair-wise combinations under laboratory and semi-field conditions.

Trial No.	Strains tested	Number of couples (avg ± SD)								Chi Square test of Independence	
		AA	AB	BA	BB	PM	MRPI	FRPI	ISI	X^2(ISI)	*P*
1	L-W(Lab)	4.5 ± 0.8Aa	0.7 ± 0.8BCa	1.2 ± 0.8Ba	0.0Ca	0.5 ± 0.1a	0.6 ± 0.2*	0.8 ± 0.2*	0.44 ± 0.10*	15.37	0.009*
	L-W(Field)	2.3 ± 0.8Ab	1.7 ± 1.0ABb	2.3 ± 1.4Ab	0.3 ± 0.5Ba	0.5 ± 0.1a	0.1 ± 0.3	0.7 ± 0.3*	-0.02 ± 0.17	10.92	0.053
2	L-S(Lab)	1.8 ± 0.8Aa	2.7 ± 0.5Aa	2.0 ± 1.1Aa	2.7 ± 0.5Aa	0.7 ± 0.2b	-0.0 ± 0.2	-0.2 ± 0.2	-0.02 ± 0.05	1.06	0.958
	L-S(Field)	2.7 ± 1.4Aa	1.0 ± 0.9Ab	1.5 ± 0.8Aa	2.5 ± 1.0Aa	0.6 ± 0.1ab	-0.1 ± 0.2	0.1 ± 0.2	0.23 ± 0.10	9.988	0.076
3	S-W(Lab)	5.3 ± 0.5Aa	0.0Ba	0.2 ± 0.5BCa	1.5 ± 0.8Ca	0.6 ± 0.1ab	0.5 ± 0.3*	0.6 ± 0.2*	0.9 ± 0.05*	51.67	0.000*
	S-W(Field)	3.3 ± 1.4Ab	1.0 ± 0.9Bb	1.5 ± 0.8ABb	0.0Cb	0.5 ± 0.1a	0.5 ± 0.3*	0.7 ± 0.3*	0.16 ± 0.17*	12.20	0.032*

PM = participation in mating; MRPI = male relative performance index; FRPI = female relative performance index; ISI = index of sexual isolation.

*Observed index significantly departs from random mating (ISI) or equal performance of each sex (MRPI and FRPI) (χ^2 test, $\alpha = 0.05$). For number of couples, means followed by the same upper case letter in each row and means followed by the same lower case letter in each sub-column are not significantly different (Tukey–Kramer's post-hoc test: $P > 0.05$). "L-W": adult treatment = non-irradiated laboratory adults vs. non-irradiated wild adults, "L-S": adult treatment = non-irradiated laboratory adults vs. irradiated laboratory adults, "SW": adult treatment = irradiated laboratory adults vs. non-irradiated wild adults. "AA" denotes mean number of matings of "A" females with "A" males for homotypic couples in the respective adult treatment. "BB" denotes mean number of matings of "B" females with "B" males for homotypic couples in the respective adult treatment. "AB" denotes mean number of matings of "A" females with "B" males for heterotypic couples in the respective adult treatment. "BA" denotes mean number of matings of "B" females with "A" males for heterotypic couples in the respective adult treatment.

ed laboratory and irradiated laboratory adults ($F = 1.134$; df = 27,280; $P = 0.296$) (Fig. 2). There was also no significant time × mating type effect ($F = 0.891$ df = 27,280; $P = 0.634$) nor location × mating type effect ($F = 1.501$; df = 3,280; $P = 0.215$) on mating frequency. In the laboratory the greatest number and earliest (between 18:00 h and 20:00 h) mating combinations consisted of the irradiated laboratory females and irradiated laboratory males ("S♀ × S♂"), non-irradiated laboratory females and irradiated laboratory males ("L♀ × S♂") and irradiated laboratory females and non-irradiated laboratory males ("S♀ × L♂") (Fig. 2). In addition the mating frequencies of these afore-mentioned combinations did not differ significantly, and there were significantly more heterotypic matings ("L♀ × S♂") and ("S♀ × L♂") than homotypic matings with non-irradiated laboratory adults ("L♀ × L♂") during this period, indicating no ill effects due to irradiation treatment. There was a small mating peak by the "L♀ × L♂" mating type at 01:00 h but it was not significantly different from the frequency of the heterotypic matings "L♀ × S♂" and "S♀ × L♂" that occurred earlier at 22:00 h and 00:00 h respectively.

In the field, the greatest mating frequencies were observed for the "S♀ × L♂", "L♀ × L♂" and "S♀ × S♂" combinations which occurred mainly around 20:00 h. During that same period there was also a high number of "L♀ × S♂" matings, which were otherwise only significantly lower than the "L♀ × L♂" combination. In the field cage most matings occurred between 19:00 h and 22:00 h, with the peak period being at 20:00 h (Fig. 2). In general the data from the field location indicated no negative effects due to irradiation.

