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General biology of *Eldana saccharina* (Lepidoptera: Pyralidae): A target for the sterile insect technique

*Angela J. Walton[1] and Des E. Conlong[2, 3,]**

Abstract

Eldana saccharina Walker (Lepidoptera: Pyralidae: Gallerinae) occurs on many graminaceous crops and several wild grasses and sedges throughout Africa. It has been reared at the South African Sugarcane Research Institute (SASRI) since the 1970s to study its biology and behavior, as a host for natural enemies and to provide insect material for the plant breeding program. Studies were completed on laboratory-reared *E. saccharina* of South African origin to assess fecundity, fertility and male and female mating frequencies. Mean fecundity of *E. saccharina* was 518 ± 27.5 (mean ± SE) eggs per female, up to a maximum of 798 eggs. Mean egg hatch (fertility) of *E. saccharina* was 63.2 ± 4.2%. In the laboratory, 56.7% of *E. saccharina* females mated only once but on average females mated 1.5 ± 0.1 times (maximum of 3). Males mated with a maximum of 6 females per male but on average males mated 3.3 ± 0.7 females. Most matings (93%) occurred on the first and second nights after male emergence, and the females oviposited most of their eggs (49.9 ± 3.9%) on the second night after emergence. *Eldana saccharina*'s high fecundity confirmed its potential as a crop pest. This study has, for the first time, confirmed that male and female *E. saccharina* were able to mate more than once under controlled laboratory conditions. This has important implications for calculating required release rates of sterilized males to obtain adequate sterile to wild male over-flooding ratios in area-wide integrated pest management programs that have a SIT component.

Key Words: fecundity; fertility; mating propensity; sugarcane borers; laboratory rearing; spermatophores; area-wide integrated pest management

Resumen

Eldana saccharina Walker (Lepidoptera: Pyralidae: Gallerinae) se presenta en muchos cultivos graminosos y varias hierbas y juncias silvestres en toda África. Se ha criado esta especie en el Instituto de Investigación de la Caña de Azúcar de Sudáfrica (SASRI) desde la década de 1970 para estudiar su biología y comportamiento, como un hospedero para enemigos naturales y para proveer material de insectos para el programa de fitomejoramiento. Se realizaron los estudios sobre especimenes de *E. saccharina* de origen sudafricano criadas en el laboratorio para evaluar la fecundidad, fertilidad y frecuencia de apareamiento de los machos y hembras. El promedio de la fecundidad de *E. saccharina* fue de 518 ± 27.5 (promedio ± DE) huevos por hembra, hasta un máximo de 798 huevos. El promedio de la eclosión de los huevos (fertilidad) de *E. saccharina* fue de 63.2 ± 4.2%. En el laboratorio, el 56.7% de las hembras de *E. saccharina* se aparearon sólo una vez, pero por lo general las hembras se aparearon 1.5 ± 0.1 veces (máximo de 3). Los machos se aparearon con un máximo de 6 hembras por macho, pero por lo general los machos se aparearon con 3.3 ± 0.7 hembras. La mayoría de los apareamientos (93%) sucedieron en la primera y segunda noches después de la emergencia de los machos, y las hembras ovipositaron la mayoría de sus huevos (49.9 ± 3.9%) en la segunda noche después de la emergencia. La alta fecundidad de *Eldana saccharina* confirmó su potencial como plaga de cultivos. Por primera vez, este estudio ha confirmado que los machos y hembras de *E. saccharina* pueden aparearse más de una vez en condiciones de laboratorio controladas. Esto tiene implicaciones importantes para el cálculo requerido de la tasa de liberación de machos esterilizados para obtener la proporción adecuada en exceso de la inundación de machos esteriles a machos salvajes en los programas de manejo integrado de plagas en toda una zona que tienen un componente de la TIE.

Palabras Clave: fecundidad; fertilidad; propensión de apareamiento; barrenadores de caña de azúcar; cría de laboratorio; espermatóforos; manejo integrado de plagas en toda la zona

Eldana saccharina Walker (Lepidoptera: Pyralidae) is indigenous to Africa and occurs on graminaceous crops (sugarcane, maize and sorghum) and on several wild grasses and sedges (Conlong 1994a, b, c). It was first described from sugarcane in Sierra Leone, West Africa in the late 1800's (Betbeder-Matibet 1981) and has also been reported from sugarcane in East Africa (Girling 1972; Conlong 2000; Conlong & Muga-

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lula 2001). After the initial outbreak in 1939 on the Umfolosi Flats, KwaZulu-Natal (Dick 1945), the pest "disappeared" from South African sugarcane, but resurfaced in the 1970's (Carnegie 1974). Conlong (1994c, 2001) reviewed the literature regarding *E. saccharina*'s host range and determined that it has only fairly recently (i.e., since 1939 to present—75 years) extended its home range to graminaceous crops in South Africa, with indigenous sedges and grasses being its primary host plants.

