Radiation biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

by
Angela Jasmin Walton

Thesis presented in partial fulfilment of the requirements for the degree Master of Science at the University of Stellenbosch

Supervisor: Prof. Desmond Edward Conlong
Co-supervisor: Dr. Pia Addison
Faculty of Science
Department of Conservation Ecology and Entomology

March 2011

PREFACE

The experimental work described in this thesis was carried out in the Department of Conservation Ecology and Entomology, Stellenbosch University; Deciduous Fruit Producers Trust Laboratories, Western Cape and the South African Sugarcane Research Institute, Mount Edgecombe, KwaZulu-Natal, from August 2007 to February 2009, under the supervision of Prof. Des Conlong.

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Date: 1 March 2011

ABSTRACT

Eldana saccharina Walker (Lepidoptera: Pyralidae) is indigenous to Africa. It has become a major pest of the sugar industry in southern Africa since the 1970's resulting in it being the subject of much research at the South African Sugarcane Research Institute (SASRI). Current control recommendations are not adequate and SASRI has shifted its focus to Area-Wide Integrated Pest Management. The use of Sterile Insect Technique (SIT) is part of an area wide approach. *Eldana saccharina* was assessed as a suitable candidate for SIT.

The biology of *E. saccharina* was reviewed. Controlled studies were completed on laboratory reared *E. saccharina* to assess fecundity, fertility and male and female mating frequency. Mean fecundity was 518 ± 27.5 (mean ± SE) and females laid up to a maximum of 798 eggs. Mean fertility was 63.2 ± 4.2%. The majority (56.7%) of females mated once but on average mated 1.5 ± 0.1 times (max = 3). Males mated up to six females but a single male mated on average 3.3 ± 0.72 females. Most mating occurred on the first (90%) and second (93%) night after male emergence. The majority of the total eggs laid (49.9 ± 3.9%) were laid on the second night after emergence.

A SIT programme requires sterilised marked adults to be released and the marker should not be detrimental to their biology. Sudan Red 7B and Calco Red N1700, were incorporated into different diets used to rear *E. saccharina.* Sudan Red reduced adult emergence by 38% and fecundity by 70%, and significantly prolonged development time compared to the control. Calco Red, in contrast, had no significant effect on the development and reproductive biology of *E. saccharina*, and is therefore a suitable marker. Both dyes marked *E. saccharina* red.

To determine *E. saccharina* radiation biology, laboratory reared males and females were exposed to increasing doses of radiation and crossed as follows: radiation treated males (T♂) mated with untreated females (N♀) and radiation treated females (T♀) mated with untreated males (N♂) at radiation doses of 0, 150, 200, 250, 300 and 350

gray (Gy). The cross of radiation treated females (T♀) mated with radiation treated males (T♂) were exposed to the same radiation doses above except 350Gy. The surviving F_1 progeny from these three crosses were mated with untreated counterparts and F_1 adults of the opposite sex from each radiation dose to assess F_1 fertility. Fertility declined significantly at increasing doses of radiation in the parental crosses and F_1 progeny of treated males. Treated females mated with laboratory males and those mated with treated males were more sensitive to radiation and completely sterile at 200Gy and 150Gy respectively, while treated males mated with laboratory females still had a residual fertility of 0.19% when exposed to 350Gy. F_1 male and female progeny of treated males were completely sterile at 250Gy, while their male parent's fertility was 0.82%.

Eldana saccharina is therefore a suitable candidate for SIT development.

OPSOMMING

Eldana saccharina Walker (Lepidoptera: Pyralidae) is inheems in Afrika. Sedert die 1970's is dit een van die vernaamste plae van die suiker bedryf in suidelike Afrika en om die rede is dit die onderwerp van vele navorsingsprojekte by die Suid Afrikaanse Suikerriet Navorsings Institut. Huidige beheer maatreëls is nie voldoende nie, en gevolglik het die navorsingsfokus vanaf 'n land-by-land basis na 'n Wye-Area Geïntegreerde Plaag Bestuur fokus verskuif. Die gebruik van Steriele Insek Tegniek (SIT) is deel van 'n wye-area benadering. *Eldana saccharina* was ondersoek as 'n geskikte kandidaat vir SIT.

Die biologie van *E. saccharina* was hersien. Gekontroleerde studies, waarin voortplantingspotensiaal, lewensvatbaarheid en manlike en vroulike paringsgedrag ondersoek was, was uitgevoer op laboratorium geteelde *E. saccharina*. Die gemiddelde voortplantingspotensiaal was 518 ± 27.5 eiers (gemiddelde ± standaard fout) en 'n maksimum van 798 eiers was gelê, waarvan die gemiddelde lewensvatbaarheid 63.2 ± 4.2% was. Die meerderheid (56.7%) wyfies het slegs eenmaal gepaar, maar die gemiddelde paringsfrekwensie was 1.5 ± 0.1 maal en 'n maksimum van 3 maal. Mannetjies het met so veel as ses wyfies gepaar, maar die gemiddelde was met 3.3 ± 0.72 wyfies. Die meeste paring het geskied gedurende die eerste (90%) en tweede (93%) nagte na die uitkoms van die mannetjies. Gedurende die tweede nag na uitkoms was die meerderheid van die totale eiers (49.9 ± 3.9%) gelê.

'n SIT program vereis dat steriele gemerkte volwasse individue vrygestel moet word en dat die merker nie nadelig ten opsigte van hulle biologiese ontwikkeling mag wees nie. Sudan Red 7B en Calco Red N1700 was toegevoeg in voedselbronne waarop *E. saccharina* geteel was. Sudan Red het die volwasse uitkoms en voortplantingspotensiaal onderskeidelik met 38% en 70% verminder en het die ontwikkelingstydperk aansienlik verleng in vergelyking met die ongemerkte kontrole *E. saccharina.* Kontrastreerend, het Calco Red geen merkwaardige uitwerking op die

ontwikkeling en reproduktiewe biologie van *E. saccharina* gehad nie, en is gevolglik ‘n geskikte merker. Beide kleurstowwe het *E. saccharina* rooi gemerk.

Die bestralingsbiologie van *E. saccharina* was ondersoek deur laboratorium-geteelde mannetjies en wyfies aan toenemende bestralingsdosisse (0, 150, 200, 250, 300 en 350 gray (Gy)) bloot te stel en daarna, vir elke bestralingsdosis, as volg te teel: bestraalde mannetjies (T♂) met onbestraalde wyfies (N♀) en bestraalde wyfies (T♀) met onbestraalde mannetjies (N♂). Die telingskombinasie van bestraalde wyfies (T♀) en bestraalde mannetjies (T♂) was blootgestel aan dieselfde bestralingsdosisse behalwe 350Gy. Die oorlewende F_1 nageslag wat verkry was vanaf die drie bogenoemde telingskombinasies was geteel met onbehandelde eweknieë en F_1 volwassenes van die teenoorgestelde geslag vanaf elke bestralingsdosis om F_1 lewensvatbaarheid te ondersoek. Lewensvatbaarheid het aansienlik afgeneem met toenemende bestralingsdosisse in the ouerlike parings en die F_1 nageslag van die bestraalde mannetjies. Bestraalde wyfies wat met laboratorium geteelde en bestraalde mannetjies gepaar was, was meer sensitief teenoor bestraling en was geheel en al steriel by 150Gy en 250Gy onderskeidelik, terwyl bestraalde mannetjies wat met laboratorium geteelde wyfies gepaar was nogsteeds ‘n residuele lewensvatbaarheid van 0.19% gehad het na blootstelling aan 350Gy. F_1 nageslag, beide manlike en vroulik, van bestraalde mannetjies (blootgestel aan 250Gy en 0.82% vrugbaar) was geheel en al steriel.

Eldana saccharina is gevolglik ‘n gepaste kandidaat vir SIT ontwikkeling.

ACKNOWLEDGEMENTS

I would like to thank the following people and organisations for their contributions towards this study:

- Prof. Des Conlong for his assistance with the radiation experiments at Stellenbosch, Western Cape and valuable comments to this manuscript.
- Mr. Matthew Addison and Mrs Dalene Stenekamp for their valuable assistance, discussions and providing laboratory space at the Deciduous Fruit Producers Trust (DFPT) laboratories, Stellenbosch, Western Cape.
- Anonymous reviewers for valuable comments to the manuscript published from Chapter 3.
- The staff at the South African Sugarcane Research Institute (SASRI) Insect Rearing Unit for their valuable assistance with the radiation experiments at SASRI, Mount Edgecombe, KZN.
- Ms. Nikki Sewpersad and Dr. Malcolm Keeping from SASRI for their assistance with statistical analysis.
- SASRI for providing the funds to complete this research.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 The problem

Eldana saccharina Walker (Lepidoptera: Pyralidae) is indigenous to Africa (Conlong, 1994a; Conlong, 1994b). Since the 1970's, it has became a major pest of the sugar industry in southern Africa (Carnegie, 1974). Its larvae bore into the sugarcane stalk and cause extensive tunnelling damage. During the 2003/2004 milling season, it was estimated that *E. saccharina* caused a R153 million loss to the South African Sugar Industry (Goebel and Way, 2007). In addition to the loss of sucrose from tissue damage from feeding larvae, the tissues around the borings turn red due to secondary infections by microorganisms (Way and Goebel, 2003). These microorganisms metabolise sucrose into glucose, which causes an overall decline in sugarcane quality, as less sucrose is extracted at the factory (mill) (Way and Goebel, 2003).

Furthermore, the sugarcane milling season in South Africa is approximately 10 months long, ranging from March to December each year (Davis et al., 2009). In order to maximize sucrose production, it is important that sugarcane in the coastal area be grown for 16-18 months. As a result, each year a portion of sugarcane planted during the current season, is kept for harvest and milling in the following season, so that there is appropriately mature sugarcane available for harvesting at the beginning of the new season (Leslie, 2003). This sugarcane is known as carry-over sugarcane and is very vulnerable to attack by *E. saccharina* (Leslie, 2003), necessitating its early cutting, and thus loss of potential production.

Varietal resistance and cultural control methods are currently the preferred control mechanisms available at present (Keeping, 2006; Conlong and Rutherford, 2009). Sugarcane varieties have been routinely screened for *E. saccharina* resistance since the 1980's and statistical interpretation of data has improved the selection process

(Leslie and Keeping, 1996; Keeping, 2006). There has also been extensive recent research on timing of insecticide applications in relation to adult moth peaks (Leslie, 1997; Leslie 2003). There are two moth peaks occurring in April/May and September/October (Leslie, 2003). At these times more eggs are laid, and thus more neonate larvae hatch from the eggs. The neonate larvae, which forage on the sugarcane stalk for the first few days after eclosion, and disperse from the canopy of sugarcane, are the only stage exposed to insecticide before they bore into the stalk (Leslie and Keeping, 1996) to continue their development through to pupae. If an insecticide application targets this neonate dispersal and foraging phase, subsequent crop damage may be reduced (Leslie, 1997).

The South African Sugarcane Research Institute (SASRI) has conducted extensive research on biological control since 1981 and more recently habitat management, and habitat conservation (Conlong, 1990; Conlong, 1994a, 1994c; Kasl, 2004, Barker et al., 2006, Smith et al., 2006). Despite the apparent lack of establishment of many biological control agents tested against *E. saccharina* in sugarcane, biological control is still considered an option, especially when linked to habitat management and conservation (Conlong, 1990; Kasl, 2004; Barker et al., 2006, Smith et al., 2006). Habitat management compliments biological control by making the habitat more favourable for parasitoids and predators. By increasing the natural habitat (Conlong, 1990) and using stimulo-deterrent diversionary tactics (Kasl, 2004, Barker et al., 2006, Smith et al., 2006), this aims to repel *E. saccharina* from sugarcane and re-attract it to its indigenous wetland areas, where indigenous parasitoids are prevalent and provide good control (Conlong, 1990).

The fact that *E. saccharina* is indigenous to Africa, occurs on many other host plants and is cryptic in nature (Conlong, 1994a; Conlong 1994b), has complicated attempts to control it through cultural methods, use of resistant varieties and insecticide applications. After more than 30 years, *E. saccharina* still remains the most economically important pest in the sugar industry (Anonymous, 2005). Traditional pest management approaches are reactive and are generally dealt with very selectively, i.e.

field by field, with very little, if any regard to the biology and ecology of the pest species (Conlong and Rutherford, 2009). If a crop is infested with a pest, insecticides or other control measures are applied and these are generally not integrated together (Vreysen et al., 2006). As the world becomes more environmentally conscious, the use of broad spectrum insecticides is losing favour. This is because improper use of insecticide results in resistance development in pest populations, outbreaks of secondary pests and suppression of beneficial insect populations. It is important that more ecologically sound pest management principles are applied (Vreysen et al., 2006).

Control strategies for *E. saccharina* at SASRI are now focused on an Area-Wide IPM strategy (AW-IPM) using conventional recommendations mentioned above (cultural control, resistant varieties, and use of insecticides where needed) in addition to new technologies. Modern strategies include delineation of species within populations, habitat management, chemical ecology, use of plant endophytic pathogens, such as use of antagonistic *Fusarium* isolates identified by McFarlane and Rutherford (2005), use of *Wolbachia* species (an entomopathogen that skews offspring sex ratio) and Sterile Insect Technique in order to make AW-IPM more effective (Conlong and Rutherford, 2009).

Modern IPM is about holistic agro-ecosystem management, based on knowledge about the complete ecology of the target pest and its interactions with environmental factors around it. It is vital that the focus in any commercial crop system is on AW-IPM, as insects do not recognise boundaries or borders. Sterile Insect Technique shows much promise for controlling insect pests, because it is an Area Wide approach and is specific to the target insect. SASRI has started preliminary studies on the use of Sterile Insect Technique for controlling *E. saccharina* (Conlong, 2007). Modern AW-IPM strategies aim to minimise environmental impacts of synthetic pesticides even further (Conlong and Rutherford, 2009).

1.2 Sterile Insect Technique

Sterile insect technique (SIT) is an area-wide approach and targets an entire pest insect population (Vreysen et al., 2006). The basis of SIT is mass rearing and release of sterilised adults of the target pest into a crop environment so they mate with their wild counterparts (Klassen, 2005). Because adults are released, this approach cannot be done on a field by field basis, as adult insects are mobile, and do not recognise field or farm boundaries (Conlong and Rutherford, 2009). This mating of sterilised adults with wilds induces sterility in the wild population and reduces pest population numbers (Klassen, 2005). The SIT concept was first conceived in the 1930s and 1940s by three independent researchers: A.S. Serebrovskii from Moscow University; F.L. Vanderplank from a Tsetse research institute in Tanzania and E.F. Knipling from the United States Department of Agriculture (Klassen and Curtis, 2005).

The use of SIT as a pest management strategy has been increasingly researched and implemented since the 1950's. Laboratory experiments conducted by Bushland and Hopkins (1951; 1953) showed that sterility in screw-worm male and female flies (*Lucilia* (*Cochliomya*) *hominivorax* Coquerel (Diptera: Calliphoridae)) could be achieved by irradiating the pupae with X- or gamma rays, with no adverse effects on their mating behaviour. Lindquist (1955) and Baumhover et al. (1955) reported that screw-worm had been completely eradicated from the island of Curacao, within three months of the commencement of releases of sterile males (Knipling, 1955; Vreysen et al., 2006). Since then, the screw-worm has been eradicated from the United States of America (USA), Mexico, Central America and Panama (Klassen and Curtis, 2005).

SIT has been successfully used world-wide against other major agricultural pests to establish areas of eradication, suppression (low prevalence) and exclusion (where the pest is prevented from entering the area). Extensive SIT programmes have been developed and successfully used to stop the spread of the Mediterranean fruit fly (*Ceratitis capitata* Weidemann (Diptera: Tephritidae)) from Central America into southern Mexico. In Chile the same pest has been eradicated. There are currently

large SIT programmes to suppress and exclude this pest in Argentina, USA, Thailand, Israel, Brazil, Portugal, Spain and Tunisia and South Africa (Klassen and Curtis, 2005). SIT has been applied to suppress the Mexican fruit fly (*Anastrepha ludens* Loew (Diptera: Tephritidae)) and the West Indian fruit fly (*Anastrepha obliqua* Macquart (Diptera: Tephritidae)) in Northern Mexico (Klassen and Curtis, 2005; Vreysen et al., 2006) and the Onion fly (*Delia antique* Meigen) in The Netherlands (Vreysen et al., 2006). The Melon fly (*Bactrocera cucurbitae* Coquillet (Diptera: Tephritidae)) has been eradicated from Okinawa and the south-western islands of Japan (Klassen and Curtis, 2005; Vreysen et al., 2006). SIT has also been used successfully against the Queensland fruit fly (*Bactrocera tryoni* Froggatt (Diptera: Tephritidae)) in Australia to suppress the pest (Vreysen et al., 2006).

Tsetse fly (*Glosinia austeni* Newstead (Diptera: Glossinidae) was eradicated from Zanzibar in 1997 (Klassen and Curtis, 2005).

Cockchafers (*Melolontha vulgaris* F. (Coleoptera: Scarabaeidae)) have been eradicated from Switzerland using SIT and there is a large AW-IPM programme integrating SIT against the sweet potato weevil *Euscepes postfaciatus* (Fiarmaire) Coleoptera: Curculionidae) on Kume island, Japan (Klassen and Curtis, 2005).

A SIT programme has been operating against the Pink bollworm (*Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae)) since 1967 to prevent its spread into southern California, USA. Codling moth (*Cydia pomonella* L. (Lepidoptera: Tortricidae)) is being suppressed in British Columbia, Canada with the use of SIT (Bloem et al., 2005; Klassen and Curtis, 2005). The use of SIT is also currently being researched to control Date moth (*Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)) (Vreysen et al., 2006) and the cactus moth (*Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae)) (Carpenter et al., 2001a, 2001b; Bloem et al., 2003; Vreysen et al., 2006). There is also a SIT programme targeting the False Codling moth (*Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)) (Hofmeyer et al., 2005; Groenewald, 2009) and the Codling moth (Addison, 2005) in South Africa.

There is thus world-wide scale use of this technology against pests of economic importance (Klassen and Curtis, 2005; Vreysen et al., 2006). Advantages of SIT are that it is environmentally friendly, does not adversely affect non-target organisms and can be easily integrated with other biological control methods, using parasitoids, predators and pathogens (Figure 1.1) (Carpenter et al., 2005; Vreysen et al., 2006).

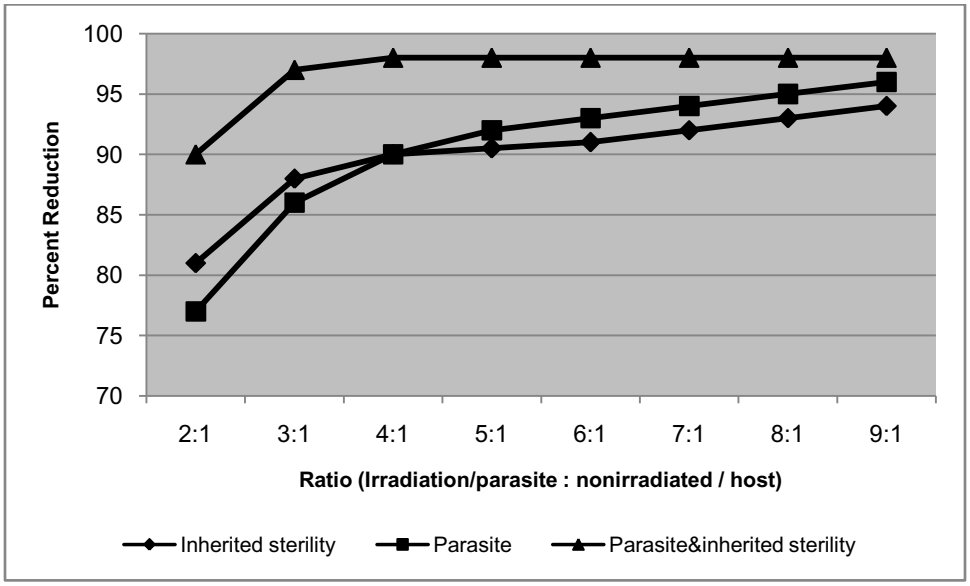

Figure 1.1. Predicted reduction in population growth when parasitoids, irradiated moths and combination of the two are released (from Carpenter et al., 2005).

Successful SIT programmes require that the target pest is present in low numbers because SIT efficacy is inversely density dependant (Figure 1.2) (Feldmann and Hendrichs, 2001; Klassen, 2005). The model described in Figure 1.2 shows that a conventional control method (e.g. an insecticide application) is more effective when a large number of insects are targeted but as a whole will achieve a very small percentage of control of the entire population. The cost to apply insecticide is the same regardless if the population is high or low. It is therefore more cost effective if insecticide is to be applied, to apply when the population is high (Feldmann and Hendrichs, 2001). Sterile Insect Technique is more efficient at suppressing a larger percentage of the pest population, when the population is low. If pest numbers are

relatively low, most wild insects are more likely to mate with released sterile insects, to thus induce sterility in the wild population (Klassen, 2005; Vreysen et al., 2006).