There was a highly significant ($F = 29.36$; df = 9,280; $P = 0.001$) (Fig. 3) 2-way interaction effect between time of mating and location on the mean number of mating pairs. The greatest mating frequency occurred in the laboratory at 18:00 h, while no matings were observed in the field during that period. In the field cage, the highest mating frequency was observed around 20:00 h with no mating in the laboratory during that same period (Fig. 3). In both locations mating frequencies at 19:00 h did not differ significantly (Fig. 3). In the field mating only began at 19:00 h and peaked at 20:00 h and declined significantly thereafter. The mating frequencies in both locations after 21:00 h were not significantly different (Fig. 3).

ADDITIVE OR SYNERGISTIC EFFECTS DUE TO LABORATORY-REARING AND IRRADIATION

There was a highly significant 3-way interaction effect ($F = 5.933$; df = 33,280; $P = 0.001$) among time of night, mating type and location on the number of matings between irradiated laboratory *E. saccharina* and their non-irradiated wild counterparts. In the laboratory, the greatest mating frequency was observed for the homotypic combination of irradiated laboratory females with irradiated laboratory males ("S♀ × S♂") peaking in the first h of the scotophase and continuing until 22:00 h (Fig. 4). There was also some mating between non-irradiated wild females and irradiated laboratory males ("W♀ × S♂") between 19:00 h and 20:00 h. Homotypic mating between non-irradiated wild females and non-irradiated wild males commenced late into the scotophase at 01:00 h, peaked at 03:00 h and ended at 05:00 h. Despite occurring at different times during the scotophase period under observation the mating frequency of the "W♀ × S♂" combination did not differ with that of "W♀ × W♂" indicating that non-irradiated wild females were as responsive to irradiated laboratory males as they were to their non-irradiated wild counterparts. There was no mating between irradiated laboratory females and non-irradiated wild males over the entire duration of the experiment in the laboratory location (Fig. 4). With respect to timing in the laboratory experiment irradiated laboratory males regardless of the females involved ("S♀ × S♂ and