The South African Sugarcane Research Institute (SASRI) has been conducting research on this pest since it was found in sugarcane (*Saccharum* spp.; Poales: Poaceae). Dick (1945) reared *E. saccharina* on an artificial diet inoculated with the mold, *Mucor hiemalis* Wehmer (Mucorales: Mucoraceae). In the 1970s, Atkinson (1978) reported that *E. saccharina* was reared on an artificial diet for biological studies. SASRI has maintained a colony since then, with numerous adaptations to the rearing procedure being made.

Atkinson (1980) published data on the number of larval instars, lower development temperature thresholds and duration of life stages of *E. saccharina*. The temperature cabinets that were used in his trial were not controlled at lower temperatures and therefore the lower development threshold temperature could not be accurately calculated. He estimated the lower larval development threshold to be 15 °C. Furthermore, the duration of the different life stages at various temperatures were calculated in days, not degree-days. The same author reported 6 to 7 larval instars for female larvae and 5 to 6 larval instars for male larvae. Anatomical differences between male and female larvae and pupae were also described and illustrated. Due to adaptations to the artificial diet, the continuous laboratory rearing of *E. saccharina*, and the acquisition of more sophisticated incubators, Way (1995) reviewed and accurately calculated the lower development thresholds for eggs (5.3 °C), larvae (10.2 °C) and pupae (10.7 °C), and calculated the development time of each life stage in degree-days. The total development time from egg to adult in *E. saccharina* amounted to 897.9 degree-days. Degree-days are a measure of heat units required over time to complete a certain stage of development at a temperature above a development threshold temperature. The advantage of using this calculation is that it is a physiological time scale and development time can be calculated at any given temperature. Way (1995) confirmed the same number of larval instars as reported by Atkinson (1980). In a prior study, Way (1994) reported on the effect of 4 different rearing temperatures on *E. saccharina* adult female longevity, mating success, oviposition and egg development. Longevity was inversely correlated with temperature, being shorter at higher temperatures and longer at lower temperatures. Fecundity was greatest at 20 °C and 25 °C, with an average of 417.7 and 432.8 eggs oviposited per female, respectively. Both at 15 °C and 35 °C, fecundity was reduced by approximately half, with 205.8 and 183.2 eggs oviposited per female, respectively. Way (1994) reported that oviposition did occur at all temperatures tested, but occurred over a longer period at 15 °C, with it occurring over progressively shorter intervals at increasing temperatures. Eggs failed to hatch at 15 °C and 35 °C.

There is a great variety of fecundity data reported in the literature. In an earlier study by Dick (1945) females oviposited on average 750 eggs, with a maximum of 1004. Mating increased the number of eggs oviposited compared with unmated females (Dick 1945; Sampson & Kumar 1985). In West Africa, Betbeder-Matibet et al. (1977) reported an average fecundity of 460 eggs oviposited per female, up to a maximum of 811 for females reared on an artificial diet. In a later paper, Betbeder-Matibet (1981) reported similar fecundity but did not state if the females were reared on artificial diet or sugarcane stalks. In East Africa, Waiyaki (1974) reported an average fecundity of 150 to 230 eggs per female and Sampson & Kumar (1985) reported an average fecundity of 327 ± 17 eggs per female reared on sugarcane stalks.

Sampson & Kumar (1985) compared adult longevity and life cycle lengths of *E. saccharina* from Ghana, West Africa with those reported by Girling (1978) from Kenya, East Africa. Observed differences made them propose the existence of 2 biotypes of *E. saccharina*. Since then, differences in field behavior and parasitoid complexes have been found between the West African, East African and southern African *E. saccharina* populations (Conlong 2001). Assefa et al. (2006) found considerable genetic diversity between these populations due to geographical isolation and confirmed 3 biotypes of *E. saccharina* in Africa.

Females oviposited most of the eggs on the second and third night post-emergence (Dick 1945; Betbeder-Matibet 1981). This was confirmed by Sampson & Kumar (1985) who reported that most eggs were oviposited within 4 days.