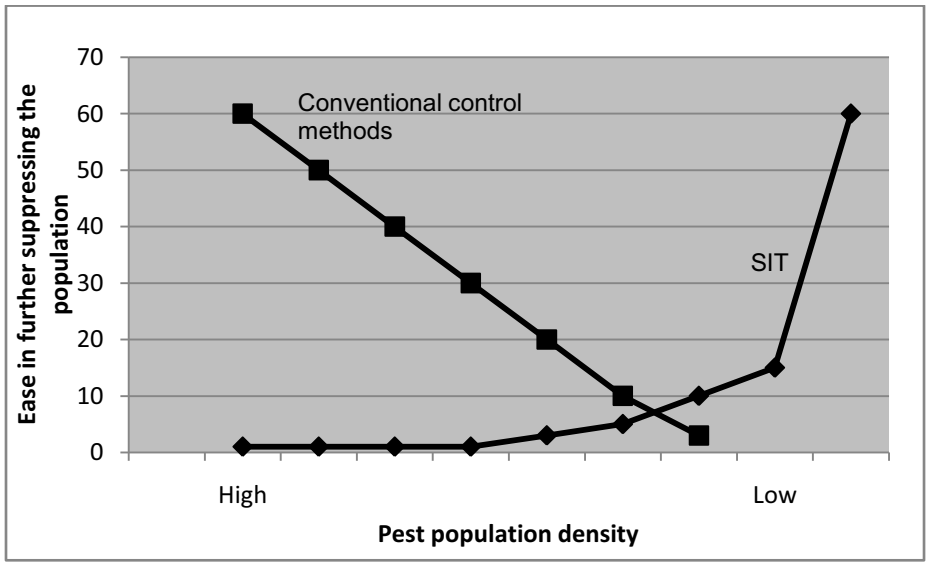

Figure 1.2. Efficiency of conventional control methods and the Sterile Insect Technique (SIT) (from Feldmann and Hendrichs, 2001; Klassen, 2005).

With correct use of resistant varieties and cultural control (correct fertiliser applications and harvesting sugarcane at the right age) on an area wide basis, combined with area wide habitat management techniques as outlined above, *E. saccharina* populations in sugarcane fields could be lowered to a population level where SIT would become viable (Conlong and Rutherford, 2009). SIT is also fully compatible with biological control efforts, as it can increase the efficacy of agents already working in crop fields (Vreysen et al., 2006). Therefore SIT can become an integral part of Integrated Pest Management (IPM), resulting in more sustainable long-term pest control (Vreysen et al., 2006; Conlong and Rutherford, 2009).

1.3 Rationale for use of SIT against *Eldana saccharina*

False codling moth (FCM) is indigenous to South Africa, Ethiopia and many islands on the African continent. It attacks a wide range of indigenous host plants and has moved

onto cultivated crops (Bloem et al., 2003; Carpenter et al., 2007). It is a major pest in the South African citrus industry and has become resistant to insecticides. Crop management methods and biological control with predators, pathogens and parasitoids have been initiated against it, but with limited success (Bloem et al., 2003; Carpenter et al., 2007). This situation is similar to that experienced in the South African sugar industry, as *E. saccharina* is also indigenous to Africa and attacks many indigenous host plants in the South African sugarcane growing region (Conlong, 1994a; Conlong 1994b; Conlong, 2004; Conlong, 2007). Despite the use of resistant varieties, shortened crop cycles and field hygiene, *E. saccharina* still remains a significant pest in the South African sugar industry (Anonymous, 2005).

A large SIT programme was initiated against FCM in the Citrusdal Citrus growing region (32° 36' 0" S, 19° 1' 0" E), in the Western Cape, South Africa in 2007 by XSIT Pty (Ltd)[1]. The programme started releases in the 2007-2008 growing season. During the 2008-2009 growing season, the wild population of FCM in the valley and the amount of infested fruit declined significantly where releases occurred. In areas where no SIT adults were released, the wild population remained high, and fruit infestation has subsequently increased (Groenewald, 2009).

Because of the similarities in ecology of FCM with *E. saccharina,* and the success of the FCM SIT programme in South Africa, the use of this approach against *E. saccharina* appears feasible. The sugar industry in southern Africa is widespread. However, *E. saccharina* occurs in pockets throughout the industry and using habitat management with SIT, reduction of these pest populations are possible. *Eldana saccharina* is also spreading into previously uninfested sugarcane in higher altitude areas and exclusion from these areas using SIT would also be possible (D. Conlong, personal communication[2]). Much the same has been done with *C. cactorum* to manage its spread into southern USA (Carpenter et al., 2001b; Bloem et al., 2007).

[1] XSIT (Pty) Ltd, P.O. Box 422, Citrusdal, 7240, Western Cape, South Africa.
[2] Des Conlong, South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe 4300, KwaZulu Natal, South Africa, November 2009. Des.Conlong@sugar.org.za

1.4 Induction of sterility

Generally sterility of an insect, i.e. one that is unable to produce viable offspring, can be achieved by exposure to gamma or X-ray irradiation or chemicals (Bakri et al., 2005; Klassen, 2005). The use of ionizing radiation from isotopic sources (Cobalt-60 or Caesium-137) has been the primary method to induce sterility for SIT (Bakri et al., 2005). High-energy electrons and X-rays may also be used to irradiate insects. Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed. The key parameter for insect sterility is the radiation dose absorbed (Bakri et al., 2005). The dose is expressed in Système International d'Unités (SI) as gray (Gy). Provided the dose is delivered correctly, the irradiation process is effective. Other advantages of using irradiation is that temperature does not rise significantly during the process; sterile insects can be released immediately after irradiation; irradiation does not add residues that are harmful to human health or the environment, and the insects are safe to handle (Bakri et al., 2005). Radiation can also pass through packaging material, allowing the insects to be packaged, for field releases, before being irradiated (Bakri et al., 2005). This is advantageous as it reduces handling stress on the insects due to be released. Radiation is preferred to chemosterilants, because most chemosterilants are carcinogenic, mutagenic and tetragenic and can lead to environmental and human-health concerns (Bakri et al., 2005). Insect resistance to chemosterilants is an additional concern (Bakri et al., 2005).

Typically there are two types of gamma irradiators used in SIT programmes, self contained dry-storage irradiators and large-panoramic irradiators. At present, in most SIT programmes the self-contained dry-storage irradiators are used. These irradiators house the radiation source within a protective shield of lead (Bakri et al., 2005). There are currently growing complexities in the procurement of replacement gamma radiation sources and the acquisition of new gamma radiation sources. Transboundary shipment of radioisotopes is becoming complicated and expensive, and there are associated fears of terrorism (Hendrichs, 2007).

Alternative technology using low energy X-rays is currently being developed (Hendrichs, 2007). The first radiation source used to irradiate screw-worm flies in the 1950's was X-rays. There have been a number of improvements since then and modern X-ray irradiators now have a number of advantages over the ionizing radiation types. They are electrically powered, so that when there is no power to the machine, they do not emit radiation. Therefore less shielding is required. The legislation is simpler and the transport costs are lower. A costly accelerator is not needed for X-rays as it is for gamma sources (Hendrichs, 2007). Conventional X-ray machines used in hospitals cannot be used for insect irradiation however, because the radiation dose that is delivered by these is about 5Gy/min, which is too low for insect sterilisation. Levels of 150-200 Gy have been shown to be effective for SIT (examples of which are reported in Arthur et al., 1993; Barbulescu and Rosca, 1993; Bloem et al., 2003; Carpenter, 1993; Carpenter et al., 2001a; Hendrichs, 2007; Mastro, 1993; Omar and Mansor, 1993; Qureshi et al., 1993; Sutrisno et al., 1993; Sallam and Ibrahim 1993; Zhang et al, 1993). Radiation would thus take too long using hospital based machines, and the insects may suffer handling stress which could affect competitiveness in the field. X-ray irradiators have recently been successfully upgraded to yield a much more uniform and higher dose rate of 100Gy/min (Hendrichs, 2007). Currently, there is a commercial X-ray irradiator for SIT available from Rad Source Technologies, Inc.[3] The model RS 2400 can deliver a rate of between 18-70Gy per minute (http://www.radsource.com/pdf/rs2400.sitbrochure.pdf). X-ray irradiators have many benefits as described above and will certainly be the source of irradiation in the future (Hendrichs, 2007).

1.4.1 Mode of Action of Radiation

The physiological mechanisms of sterility in insects may be caused in one of four ways: 1) the females are unable to lay eggs, i.e. they are infecund; 2) the males are unable to produce sperm, (apspermia), or the sperm is unable to function, (sperm inactivation); 3)

[3] Rad Source Technologies Inc., 480 Brogdon Road, Suite 500, Suwanee, Georgia 30024, USA, www.radsource.com

the adults are unable to mate or 4) there are dominant lethal mutations in the reproductive cells of either the male or female (Klassen, 2005; Lance and McInnis, 2005; Robinson, 2005). Dominant lethal mutations in embryonic development are the most important for SIT (Proverbs, 1969; Robinson, 2005). These are caused by chromosomal breaks in the germ cells (Robinson, 2005; Klassen, 2005). In addition, it is important that the radiation dose must destroy the spermatogonia of the male to prevent recovery of fertility and must also destroy the oogonia or trophocytes of the female, which are required for egg formation (Klassen, 2005). The affected sperm fertilizes the egg normally, but the dominant lethal mutations kill most embryos during the first few cleavage divisions of embryonic development (Klassen, 2005; Robinson, 2005). When a break occurs in the chromosome of a mature sperm cell, it remains in this condition until the chromosome has entered the egg. During early prophase the broken chromosome undergoes normal replication. During metaphase the broken ends fuse, which then results in a dicentric chromosome and an acentric fragment, which is lost. The dicentric fragment forms a bridge at anaphase and this leads to another chromosomal break. This whole process repeats itself, which results in serious imbalances in the daughter cells and eventually death of the zygote (Robinson, 2005).

1.5 Implementation of a SIT programme against the target pest

A number of procedures need to be followed in order for a SIT programme to be successfully implemented against a pest insect. The biology and ecology of the pest must be known. An informed decision on the radiation dose applied to the target insect requires accurate data on how factors such as dose, insect stage, age and various processing and handling techniques during mass rearing affect levels of sterility and insect quality. Published data on the radiation biology of the same or similar species can provide guidance. However, in many cases data are of limited value because dosimetric procedures, dose-measurement traceability, dose distribution and other important information are often not published (Bakri et al., 2005).

It is vital to a SIT programme that high quality insects in large enough numbers are produced (Bloem and Bloem, 1998; Parker, 2005) to target the wild population. Insect rearing technology is needed in order to achieve this. The insect's sensitivity to radiation doses must be known so that an appropriate radiation dose can be applied, without any deleterious effect on the insect's biology or behaviour. Once this has been determined, it is necessary to measure the ratio of released irradiated males to wild males, released male flight distances and to gauge the success of a SIT programme against the target pest through population monitoring (Knipling, 1955; Lance and McInnis, 2005; Parker, 2005; Vreysen et al., 2006). To measure these criteria, laboratory-reared insects are commonly marked, released and recaptured in traps that have been set up in the field, which then capture both marked and unmarked wild individuals (Southwood, 1966; Hagler and Jackson, 2001; Qureshi et al., 2004). It is important that the marking technique used to do this also has no detrimental effects on the target insect's biology, so that sterile insects released remain competitive with their wild counterparts.

Once biological studies have been completed, and a decision is made to initiate a large scale SIT programme against a target pest, public relation efforts, organisational and government assistance is vital (Bloem and Bloem, 1998). As mentioned above, SIT is an area-wide approach that targets the entire insect population (Vreysen et al., 2006). The target pest may also occur in alternate hosts and it is important that these areas are included along with the crop in releases of sterile insects (Bloem and Bloem, 1998; Lindquist, 1998).

1.6 Objectives

The objectives of this study were threefold:

- To review the general biology of *E. saccharina* and to assess adult mating frequency.

- To determine the effects of two oil soluble dyes incorporated into the routine artificial diet used at SASRI, on *E. saccharina*'s biology for the purposes of mark and release recapture studies, and monitoring of the success of the SIT.
- To assess the effects of increasing doses of radiation on newly emerged *E. saccharina* adults' biology, and the induction of sterility in the F_1 generation.

The chapters in this thesis were written in the format of scientific papers, with the intention to submit to relevant peer reviewed journals. Therefore there is repetition between chapters, particularly in the materials and methods sections.

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CHAPTER 2

ELDANA SACCHARINA WALKER (LEPIDOPTERA: PYRALIDAE) GENERAL BIOLOGY

2.1 Abstract

Eldana saccharina occurs on many graminaceous crops and on several wild grasses and sedges throughout Africa. Its biology and behaviour in South Africa has been researched at the South African Sugarcane Research Institute (SASRI) since the 1970's and has been in colony at SASRI since. *Eldana saccharina*'s fecundity has been reported to be variable and dependent on nutritional status, place of origin and temperature. Controlled studies were carried out on laboratory reared South African *E. saccharina* to assess fecundity, fertility and male and female mating frequency. Mean fecundity of *E. saccharina* was 518 ± 27.5 (mean ± SE) eggs up to a maximum of 798 eggs. Mean fertility of *E. saccharina* was 63.2 ± 4.2%. The majority (56.7%) of *E. saccharina* females mated once but on average mated 1.5 ± 0.1 times (maximum of 3). Males mated up to six females and mated on average 3.3 ± 0.72 females. Most mating occurred on the first (90%) and second (93%) night after male emergence and the majority of eggs laid by the females were on the second (49.9 ± 3.9%) night after emergence.

Eldana saccharina's high fecundity confirmed its potential as a crop pest. This study has, for the first time confirmed that males and females are able to mate more than once under controlled laboratory conditions. This has important implications for calculating release ratios of laboratory sterilised males to wild males, to ensure sterile males mate with wild females in an Area-Wide Sterile Insect Release programme.

2.2 Introduction

Eldana saccharina Walker (Lepidoptera: Pyralidae) is indigenous to Africa. It occurs on graminaceous crops (sugarcane, maize and sorghum) and on several wild grasses and sedges (Conlong, 1994a; 1994b; 1994c). It was first recorded in South African sugarcane in 1939 on the Umfolozi Flats, KwaZulu-Natal (Dick, 1945). *Eldana saccharina* was first described from West Africa in the late 1800's from sugarcane in Sierra Leone (Betbeder-Matibet, 1981) and has also been reported from sugarcane in East Africa (Girling, 1972; Conlong, 2000; Conlong and Mugalula, 2001). In South Africa, after the initial outbreak in 1939 reported by Dick (1945), the pest disappeared and 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 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 the 1970's (Carnegie, 1974). Atkinson (1978) reported that *E. saccharina* at that time were reared on an artificial diet for biological studies. SASRI has maintained a colony since, with numerous adaptations to the rearing procedure being made. The current artificial diet is based on that reported by Gillespie (1993) with modifications to the diet previously described by Graham and Conlong (1988), and with ferric citrate and formaldehyde having since been removed (Table 2.1). Plastic multicell trays (32 cavity) containing 8 ml artificial diet per cell are inoculated with neonate larvae and held in rearing rooms maintained at 28 ± 2 °C; 75 ± 5% RH and a 0:24 L:D photophase. Pupal production peaks at 619 day degrees (DD). The pupae are then harvested from the diet and moved to an adult emergence and oviposition room (27 ± 2 °C; 75 ± 5% RH; 8:16 L:D photophase), where adults emerge. Adults are collected using vacuum, and are paired for mating. Eggs are laid on paper towelling. The paper towelling and eggs are placed into incubators (24 ± 2 °C; 75 ± 5% RH; 0:24 L:D photophase) for 119 DD, after which the neonates eclose from the eggs (Way, 1995).

Table 2.1. 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	3000.0g
Ground chickpea	1500.0g
Yeast extract	45.0g
Casein	257.1g
Sodium propionate	137.1g
Ascorbic acid	50.1g
Calcium lactate	17.1g
Tri-sodium citrate	34.4g
Sodium chloride	8.6g
Citric acid	34.4g
Methyl-p-hydroxybenzoate	30.0g
Dithane M45[4]	2.6g
Denol (70 %)[5]	525.0ml
Agar	75.0g
Water (for agar)	10 000.0ml
Water (balance)	5000ml

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 he could not accurately calculate the lower development threshold temperature for *E. saccharina*. He thus estimated the lower larval development threshold to be 15 °C and the duration of the different life stages at various temperatures were calculated in days, not day degrees. The same author reported six to seven larval instars for female larvae and five to six larval instars for male larvae. Anatomical differences between male and female larvae and pupae were also

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

described and illustrated. Due to adaptations in the artificial diet, the continual laboratory rearing of *E. saccharina*, and the acquisition of more sophisticated incubators, Way (1995) reviewed and accurately calculated the lower development thresholds for egg (5.3 °C), larvae (10.2 °C) and pupae (10.7 °C) and calculated day degrees. The development time from egg to adult in *E. saccharina* was 897.9 Day Degrees (DD) (Way, 1995). Day degrees are a measure of heat units required over time to complete a certain stage of development above a development threshold temperature (Way, 1995). 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 number of larval instars is the same as reported by Atkinson (1980). In a prior study, Way (1994) reported on the effect of four different temperatures on *E. saccharina* adult female longevity, mating success, oviposition and egg development. Longevity was inversely dependant on temperature, being shorter at higher temperatures and longer at lower temperatures. Fecundity was highest at 20 °C and 25 °C, with an average of 417.74 and 432.84 eggs laid per female respectively. At temperatures of 15 °C and 35 °C, fecundity was approximately half, with 205.78 and 183.19 eggs laid 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 being progressively shorter at increasing temperatures. Eggs failed to hatch at 15 °C and 35 °C (Way, 1994).

Dick (1945) reared *E. saccharina* on an artificial diet inoculated with the mould *Mucor hiemalis* Wehmer. It was found that the average number of eggs laid per female was 750 with a maximum of 1004. Mating was found to increase the number of eggs laid compared to unmated females (Dick, 1945; Sampson and Kumar, 1985). In West Africa, Betbeder-Matibet et al. (1977) reported an average fecundity of 460 eggs laid per female up to a maximum of 811. These females were reared on 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 and Kumar (1985) reported an average fecundity of 327 ± 17 eggs per female reared on

sugarcane stalks. Fecundity of *E. saccharina* was therefore very variable, temperature and nutritional status played important roles. The majority of eggs were laid on the second and third nights after emergence of the female (Dick, 1945; Betbeder-Matibet, 1981). Sampson and Kumar (1985) reported that most eggs were laid within 4 days.

Eldana saccharina is a cryptic insect (Conlong, 1994a; Conlong 1994b). Females have a prehensile and flexible ovipositor, with sensory hairs on its tip, which all need to be stimulated before oviposition occurs (Wallade, 1982) (Figure 2.1). This ensures that the eggs are cryptically placed. Eggs are deposited mainly between the dead leaf sheaths and the mature sugarcane stalk (Sampson and Kumar, 1985). In the South African sugar industry, eggs are laid on dead leaf material on the lower third of the stalk where this material is more abundant (Leslie, 1990).

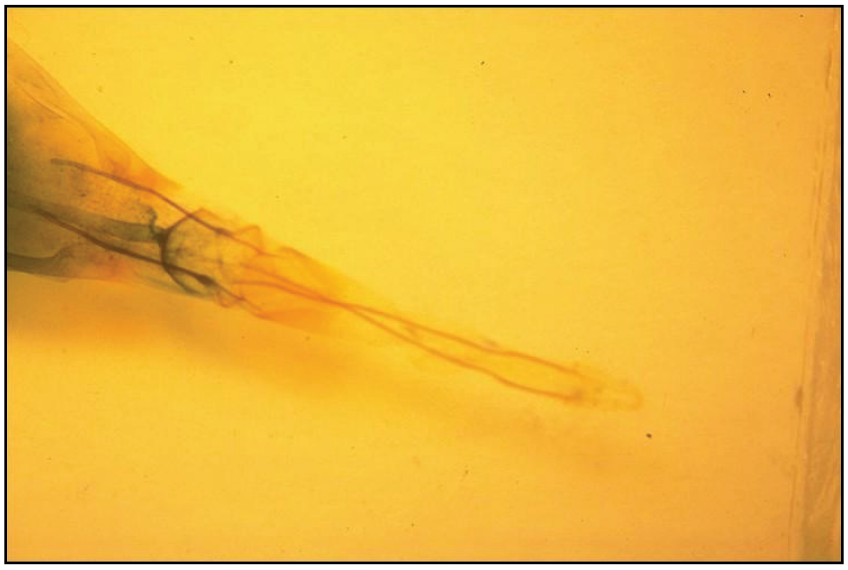

Figure 2.1. Dissected and extended ovipositor of an *Eldana saccharina* female (x100).

Atkinson (1981) described the mating behaviour of *E. saccharina* in sugarcane and in the laboratory in detail. In the field, males in groups of three to six, aggregated in the sugarcane canopy and called females by flapping their wings with their abdomens recurved and pencil hairs extended from the end of the abdomen. Females responded

by approaching 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) determined in one experiment, that one male out of 20 males was able to fertilise two females. The first female was fertilised on the night of emergence of the male and the second female was fertilised two nights later. He conducted a further experiment to quantify the number of times a male could mate, by placing 20 males with four females. Only one of these females laid 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) reported that all adult males mated more than once, and most matings occurred in the first three 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 on 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 females reproductive system, and because only one had ever been found per female, Atkinson (1981) concluded that females mate once. Atkinson (1981) did not describe any controlled experiment to determine this and it can therefore be assumed that females from the field and laboratory mating boxes were dissected at random by him.

Due to variability in *E. saccharina* fecundity reported by previous authors and because a controlled experiment to determine female and male mating frequency has not been reported on, this chapter aims to confirm fecundity, and female and male mating frequency.

2.3 Materials and Methods

Colony rearing conditions

Eldana saccharina is routinely reared at SASRI as described in this chapter, section 2.2. For the purposes of the trials completed to produce the results of this chapter, pupae were then processed as described below.