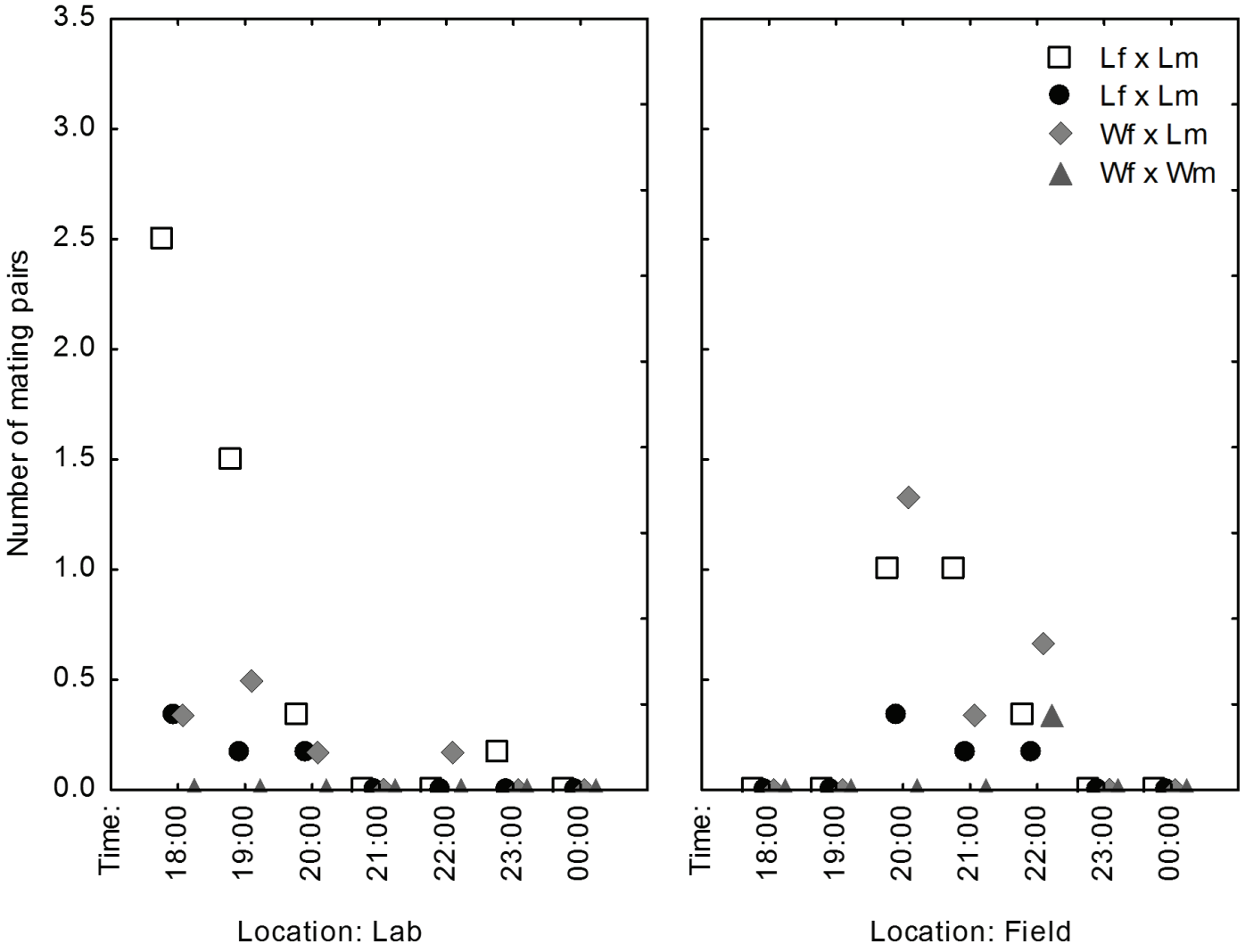

Fig. 1. The mean number of matings in a pair-wise comparison between non-irradiated laboratory and wild *Eldana saccharina* adults showing a significant 3-way interaction across time of night, type of cross and location of trials. The expected possible mating combinations were: (i) non-irradiated laboratory female and non-irradiated laboratory male (Lf x Lm); (ii) non-irradiated laboratory female and non-irradiated wild male (Lf x Wm); (iii) non-irradiated wild female and non-irradiated laboratory male (Wf x Lm); and/or (iv) non-irradiated wild female and non-irradiated wild male (Wf x Wm).

"W♀ × S♂") were more active during the early part of the scotophase while the non-irradiated wild males only commenced mating late in the scotophase period of the experiment (Fig. 4).

In the field cage mating did not commence until 20:00 h which was also the time when the greatest number of matings of the "S♀ × S♂" combination occurred (Fig. 4). From this period until 23:00 h, there were also matings of the "S♀ × W♂ and "W♀ × S♂" combinations albeit significantly less than those of the homotypic "S♀ × S♂" pairing. No homotypic matings of wild moths (i.e., "W♀ × W♂") were observed in the field location (Fig. 4).

The detailed summary of the mean mating frequencies of each of the mating combinations reported in all the above mentioned trials is given in Table 1.

MATING COMPATIBILITY TESTS

Mating was observed in all 4 possible combinations in the following pair-wise comparisons and locations: "L-W" (field) and "L-S" (laboratory and field) (Table 1). Likewise, the afore mentioned absence of mating barriers was confirmed by the chi-square test of independence which showed that the mean ISI values of all mating combinations in the respective comparisons were not significantly different from zero (Table 1). However, there were no homotypic "W♀ × W♂" matings in the "L-W" laboratory test and the "S-W" field test, and also no "S♀ × W♂" heterotypic matings in the "S-W" laboratory test (Table 1). This was reflected in the mean ISI values of these mating tests which were significantly different from zero. There was a significantly greater participation of irradiated and non-irradiated adults compared with wild adults in the "L-W" (laboratory) and the "S-W" (laboratory and field) tests as reflected in the relatively high MRPI (0.6, 0.5 and 0.5, respectively) and FRPI (0.8, 0.6 and 0.7, respectively) values.

In the "L-W" and "S-W" laboratory tests, there were significantly more "L♀ × L♂" and "S♀ × S♂" homotypic pairs than heterotypic pairs, indicating a laboratory environment artefact rather than inferiority in quality of wild adults (Table 1). In the field tests, the number of heterotypic pairs did not differ significantly from that of the homotypic pairs, indicating equal participation between laboratory adults and their non-irradiated wild counterparts, indicating no negative effects due to laboratory rearing or the irradiation treatment and also suggesting no incompatibility between the wild strain and the laboratory adults.