Eldana saccharina is a cryptic insect (Conlong 1994a, b) and females have a prehensile, extendable and flexible ovipositor, with sensory hairs on its tip, all of which need to be stimulated before oviposition occurs (Wallade 1982). This ensures that eggs are cryptically deposited mainly between the dead leaf sheath and the mature sugarcane stalk (Sampson & Kumar 1985). In South African sugarcane, eggs are mainly oviposited on dead leaf material on the lower third of the stalk where this material is more abundant (Leslie 1990).

Atkinson (1981) described the mating behavior of *E. saccharina* in sugarcane and in the laboratory in detail. In the field, males aggregated in groups of 3 to 6 in the sugarcane canopy and called females by flapping their wings each with his abdomen recurved and pencil hairs extended from the end of the abdomen. Females approached the males and flapped their wings just before mating. *Eldana saccharina* is one of a few Lepidoptera where males call the females and not vice versa. Male pheromone release has reported to be common amongst the subfamily Gallerinae to which *E. saccharina* belongs (Atkinson 1981). Dick (1945) reported results of 2 experiments on mating frequency. In the first experiment, one male out of 20 males fertilized 2 females. The first female was fertilized on the night of emergence of the male and the second female was fertilized 2 nights later. In the second experiment, he placed 20 males with 4 females. Only one of the 4 females oviposited fertile eggs. From both of the above experiments, Atkinson (1981) concluded that while males could mate more than once, it did not usually occur. Betbeder-Matibet et al. (1977) however, reported that all adult males mated more than once, and most matings occurred in the first 3 days of the life of the male. The large chitinous spermatophore that is deposited by *E. saccharina* males into the bursa copulatrix of the female during mating, and the structure of the female reproductive system was described and illustrated by Atkinson (1981). Due to the size of the spermatophore contained within the female reproductive system, and because only one has ever been found per female, Atkinson (1981) also concluded that females mate once. He did not describe any controlled experiment to determine this conclusion and it can therefore be assumed that females from the field and laboratory mating boxes were dissected at random by him.

Thus, it is clear that there are large variations in *E. saccharina* fecundity and fertility as reported by previous authors working on this insect, and summarized above. Furthermore, because a controlled experiment to determine female and male mating frequency was never carried out, this paper aims to confirm fecundity, fertility and female and male mating frequency under controlled laboratory conditions.

Materials and Methods

COLONY REARING CONDITIONS

Eldana saccharina was routinely reared at SASRI using an artificial diet based on that of Gillespie (1993), which was modified from that previously described by Graham & Conlong (1988), and had ferric citrate and formaldehyde removed (Table 1). Plastic multicell trays (32

Table 1. Composition of the current SASRI diet for rearing *Eldana saccharina*. Quantities are sufficient to yield approximately 15 L of diet.

Ingredients	Quantity
Dried crushed cane stalk	3,000 g
Ground chickpea	1,500 g
Yeast extract	45 g
Casein	257.1 g
Sodium propionate	137.1 g
Ascorbic acid	50.1 g
Calcium lactate	17.1 g
Tri-sodium citrate	34.4 g
Sodium chloride	8.6 g
Citric acid	34.4 g
Methyl-p-hydroxybenzoate	30 g
Dithane M45[1]	2.6 g
Denol (70 %)[1]	525 mL
Agar	75 g
Water (for agar)	10,000 mL
Water (balance)	5,000 mL

[1]Dithane M45®, Grovida Horticultural Products, P.O. Box 18163, Dalbridge, 4014, KZN, South Africa
[2]Denol®, Polychem Supplies, P.O. Box 17254, Congella, 4013, KZN, South Africa

cavity) containing 8 mL artificial diet per cell were inoculated with neonate larvae and held in rearing rooms maintained at 28 ± 2 °C, 75 ± 5% RH and a 0:24 h L:D photoperiod. Pupal production peaked at 619 degree-days. The pupae were harvested from the diet and moved to an adult emergence and oviposition room maintained at 27 ± 2 °C, 75 ± 5% RH, and a 8:16 h L:D photoperiod. Adults were collected using vacuum, and paired for mating. Eggs were oviposited on paper towels, which were placed in incubators (24 ± 2 °C; 75 ± 5% RH; 0:24 h L:D photoperiod) for 119 degree-day, after which neonates eclosed from eggs (Way 1995).