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®[6]) which was aerated with a pin prick above each cell. This ensured that virgin males were available to be paired with virgin females for the experiment, as each sex emerged singly in separate cells. Freshly emerging adults were collected from the cells they eclosed in, sexed and a single male and female moth pair was placed into a 500 ml paper drinking cup, containing a pleated cardboard oviposition substrate (50x10 mm when pleated five times), held together with a paper clip, and a 10 mm dental cotton "wick" soaked with water for adults to drink from (Figure 2.2). Plastic lids were then placed on the paper cups. Oviposition substrates were changed daily for 5 days, after which females were killed 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; 8:16 L:D photophase. The collected oviposition substrates were incubated at 24 °C and 75% RH 0:24 L:D photophase. Eggs laid on the oviposition substrates were counted to determine fecundity, as were neonate larvae emerging from the eggs to determine fertility of the eggs laid. Ten pairs were used to assess mean fecundity per *E. saccharina* female. After dissection of the bursa copulatrix mated females were used to assess mean fertility. The above experiment was repeated twice.

[6] Handywrap®, Chipkins Catering Supplies, P.O. Box 12767, Jacobs, 4026, KZN, South Africa.

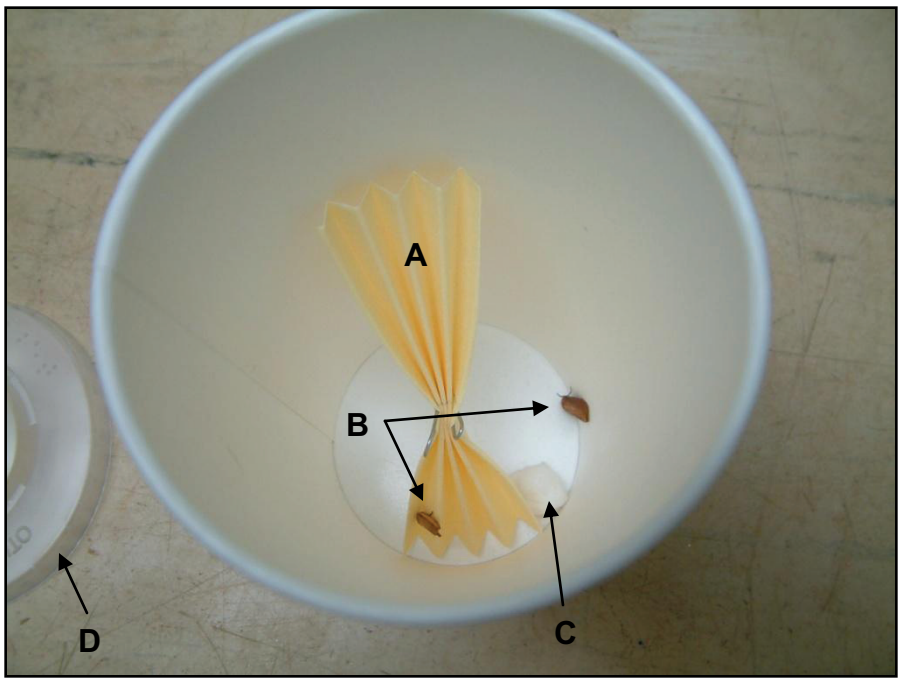

Figure 2.2. Prepared paper cups for mating *Eldana saccharina* showing pleated egg substrate (A), the moth adult pair (B), dental cotton wick (C) and lid (D).

Female mating frequency

Single harvested pupae were placed 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 pair was placed into 500 ml paper cups, prepared as described above. Oviposition substrates and the male were removed daily. A new oviposition substrate and freshly emerged male were placed into the paper cup with the remaining female and left to mate overnight. This procedure was repeated until the female died or for a further four days (whichever soonest) after which the female was killed by freezing and dissected to assess mating frequency by counting the spermatophores within her bursa copulatrix. A total of 30 females were prepared as outlined above and received a newly emerged male daily.

To ensure proper identification of spermatophore number, an additional five females were prepared in the same manner. However, these females were allowed to mate for one night only, after which 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 one mating opportunity, the spermatophore within the bursa copulatrix was clearly identified and spermatophores found in females with more than one mating opportunity could thus be clearly and unambiguously identified. The pupae and mating adults were held at 27 ± 2 °C; 75 ± 5 % RH; 8:16 L:D photophase.

Male mating frequency

Single harvested pupae were placed into individual cells of the multicell trays and treated as described above thus ensuring virgin adult pairings for the experiment. A freshly emerged male adult 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 according to the above procedure. Oviposition substrates and the female were removed daily. A new oviposition substrate and freshly emerged female were placed into the paper cup and left to mate overnight. The female that was removed, was killed by freezing and placed into a plastic resealable bag labelled with the male she was paired with and the date she was placed with the 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; 8:16 L:D photophase. From successful mating of a female and using the date that the female was placed with the male, data was obtained 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) as there was no treatment or control to conduct comparative tests. For the fecundity and fertility data, the data was pooled and a minimum, maximum and mean fecundity and fertility was obtained. From the fecundity data, the % of eggs laid per night after emergence was calculated. For the female mating status data, maximum, minimum and mean number of spermatophores per female was calculated. For the male mating status, maximum, minimum and mean number females

mated per male were calculated. From this data it was possible to determine when most matings occurred after male emergence.

2.4 Results

Fecundity and Fertility

Eldana saccharina females laid a mean of 518 ± 27.5 eggs (mean ± SE; n=20) per female over the five nights. Maximum fecundity was 798 and minimum fecundity was 353. On the first night of emergence and after mating, *E. saccharina* females laid very few eggs. The majority, 49.9 ± 3.9% (mean ± SE; n=20) of the total eggs were laid on the second night of emergence (Figure 2.3).

All females were mated. Mean fertility was 63.2 ± 4.2% (mean ± SE; n=20) per female. Maximum fertility was 100%, while the minimum fertility was 19.9%. There was some neonate mortality on hatching. A mean of 8.7 ± 0.9% neonate larvae died shortly after emergence per female ovipositing.

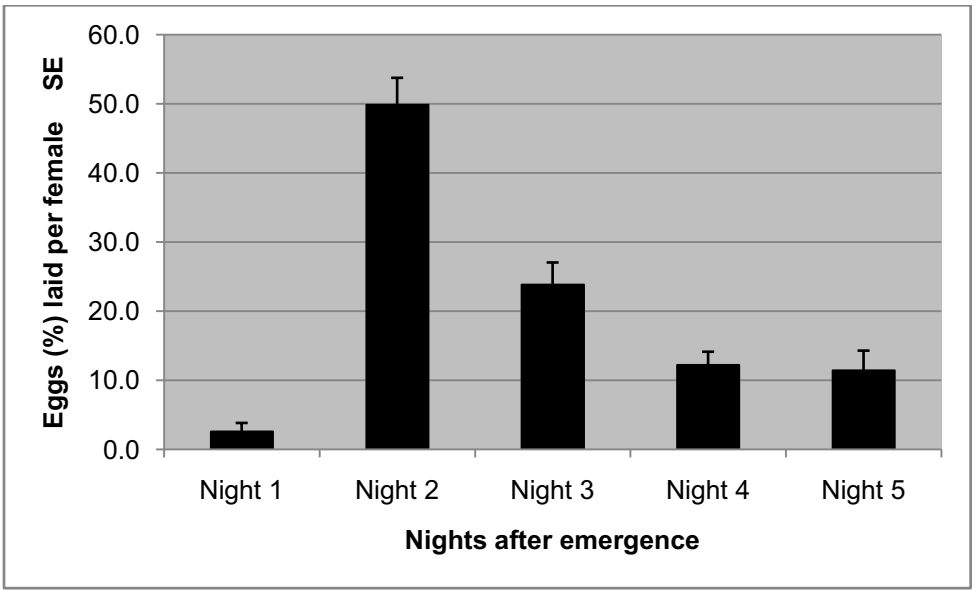

Figure 2.3. Mean proportion of eggs laid by *Eldana saccharina* adult females per night after emergence.

Female mating status

Eldana saccharina females that were given one mating opportunity had one spermatophore present in their bursa copulatrix (80% mating, n=5). Those females that were given more than one mating opportunity (i.e. had a freshly emerged virgin male placed with them every night on removal of the previous male) mated more than once. The average number of spermatophores present in their bursa copulatrix was 1.5 ± 0.1 (mean ± SE; n=30) with a minimum of 1 and a maximum of 3 spermatophores found in dissected females. However, even with the daily supply of virgin males, the majority of females still mated only once (56.7%), but 36.7% mated twice and 6.7% mated three times. Because the females were only assessed after five days, it could not be determined on which night successful matings occurred. Figure 2.4 illustrates the bursa copulatrix and number of spermatophores found within the bursa copulatrix after dissection.

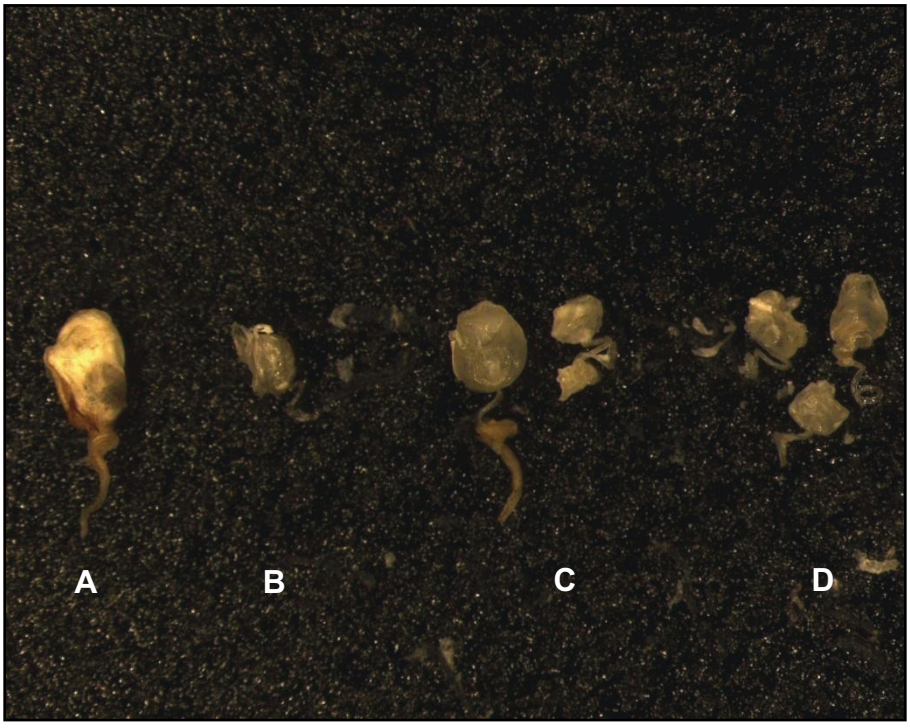

Figure 2.4. Left to right: Bursa copulatrix (A), one (B), two (C) and three (D) spermatophores dissected from the bursa copulatrix of mated *Eldana saccharina* females respectively (x80). The spermatophores in C and D contain some remnants of the bursa copulatrix.

Male mating status

Eldana saccharina males were shown to mate more than once, should they be presented with freshly emerged virgin females to replace the ones from the previous day during their life span, and mated up to a maximum of six different females. The majority of males mated two to three females each (23.3% and 23.3% respectively), 20% mated five females and 16.7% mated four females (Figure 2.5). The average number of virgin females that a male mated with was 3.3 ± 0.72 (mean ± SE; n=30). Males lived for an average of 4.3 ± 0.30 days (mean ± SE; n=30), up to a maximum of 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 43.33% on the fourth night after emergence (Figure 2.6).

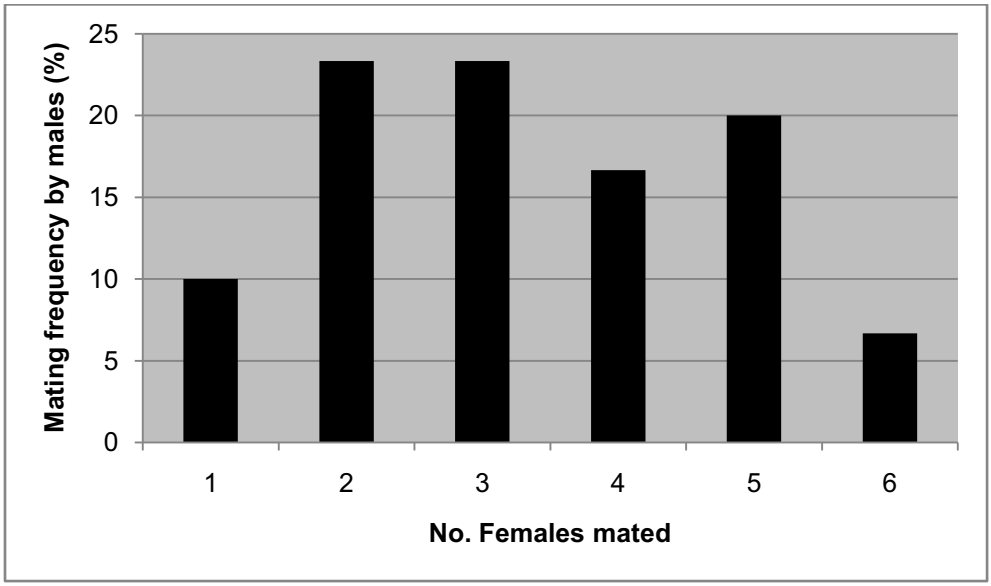

Figure 2.5. Percentage of *Eldana saccharina* males exhibiting mating frequencies of one to six females.

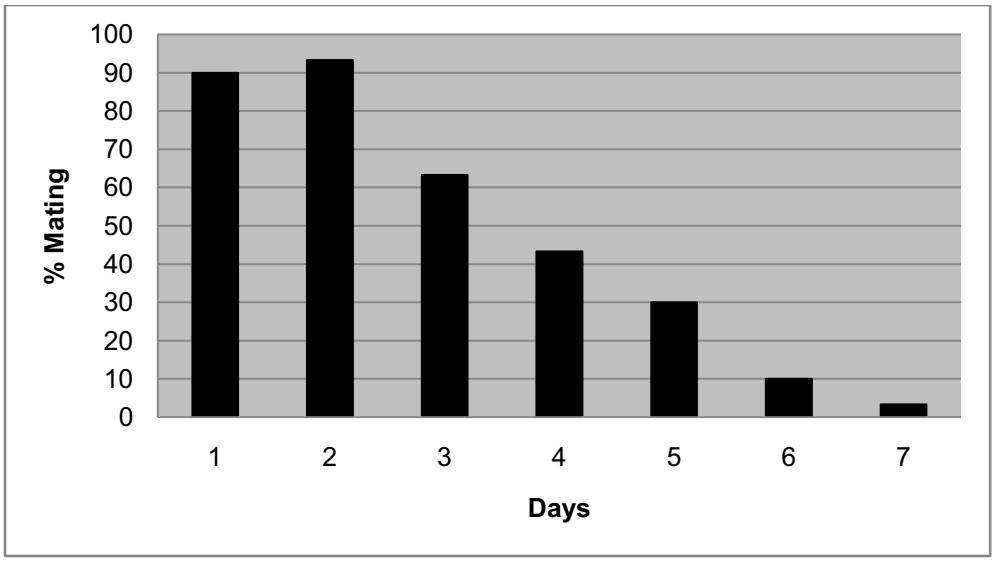

Figure 2.6. Frequency of mating by *Eldana saccharina* adult males per night after emergence.

2.5 Discussion

Fecundity and Fertility

Sampson and Kumar (1985) compared life cycle traits of *E. saccharina* in Ghana, West Africa with those reported from Kenya, East Africa by Girling (1978). Due to differences in general life cycle traits, predominantly regarding adult longevity and life cycle length, they suggested that two biotypes of *E. saccharina* exist. Since then, field behaviour and parasitoid complexes of *E. saccharina* have been found to be different between the West African, East African and southern African *E. saccharina* populations (Table 2.2) (Conlong, 2001). Assefa et al. (2006) confirmed that there is considerable genetic diversity between these populations due to geographical isolation and confirmed three biotypes of *E. saccharina* in Africa. From these results and the results of other authors (Dick, 1945; Waiyaki, 1974; Betbeder-Matibet, 1977; 1981; Way, 1994), it can be expected that *E. saccharina* fecundity may be variable, with temperature, nutritional status and biotype most likely to influence average fecundity. The mean female fecundity results obtained in this study (518 ± 27.5 eggs) compared with what was found in other studies on the southern African population. 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. Dicks' (1945) diet may have been more nutritious than the current diet as his *E. saccharina* which were reared on an artificial diet inoculated with the mould *Mucor hiemalis* had many more eggs (Mean number of eggs laid per female =750 with a maximum of 1004).

Table 2.2. General life cycle traits and behavioural patterns between West African, East African and southern African *Eldana saccharina* populations.

Characteristic	Southern Africa	East Africa	West Africa	Source
Adult longevity	-	14 days	Mated females: 8.65 ± 0.07 days Mated males 8.25 ± 0.08 days (mean ± SE)	Sampson and Kumar (1985)
Life cycle length	-	36 to 62 days	60 to 90 days	Sampson and Kumar (1985)
Indigenous host plants attacked	Cyperaceae, Graminae and Juncaceae	Cyperaceae and Graminae	Predominantly Graminae (more commonly crop hosts)	Conlong (2001)
Position of stalk attacked	Lower third of the stalk	Top and middle third	Top and middle third	Conlong (2001)
Parasitoid complex	10 parasitoid and pathogen species attacking *E. saccharina* in Cyperaceae and not in sugarcane	Parasitoid complex similar to that of southern Africa with additional species not collected in southern Africa	Parasitoid complex completely different to southern Africa and East Africa.	Conlong (2001)

Although fecundity of *E. saccharina* was found to be variable, depending on the nutritional status of the substrate the larvae fed on, the females ability to produce in excess of 500 eggs, the majority of which were fertile, confirms its potential as a crop pest, whose populations could grow quickly should they not be controlled. In addition, when compared with other lepidopteran pests that currently have Sterile Insect Technique (SIT) pest management programmes to control them, they have much lower fecundities compared to *E. saccharina* yet they are also considered pests. The cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), an invading pest of native *Opuntia* species in south western USA and Mexico, has a mean fecundity of 119.8 ± 68.9 (mean ± SD) (Carpenter et al., 2001). The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a major pest of apples and pears in Canada, USA and South Africa, has a mean fecundity of 200 eggs per female (Bloem et al., 1999). The false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), which is a major pest of citrus fruit in South Africa, has a mean fecundity of 400 eggs per female (Bloem et al., 2003).

Results reported here also confirm with authors Dick (1945), Betbeder-Matibet (1981) and Sampson and Kumar (1985) that the majority of eggs are laid on the second and third night after emergence. Biotype, nutritional status and temperature therefore do not impact on this behaviour. Females of the Naval Orange worm *Amyelois transitella* Walker (Lepidoptera: Pyralidae) laid most of their eggs in the first day after mating (one to two days after emergence) (Landolt and Curtis, 1991). *Cactoblastis cactorum* females oviposited soon after emergence and mating, and did not show any additional mating behaviour 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 females are able to mate more than once, 56.7% mated only once. Furthermore, *E. saccharina* has a relatively short lifespan of 5 to 7 days in the laboratory (Atkinson, 1981). For the purposes of SIT this is advantageous. If released irradiated *E. saccharina* males successfully mate with a wild

female and oviposition starts soon thereafter, it is unlikely that these wild females will seek out other mates and possibly mate with a wild male.

Fertility was found to be variable in this study, which ranged from 19.9% to 100% (mean 63.2 ± 4.2%). Other authors have reported variable fertility of *E. saccharina* eggs. Dick (1945) reported 98.3% fertility. 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 was reported to range from 79.8% to 95.9%. Way (1994) reported variable fertility at different temperatures. Fertility was 54.9% at 20 °C and 49.3% at 25 °C. At 15 and 35 °C, eggs failed to hatch. Variability in fertility reported found in the current study could be due to the mating frequency of the female. The possible reasons why lepidopteran females mate more than once were listed by Byers (1982 cited by Gomez et al., 2000). One of the reasons proposed was that sperm was inadequate (in addition to improving genetic diversity of offspring, spermatophores contributing towards a nutritional requirement by the female and increase phenotypic variation). The lowest fertility attained during the current study of 19.9% was in one female out of 20 mated females. 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 SIT.

Female mating frequency

The description of spermatophores found within the bursa copulatrix (Figure 2.4) is the same as what was reported by Atkinson (1981). In the above controlled experiment, although not common, up to three spermatophores were present in the bursa copulatrix of a mated female. Controlled studies on mating frequency of *E. saccharina* females specifically have not been documented. It has been assumed, based on Atkinson (1981) that because the spermatophore within the bursa copulatrix is large, females mate only once. The current study shows that females are able to mate more than once and mated up to three times in the laboratory, which is in contrast to that reported by Atkinson (1981). It is also common among Lepidoptera that females are able to mate more than once and reasons have been proposed for this as mentioned above.

Copitarsia conseuta Walker (Lepidoptera: Noctuidae) (a polyphagous insect occurring in Mexico) females and *Cactoblastis cactorum* male and females were reported to mate more than once (Gomez et al., 2000; Carpenter et al., 2009). *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), is a serious cotton pest in the United States of America (USA) and under control with SIT. Here females were reported to mate more than once (LaChance et al., 1975). *Galleria mellonella* L. (Lepidoptera: Pyralidae) females (a serious pest of bee hives) were also reported to mate more than once (Flint and Merkle, 1983) and these authors also found absence of the spermatophore did not indicate that the females were not mated, as sperm was still found in the bursa copulatrix. Mating behaviour in *G. mellonella* males are similar to *E. saccharina* in that males emit pheromones and call females for mating.

Male mating frequency

This study confirmed that *E. saccharina* males are 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 lifespan, 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 overflooding ratios for SIT. For SIT compatibility, it is not essential that females are monogamous (Barclay, 2005; Calkins and Parker, 2005). It is however, vital that mating is random with sterile and wild males and that mass reared and released sterile males are as competitive as wild males for mates (Barclay, 2005). The results of female mating frequency reported in the current study are based in the laboratory where adults were confined to small cages. Even under these conditions, the majority of *E. saccharina* females mated once (56.7%). It is likely that females in the field will thus mate once. The ability of *E. saccharina* males to mate more than once could possibly reduce the overflooding ratio of sterile males to wild males and thus decrease rearing costs, provided released males are as competitive with wild males. The effects of oviposition on the mating frequency of *E. saccharina*

females and radiation on mating competitiveness of sperm need to be further investigated.