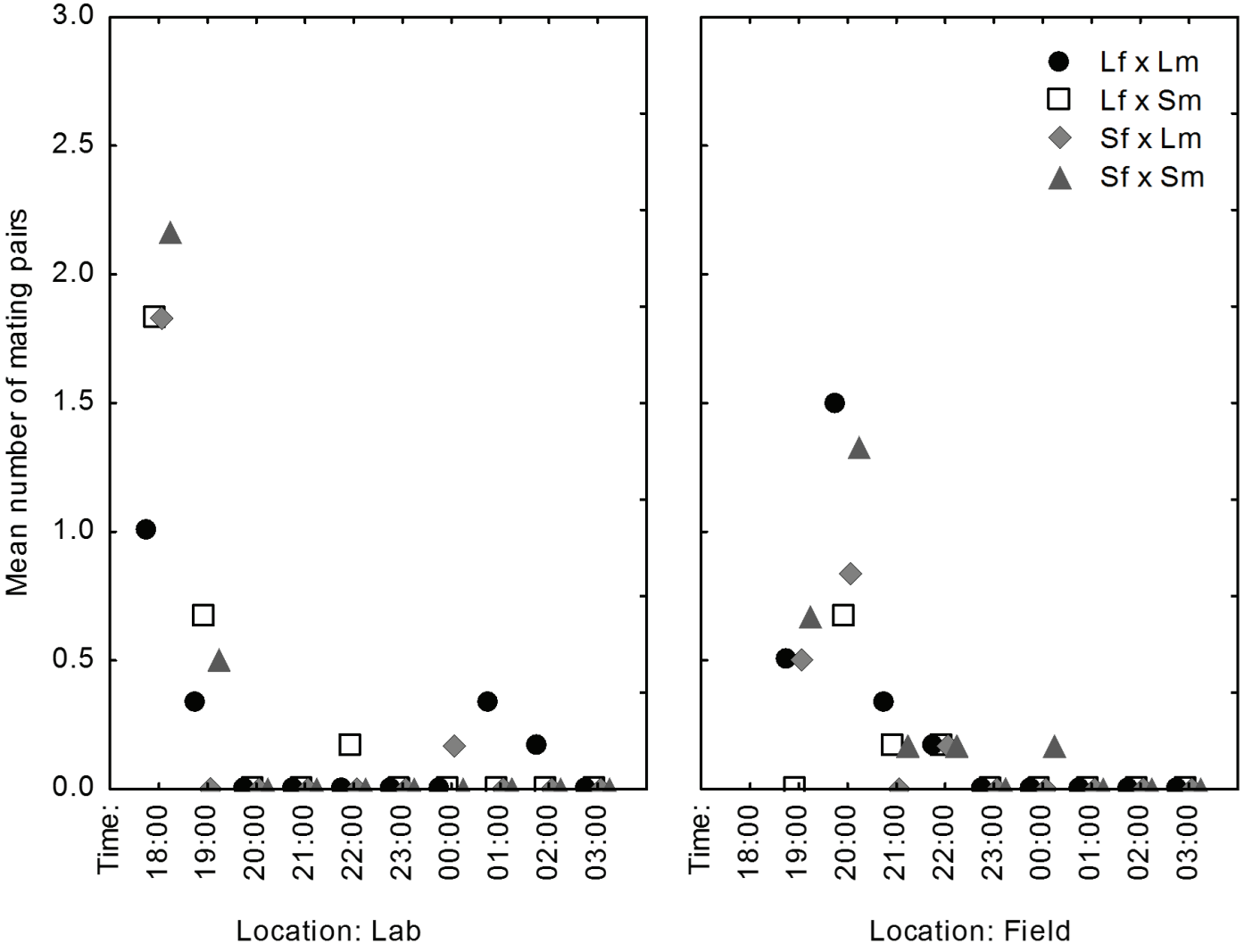

Fig. 2. The mean number of matings in a pair-wise comparison between non-irradiated and irradiated laboratory reared *Eldana saccharina* adults. The expected possible mating combinations were: (i) non-irradiated laboratory female and non-irradiated laboratory male (Lf x Lm); (ii) non-irradiated laboratory female and irradiated laboratory male (Lf x Sm); (iii) irradiated laboratory female and non-irradiated laboratory male (Sf x Lm); and/or (iv) irradiated laboratory female and irradiated laboratory male (Sf x Sm).

In the "L-S" mating tests, irradiated and non-irradiated adults showed equal participation in mating by both sexes in both locations as reflected in the MRPI and FRPI values, which did not differ significantly from zero (Table 1). The number of heterotypic and homotypic pairs was also similar, indicating that there were no mating barriers between fertile laboratory and irradiated laboratory adults. The chi-square test of independence also showed that the ISI values for the "L-S" comparison in both locations did not depart significantly from zero confirming random mating between the strains.

Discussion

EFFECT OF LABORATORY REARING ON MATING COMPETITIVENESS

The current study reports on mating competitiveness and compatibility between mass-reared, sterile and wild populations of *E. saccharina* in laboratory and field cages. There was a significant interaction across time of mating, test location and type of mating combination in the pair-wise test of non-irradiated laboratory reared and wild adults (L-W). This can be attributed to the origin of the respective strains, which may have significantly influenced the behavior outside the natural environment in which they were reared. According to Rössler (1975b) it is generally difficult to induce wild insect populations to mate and reproduce in the laboratory because conditions significantly differ from those in the wild, which inevitably influences their behavior. The non-irradiated wild adults used in this study were obtained from sugarcane host plants in an area with warmer subtropical climatic conditions [Tinley Manor: long-term mean (LTM_{min}) = 15.1 °C, LTM_{max} = 26.2 °C, LTM = 20.7 °C; SASRI weatherweb – Tongaat-Klipfontein meteorological station], while both the irradiated and non-irradiated adults were produced under constant and controlled abiotic conditions.