FECUNDITY AND FERTILITY

Harvested pupae were placed singly into the individual cells of the multicell trays. The multicell tray was wrapped with cling wrap (Handywrap®[1], Chipkins Catering Supplies, P.O. Box 12767, Jacobs, 4026, KwaZulu-Natal, South Africa), which was aerated with a pin prick above each cell. Each moth emerged singly in the separate cells and this ensured that virgin males were available to be paired with virgin females for the experiment. Freshly emerged adults were collected from the cells, sexed and a single male and female moth pair was placed into a 500 mL paper drinking cup, containing a pleated cardboard oviposition substrate (50 × 10 mm when pleated 5 times) that was held together with a paper clip, and a 10 mm dental cotton "wick" soaked with water for adults. Plastic lids were placed on the paper cups and oviposition substrates were changed daily for 5 days. Because females laid the majority of their eggs 2 to 4 days after emergence (Dick 1945; Betbeder-Matibet 1981; Sampson & Kumar 1985), they were killed after 5 days by freezing, and dissected to assess mating status by the presence of a spermatophore. The pupae and mating adults were held at 27 ± 0.65 °C, 70 ± 4.13% RH and a 8:16 h L:D photoperiod. The collected oviposition substrates were incubated at 24 °C and 75% RH, and 0:24 h L:D photoperiod. Eggs oviposited on the substrates were counted to determine female fecundity, as were eclosed neonate larvae to determine percentage egg hatch (fertility). Ten mating pairs were used to assess mean fecundity per *E. saccharina* female. The bursa copulatrix was dissected from each mated female. The mated status was confirmed by the presence of a spermatophore, and these mated females were used to assess mean fertility. The above experiment was repeated twice.

FEMALE MATING FREQUENCY

Harvested pupae were placed singly into individual cells of the multicell trays and treated as described above, thus ensuring virgin adult pairings. A freshly emerged male moth adult was paired with a freshly emerged female moth. The pairs were placed into 500 mL paper cups, prepared as described above. Oviposition substrates and 24 h-old male moths were removed daily and each replaced with a new oviposition substrate and a freshly emerged male that was left to mate overnight with the remaining female. This procedure was repeated until the female died or for 4 additional days (whichever occurred first), after which the female was killed by freezing and dissected to assess mating frequency by counting the number of spermatophores within her bursa copulatrix. A total of 30 females were prepared as outlined above and each received a newly emerged male daily.

To ensure proper identification of more than 1 spermatophore present, an additional 5 females were prepared in the same manner, but they were allowed to mate for 1 night only. Thereafter, they were killed by freezing and dissected to assess mating success by the presence of a spermatophore. This ensured that in those females that had 1 mating opportunity, the spermatophore within the bursa copulatrix was clearly identified and spermatophores found in females with more than 1 mating opportunity could thus be clearly and unambiguously identified. The pupae and mating adults were held at 27 ± 2 °C, 75 ± 5% RH, and a 8:16 h L:D photoperiod.

MALE MATING FREQUENCY

Harvested pupae were placed singly into individual cells of the multicell trays and treated as described above, thus ensuring virgin adult pairings for the experiment. A freshly emerged adult male was paired with a freshly emerged female, and the pair placed into a 500 mL paper cup, as described above. A total of 30 pairs were mated following the above procedure. Oviposition substrates and the 24 h-old females were removed daily and each replaced with a new oviposition substrate and a freshly emerged female, which was left to mate overnight. Each female that was removed, was killed by freezing and placed into a plastic re-sealable bag labelled with the male number she was paired with and the date she was placed with that particular male. She was then dissected to assess mating status. This procedure was repeated until the male died. Pupae and mating adults were held at 27 ± 2 °C, 75 ± 5% RH, and a 8:16 h L:D photoperiod. The number of successful matings with a female, combined with the date that the female was placed with the male provided data on when most of the matings occurred after male emergence.

STATISTICAL ANALYSIS

Microsoft® Excel (2007) was used for statistical analysis. The data for all trials were subjected to descriptive statistics (minimum; maximum and mean ± standard error), because there was no treatment or control to conduct comparative tests. Fecundity and fertility data were pooled and the minimum, maximum and mean fecundity and fertility values were obtained. From the fecundity data, the percentage of eggs oviposited per night after emergence was calculated. For the female mating status data, the maximum, minimum and mean number of spermatophores per female were calculated. For the male mating status, the maximum, minimum and mean number females mated per

[1]No footnote supplied.

male were calculated. From these data it was possible to determine when most of the matings had occurred after male emergence.