2.6 Conclusion

This study confirmed that *E. saccharina* males are able to mate more than once and for the first time has shown that *E. saccharina* females are able to mate more than once. This study also confirmed that *E. saccharina* females lay higher numbers of eggs compared to many other pests and thus confirmed its crop pest potential. Calculation of overflooding ratios of release irradiated males to wild males can be made more accurate with the use of this information.

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CHAPTER 3

THE EFFECT OF OIL SOLUBLE DYES ON THE BIOLOGY OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

3.1 Abstract

Behavioural, population and ecological studies of animals and insects employ mark-release-recapture techniques. Dyes incorporated into edible oil have been used to mark many lepidopteran pests. Two oil soluble dyes, Sudan Red 7B and Calco Red N1700, were incorporated into the diet used to rear *Eldana saccharina*, the most serious pest of sugarcane in South Africa. The dyes, which are taken up in the fat bodies of the target insect, allow laboratory-reared adults to be distinguished from their wild counterparts caught in traps. However, the use of dyes can have detrimental effects on the developmental biology, fecundity and fertility of insects. It was found that Sudan Red reduced *E. saccharina* adult emergence by 38% and fecundity by 70%, and significantly prolonged development time in the diet until pupation. In addition, pupation was reduced by 46% in the Sudan Red treatment compared to the control, although sex ratio and fertility were not significantly affected. Calco Red, in contrast, had no significant effect on the developmental and reproductive biology of this insect, and is therefore more suitable for marking *E. saccharina*.

This breakthrough allows marked adults to be used in mating, field dispersion and population estimation studies. Such information is important for the formulation of sterile insect technology and mating disruption control options for *E. saccharina*.

3.2 Introduction

For behavioural, population and ecological studies of insects, as well as for monitoring, mark-release-recapture techniques are commonly employed (Southwood, 1966; Hagler and Jackson, 2001). Laboratory-reared insects are marked, released and recaptured in

traps that have been set up in the field, which then capture both marked and unmarked wild individuals (Qureshi et al., 2004).

Various methods of marking adult insects have been used. These include dust, paint, mutilation, internal and external dyes, genetic markers and radio-isotopes (Hagler and Jackson, 2001; Parker, 2005). Ideally, the marker should persist on the insect without affecting its normal biology, and be safe, cost effective and easy to use (Hagler and Jackson, 2001; Qureshi et al., 2004). Ease of marker detection is also an important criterion. However, a marker used with success on one insect species may not be suitable for another, and it is therefore necessary to test potential markers on the target insect's normal biology (Hagler and Jackson, 2001; Qureshi et al., 2004, Parker, 2005).

Dyes dissolved in edible oil and added to insect diets have been used to mark adults of many lepidopteran pests (Hagler and Jackson, 2001; Qureshi et al., 2004) and in particular for those that have been reared for Sterile Insect Technique (SIT) programmes (Parker, 2005). These dyes accumulate in the insect's fat bodies and can be seen through the integument of developing larvae and pupae, and through the intersegmental membranes in the adult stage. As the dyes do not mark scales, some Lepidoptera adults need to be dissected or crushed to reveal the dye in the internal organs (Hagler and Jackson, 2001; Qureshi et al., 2004). A key advantage in the use of dyes to mark insects internally is that expensive equipment is not needed to identify these insects and that they are easy to administer (Parker, 2005). Internal dyes do sometimes have a number of drawbacks, as they show varying marking efficiencies between different species. In addition, these internal dyes may be toxic and cause development problems or behavioural changes such as not calling for mates or not responding to calling mates. The marker may also not be present in subsequent generations, i.e. the F_1 generation will be impossible to identify. It is therefore important to establish if the chosen marker has detrimental effects on the insect's biology before radiation is applied (Hagler and Jackson, 2001; Qureshi et al., 2004, Parker, 2005; Zhao et al., 2008).

External dye marking has been used, where a fluorescent dye is dusted or sprayed onto insects, but there are no published accounts showing the impact of dusts on the behaviour or longevity of insects (Parker, 2005). However, Hagler and Jackson (2001) and Parker (2005) point out that it is important that minimum dust is applied as too much could cause mortality, decreased mobility or interference with sensory organs. The process of applying an external dye will also add an additional step to the rearing process (Parker, 2005).

New marking technologies include genetic mutation, where insects are marked by eye or body mutations, and/or biochemical genetic marking, where markers are incorporated into the insect (Hagler and Jackson, 2001). Genetic mutation could interfere with mating ability, which is vital for the success of a SIT programme. Biochemical genetic marking would need extensive preparation in the laboratory before fieldwork could be conducted (Hagler and Jackson, 2001).

The internally administered dye Calco Red N1700 (Pylam Products) has been used extensively for marking Lepidoptera for SIT programmes, e.g. the pink bollworm *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) and the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Bloem et al., 2001; Parker, 2005). Similarly, Qureshi et al. (2004) successfully marked the south-western corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), with Sudan Red 7B and Sudan Blue 670 (Sigma-Aldrich), and Zhao et al. (2008) successfully marked *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) with Sudan Red 7B but not Sudan Blue 670. Previous to these studies, Wilkinson et al. (1972) used Calco Red N1700 to successfully marked *Pieres rapae* L. (Lepidoptera: Pieridae), *Heliothis zea* Boddie (Lepidoptera: Noctuidae) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). Although Wilkinson et al. (1972) found that oviposition was delayed by one day, and longevity of adults were reduced, the authors did not think that this was significant enough to prevent the use of Calco Red for field studies. These examples illustrate the importance of testing the dye before use, so that deleterious effects are detected before being used for field studies.

In these studies on the impact of the above internally administered dyes on *Eldana saccharina* Walker (Lepidoptera: Pyralidae) development time and survival, pupal weight, adult emergence, sex ratio, fecundity and fertility are described. Data reported here have been published in a peer reviewed conference proceedings (Walton and Conlong, 2008).

3.3 Materials and Methods

Colony rearing conditions

Eldana saccharina is routinely reared at SASRI, based on the methods described by Graham and Conlong (1988). The modified *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) diet described by Graham and Conlong (1988) was modified further by Gillespie (1993). Since then, the components ferric citrate and formaldehyde have been removed (Chapter 2, Table 2.1). Plastic multicell trays (32 cavity) containing 8 ml of artificial diet each and developing larvae are held in rearing rooms (28 ± 2°C; 75 ± 5% RH; 0:24 L:D photophase) for approximately 619 day degrees (DD), which is the time for peak pupal production (Way, 1995). Pupae are harvested from the artificial diet and transferred to an adult emergence room (27 ± 2°C; 75 ± 5% RH; 8:16 L:D photophase) where adults emerge and are paired for mating. Eggs are laid on paper towelling. The paper towelling and eggs are collected and placed into incubators (24 ± 2°C; 75 ± 5% RH; 0:24 L:D) for 119 DD, until neonate larvae eclose from the eggs (Way, 1995).

Sudan Red 7B

Sudan Red 7B (3 g) was dissolved in 30 ml sunflower oil and added to 15 L of the *E. saccharina* artificial diet. This was the concentration used by Qureshi et al. (2004) for *D. grandiosella*. Neonate *E. saccharina* larvae were inoculated onto the diet in the multicell trays using a mechanical inoculator, and allowed to develop in the environmentally controlled larval growth rooms (28 ± 2°C; 75 ± 5% RH; 0:24 L:D photophase) for 604 DD. At this time, five trays were removed from the larval growth room. The diet and contents were emptied onto a large flat surface and pupae and

larvae in the trays were removed and counted to assess for number dead larvae, number of pupae and number of larvae. From these parameters % mortality and % pupation were calculated. The parameter % pupation served as an indication of development time. Thirty pupae from each repetition were sexed according to Atkinson (1980) and weighed in milligrams. Pupae were placed singly into individual cells of the multicell trays. The multicell tray was wrapped with cling wrap (Handywrap®[7]) which was aerated with a pin prick above each cell. This ensured that virgin adults were available to be paired for the experiment, as each sex emerged singly in separate cells. Adult emergence and sex ratio were calculated from the adults emerging from these 30 pupae. Adults were paired at emergence for fecundity and fertility measurements. Each pair was placed into a 500 ml paper drinking cup containing a pleated cardboard oviposition substrate (50x10 mm when pleated five times) and held together with a paper clip, and a 10 mm dental cottonwool wick soaked with water for the adults to drink from (Chapter 2; Figure 2.2). The contents were then secured with plastic cup lids. Oviposition substrates were changed daily until females died. Eggs were counted to measure fecundity, as were neonate larvae emerging from the eggs to measure fertility. One repetition comprised 10 pairs of adults emerging from pupae reared on Sudan Red diet. This process was replicated three times. A routine diet without dye, prepared and inoculated on the same day as the Sudan Red diet, served as a control, and was assessed as described above for the Sudan Red reared insects.

Calco Red N1700

Calco Red N1700 was obtained from the Deciduous Fruit Producers Trust (DFPT) SIT rearing facility in Stellenbosch. The dye (0.62 g) was dissolved in 18 ml sunflower oil and added to 15 L of the *E. saccharina* artificial diet. This concentration was used by DFPT to mark *C. pomonella* (D. Stenekamp, personal communication[8]). Neonate larvae were inoculated onto the diet as described for Sudan Red 7B, and allowed to develop for approximately 608 DD. Ten multicell trays were removed from the environmentally controlled larval growth room (28 ± 2°C; 75 ± 5% RH; 0:24 L:D

[7] Handywrap®, Chipkins Catering Supplies, P.O. Box 12767, Jacobs, 4026, KZN, South Africa.
[8]Daleen Stenekamp, Entomon Technologies, P.O. Box 12669, Die Boord, 7613, Stellenbosch, Western Cape, South Africa, May 2007. daleen@entomon.co.za

photophase), and pupae and larvae in the trays were collected, counted and assessed for % mortality and % pupation as described for Sudan Red 7B. Thirty pupae from each repetition were sexed and weighed in milligrams and adult emergence and sex ratio calculated as described for Sudan Red 7B. Pupae were placed singly into individual cells of the multicell trays as described for Sudan Red 7B to ensure virginity before mating. Fecundity and fertility assessments were conducted as described for Sudan Red 7B above. This process was replicated five times. A routine diet, prepared and inoculated on the same day as the Calco Red diet, served as a control, and was assessed as described for the Calco Red reared insects.

Statistical analysis

Sigmastat 3.11© (2004) was used for statistical analysis.

For the Sudan Red and Calco Red trials, results from the treatments and controls that conformed to the normal distribution were subjected to an unpaired *t*-test. All results conformed to the normal distribution, except the % mortality control data for Calco Red. The data were therefore square root transformed to attain normality for the % mortality in the Calco Red treatment and corresponding control. The % survival was calculated from % dead in order to present survival more clearly in Figure 3.1.

Because the Sudan Red and Calco Red experiments were conducted at different times, the day degrees were four day degrees apart. Because the data did not conform with the normal distribution a Mann Whitney U test was performed to check for statistical significance.

3.4 Results

Development time and survival

The day degrees at which the Sudan Red and Calco Red trials were assessed, were not significantly different (Mann Whitney U test: P=1). Figure 3.1 compares the % pupation, and mortality of the larvae in the control diet, to those in the Sudan Red and

Calco Red diets at 604 and 608 DD, respectively. There was no significant difference in % pupation of the larvae reared in the Calco Red and control diets (*t*=-0.41; df=8; P=0.69). Calco Red had no significant effect on the survival of larvae compared to the controls (*t*=0.78; df=8; P=0.46). The larvae reared in the Sudan Red diet, however, took significantly longer to develop, as only 42 ± 21.25% reached the pupal stage (as reflected in their % pupation), compared to those reared in the control diet (88.2 ± 0.69%) (*t*=-3.76; df=4; P=0.02) at the time of sampling at 604 DD. In contrast though, no mortality of the slower developing larvae were recorded (Figure 3.1).

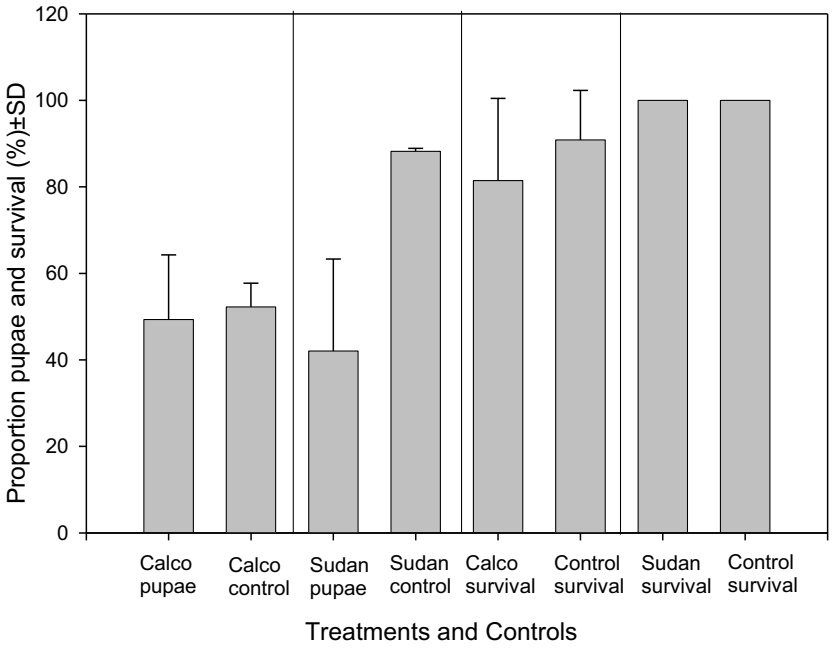

Figure 3.1. Development of *Eldana saccharina* larvae, as measured by % pupation, and % survival on control, Calco Red N1700 and Sudan Red 7B diets after 608 DD.

Male and female pupal weights

Calco Red had no significant effect on female or male pupal weights (Figure 3.2: *t*=0.38; df=8; P=0.71 and *t*=0.62; df=8; P=0.55, respectively) compared to those reared on control diets. Sudan Red also had no significant effect on the female or male pupal weights (Figure 3.2: *t*=-1.471; df=4; P=0.22 and *t*=-1.09; df=4; P=0.34 respectively), compared to those reared on control diets.

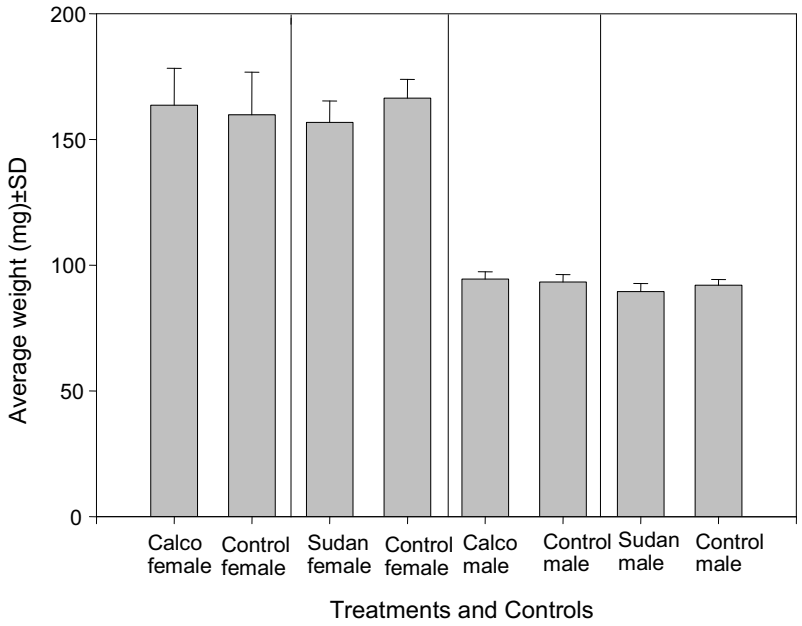

Figure 3.2. Pupal weights of *Eldana saccharina* reared on diets containing the oil soluble dyes, Calco Red N1700 and Sudan Red 7B, and on the control diets.

Adult emergence

Adult percentage emergence from pupae collected at 608 DD for Calco Red diet (87.2 ± 10.6%) and its control diet (89.2 ± 6.5%), was not significantly different (Figure 3.3: *t*=-0.36; df=8; P=0.73). However, adult emergence from pupae obtained from the Sudan Red diet (17.0 ± 3.6%) at 604.2 DD was significantly reduced compared to those emerging from pupae obtained from the control diet (55.7 ± 4.9%) (Figure 3.3: *t*=-10.96; df=4; P<0.001).

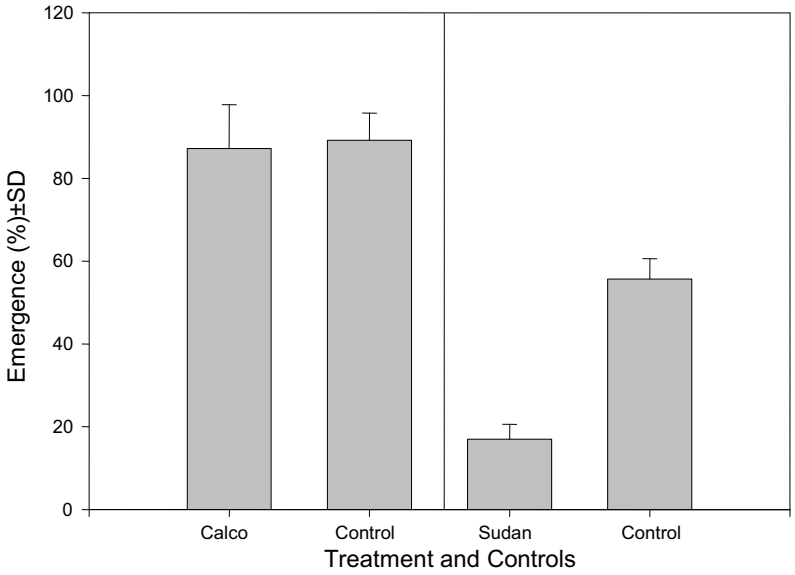

Figure 3.3. Adult emergence of *Eldana saccharina* reared on diets containing the oil soluble dyes, Calco Red N1700 and Sudan Red 7B, and on the control diets.

Sex ratio

Calco Red (Figure 3.4: *t*=-1.537; df=8; P=0.16) and Sudan Red (Figure 3.4: *t*=-1.26; df=4; P=0.28) both had no significant effect on the sex ratio of adults emerging from pupae reared on diets containing these dyes compared to those emerging from pupae reared on their respective control diets.

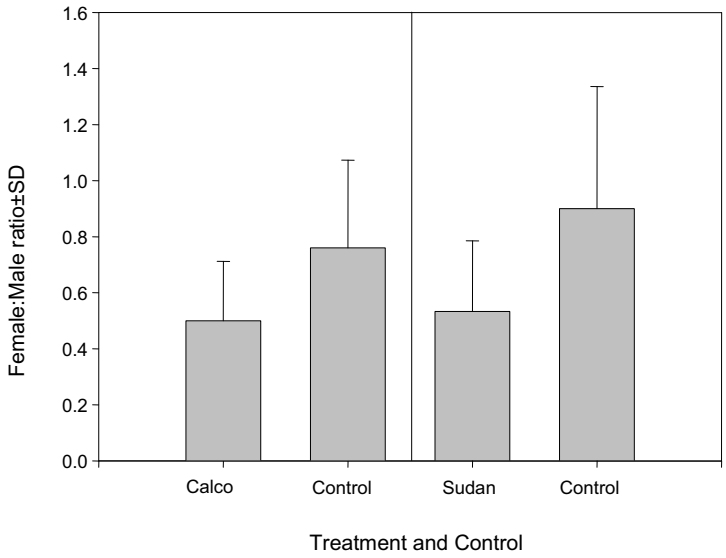

Figure 3.4. Sex ratio of *Eldana saccharina* adults reared on diets containing the oil soluble dyes, Calco Red N1700 and Sudan Red 7B, and on the control diets.

Fecundity

Calco Red did not have a significant effect on the fecundity of *E. saccharina* (Figure 3.5: t=-0.74; df=8; P=0.48), whereas Sudan Red significantly lowered its fecundity (Figure 3.5: t=-8.78; df=4; P<0.001), compared to those reared on their respective control diets. The mean number of eggs laid per *E. saccharina* female reared on Sudan Red was 337.67 $\pm$ 106.2 compared to a mean of 1141.5 $\pm$ 117.1 eggs per *E. saccharina* female on the control diet.

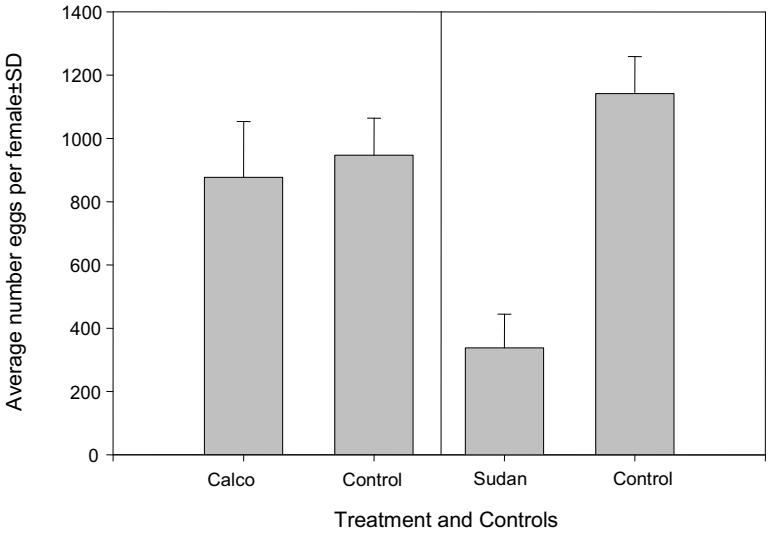

Figure 3.5. Fecundity of mated *Eldana saccharina* adult females reared on diets containing the oil soluble dyes, Calco Red N1700 and Sudan Red 7B, and on the control diets.