The results of these assays show that males from the laboratory-reared population mated significantly earlier in the laboratory than they did in the field cage, while wild males called and mated much earlier in the field location than they did in the laboratory. Under mass-rearing, the requirements for appropriate mating "behavior" are removed as cost effective production processes demand that important compromises be made in the environmental arena presented to the moths for

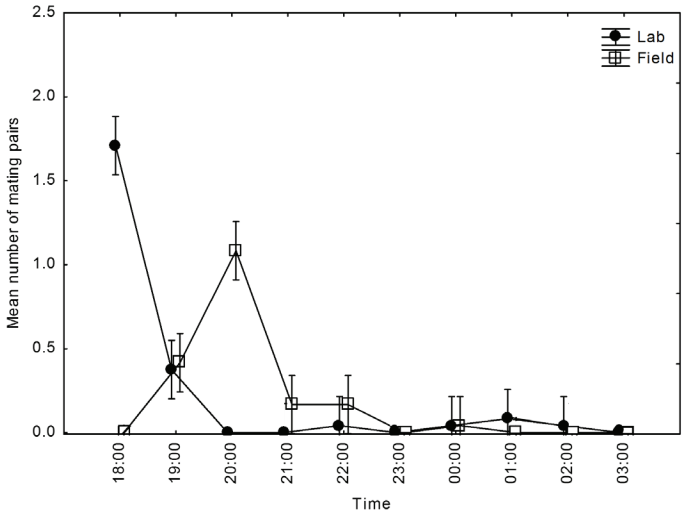

Fig. 3. The mean number of *Eldana saccharina* matings in a pair-wise comparison between non-irradiated laboratory adults and irradiated laboratory adults showing a significant 2-way interaction between time of night and location of trials. Data were pooled across the entire observation period to obtain total matings irrespective of cross type. Error bars denote 95% confidence limits.

mating (Robinson et al. 2002). First, the adult density in production cages often leads to degeneration in most aspects of normal mating behavior, such as early initiation of calling and mating (Cayol 2000). Second, the abiotic conditions under mass-rearing—such as constant light, moisture and temperature regimes and an artificial larval diet that is much richer in proteins compared to diets in nature—significantly differ from those in the field (Robinson et al. 2002). On the other hand, the thermal background and feeding status of the field collected wild insects used in this study were different from those of their mass-reared laboratory counterparts. From an evolutionary perspective, these differences could result in natural selection for physiological changes that result in traits being associated with local microclimates (Kellermann et al. 2012). These changes therefore may impact directly on mating behaviors exhibited by the different strains under both laboratory and field cage conditions. Space, for instance, is drastically restricted under laboratory conditions, such that frequent and random interactions result in successful mating by the laboratory adapted strains compared with their wild counterparts, even in the case of males that are less sexually motivated or less competitive (Lux et al. 2002).

Nevertheless, it is important to note that even though laboratory-reared males commenced mating later in the field cage (i.e., 20:00 h) than they did in the laboratory (i.e., 18:00 h), their mating frequency was similar to that of the wild strain regardless of the type of female involved. Mass-rearing conditions have been reported to increase male aggressiveness and favor faster mating and shortened courtship (Calcagno et al. 2002)—traits that may have evolved to avoid interruptions during mating and to increase the likelihood of securing mates under conditions of overcrowding (Briceno & Eberhard 2002). It may therefore be advantageous for males to begin calling earlier in the presence of intense competition from other males, but success of this mating strategy would depend on co-evolution in female choice criteria within the laboratory population (Briceño & Eberhard 2002).

The onset of scotophase in the laboratory is easy to determine as the light snaps to full power in an instant while in nature, it depends on the insect's light-detection and switching mechanisms—as these conditions could be anywhere from slight pre-sunset dimming to post-sunset glow. This is important in interpreting the differences found in timing of mating. Diaz-Fleischer & Arredondo (2011) demonstrated that divergent wavelengths provoked sensory system differences that, in turn, reduced random mating in Mexican fruit flies, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), that originated from environments of variable light regimes. Assortative mating has also been shown to take place according to light spectrum in polymorphic fishes, whereby some morphs were successful under specific light conditions (Fuller et al. 2005). While *E. saccharina* is one of the unique lepidopteran species that employs a complex male lek-based mating system (Atkinson 1981; Zagatti 1981) the ontogenetic effect of light regimes on sexual selection has never been explored. It is known that long-term rearing conditions modify insect mating schedules and behavior, and thereby reduce inter-breeding between wild and laboratory flies (Cayol 2000; Briceño & Eberhard 2002). For example Diaz-Fleischer & Arredondo (2011) showed that light conditions during rearing affected mating success of *A. ludens.* The laboratory adults used in the present study were reared in an artificially regulated environment for many years, a condition that standardizes many individual traits (Cayol 2000). So, in light of the variations in onset times of mating between the laboratory and field cage, it could be inferred that light spectrum may have an ontogenetic effect on mating behavior of the moths from different origins. The modification in ontogenetic development could be manifested by differences in male signalling displays and female visual signalling receptions, which in turn, represent disadvantages when competing for mates.