Results

FECUNDITY AND FERTILITY

Eldana saccharina females oviposited a cumulative average of 518 ± 27.5 eggs (mean ± SE; *n* = 20) per female over the 5 nights. Maximum fecundity was 798 eggs and minimum fecundity was 353 eggs per female. On the first night after emergence and after mating, *E. saccharina* females oviposited very few eggs. The largest number of eggs (49.9 ± 3.9% (mean ± SE; *n* = 20) of the total) were oviposited on the second night after emergence (Fig. 1).

All 20 females were mated, as evidenced by the presence of 1 or more spermatophores in their bursa copulatrix. Mean fertility of the females was 63.2 ± 4.2% (mean ± SE; *n* = 20) eggs/female. Maximum fertility was 100%, while the minimum fertility was 19.9%. An average of 8.7 ± 0.9% neonate larvae died shortly after eclosion (as proportion of total eggs produced per ovipositing female).

FEMALE MATING STATUS

Eighty percent of 5 *Eldana saccharina* females that were given 1 mating opportunity had 1 spermatophore present in their bursae copulatrices (*n* = 5). Of those females that were given more than 1 mating opportunity (i.e., had a freshly emerged virgin male placed with them every night after removal of the previous male) 56.7% mated once, 36.7% mated twice and 6.7% mated 3 times. An average of 1.5 ± 0.1 (mean ± SE; *n* = 30) spermatophores were found in their bursae copulatrices (a minimum of 1 and a maximum of 3 spermatophores). Because the females were dissected only at 5 days after emergence, it could not be determined on which night successful matings had occurred.

MALE MATING STATUS

Each *E. saccharina* male showed the ability to mate more than once when presented with a freshly emerged virgin female on consecutive nights (to replace the one from the previous day) during their life span, and mated up to a maximum of 6 different females. The largest number of males mated 2 (23.3%) and 3 females (23.3%), 16.7% mated 4 females and 20% mated 5 females (Fig. 2). On average, males mated with 3.3 ± 0.7 (mean ± SE; *n* = 30) virgin females. Males lived an average of 4.3 ± 0.3 days (mean ± SE; *n* = 30) and the maximum lifespan recorded in the laboratory was 7 days. Most matings occurred in the first few days of life of the male, 90% on the first night, 93% on the second night, 63.3% on the third night and 43.3% on the fourth night after emergence (Fig. 3).

Discussion

FECUNDITY AND FERTILITY

The mean female fecundity obtained in our study (518 ± 27.5 eggs/female) was very similar to that found in other studies with the southern African population, i.e., Way (1994) reported an average of 432 eggs per female. This and the current study used adults reared on the diet of Gillespie (1993), so trials were completed using individuals that had fed on diets of similar nutritional status in similar rearing conditions. Dick's (1945) diet, which was inoculated with the mold *M. hiemalis*, may have been more nutritious than our diet, which was reflected in more productive females, i.e., the mean number of eggs oviposited per female was 750 with a maximum of 1,004. From these results and the results of other authors (Dick 1945; Waiyaki 1974; Betbeder-Matibet et al. 1977; Betbeder-Matibet 1981; Way 1994), it can be expected that *E. saccharina* fecundity may vary with temperature, nutritional status and biotype.

Although fecundity of *E. saccharina* varied with the nutritional content of the substrate the larvae fed on, the female's ability to produce in excess of 500 eggs, the majority of which were fertile, confirms its potential as a crop pest, whose populations could explode without proper control. In addition, other lepidopteran pests that are being controlled with pest management programs that have a SIT component, have smaller fecundities than *E. saccharina,* yet they are considered pests. The cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), an invading potential pest of native *Opuntia* species in southeastern USA and Mexico, showed a mean fecundity of 119.8 ± 68.9 (mean ± SD) eggs/female (Carpenter et al. 2001). The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a major pest of apples and pears in Canada, Europe, USA and South Africa, had a mean fecundity of 200 eggs per female (Bloem et al. 1999). The false codling