Fertility

Calco Red did not significantly affect fertility of eggs laid by mated *E. saccharina* adult females (Figure 3.6: *t*=-0.480; df=8; P=0.64). Sudan Red also did not have a significant effect of fertility of eggs laid by mated *E. saccharina* adult females (Figure 3.6: *t*=-1.845; df=4; P=0.14) compared to that of the *E. saccharina* reared on the respective control diets.

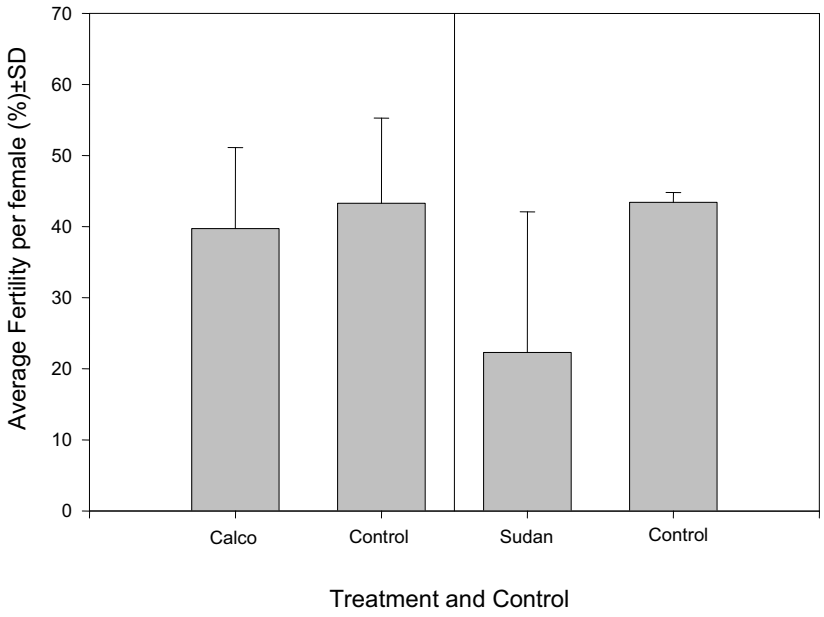

Figure 3.6. Fertility of eggs laid by mated *Eldana saccharina* adult females reared on diets containing the oil soluble dyes, Calco Red N1700 and Sudan Red 7B, and on the control diets.

3.5 Discussion

Both the Sudan Red 7B and Calco Red N1700 dyes could easily be detected in *E. saccharina* larvae and pupae (Figure 3.7A). Larvae and pupae which had been fed on diet containing one or the other of these dyes retained a red colour through to adulthood (Figure 3.7B). This result was similar to those of Gast and Landin (1966) and Daum et al. (1969), who tested Calco Red on the adult boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Daum et al. (1969) found it impossible to distinguish between the Sudan Red and Calco Red dyes, when these were used to mark the adult boll weevils. This effect was also found in this study, as both Sudan Red and Calco Red marked *E. saccharina* with a similar red colour. *Eldana saccharina* adults retained both dyes well, which could be seen either by dissection to observe fat bodies or by separation of the intersegmental membranes. The colour effects in females fed on these dyes were carried over to their eggs and to the resulting neonate

larvae (Figure 3.7C). This was similar to results obtained by Qureshi et al. (2004) for *D. grandiosella* marked with Sudan Red. However, subsequent feeding on a diet containing no dye reduced the amount of colour that could be observed in the developing larvae (unpublished results). This was also found by Qureshi et al. (2004) for *D. grandiosella* marked with Sudan Red.

Figure 3.7A. *Eldana saccharina* life stages marked with oil soluble dye compared to unmarked individuals of the same stage. Top row: fifth to sixth instar larvae and pupae not marked; Bottom row: fifth to sixth instar larvae and pupae marked with dye.

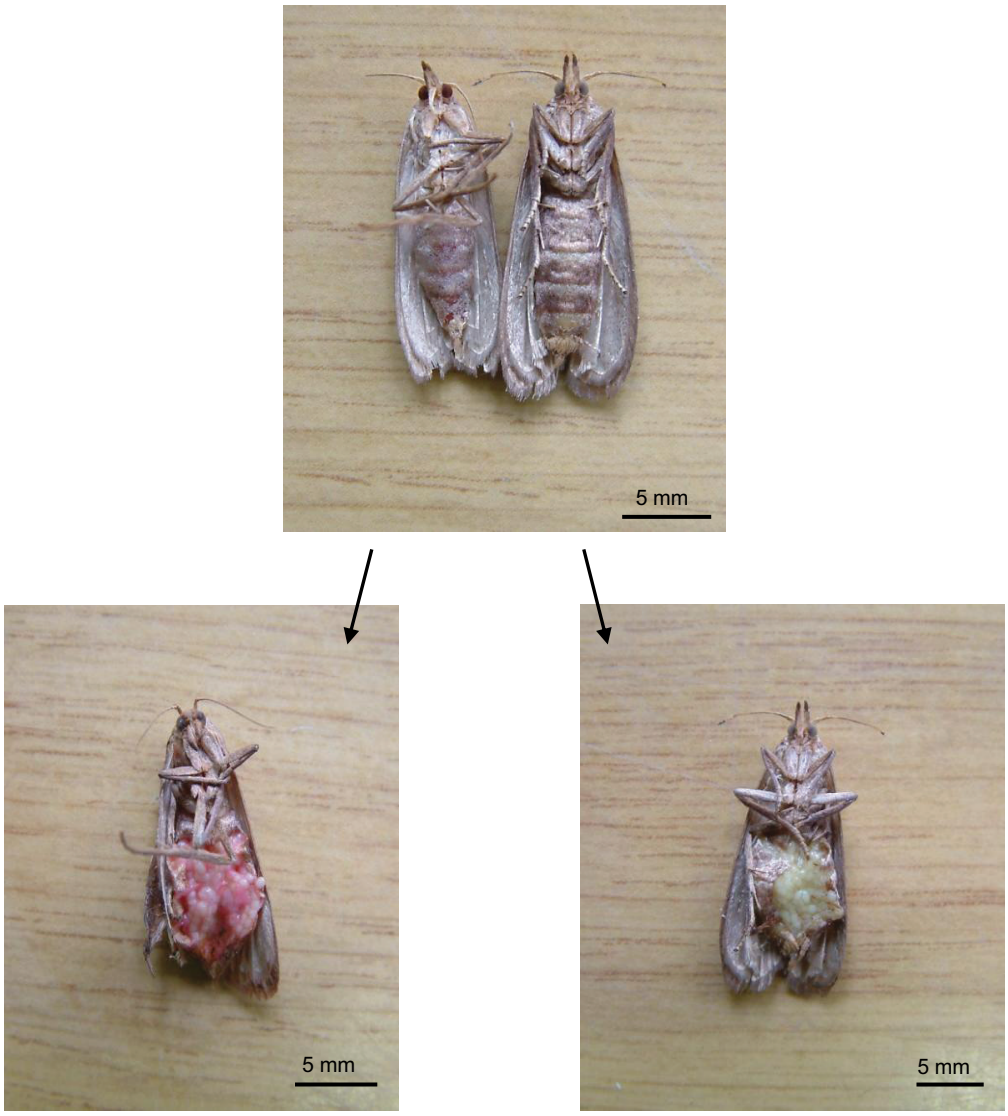

Figure 3.7B. ***Eldana saccharina*** **life stages marked with oil soluble dye compared to unmarked individuals of the same stage. Top: adult marked with dye (left) and adult not marked (right); Bottom left: dissected female marked with dye; Bottom right: dissected female not marked.**

Figure 3.7C. *Eldana saccharina* life stages marked with oil soluble dye compared to unmarked individuals of the same stage. Top: eggs and neonate larvae not marked; Bottom: eggs and neonate larvae marked with dye.

Sudan Red 7B prolonged development time from the neonate stage to the pupal stage, and, from the *E. saccharina* pupae produced, adult emergence and fecundity were significantly reduced. Figure 3.6 shows that mean fertility for *E. saccharina* reared on the Sudan Red diet was 22.30 ± 19.77, while that for the control was 43.41 ± 1.4. The mean was skewed in the Sudan Red treatment; however, the difference was not significant (P=0.14).

Qureshi et al. (2004) found that female larval development time was prolonged and male development time was shortened in *D. grandiosella* when reared on diet

containing Sudan Red diet. This study did not measure female and male development times separately. However, the adults emerging from the Sudan Red diet had a mean number of females per male of 0.53 ± 0.25 compared to 0.90 ± 0.43 in the control diet. It can therefore be concluded from the sex ratio of emerging adults, that pupae harvested were male biased in the Sudan Red treatment. This reflects the results of Qureshi et al. (2004), as the development times of female *E. saccharina* larvae in this study were also slower in the Sudan Red diet compared to that of the control. There was also a significant reduction in fecundity in *E. saccharina* females reared on Sudan Red compared to its control. Qureshi et al. (2004) found no significant effect on fecundity in *D. grandiosella* reared on Sudan Red diet compared to the control.

Calco Red N1700 had no effect on the developmental and reproductive biology of *E. saccharina.* This was similar to results obtained by Gast and Landin (1966) and Daum et al. (1969) for *A. grandis*. Calco Red is also used extensively in SIT programmes for *P. gossypiella* and *C. pomonella* (Parker, 2005). Because Calco Red had no detrimental effects on *E. saccharina*, it is a suitable marker. In addition, less Calco Red dye than Sudan Red is required per litre of diet, thereby reducing the quantity of dye *E. saccharina* is exposed to while feeding on the artificial diet.

3.6 Conclusion

The ability to mark *E. saccharina* with Calco Red N1700 dye without affecting its life cycle parameters and behaviour allows field population studies to be undertaken using mark-release-recapture techniques. In the proposed SIT programme against *E. saccharina*, irradiated moths released into the field will be marked to differentiate them from the naturally occurring moths, so it is important that the marking technique does not further reduce their fitness, as radiation does. The success of a SIT programme is measured by monitoring adults in the release areas. When only marked moths are recovered from the monitoring traps, this will indicate that the wild population has been successfully reduced to negligible levels.

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CHAPTER 4

PARENTAL AND F_1 STERILITY OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

4.1 Abstract

Lepidoptera are radiation resistant and high doses of radiation are required to completely sterilise males for Sterile Insect Technique (SIT). These high doses can compromise their fitness, making competition for mates with wild males difficult. However, partial sterility of males, using a lower radiation dose, provides a viable solution for effective SIT. It is common in Lepidoptera that F_1 (first filial) progeny of partially sterilised males are more sterile than their fathers, have a longer development time from neonates to adult emergence, and their offspring are predominantly male. This is known as F_1 sterility.

In order to determine the radiation biology of *Eldana saccharina*, and to assess its suitability for SIT as part of an area wide integrated pest management programme, laboratory reared males and females were exposed to increasing doses of radiation, crossed with non-irradiated counterparts and irradiated counterparts at the same radiation dose. The following crosses were made, radiation treated males (T♂) mated with untreated females (N♀) and radiation treated females (T♀) mated with untreated males (N♂) at radiation doses of 0, 150, 200, 250, 300 and 350 gray (Gy). The cross of radiation treated females (T♀) mated with radiation treated males (T♂) were exposed to the same radiation doses above except 350Gy. The surviving F_1 progeny from these three crosses were mated with untreated counterparts and F_1 adults of the opposite sex from each radiation dose to assess F_1 fertility. Initially survival of treated female (200Gy) offspring and fertility of F_1 progeny of treated male parents at higher radiation doses (300Gy and 350Gy) mated with untreated females were unexpectedly high and comparable to the controls. Radiation experiments were repeated where treated females (T♀) were exposed to 200Gy of radiation and mated with untreated males (N♂)

and treated males (T♂) were exposed to 200, 250, 300 and 350Gy of radiation and mated with untreated females (N♀). Fertility declined significantly at increasing doses of radiation in the parental crosses and F_1 progeny of treated males. Treated females mated with untreated males and those mated with treated males were more sensitive to radiation and were completely sterile at 200Gy and 150Gy respectively, while treated males mated with untreated females still had a residual fertility of 0.19% when exposed to 350Gy of radiation. F_1 male and female progeny of treated males mated with untreated counterparts were completely sterile at 250Gy, while their male parent's fertility was 0.82%. Due to poor survival of F_1 progeny from the parental crosses a male biased sex ratio and longer development time of F_1 larvae could not be observed.

Therefore *E. saccharina* is susceptible to ionising radiation and is a suitable candidate for the further development of a SIT programme against it, using radiation doses of 200Gy and 250Gy.

4.2 Introduction

Ionizing radiation is the primary method used to induce sterility in insects targeted for Sterile Insect Technique (SIT) (Bakri et al., 2005). After insects have been exposed to radiation, dominant lethal mutations caused by chromosomal breaks in embryonic development occur (Klassen, 2005; Robinson, 2005). The affected sperm fertilizes the egg normally, but the dominant lethal mutations kill most embryos during the first few cleavage divisions of embryonic development (Klassen, 2005; Robinson, 2005). Subsequently the eggs do not hatch.

For an effective SIT programme to be implemented against a target insect pest, an informed decision needs to be made on the correct radiation dose to induce sterility in the wild population (Bakri et al., 2005). Dose responses are species-specific (Robinson, 2005) and therefore need to be tested on each species considered a target before any SIT programme can be implemented against a pest insect. Dose response curves for sterility by exposure to different doses of radiation are measured by fecundity and

fertility, from pairs of adults mated in the following way, 1) irradiated females with non-irradiated males and 2) non-irradiated females with irradiated males. The shape of a dose response curve, which is calculated by measuring fertility of insects exposed to increasing doses of radiation, is characteristic of the types of initial chromosomal lesion that occurs (Robinson, 2005). There is a linear relationship between the dose response and the induction of dominant lethal effects. At higher doses, the curve reaches 100% sterility asymptotically, due the induction of multiple lethal events in the same cell. The shape of the curve also assists in selecting a dose for release of sterile insects (Robinson, 2005).

It is important that if females are released, there is no residual fertility, as this may increase the wild population. Residual fertility in males is less important as this will reduce the rate at which the population is suppressed (Robinson, 2005). Male and female insects respond differently to radiation because sperm are haploid post-meiotic cells and eggs are pre-meiotic. In the female, mature eggs are much more resistant to radiation compared to oocytes. Consequently, a radiation dose that induces full sterility in mature eggs can lead to cessation of oogenesis and an increase in lifespan in the female. This is generally not a problem, unless the female stage is a pest such as Tstese fly (*Glossina* spp. Weidemann (Diptera: Glossinidae)) (Robinson, 2005). Generally females are more sensitive to radiation than males. It is therefore possible to select the radiation dose that is acceptable for males, as females will be sterile. The use of this dose will ensure that all released females are fully sterile (Robinson, 2005). In order to maximise genetic damage to mature germ cells and minimise somatic damage, insects should be irradiated as late as possible in the development pathway, ideally as fully differentiated adults, where insects are most resistant to somatic damage. Certain somatic cells, especially in the gut, continue to divide and this can compromise fitness (Robinson, 2005).

The proportion of wild females rendered sterile by a given number of sterile males released depends on both the released male's sterility and their success in competing with other wild males to mate with wild females. Therefore, to optimize the balance

between fitness and genetic sterility, it is better to choose radiation doses that maximize the genetic load introduced into the wild populations. Therefore chosen optimal radiation doses to achieve this may give sterility levels below 100% (Robinson, 2005).

When exposed to radiation, insects can be divided into two major groups based on their sensitivity to radiation and resultant induction of dominant lethal chromosome mutations. Diptera, Hymenoptera and Coleoptera are radiation sensitive, while Lepidoptera, Homoptera and Hemiptera are radiation resistant, and higher doses are required to induce full sterility (Carpenter et al., 2005; Robinson, 2005). The former group have a localized centromere on the chromosome that is classified as monokinetic. The latter group have a diffuse centromere on the chromosome and are classified as holokinetic. This plays a major role in radiation sensitivity between the orders described above (Carpenter et al., 2005). However there is some disagreement and some authors suggest that the lepidopteran chromosome is intermediate between holokinetic and monokinetic (Carpenter et al., 2005).

Resistance to radiation in Lepidoptera is largely due to their chromosomes being holokinetic in “nature” even if they are not truly holokinetic (Carpenter et al., 2005). Most lepidopteran species have on average 30 haploid chromosomes (n = 28 – 32). Their chromosomes are usually spherical and uniform and therefore not much is known about their morphology, kinetic organisation or behaviour during mitotic and meiotic cell division (Carpenter et al., 2005). Lepidopteran chromosomes lack distinct centromeres (primary constrictions). As a result the chromotids separate by parallel disjunction during mitotic metaphase (Carpenter et al., 2005). Lepidopteran chromosomes also possess a localised kinetochore plate where the spindle microtubules attach during cell division. These plates are large and cover a significant portion of the chromosome length. This ensures that most breaks in lepidopteran chromosomes following radiation will not lead to the loss of chromosome fragments. These losses of chromosome fragments are typical in species with monokinetic chromosomes after exposure to radiation (Carpenter et al., 2005; Robinson, 2005). In addition, lepidopteran chromosomes, can tolerate telomere loss without the drastic effects that this has on

chromosomes of other orders (Robinson, 2005). Figure 4.1 illustrates the differences between holokinetic and monokinetic chromosomes during mitotic metaphase.

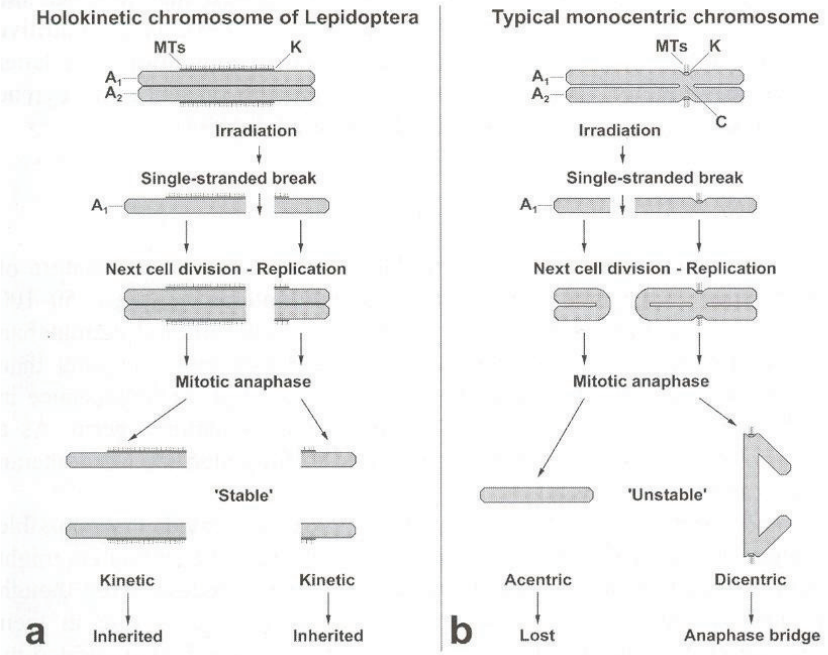

Figure 4.1. Lepidopteran chromosome structure during mitotic metaphase and the consequences of chromosome breakage. a: Holokinetic chromosome with 2 chromotids (A1 and A2) and Kinetochore plate (K). Spindle microtubules (MT) are attached to the kinetochore. b: monocentric (monokinetic) chromosome. Chromotids are joined with a centromere (C). The kinetochore is located on the centromere (from Carpenter et al., 2005).

Multiple chromosome rearrangements must be induced in lepidopteran males to appear as dominant lethal mutations, which explains why lepidopteran males require very high radiation doses (350 – 500Gy) to be completely sterilized (Carpenter et al., 2005) compared to Diptera which are generally completely sterilised at doses below 100Gy. For example, the screw worm fly (*Lucilia* (*Cochliomya*) *hominivorax* Coquerel (Diptera: Calliphoridae)) is 100% sterile at 75Gy (Klassen, 2005). Lepidopteran females are much more sensitive to radiation. This is because meiosis in females stops at the development of the oocytes and only resumes after fertilization, whereas spermatogenesis in the males is complete when adults emerge from the pupal stage.

Various secondary effects are expected to occur in the oocytes, which have a large amount of cytoplasm necessary for embryonic development, compared to sperm which does not contain much cytoplasm (Carpenter et al., 2005).

Because Lepidoptera are radiation resistant, higher doses of radiation may result in less competitive individuals and can induce unfavourable physiological and behavioural changes. These include failure of irradiated males to disperse widely enough, seek out appropriate niches, compete with wild males for mates, respond to calling mates and to mate, to form the spermatophore and to transfer sufficient sperm to the female (North, 1975; Omar and Mansor, 1993; Carpenter et al., 2005).

Lepidoptera have two types of sperm, eupyrene (nucleate) sperm, which are needed to fertilise the egg, and apyrene (anucleate) sperm which are necessary for the transport of eupyrene sperm from the spermatophore down the seminal duct into the spermatheca of the female. Eupyrene sperm are more easily damaged by radiation (Klassen, 2005; Carpenter et al., 2009). It has been found in F_1 male progeny that many eupyrene sperm exhibit structural abnormalities (Klassen, 2005; Carpenter, et al., 2009). Klassen (2005) reported that it has been found that only normal appearing sperm are found in the spermatheca of females that mated with a F_1 male. This implies that less sperm is transferred by irradiated males compared to non-irradiated males. Koudelová and Cook (2001) found that treated males of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) transferred less sperm to females than non irradiated males. It is therefore vital that irradiated males are able to compete with wild males in terms of good sperm production in order to induce sterility in the wild population and therefore fitness should not be compromised as a result of radiation.