Therefore, under field conditions early mating of the mass-reared sterile *E. saccharina* males might be desirable for an SIT program because many wild females could be mated by sterile males before wild males had the opportunity to do so, thereby achieving the number of sterile matings required for population reduction.

Another observation noted in the pair-wise experiment between non-irradiated wild vs. non-irradiated laboratory-reared (L-W) adults was that laboratory-reared females were more prone to mate than wild females regardless of the male type involved in both locations. This is a common characteristic of mass-reared individuals when they are confined together in competition tests with wild strains (Harris et al. 1986). According to Cayol et al. (2002) laboratory females tend to be less "choosy" than their wild counterparts. This is because traits that favor simpler, less discriminating and earlier courtship sequences to ensure copulation success and changes in sexual competitiveness may be selected under mass-rearing conditions in females (Cayol et al. 2002). On the other hand, *E. saccharina* has a complex lek polygyny (Atkinson 1981; Zagatti 1981), such that mass-rearing conditions may represent a different environment where lek formation might not be as important as in nature. This was observed in similar assays under field cage conditions with the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), a species with a lek polygyny (Rodriguero et al. 2002). In the field location, laboratory-reared males and females mated with both members of their own population and those of the wild strain, while in the laboratory assay there were significantly more homotypic "L♀ × L♂" matings than heterotypic combinations. A possible explanation for these observations could be that where mass-produced males and females are released together, they tend to mate amongst themselves before having an opportunity to mate with their non-irradiated wild counterparts (Moreno et al. 1991). This can also be linked to laboratory adaptation, mass-rearing and irradiation, all of which produce genetic and physiological effects in conventional strains (Benedict & Robinson 2003), thereby resulting in variable mating behavior.

EFFECT OF RADIATION TREATMENT ON MATING COMPETITIVENESS

In the case of the non-irradiated laboratory reared and sterile comparison (L-S), the mean number of matings was similar regard-