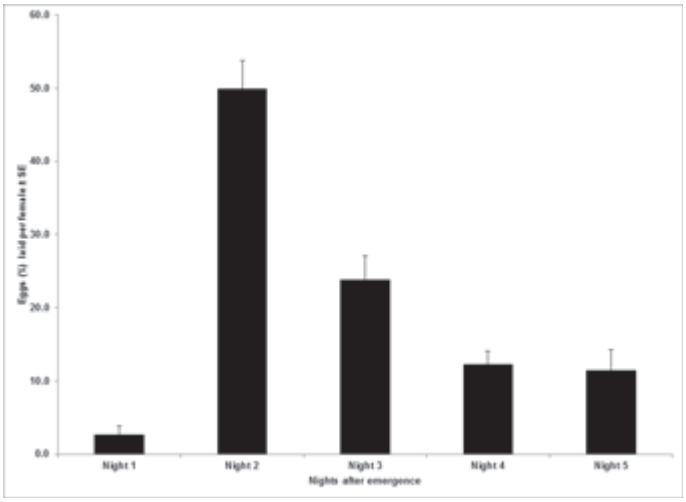

Fig. 1. Mean percentage of eggs (± SE; *n* = 20) oviposited by *Eldana saccharina* adult females per night after emergence.

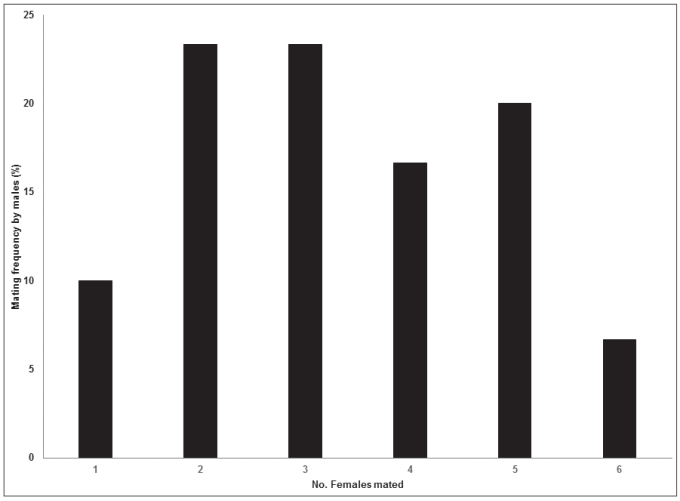

Fig. 2. Frequency distribution of *Eldana saccharina* males having mated 1 to 6 females (*n* = 30).

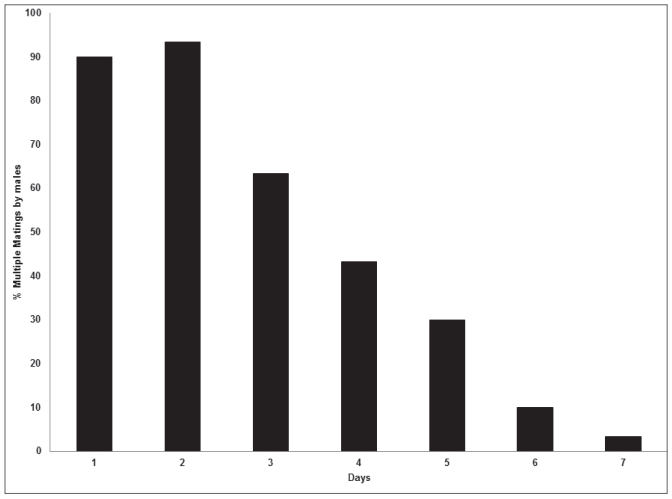

Fig. 3. Frequency distribution of multiple matings of *Eldana saccharina* males during 1 to 7 days after emergence (*n* = 30).

moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), which is a major pest of citrus fruit in South Africa, had a mean fecundity of 400 eggs per female (Bloem et al. 2003).

Results reported here also confirm earlier data (Dick 1945; Betbeder-Matibet 1981; Sampson & Kumar 1985) that most eggs were oviposited on the second and third night after emergence. Biotype, nutritional status and temperature, therefore, did not alter this behavior. Females of the navel orange worm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae) oviposited most of their eggs on the first day after mating (1 to 2 days after emergence) (Landolt & Curtis 1991). *Cactoblastis cactorum* females oviposited soon after emergence and mating, and did not show any additional mating behavior once oviposition started (Hight et al. 2003). The fecundity and mating frequency experiments for *E. saccharina* conducted in the current study were independent of each other and did not take into account whether the start of oviposition prevented females accepting another mate. Although female *E. saccharina* were able to mate more than once, 56.7% mated only once. Furthermore, the moths had a relatively short lifespan of 5 to 7 days in the laboratory (Atkinson 1981). This is advantageous for the purposes of the SIT, because soon after a released irradiated *E. saccharina* male has successfully mated with a wild female, she will oviposit, and therefore the probability that such once-mated wild females would seek out other wild males and mate with them is small.