Inherited sterility in Lepidoptera was first reported in the 1930's in the Soviet Union in the silkworm (*Bombyx mori* L. (Lepidoptera: Bombycidae)) and the greater wax moth (*Galleria mellonella* L. (Lepidoptera: Pyralidae)). In North America during the 1960s Proverbs (cited by North, 1975; Carpenter et al., 2005) documented inherited sterility in the codling moth (*Cydia pomonella* L. (Lepidoptera: Tortricidae) and found that when

males were exposed to sub sterilising radiation doses and then mated with non radiated females, females produced reduced numbers of F_1 progeny, with a skewed sex ratio towards males that had very low fertility (North, 1975; Carpenter et al., 2005). In Lepidoptera some dominant lethal mutations express just prior to eclosion of neonate larvae, and most of the broken chromosomes pass onto the F_1 generation. Dominant lethal mutations are then predominantly expressed in the F_1 generation (Carpenter et al., 2005; Klassen, 2005).

Factors common to Lepidoptera with inherited sterility are that F_1 male and female offspring are more sterile than the parental irradiated generation, and more F_1 male progeny than female progeny are produced. Other characteristics may include longer larval development time and eclosion to adults as well as reduced sperm quality in the F_1 generation (North, 1975; Carpenter et al., 2005). In terms of control of lepidopteran pests such as *Eldana saccharina* Walker (Lepidoptera: Pyralidae), the skewed sex ratio towards males in the F_1 generation as well as increased development time is an advantage. More males will be available to mate with wild females, which will lay an increase proportion of sterile eggs. The eggs that do hatch will be of reduced quality and will be exposed to more environmental pressures with less chance of survival.

True genetic sterility in irradiated released males require that they are able to function as a normal insect which can produce fully functional sperm that can fertilise eggs and initiate zygote development. Radiation induced F_1 sterility actually occurs in the generation that follows the release of the males, by the production of F_1 males that produce gametes that result in non-development of zygotes. A male insect that cannot mate, that does not produce sperm or that transfers non-functional sperm is sterile but is not effective for a SIT programme (Robinson, 2005). It is essential that irradiated males are functional so that females do not call for another mate (Robinson, 2005). In *E. saccharina* males form leks and call the females (Chapter 2). Therefore, *E. saccharina* males still need to be competitive with wild males in order to successfully mate with a wild female. In species that do re-mate, radiation must not affect the sperm's function to compete with other male's sperm (Robinson, 2005). This will be

important for *E. saccharina* as males and females have been shown, under laboratory conditions, to mate more than once (Chapter 2). Sperm competition is not being tested in this study.

A lower dose of radiation used to induce F_1 sterility is preferred because it ensures the quality and the competitiveness of the released insects is not compromised (Bloem et al., 2001) as measured by improved dispersal after release (Carpenter et al., 2005). The progeny of irradiation of parents at sub sterilising doses have also been shown to be more sterile than that of the parents (North, 1975; Carpenter et al., 2005). Proverbs (1970, cited by Carpenter et al. 2005) found that the release of partially sterile insects offered greater suppressive control than the release of fully sterile insects.

Chapter 1, section 1.3 describes the similarities in ecology of False Codling Moth (FCM) (*Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)) and *E. saccharina.* The effect of gamma radiation on the biology and inherited sterility on FCM was studied by Bloem et al. (2003). The authors found that females were 100% sterile when exposed to a radiation dosage of 200Gy. Newly emerged males radiated at 200Gy had a residual fertility of approximately 25%. Newly emerged males irradiated at 350Gy still had a residual fertility of 5.2% when mated with untreated females. This fertility at such a high radiation dose confirms that Lepidoptera are much more resistant to radiation compared to other insect orders. With increasing doses of radiation applied to male parents, a decrease in fecundity, fertility and increased F_1 mortality with a sex ratio in favour of males was found. These factors are common to F_1 Lepidoptera as previously explained. The dosage where almost all offspring were male, and F_1 males 100% sterile was found to be 150Gy. This confirmed that F_1 males are more sterile than parent males, a factor common to F_1 sterility (North, 1975; Carpenter et al., 2005).

This chapter aims to assess the fertility of the parent generation and the F_1 generation of *E. saccharina* after exposure of the parents to increasing doses of radiation. This will indicate whether *E. saccharina* exhibits F_1 male sterility making it a suitable candidate

for development of a SIT programme as an Area-Wide Integrated Pest Management approach.

4.3 Parental Sterility

4.3.1 Materials and Methods

Colony rearing conditions

Eldana saccharina is routinely reared at SASRI, based on the methods described by Graham and Conlong (1988). The modified *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) diet described by Graham and Conlong (1988) was modified further by Gillespie (1993). Since then, ferric citrate and formaldehyde have been removed (Chapter 2, Table 2.1). Plastic multicell trays (32 cavity) containing 8ml artificial diet in each cell and developing *E. saccharina* larvae are routinely held in rearing rooms (28 $\pm$ 2°C; 75 $\pm$ 5% RH; 0:24 L:D photophase) for approximately 619 day degrees (DD), which is the time for peak pupal production (Way, 1995).

This experiment was conducted in August 2007. For the assessment of parental sterility, pupae were harvested from the artificial diet after approximately 619 day degrees of development and placed singly into individual empty cells of the multicell trays. The trays were wrapped with cling wrap (Handywrap®[9]) and the wrap was aerated with a pin-prick above each cell. This ensured that virgin adults were available to be paired, as each sex emerged singly in the separate cells. The packed *E. saccharina* pupae were transported by air and road to the Deciduous Fruit Producer's trust (DFPT) mass rearing laboratories, Stellenbosch, Western Cape, South Africa (33° 55' 26" S, 18° 52' 25" E) where the irradiator is located in the Agricultural Research Council (ARC) Infruitec grounds. Emerged adults of the same sex < 24 hours old were placed in groups of 10 and exposed to increasing doses of Gamma radiation delivered at a rate of 375.36 rads/min from a ^{60}Co source. For the purpose of radiation, each moth was placed into a single cell in a multicell tray. The multicell tray was wrapped

[9] Handywrap®, Chipkins Catering Supplies, P.O. Box 12767, Jacobs, 4026, KZN, South Africa.

with cling wrap and aerated with a pin-prick above each cell. Radiation doses that the groups of moths were exposed to were 100; 150; 200; 250; 300 and 350 gray (Gy). Five males (♂) from each irradiated group and five females (♀) from each irradiated group (labelled “T” for “Treatment”) were mated with adults of the opposite sex that did not receive any radiation (labelled “N” for “Normal”). The remaining five males from each irradiated group were mated with the remaining females that were irradiated at the same radiation dose. Crosses were therefore made as follows at each radiation dose, T♂xN♀; T♀xN♂; T♀xT♂. This gave five repetitions for each cross at each radiation dose. There was one exception. At 350Gy, the T♀xT♂ cross was not made based on unpublished data acquired the previous year that no progeny emerged from this cross. Five pairs of untreated adults were crossed as a control, N♀xN♂.

For mating, pairs were then placed individually into a 500 ml paper cup, with a pleated cardboard oviposition substrate (50x10 mm when pleated five times), secured with a paperclip to maintain the pleats, and a 10 mm cotton dental wick soaked with water for the adults to drink from (Chapter 2; Figure 2.2). Plastic lids were then placed on the paper cups. Moths were held at the DFPT laboratories (26 ± 2°C; 65 ± 5% RH; 16:8 L:D photophase) for mating and oviposition. Oviposition substrates were changed daily until the female died or until the pair was five days old (as by then the majority of eggs were laid) (Dick, 1945; Betbeder-Matibet, 1981; Sampson and Kumar, 1985). The removed oviposition substrates were placed into re-sealable transparent plastic bags and labelled with the date, sex irradiated (either male or female) and radiation dose received. After oviposition was complete, females were killed by freezing and dissected to detect a spermatophore in the bursa copulatrix to assess mating status (Figure 2.4, Chapter 2). To assess fecundity, the eggs laid on the substrates were counted and the substrates were placed back into their corresponding plastic bag for neonate emergence. The eggs on the substrates were packaged in their labelled plastic bags, into a cardboard box and transported back to SASRI at Mount Edgecombe, KwaZulu-Natal (29° 42’ 24” S, 31° 02’ 45” E) by air and road. Eggs were placed into an incubator at SASRI (26 ± 2 °C; 60 ± 5% RH; 0:24 L:D photophase) to allow for hatching. To assess fertility, the neonate larvae emerging from the eggs were counted. For the

assessment of development time, survival and sex ratio, emerging neonate larvae were inoculated onto the same artificial diet described above, dispensed in 25 ml plastic vials with aerated screw-cap lids, to prevent larvae from different treatments escaping. Due to space constraints and to avoid mixing the experimental larvae with the routine colony at SASRI, the plastic vials containing the neonate larvae were placed in a separate temperature controlled rearing room (26 ± 2 °C; 70 ± 5% RH; 8:16 L:D photophase). Developing larvae were checked daily for pupation and adult emergence.

Statistical analysis

Genstat 12.1© (2009) was used for REML (Residual Maximum Likelihood) variance component analysis. The statistic is reported with a χ^2 value. Sigmaplot 9.0© (2004) was used for regression analysis and calculation of mean development time, % survival and sex ratio.

Assessment of Parental fecundity and fertility

Because not all *E. saccharina* pairs were mated (Table 4.1), the data were unbalanced and an Anova test could not be used. A REML variance component analysis was performed on the fecundity and fertility data from these pairs with dose and type of cross as variables. The fecundity data were normal, while the fertility data were square root transformed to attain normality. For fertility, where a dose by type of cross interaction was found to be significant, data from each type of cross were subject to a polynomial regression.

Table 4.1. Number of successfully mated *Eldana saccharina* pairs with one of either sex irradiated with increasing doses of gamma radiation (T= Treatment, irradiated; N= Normal).

Dose	N♂xN♀	T♂xN♀	T♀xN♂	T♀xT♂
0	5			
100		5	4	5
150		5	5	5
200		5	4	5
250		5	4	4
300		5	5	3
350		5	5	

Development time of F_1 neonates to adulthood

Individual F_1 development time from each parental cross pair was not followed to adult emergence, instead the data were pooled at each radiation dose for each type of parental cross. The average development time at each dose for each type of cross was not normal and could not be transformed to attain normality for the purposes of a regression analysis. Because of this, no statistical tests could be used on the data.

Survival of F_1 neonates to adulthood

As for development time individual repetitions were not followed through for this assessment. Total survival to adulthood was calculated from neonates placed onto artificial diet for each radiation dose.

Sex ratio of F_1 adults

As for survival of F_1 neonates, total % males in the surviving offspring was calculated collectively for each radiation dose from the total adults that emerged from the neonates placed onto artificial diet for each radiation dose.

4.3.2 Results

Parental fecundity

There was a significant interaction between the dose and type of cross on mean fecundity per female at increasing doses of radiation (Figure 4.3.1: χ^2=18.66, df=9, P=0.05). Females were more sensitive to radiation compared to males as treated females mated with normal males laid fewer eggs at increasing doses of radiation compared to the normal females mated with males exposed to increasing radiation doses. At 200Gy, mean fecundity per treated females crossed with normal males was 280 eggs, and those crossed with treated males was 292 eggs, half that of the control (448 eggs) and treated males crossed with normal females (486 eggs). At 250Gy, fecundity was similar to 200Gy. Surprisingly, at 350Gy treated females crossed with treated males and at the lower 300Gy treated females crossed with normal and treated males (both crosses) fecundity increased to levels similar to that of the control (Figure 4.3.1).

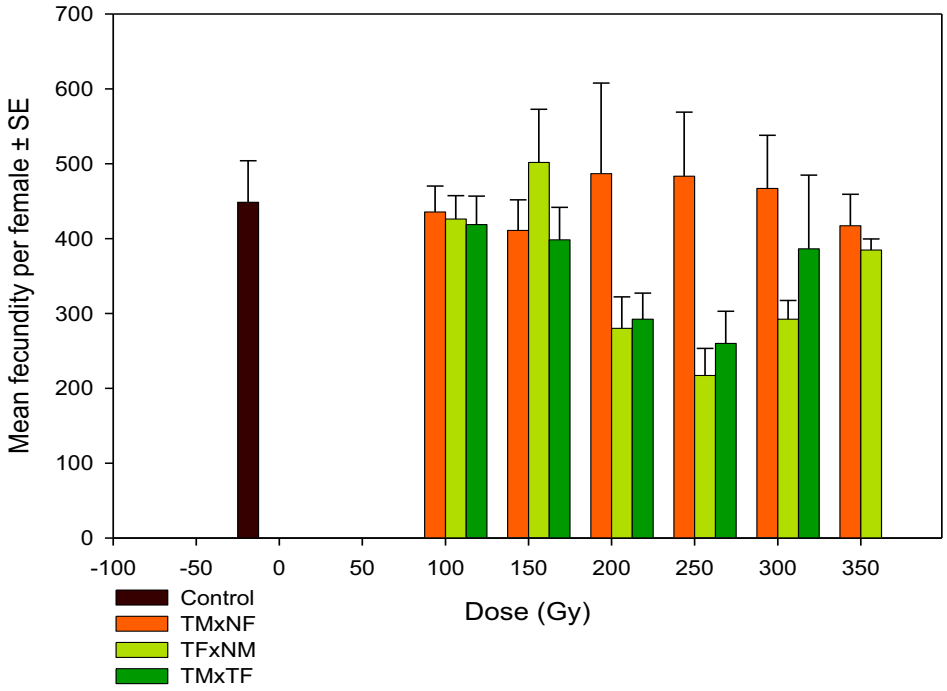

Figure 4.3.1. Mean fecundity per *Eldana saccharina* female from three parental crosses at increasing radiation doses (T= Treatment, irradiated; N= Normal; M= Male; F= Female).

Parental fertility

There was a significant interaction of dose and type of cross on fertility (χ^2=25.92, df=9, P=0.006). In the Treated male x Normal female cross, fertility declined significantly (y=7.41-0.019x; F=60.97; df=34, P<0.001, R^2=0.65) at increasing radiation doses, from 31.32% at 100Gy to 3.11% at 350Gy compared to the control where fertility was 65.35% (Figure 4.3.2). In the Treated female x Normal male cross, fertility declined significantly at increasing doses of radiation, as treated females laid even fewer fertile eggs than normal females mated with treated males (from 16.43% at 100Gy to 0.16% at 200Gy and 0.04% at 300Gy compared to the control (65.35%) (Figure 4.3.2) (y=8.055-0.0611x+0.00112x^2, F=195.27, df=31, P<0.001, R^2=0.93). In the Treated female x Treated male cross, fertility was significantly reduced at increasing doses of radiation and was lower than the Treated female x Normal male cross (from 0.23% at 100Gy and 0% at 200Gy compared to the control (65.35%) (Figure 4.3.2) (y=7.698-

$0.0826x+0.000202x^2$, F=192.336, df=26, P<0.001, R^2=0.94). It is clear from these results that % fertility in all crosses declined significantly at increasing doses of radiation and that females were more sensitive to radiation than males.

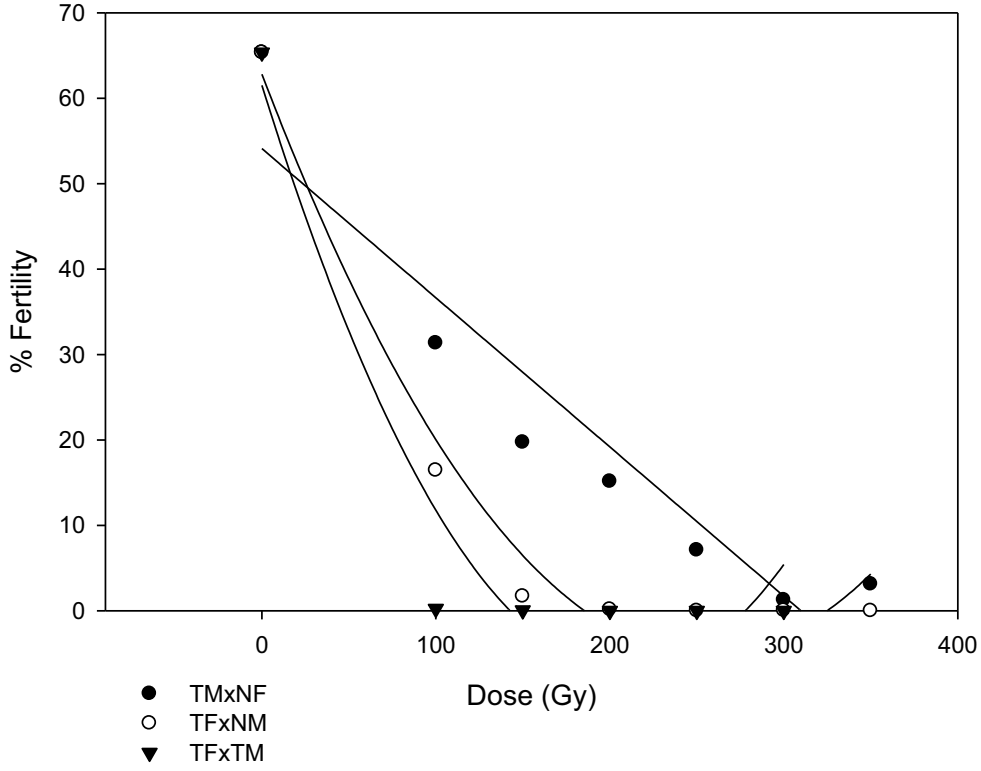

Figure 4.3.2. Mean fertility of *Eldana saccharina* females from three parental crosses at increasing radiation doses (T= Treatment, irradiated; N= Normal; M= Male; F= Female).

Development time of F_1 neonates to adulthood

In the Treated male x Normal female cross, the resulting F_1 neonates showed an increased mean development time at 250Gy and 350Gy of 69.18 and 68.50 days respectively compared to the control (59.25 days) (Figure 4.3.3). However at 350Gy, mean development time was 60.50 days which was similar to the control (59.25 days) (Figure 4.3.3). The development time, in days, of F_1 neonates from the Treated female x Normal male cross also increased at the higher radiation doses up to an mean of 65.50 days and 68.0 days at 150Gy and 200Gy respectively compared to the control where mean development time was 59.25 days (Figure 4.3.4). There was just one F_1

neonate that completed development from the Treated female x Treated male cross at 100Gy. Development time for this individual was 63 days compared to the control of 59.25 days. Data for this Treated female x Treated male cross is thus not presented in graph format as there was only one data point.

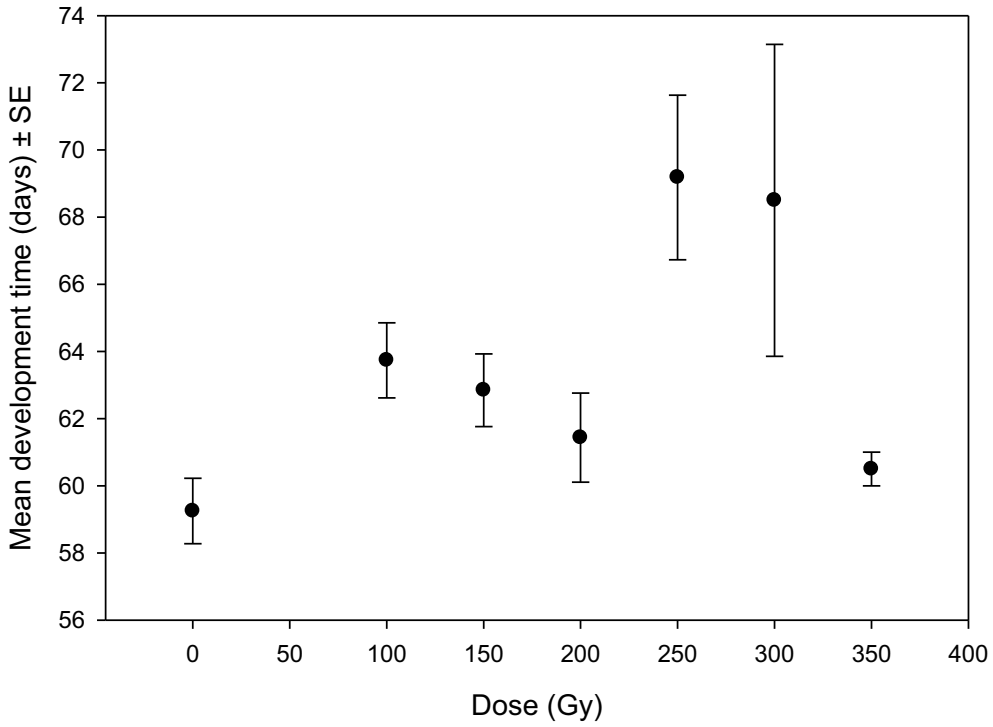

Figure 4.3.3. Mean development time of *Eldana saccharina* F_1 neonates from males treated at increasing radiation doses mated with normal females.