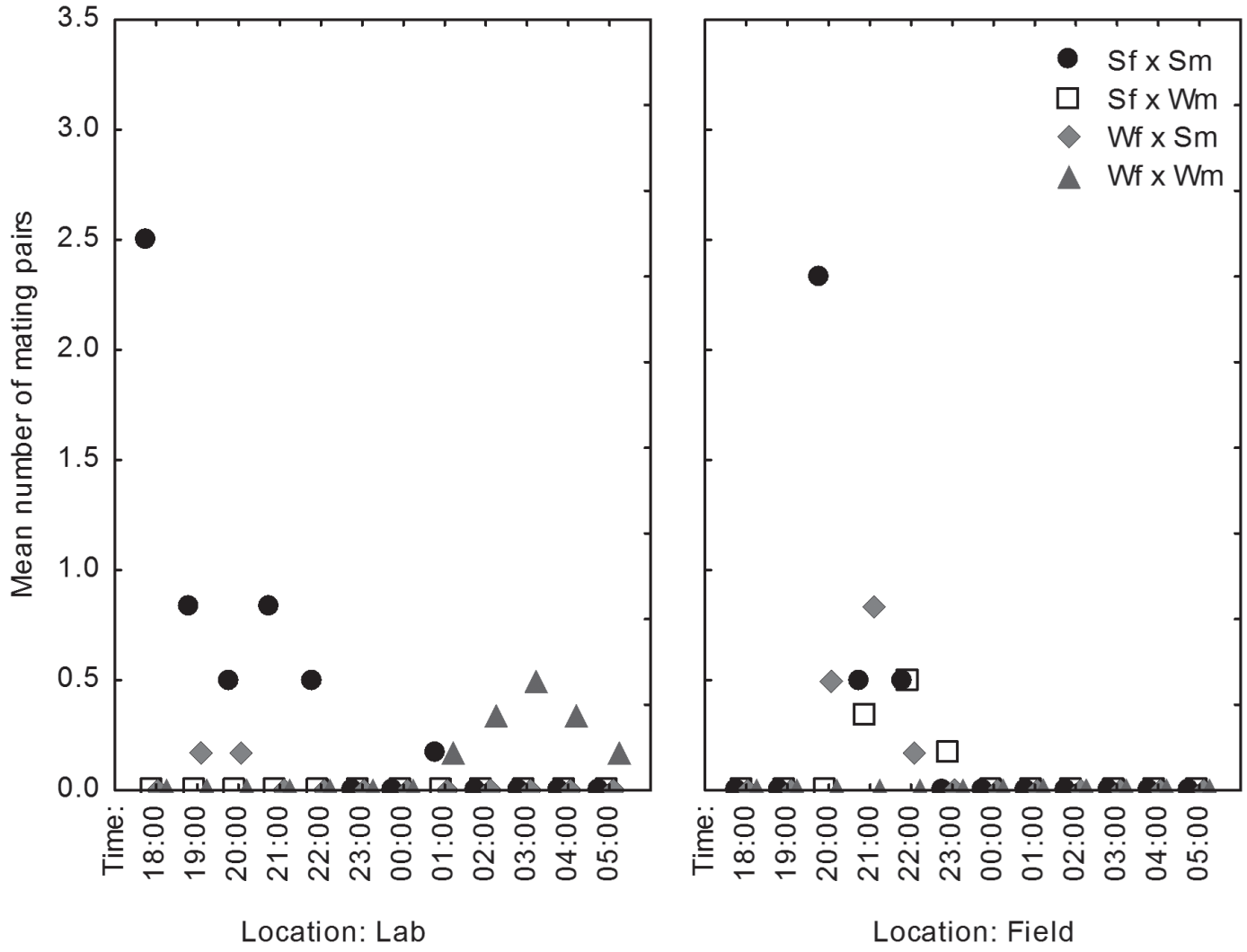

Fig. 4. The mean number of matings in a pair-wise comparison between irradiated laboratory reared and non-irradiated wild *Eldana saccharina* adults showing a significant 3-way interaction across time of night, type of cross and location of trials. The expected possible mating combinations/cross types were: (i) irradiated laboratory female and irradiated laboratory male (Sf x Sm); (ii) irradiated laboratory female and non-irradiated wild male (Sf x Wm); (iii) non-irradiated wild female and irradiated laboratory male (Wf x Sm); and/or (iv) non-irradiated wild female and non-irradiated wild male (Wf x Wm).

less of location. No negative effects on the mass-reared strain due to irradiation were detected. Both irradiated and non-irradiated laboratory adults were from the same origin, and hence the traits selected in the laboratory environment were also expressed in the irradiated strain. There was no evidence of variations in times of mating or discrimination amongst the strains in each of the experimental locations. These results indicate that the radiation dose of 200 Gy is still within the range that will not compromise the performance of the treated adults. Any negative effect due to radiation would be a result of an increased radiation dose, since the use of high ionizing radiation reduces sexual motivation and overall mating performance (Weldon 2005). Lux et al. (2002), for example, reported that irradiated male *C. capitata* were more passive, less vigorous and less sexually motivated than non-irradiated, mass-reared males.

However, there was a significant interaction between location and time of mating in the pairwise comparison of non-irradiated versus irradiated laboratory adults. This was attributed to significant differences in peak mating times and mean number of matings achieved in the laboratory compared to the field location. In the former, most matings occurred during the early part of the night (18:00 h) while in the latter, peak mating was attained only 2 h later (20:00 h), and there were significantly fewer matings. Mating behavior characteristics of mass-reared and sterile insects in a natural environment may be influenced by acclimatization to outdoor conditions (Pereira et al. 2007). For example, Judd & Gardiner (2006) state that temperature and light transitions are common in the field as the night progresses and hence may be responsible for differences in response of mass-reared and sterile strains to these changes when released into the field. They further state that mass-rearing could possibly affect temperature thresholds for general activity or dispersal from release locations. Pereira et al. (2007) reported that intense selection pressures imposed by rearing conditions lead to shift the sequence and timing of mating away from that which is normally exhibited in the wild. The significantly early mating and large number of matings for both non-irradiated laboratory and irradiated laboratory adults in the laboratory compared with the field are indicative of adaptation to laboratory conditions, where emphasis is on high reproduction rate, earlier and shorter mating (Iwahashi 1996; Matos et al. 2000). According to Simmons et al. (2010) a high quality and productive insect in the mass-rearing facility is not necessarily a good performer in the field because the production facil-

ity results in the selection of traits that enable an organism to adapt and persist in the artificial environment in which it is produced. Overall, irradiation did not impact negatively on mating of laboratory reared *E. saccharina*.

ADDITIVE OR SYNERGISTIC EFFECTS OF LABORATORY REARING AND IRRADIATION ON MATING COMPETITIVENESS

In the case of the "S-W" mating trial between irradiated laboratory and non-irradiated wild adults, irradiation did not affect the performance of *E. saccharina*. Irradiated male and female individuals did not discriminate against their wild counterparts in either the laboratory or field locations. These results show that *E. saccharina* treated with the sub-sterile gamma radiation dose of 200 Gy were as competitive as the non-irradiated wild strain. The latter is the optimum dose recommended by Walton (2011) for a F_1 (inherited) sterility program against *E. saccharina*. While irradiation of adults did not negatively impact the mating competitiveness of *E. saccharina*, as confirmed in "L-S" trials there was no evidence of additive or synergistic effects due to laboratory rearing and irradiation. Results from the pairwise comparisons of the "L-W" and "S-W" showed that the responses of the non-irradiated wild females to either the non-irradiated or irradiated laboratory males were similar. There are 2 probable explanations for this occurrence. First, both the sterile and the non-sterile adults originate from the same source, and, hence, share a similar thermal and life history. Therefore, had there been variation in timing and mating frequency, it would be attributed to irradiation treatment. Secondly, these findings imply that the gamma radiation dose of 200 Gy to which the sterile moths in this study were exposed was below the threshold above which ill-effects due to radiation begin to show. It is known that irradiation degrades insect quality (Lux et al. 2002; Calkins & Parker 2005). The greater the dosage the more deleterious its effects are on the target insect as manifested by reduced ability to participate in lek formation, lethargy, diminished tendency to sexual behavior, mating confusion and reduced performance (Lux et al. 2002; Calkins & Parker 2005). Therefore the results of this study indicate that the recommended dose of 200 Gy had no negative impact on *E. saccharina* mating behavior and performance. This bodes well with regard to the end goal of F_1 (inherited) sterility because the dosage does not cause full parental male sterility (Walton 2011). Therefore the major suppressive effect of the technique will be primarily due to enhanced mating competitiveness of partially sterile parents as well as the dominant lethal genes and radiation-induced chromosomal aberrations characteristic of the F_1 progeny (Soopaya et al. 2011).