Fertility was found to be variable in this study, which ranged from 19.9% to 100% (mean of 63.2 ± 4.2% egg hatch). Other authors have reported variable fertility of *E. saccharina* eggs, i.e., Dick (1945) reported 98.3% fertility, and Betbeder-Matibet et al. (1977) reported a maximum fertility of 96.5% and a minimum fertility of 39.7%. In a later study by Betbeder-Matibet (1981) fertility ranged between 79.8% and 95.9%. Way (1994) reported variable fertility at different temperatures, i.e., 54.9% egg hatch at 20 °C, 49.3% egg hatch at 25 °C, and no egg hatch at 15 and 35 °C. Variability in fertility found in our current study could be due to the mating frequency of the female. Byers (1982 cited by Gomez et al. 2000) listed the possible reasons for lepidopteran females to mate more than once. These include inadequate sperm, improved genetic diversity of offspring, and spermatophores possibly contributing towards female nutrition to extend female longevity (Torres-Vila & Jennions 2005). One out of 20 mated females in this study showed only 19.9% egg hatch. It may be that insufficient sperm was transferred to the female or the quality of the sperm was poor. This further supports the importance that released males must be fit enough to successfully secure a mating with a wild female for the purposes of the SIT.

FEMALE MATING FREQUENCY

In the described controlled experiment, up to 3 spermatophores per mated female were found in the bursa copulatrix. Controlled studies on mating frequency of *E. saccharina* females specifically had not been documented, and it was assumed that females mate only once because the spermatophore in the bursa copulatrix was large (Atkinson 1981). The current study showed that females were able to mate up to 3 times in the laboratory, which is in contrast to the reports of Atkinson (1981). Multiple matings are common among Lepidoptera females, and reasons for this have been proposed as mentioned above. Females of the polyphagous *Copitarsia consueta* Walker (Lepidoptera: Noctuidae) in Mexico and male and female *C. cactorum* were reported to mate more than once (Gomez et al. 2000; Carpenter et al. 2009). Also, females of the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae)—once a major cotton pest in the USA that was the target of an AW-IPM program combining *Bt* cotton with the release of sterile insects—were reported to mate more than once (LaChance et al. 1975). Multiple matings were observed in *Galleria mellonella* L. (Lepidoptera: Pyralidae: Gallerinae) females (a serious pest of bee hives) (Flint & Merkle 1983). Mating behavior of *G. mellonella* males is similar to that of *E. saccharina,* in that males emit pheromones and call females for mating.

MALE MATING FREQUENCY

This study confirmed that *E. saccharina* males were able to mate more than once and confirmed the results obtained by Dick (1945) and Betbeder-Matibet et al. (1977). Most matings in this study were accomplished in the first few days of the adult male's life, similar to that reported by Betbeder-Matibet et al. (1977).

The ability of both *E. saccharina* females and males to mate more than once has important implications for calculating over-flooding ratios for the SIT. It is not essential that females are monogamous for the SIT to be successful (Barclay 2005; Calkins & Parker 2005), but mating of sterile and wild males has to be random and it is helpful if the competitiveness of released sterile males is close to that of the wild males (Barclay 2005). Our results of female mating frequencies were based on laboratory studies in which adults were confined to small cages. Even under these conditions, most *E. saccharina* females mated only once (56.7%). It is likely that females in the field will mate once, because Atkinson (1980) found only single spermatophores in the bursae copulatrices of wild females. Torres-Vila & Jennions (2005) found that in Lepidoptera, virgin males produced larger spermatophores than previously mated males and that females mated to virgin males were more fertile than those mated to previously mated males. In a sterile insect release program against *E. saccharina,* ensuring that sterilized virgin males are released, will decrease the chances of wild females looking to re-mate, and increase the program's effectiveness. However, the ability of *E. saccharina* males to mate more than once could possibly reduce the over-flooding ratio of sterile to wild males and thus decrease rearing costs, provided released males remain as competitive as wild males. The effects of oviposition on the mating frequency of *E. saccharina* females, whether males are able to mate with more than one female per night, and the effect of radiation on competitiveness of sperm need to be further investigated.

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