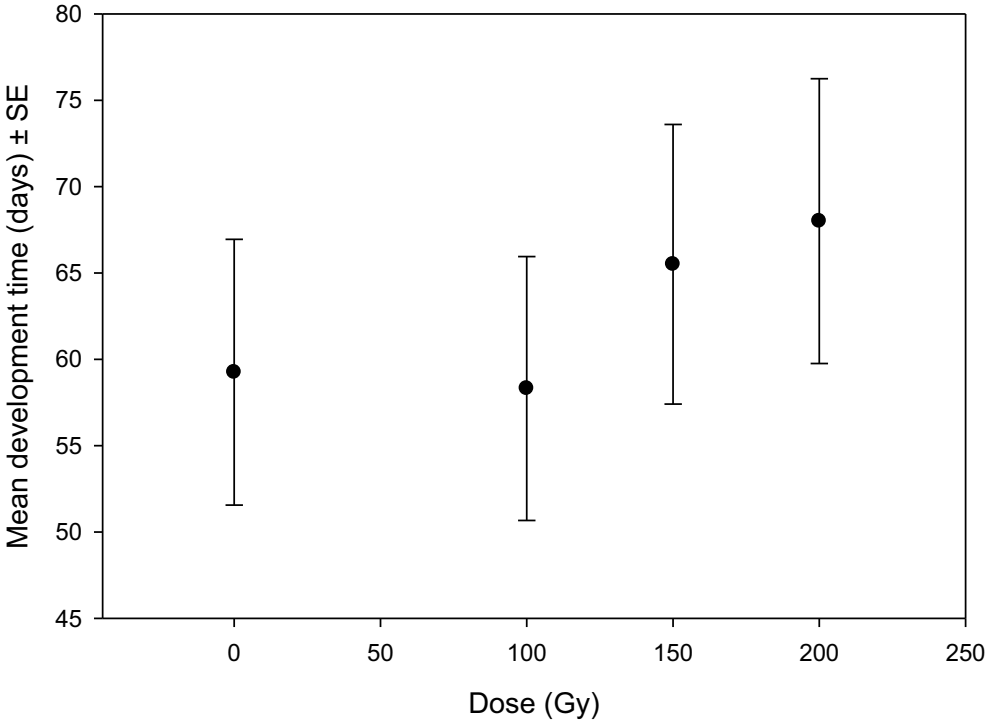

Figure 4.3.4. Mean development time of *Eldana saccharina* F_1 neonates from females treated at increasing radiation doses mated with normal males.

Survival of F_1 neonates to adulthood

The total survival of F_1 neonates in the Treated male x Normal female cross declined significantly at increasing doses of radiation from 31.14% in the control to 11.76% at 350Gy (Figure 4.3.5) ($y=29.445-0.579x$; $F=14.64$; $df=1$, $P=0.012$, $R^2=0.70$). In the Treated female x Normal male cross, at 200Gy survival increased to 66.6%, which was unexpected, but then declined to 0% at 250Gy. Of the three neonate larvae that hatched from eggs laid by females irradiated at 200Gy, two adults emerged. In the Treated female x Treated male cross survival in the control and at 100Gy remained similar, 31.14% and 33% respectively but at 150Gy declined to 0% (Figure 4.3.5). At a radiation dose of 100Gy in this cross, of the three F_1 larvae inoculated onto artificial diet, one individual emerged which indicated a survival of 33%, which was similar to the control (31.14%).

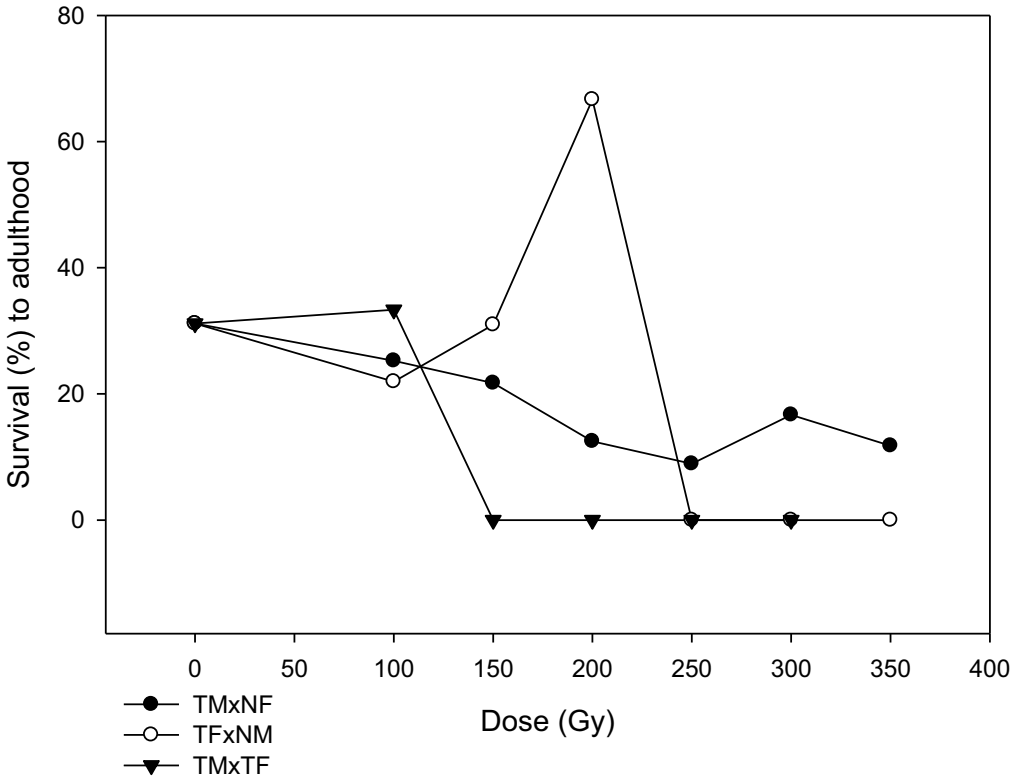

Figure 4.3.5. Percentage survival of *Eldana saccharina* F_1 larvae from neonate to adulthood of the three crosses (T= Treatment, irradiated; N= Normal; M= Male; F= Female).

Sex ratio of F_1 adults

The proportion of males emerging from F_1 neonate inoculations in the Treated male x Normal female cross increased from 52.78% in the control to 60.71% at 150Gy, but then dropped to 35.71% at 250Gy, and was 50.0% at both 300Gy and 350Gy (Figure 4.3.6). In the Treated female x Normal male cross, the proportion of males in the surviving adults declined to 0% at 200Gy. In the Treated female x Treated male cross, at 100Gy, offspring was 100% male (Figure 4.3.6).

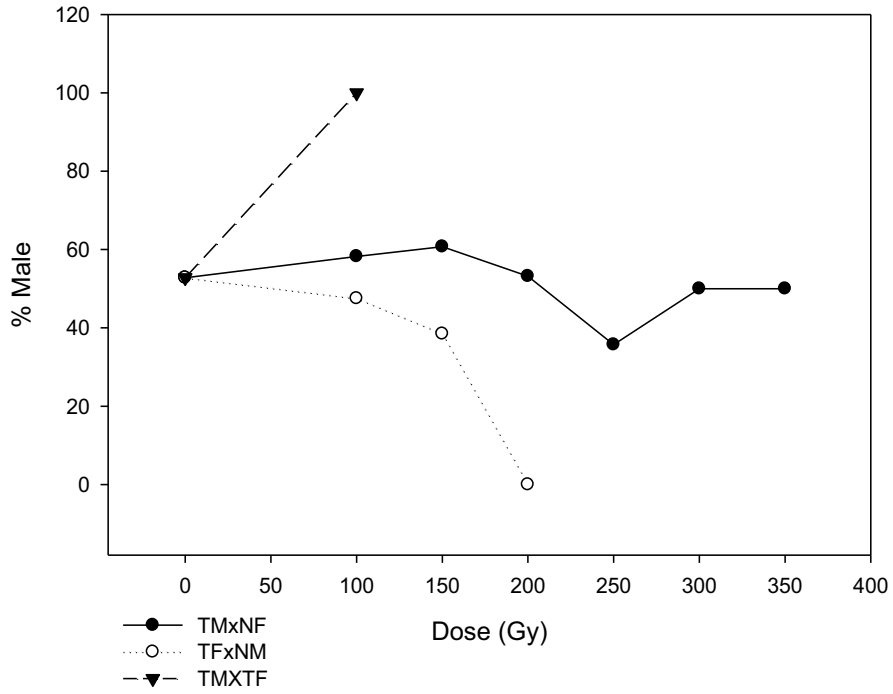

Figure 4.3.6. Percentage males in the total *Eldana saccharina* F_1 larvae surviving to adulthood from three crosses at increasing radiation doses (T= Treatment, irradiated; N= Normal; M= Male; F= Female).

4.3.3 Discussion

Parental fecundity

Generally, radiation treated *E. saccharina* females mated with normal males laid fewer eggs than normal females (N) mated with treated males and the control, at radiation doses of 200Gy to 250Gy (Figure 4.3.1). Thus, the fecundity of normal *E. saccharina* females mated with treated males was not affected by radiation in *E. saccharina.* This is consistent with the earlier published radiation biology studies on codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Bloem et al. 1999; Blomefield et al., 2010) and FCM (Bloem et al., 2003), but not for the closely related sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Crambidae), where treated males mated with normal females produced significantly fewer eggs than the control (Sanford, 1976). However,

in both the Tortricid species, treated female fecundity decreased almost linearly in response to increasing doses of radiation. In contrast, this type of cross in *E. saccharina*, especially with females treated at higher radiation doses (300 and 350 Gy; Figure 4.3.1), did not follow the trend reported for the tortricid species. The apparent lack of effect of increasing radiation dose on treated female fecundity may be a function of lepidopteran family. In the cactus moth *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) female fecundity was not affected by increasing doses of radiation. This response was found for treated males and for treated females mated with their normal counterparts respectively in *C. cactorum* (Carpenter et al., 2001). *Eldana saccharina,* like *C. cactorum*, belongs to the family Pyralidae. A non-significant response of treated female fecundity of *E. saccharina* to increasing radiation dose indicates that there may be differences in fecundity response to radiation between insect families and further investigation on this aspect is necessary.

However, a non-decline in fecundity in response to radiation in *E. saccharina* is not detrimental to the development of a SIT programme. This is because fertility is a much more reliable assessment of sensitivity to radiation. The aim of SIT is to induce sterility in the wild population. Therefore the number of eggs laid in the field is irrelevant provided they do not hatch and cause damage to crops. The presence of increased numbers of infertile eggs could even be regarded as a benefit, especially where natural enemies can be used in conjunction with SIT (Carpenter et al., 2005; Vreysen et al., 2006; Chapter 1.2 Figure 1.1). These authors show that non fertile eggs provide an additional host for egg parasitoids, or food source for predators.

Parental fertility

A linear decline in fertility has been observed for irradiated males of Indian meal moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) (Ashcraft et al., 1972; Brower, 1979); pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) (LaChance et al., 1973; Henneberry and Clayton, 1988); sugarcane borer *D. saccharalis* (Sanford, 1976; 1977); European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Nabors and Pless, 1981) and common cutworm *Spodoptera*

litura F. (Lepidoptera: Noctuidae) (Seth and Sharma, 2001). These authors assessed irradiated male fertility only and did not assess irradiated female fertility at increasing doses of radiation.

Where radiation effects have been assessed on both moth sexes, a linear decline in fertility in response to increasing doses of radiation was observed for both irradiated males and irradiated females in Range caterpillar *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae) (Ward et al., 1986); *C. pomonella* (Bloem et al., 1999; Blomefield et al., 2010); *C. cactorum* (Carpenter et al., 2001); and FCM (Bloem et al., 2003). These authors reported that females were more sensitive to increasing doses of radiation than males. This is a common factor in Lepidoptera, where fertility is regarded as a better indication of radiation sensitivity rather than fecundity (North, 1975; Carpenter et al., 2005). *Eldana saccharina* responded similarly, in that fertility was significantly reduced at increasing doses of radiation in all parental crosses (Figure 4.3.2). It was also found that *E. saccharina* females are much more sensitive to radiation than males, as fewer eggs laid by treated females hatched (Figure 4.3.2). Radiation sensitivity in *E. saccharina* females is an advantage to a potential SIT programme over the above species due to *E. saccharina*'s mating behaviour. In this species males call females (Atkinson, 1981). Released sterilised females will thus be able to respond to wild males, thereby preventing wild males from mating with wild females.

Development time, survival and sex ratio

Other factors common to male Lepidoptera following radiation are extended F_1 development time, increased F_1 mortality during development and a sex ratio in favour of males in the F_1 progeny (North, 1975). These attributes have been reported for *D. saccharalis* (Sanford, 1976; 1977); *P. gossypiella* (LaChance et al., 1973; Henneberry and Clayton, 1988); *C. pomonella* (Bloem et al., 1999) and FCM (Bloem et al., 2003). The data on these aspects obtained for *E. saccharina* were however quite variable because of the substantial reduction in parental fertility and poor survival of F_1 larvae at

increasing doses of radiation (Figure 4.3.5). More development time, survival and sex ratio data is therefore needed to test for statistical significance.

Some significant differences were found for example in F_1 survival, in results obtained for *E. saccharina* compared to other published literature. In the Treated female x Normal male cross, *E. saccharina* F_1 survival was 66.6% at 200Gy (Figure 4.3.5). North (1975) in contrast, reported that survival of Lepidopteran F_1 progeny from irradiated parents was poor. However, data in the *E. saccharina* study was distorted because of the three larvae that hatched from this cross (Treated female x Normal male fertility was 0.16% at 200Gy), two adults emerged (66.6% survival) and both were female (indicating 0:1 male:female sex ratio at 200Gy; Figure 4.3.6).

Sex ratio of progeny from irradiated Lepidoptera males is generally male-biased, (North, 1975). In contrast, in the Treated male x Normal female cross, *E. saccharina* F_1 adult sex ratio remained relatively equal between males and females (Figure 4.3.6). Carpenter et al. (2001) similarly found that after radiation studies on *C. cactorum*, F_1 development time was not significantly affected and a shift in sex ratio towards males did not occur. In their study, they proposed that the decline in survival of the F_1 larvae at higher radiation doses affected their results. This is similar to that found in this study in *E. saccharina* as significantly few F_1 larvae survived to adulthood at higher radiation doses from the treated male parents, and so fewer repetitions were completed. It is likely in the field that any larvae hatching from eggs laid by a female treated with 200Gy of radiation or higher would probably die due to predation (Leslie, 1982) or other biotic and abiotic factors before completing development, because of their reduced fitness.

4.4 F_1 Sterility

The F_1 adults emerging from the parental sterility assessment reported in 4.3, were used for assessment of F_1 sterility in *E. saccharina.* It is important to remember that the lower dose of radiation used to induce F_1 sterility is preferred because it ensures that the quality and competitiveness of the released insects is not compromised (Bloem et

al., 2001; Carpenter et al., 2005). In addition, progeny of irradiation of parents at sub sterilising doses have been shown to be more sterile than that of the parents (North, 1975; Carpenter et al., 2005), and it has been found that the release of partially sterile Lepidoptera offered greater suppressive control in the field than the release of fully sterile insects (Proverbs, 1970 cited by Carpenter et al. 2005). The following studies assess which dose induces F_1 sterility in *E. saccharina*, the lowest possible dose being the most desirable.

4.4.1 Materials and Methods

Developed F_1 pupae from each of the parental crosses (Treated male x Normal female; Treated female x Normal male; Treated female x Treated male; Normal female x Normal male) that survived the increasing radiation doses the parents were exposed to, were placed singly into individual cavities of 32 cavity plastic multicell trays. The multicell trays were covered with plastic cling wrap and aerated with a pin-prick to ensure virgin adults were available for mating. Emerged adults from pupae from each of the parental crosses above at each radiation dose tested were mated with normal adults of the opposite sex as follows: F_1 male x normal female (F_1♂xN♀); F_1 female x normal male (F_1♀xN♂) and F_1 female x F_1 male (F_1♀ x F_1♂) (Table 4.2). The normal female x normal male (N♀xN♂) F_1 crosses served as controls. Pairs were placed individually into a 500 ml paper cup, with a pleated cardboard oviposition substrate (50x10 mm when pleated five times), secured with a paperclip to maintain the pleats, and a 10 mm cotton dental wick soaked with water for the adults to drink from (Chapter 2; Figure 2.2). Plastic lids were then placed on the paper cups. Oviposition substrates were changed daily until females died or until the pair was five days old (whichever occurred soonest). The oviposition substrates were placed into re-sealable transparent plastic bags and labelled with date removed, parental radiation dose and radiated parent sex. After oviposition was complete, females were then killed by freezing and dissected to assess mating status by the presence of a spermatophore in the bursa copulatrix. Mating pairs were held in a temperature controlled room separate from the routine colony (26 ± 2 °C; 70 ± 5% RH; 8:16 L:D photophase). A maximum of 10 adults

were paired for each cross. However, 10 repetitions were not obtained at the higher radiation doses due to poor survival (Table 4.2). The plastic bags containing the oviposition substrates were held in an incubator (26 ± 2 °C; 60 ± 5% RH; 0:24 L:D photophase). The eggs laid on the substrates were counted, as were neonate larvae emerging from the eggs to assess F_1 fecundity and F_1 fertility respectively.

Table 4.2. Number of successfully mated F_1 *Eldana saccharina* offspring with normal adults of the opposite sex, following irradiation to the parents with increasing doses of gamma radiation (F_1= offspring from irradiated parent; T= Treatment, irradiated; N= Normal; ♂= male;♀= Female; - = no adult emergence).

	N♂xN♀			T♂xN♀			T♀xN♂			T♀xT♂		
Dose (Gy)	**F_1♂x N♀**	**F_1♀x N♂**	**F_1♀x F_1♂**	**F_1♂x N♀**	**F_1♀x N♂**	**F_1♀x F_1♂**	**F_1♂x N♀**	**F_1♀x N♂**	**F_1♀x F_1♂**	**F_1♂x N♀**	**F_1♀x N♂**	**F_1♀x F_1♂**
0	10	10	10									
100				9	10	8	9	9	6	1	-	-
150				10	10	8	5	7	-	-	-	-
200				7	8	2	-	2	-	-	-	-
250				4	8	-	-	-	-	-	-	-
300				2	2	-	-	-	-	-	-	-
350				1	1	-	-	-	-			

Statistical analysis

Genstat 12.1© (2009) was used for REML variance component analysis for both F_1 fecundity and fertility data. The statistic is reported with a χ^2 value.

F_1 fecundity and fertility

Fecundity and fertility data for F_1 offspring from both parental crosses, Treated male x Normal female and Treated female x Normal male were normal. Because F_1 sterility experiments were done using offspring from the parental experiments (section 4.3), there was not an even number of repetitions and not all pairs mated (Table 4.2). Therefore data were unbalanced and were analysed using REML variance component

analysis to test for dose and type of cross interaction. Table 4.2 shows that only one male emerged from the parental Treated female x Treated male cross. This F_1 male produced no offspring when mated with a normal female. Therefore no results are presented for this cross.

4.4.2 Results

4.4.2.1 Irradiated males mated with normal females (T♂xN♀)

F_1 fecundity

There was a significant interaction between type of F_1 cross and radiation dose (Figure 4.4.1: χ^2=32.3, df=6, P<0.001). Fecundity of F_1 female offspring x Normal males remained significantly lower than the control (566 eggs) when their male parents were treated with increasing doses of radiation, i.e 442 eggs at 100Gy to 335 eggs at 350Gy. Normal females x F_1 males also laid significantly fewer eggs than the control (538 eggs) when their male parents were treated with 100Gy to 250Gy (i.e. 361 eggs at 100Gy to 365 eggs at 250Gy). However, when their male parents were treated with radiation doses of 300Gy and 350Gy, mean number of eggs laid per female were similar to that of the control, i.e. 506 and 662 eggs respectively (Figure 4.4.1). F_1 females x F_1 males laid significantly fewer eggs than the control (594) when their male parents were treated with 100Gy to 250Gy, i.e. 280 to 273 eggs per female respectively. Because of poor survival of the F_1 progeny from the previous results reported in 4.3 (Table 4.2), pairs of F_1 males and F_1 females could not be mated for radiation doses 250Gy to 350Gy.

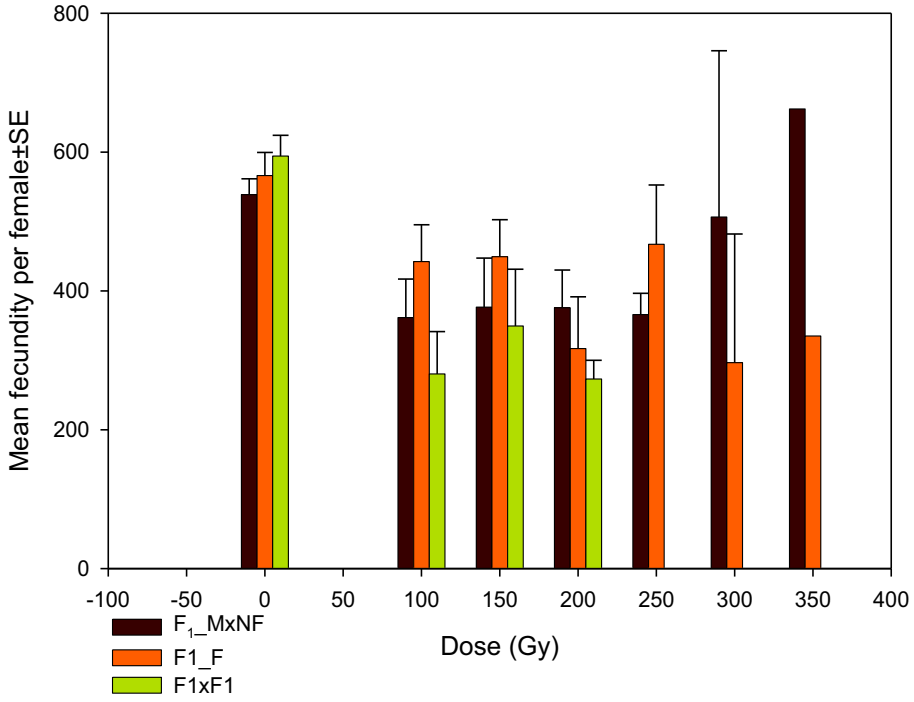

Figure 4.4.1. Mean fecundity per *Eldana saccharina* female of F_1 crosses from parent males treated at increasing radiation doses mated with normal females (F_1= offspring from irradiated parent; N= Normal; M= Male; F= Female).