MATING COMPATIBILITY TESTS

The mating indices generated from these data demonstrate that mass-reared *E. saccharina* in South Africa have not yet evolved sexual behaviors suggestive of incipient pre-mating isolation barriers with respect to the local wild strain under semi field conditions. While the more controlled but limited laboratory assessments show a greater propensity of the laboratory strains to mate with members of their own population as evidenced by the high and positive ISI values, the more robust field tests (Vreysen et al. 2009; Simmons et al. 2010) have shown no evidence of sexual incompatibility between them and their wild counterparts. This indicates a laboratory environment artefact rather than inferiority in quality of adults. The combined data of the different indices (ISI, MRPI and FRPI) complement each other very well and illustrate the sexual competitiveness and compatibility between the laboratory strains and the wild *E. saccharina* population.

In all experiments where the laboratory population (non-irradiated or irradiated) was involved with the control (wild) population (i.e., "L-W" and "S-W" comparisons), positive and highly significant FRPI and MRPI values were obtained irrespective of location. This indicated that the males and females of the laboratory populations mated more than their wild counterparts. On the other hand, lower participation of sterile females in heterotypic matings (i.e., S♀ × W♂) suggests that sterile males have a greater opportunity to mate with wild females. Despite the high propensity of laboratory females to mate with their laboratory male counterparts, our findings corroborate that such performance may have no obvious implications for SIT success but, may have caused experimental bias during evaluation of male performance (Rull et al. 2012). Therefore, a design in which laboratory and wild males compete for wild females only (i.e., in the absence of laboratory females) may be more informative on relative male performance despite the one employed here being well suited for testing sexual compatibility between populations or strains. Nevertheless, the high participation of sterile females in homotypic matings (i.e., S♀ × S♂) indicates that caution must be taken when releasing both sterile males and females together, as they may mate amongst themselves before having the opportunity to mate with wild counterparts (Moreno et al. 1991). Because copulating pairs were removed from the mating arena and not replaced after separation, it could be the logical explanation for absence of wild homotypic matings in the "L-W" (laboratory) and "S-W" (field) comparisons. This suggests that wild females are responsive to the courtship of laboratory reared sterile males even in the presence of wild males. The indices in "L-S" comparison confirm random mating and equal performance between sexes and strains thereby ruling out incompatibility issues resulting from effects of radiation treatment.

AW-IPM programs that include an SIT component, usually produce males that are of lower quality than wild males (Cayol 2000) due to the negative effects of sterilization by gamma irradiation. However, from the results of this study, it appears that the sub-sterilizing dose of 200 Gy does not cause sufficient physiological damage to alter adult male mating behavior of *E. saccharina.* The absence of sexual incompatibility between the mass-reared sterile strain and its wild counterparts suggests that the former are still as competitive in mating with wild females as the latter. These findings therefore add to a now growing number of studies (Lux et al. 2002; Rull et al. 2012) indicating that a low radiation dose will not compromise the performance of sterile males in programs that have a sterile male release component. On the other hand, the probability of developing pre-mating isolation and sexual incompatibility is greater in species with lek-based mating systems due to their complexity rather than in those with simple non-resource based systems (Taret et al. 2010). This is further compounded by mass-rearing conditions (Rodriguero et al. 2002) that impose intense unnatural selection pressures (Iwahashi 1996; Matos et al. 2000), and that, in turn, promote assortative mating preferences (Calkins & Parker 2005; Rull et al. 2012). Despite the fact that *E. saccharina* employs a complex lek polygyny, the results of this study have shown that there is no evidence of the above negative effects either due to mass-rearing or to irradiation with 200 Gy. The data presented here therefore provide the necessary evidence and confidence that the mass-reared *E. saccharina* strain currently produced at the SASRI insect rearing unit is suitable for use in SIT-based projects. Thus there is scope for the development of the SIT as an addition to the arsenal of tactics used in the integrated management of this economic pest.

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