F_1 fertility

There was a significant dose by cross interaction on fertility of F_1 offspring from the parental Treated male x Normal female cross (Figure 4.4.2: χ^2=18.65; df=9, P=0.039). This significant interaction implies that there are differences between fertility amongst the F_1 male and F_1 female offspring from the Treated male parent as radiation dose increases. F_1 males were sterile at 150Gy, but 5.95% fertile at 200Gy and 2.14% fertile at 250Gy. There was an increase in F_1 male fertility to 26.08% at 300Gy and 47.43% at 350Gy compared to the lower radiation doses. F_1 female fertility also increased to 60% at 350Gy. The increase in fertility at the higher radiation doses were comparable to results obtained in the control, and were considered erroneous. It was therefore not considered worthwhile to perform a regression analysis on the data. The F_1 female x F_1 male offspring, were sterile at 200Gy.

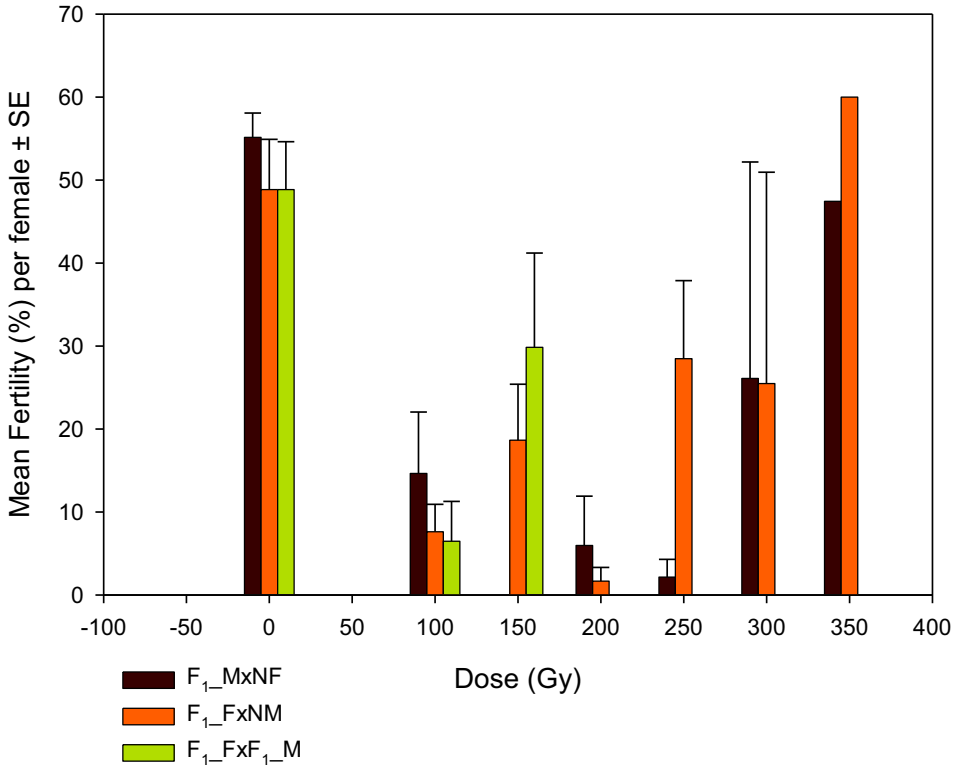

Figure 4.4.2. Mean fertility per *Eldana saccharina* female of F_1 crosses from parent males treated at increasing radiation doses mated with normal females (F_1= offspring from irradiated parent; N= Normal; M= Male; F= Female).

4.4.2.2 Irradiated females mated with normal males (T♀xN♂)

F_1 fecundity

The interaction between dose and cross was not significant (Figure 4.4.3: χ^2=6.12, df=3, P=0.118). All emerging F_1 progeny laid similar number of eggs to that of the control (approximately 500 eggs per female) except F_1 males mated with F_1 females and normal males mated with F_1 females at 100Gy (360 eggs) and 150Gy (319 eggs) respectively. Because of poor survival of F_1 progeny from the parental crosses, pairs of F_1 females and F_1 males could not be crossed at 150Gy and 200Gy.

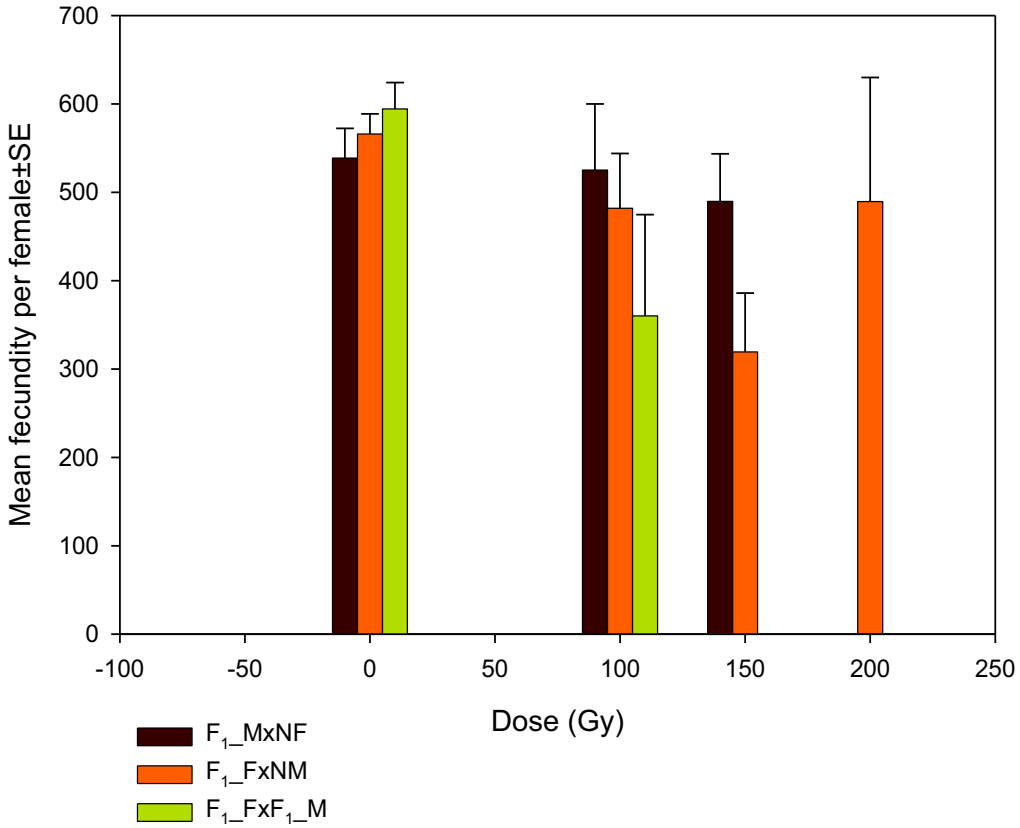

Figure 4.4.3. Mean fecundity per *Eldana saccharina* female of F_1 crosses from parent females treated at increasing radiation doses mated with normal males (F_1= offspring from irradiated parent; N= Normal; M= Male; F= Female).

F_1 fertility

The increasing radiation dose administered to the parent females significantly reduced all F_1 fertility as significantly fewer eggs hatched at 100, 150 and 200Gy compared to the controls (Figure 4.4.4: χ^2=36.96, df=3, P<0.001). F_1 females mated with normal males had a residual fertility of 28%; 29% and 22% at 100, 150 and 200Gy respectively compared to the control (48%). F_1 males mated with normal females were 20% and 32% fertile at 100Gy and 150Gy respectively compared to the control (55%) and F_1 females mated with F_1 males at 100Gy were 9% fertile compared to the control (48%).

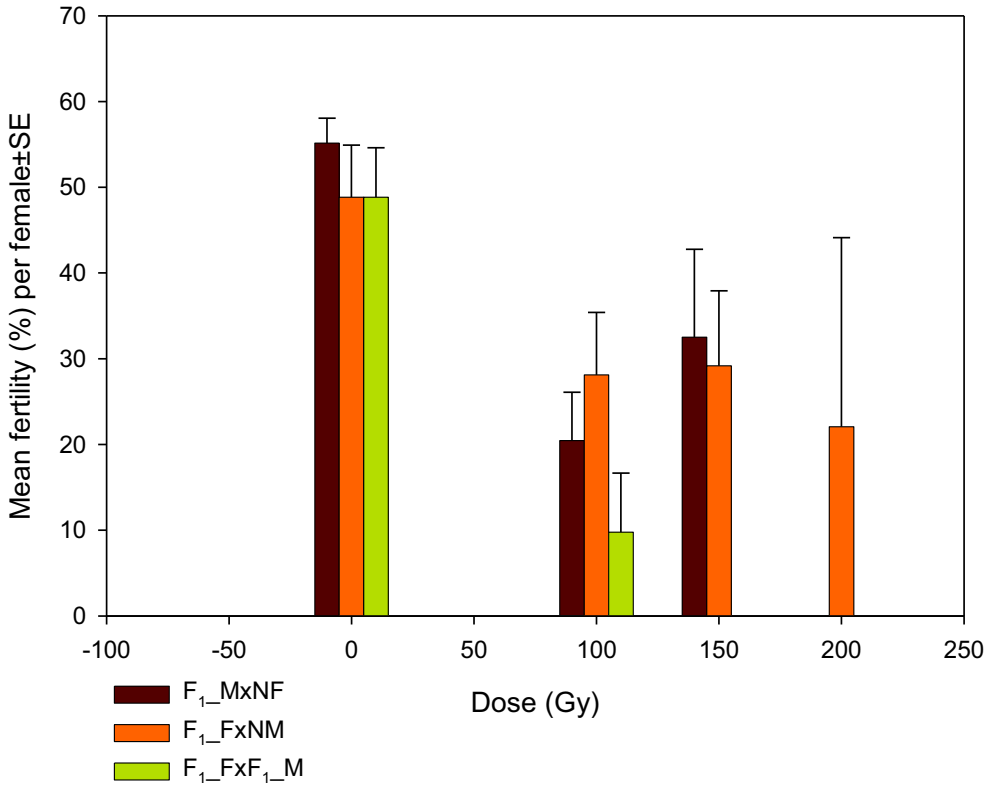

Figure 4.4.4. Mean fertility per *Eldana saccharina* female of F_1 crosses from parent females treated at increasing radiation doses mated with normal males (F_1= offspring from irradiated parent; N= Normal; M= Male; F= Female).

4.4.3 Discussion

4.4.3.1 Irradiated males mated with normal females (T♂xN♀)

F_1 Fecundity

Bloem et al. (1999) found a significant decline in fecundity for *C. pomonella* F_1 females mated with normal males, but not F_1 males mated with normal females. In contrast *E. saccharina* F_1 fecundity of normal females mated with F_1 male offspring, and F_1 female offspring mated with normal males was significantly affected by the radiation dose applied to parent males and females (Figure 4.4.1). Results obtained in this study are similar to F_1 sterility work on *D. saccharalis* (Sanford, 1976; 1977), *S. litura* (Seth and Sharma 2001) and FCM (Bloem et al. 2003) who reported a significant decline in

fecundity of F_1 males and F_1 females of the Lepidoptera they worked on, at increasing doses of radiation applied to parent males. However, for *E. saccharina* exposed to 300Gy and 350Gy fecundity of F_1 males mated with normal females increased to levels similar to that of the control, which was considered erroneous. However, as mentioned in section 4.3, assessment of fertility is more important than fecundity for the development of a SIT programme.

F_1 Fertility

A factor common to F_1 sterility is that progeny of F_1 males are more sterile than their male parents (North, 1975; Carpenter et al., 2001). *Eldana saccharina* F_1 males were more sterile than their parents radiated at 100Gy to 250Gy. However at 300Gy and 350Gy, they were more fertile, not significantly different from the control fertility (Table 4.3), contradicting the work of North (1975) and Carpenter et al. (2001). This is unusual and has not been reported by other authors and was therefore considered erroneous. A polynomial regression was therefore not considered worthwhile. Furthermore, because *E. saccharina* F_1 male fertility increased at higher radiation doses (Table 4.3), it contradicted previous published research that showed F_1 females crossed with normal males were more fertile than F_1 males crossed with normal females. This has been observed in *P. interpunctella* (Brower, 1979); *O. nubilalis* (Nabors and Pless, 1982); *P. gossypiella* (LaChance et al., 1973; Henneberry and Clayton, 1988); C*. pomonella* (Bloem et al., 1999); *E. kuehniella* (Marec et al., 1999); *C. cactorum* (Carpenter et al., 2001); *S. litura* (Seth and Sharma, 2001) and FCM (Bloem et al., 2003).

Table 4.3. Mean fertility of *Eldana saccharina* irradiation treated parent male and $_1$ progeny at increasing radiation dose (T= Treatment, irradiated; N= Normal;♂= male; ♀= Female; n= sample size).

Dose (Gy)	% Fertility T♂xN♀ (n)	% Fertility F_1♂x N♀ (n)	% Fertility F_1♀xN♂ (n)	% Fertility F_1♀xF_1♂ (n)
0	65.35 (5)	55.15 (10)	48.84 (10)	48.84 (10)
100	31.32 (5)	14.63 (9)	7.6 (10)	6.46 (8)
150	19.70 (5)	0 (10)	18.64 (10)	29.84 (8)
200	15.12 (5)	5.95 (7)	1.66 (8)	0 (2)
250	7.10 (5)	2.14 (4)	28.46 (8)	
300	1.26 (5)	26.08 (2)	25.47 (2)	
350	3.11 (5)	47.43 (1)	60.00 (1)	

4.4.3.2 Irradiated females mated with normal males (T♀xN♂)

F_1 Fecundity

Fecundity of male and female *E. saccharina* progeny from the parent female irradiated at increasing doses of radiation was not significantly affected when they were mated with normal adults of the opposite sex (Figure 4.4.3).

F_1 Fertility

Eldana saccharina fertility was, however, significantly reduced for all types of F_1 male and female offspring crosses when their female parents were treated with increasing doses of radiation (Figure 4.4.4). Bloem at al. (1999) did not report on F_1 fecundity and fertility of progeny of treated *C. pomonella* females as treated females parents were sterile at 100Gy. Furthermore, Bloem et al. (2003) did not examine F_1 sterility of treated parent females. They found that parent females were 100% sterile at 200Gy. Carpenter et al. (2001) recorded similar results with *C. cactorum* at 200Gy. This is in contrast to that found for *E. saccharina,* as females treated at 200Gy still had a small residual fertility (0.16%). Because of poor survival of developing F_1 larvae, the number of repetitions achieved at 200Gy was two.

4.5 Conclusion

Development of a SIT programme requires that released females must be 100% sterile to ensure they do not produce progeny and contribute to population growth of the pest. In the results presented and discussed so far for assessing the radiation biology of *E. saccharina*, it was very difficult to determine this fact. In addition, a number of further anomalies arose in what has been described in this chapter thus far, which was not supported from current literature published and available on radiation biology studies of other Lepidoptera. In the parental radiation treatments, the 66% survival of treated *E. saccharina* female progeny at 200Gy was unexpected, and a male-biased sex ratio in the progeny of treated males was not recorded. More prominent though, was the similar fertility attained of the F_1 progeny from the parental radiation treated males at the higher radiation doses, to that of the controls. It would appear from all literature on radiation effects on insects quoted in this chapter, that this is impossible, especially at high radiation doses.

It was suspected that the oviposition substrates of the high radiation dose parents were contaminated with neonate larvae escaping from the plastic bags housing the control and very low radiation dose oviposition substrates used in the parental radiation biology trials. If this was the case, then these escapees could have produced the erroneous results recorded at high radiation doses. It was assumed that the self sealing plastic bags used to house the oviposition substrates would have a secure enough seal to restrict neonate larval movement, which clearly was not the case.

This is further supported by recent studies conducted by Conlong et al. (2007), which demonstrated that neonate *E. saccharina* larvae are extremely active in host food selection and disperse readily to ensure survival. Because of their cryptic nature (Conlong, 1994a; Conlong 1994b) and their ability to disperse (Conlong et al., 2007) it is extremely likely that oviposition substrates from the higher radiation doses were contaminated with larvae from the control and thus counted as hatched larvae from the higher radiation doses. In addition, because *E. saccharina* larvae forage for the first few

days after hatching, before entering the sugarcane stalk in the field (Leslie and Keeping, 1996), they are able to feed on the egg chorion on the oviposition substrates, making it difficult to determine whether the larvae hatched or not from inspecting eggs for larval emergence holes.

These anomalous results thus necessitated that certain of the radiation dose treatments be repeated on *E. saccharina* adults to clarify parental and F_1 sterility radiation effects, and to determine if *E. saccharina* is a candidate for SIT.

4.6 Investigation of Anomalies

Anomalies found in the previous experiment included high survival of F_1 progeny from the parental Treated females irradiated with 200Gy and mated with normal males, and fertility of F_1 progeny of the treated male parents at the higher radiation doses of 300 and 350Gy being comparable to the controls. The experiment was repeated to investigate these anomalies.

Parental and F_1 fecundity

Because fertility was calculated using fecundity and number of eggs hatched, it was necessary to repeat the fecundity experiments.

Parental and F_1 fertility

An increase in F_1 fertility at higher radiation doses was the most prominent anomaly found in the previous study. Parental crosses had to be made in order to use the F_1 offspring for F_1 fertility assessments. Therefore fertility of the parental crosses was re-assessed, in addition to F_1 fertility.

Development time of F_1 neonates to adulthood, F_1 survival of neonates and sex ratio of F_1 offspring was not re-investigated. The repeated experiment was conducted in the same manner as the previous experiment and poor survival was expected. Because of

poor survival, definitive results for these aspects could not be obtained similar to the previous experiment.

4.6.1 Materials and Methods

The materials and methods described in section 4.3.1 and 4.4.1 were repeated exactly as described in August 2008 for selected radiation doses to re-assess parental and F_1 sterility. The radiation doses repeated for the Treated male x Normal female parental cross (T♂xN♀) were 200; 250; 300 and 350Gy. The radiation dose repeated for the Treated female x Normal male parental cross (T♀xN♂) was 200Gy. The parental cross Treated female x Treated male was not repeated. The control cross (N♀xN♂) was repeated. Extra care was taken to keep the oviposition substrates for each radiation dose separate, by keeping the plastic re-sealable bags in large brown envelopes and separate for each radiation dose, to avoid cross contamination.

Statistical analysis

Assessment of Parental fecundity fertility and F_1 fecundity and fertility

Parental and F_1 fecundity and fertility data were unbalanced as not all adult pairs mated (Table 4.4; Table 4.5) and an Anova test could not be used. A REML variance component analysis was performed on the fecundity and fertility data with dose and type of cross as variables, using Genstat 12.1© (2009). Parental and F_1 fecundity data were normal. Parental and F_1 fertility data were log transformed to attain normality. Where a significant interaction was found the data was subject to polynomial regression.

Table 4.4. Number of successfully mated *Eldana saccharina* pairs with one of either sex irradiated with increasing doses of gamma radiation (T= Treatment, irradiated; N= Normal).

Dose	N♂xN♀	T♂xN♀	T♀xN♂	T♀xT♂
0	4			
200		5	5	
250		3		
300		4		
350		5		

Table 4.5. Number of successfully mated F_1 *Eldana saccharina* offspring with normal adults of the opposite sex, following irradiation to the parents with increasing doses of gamma radiation (F_1= offspring from irradiated parent; T= Treated, irradiated; N= Normal; ♂= male; ♀= Female; - = no adult emergence).

	N♂xN♀			T♂xN♀			T♀xN♂		
Dose (Gy)	F_1♂x N♀	F_1♀x N♂	F_1♀x F_1♂	F_1♂x N♀	F_1♀x N♂	F_1♀x F_1♂	F_1♂x N♀	F_1♀x N♂	F_1♀x F_1♂
0	10	10	10						
200				10	11	-	-	-	-
250				1	1	-	-	-	-
300				-	-	-	-	-	-
350				-	-	-	-	-	-

4.6.2 Results

Parental fecundity at 200-350 Gy

The females from crosses Normal female x Treated male and Treated female x Normal male (treated with 200Gy of radiation) laid a fewer eggs (means of 389 and 386 respectively), compared to the control (577 eggs). Females from the Treated male x Normal female crosses treated with radiations of 250 to 350Gy laid a mean number of eggs similar to that of the control (i.e. 631 eggs per female to 470 eggs per female

respectively). The dose by cross interaction on fecundity of irradiated parents was not significant (χ^2=10.35, df=5, P=0.112).

Parental fertility at 200-350 Gy

There was a significant dose by cross interaction on fertility of irradiated parent males and females mated with normal counterparts as radiation dose increased (χ^2=78.45, df=5, P<0.001). Treated females were more sensitive to radiation than treated males (Figure 4.6.1). In the Treated male x Normal female crosses, fertility declined significantly at increasing doses of radiation (y=1.698-0.00516x; F=50.78; df=20, P<0.001, R^2=0.73). At 200, 250, 300 and 350Gy fertility was 10.48%, 0.83%, 1.25% and 0.19% respectively. Figure 4.6.1 compares fertility of treated male and female parents obtained in the repeated experiment with that obtained in the first. Fertility decline in the treated male crosses was similar to the first experiment, in that treated male parents still had residual fertility at higher radiation doses. However, in contrast to the 66% survival recorded in the first study, when females were irradiated at 200Gy in the repeated experiment, they were 100% sterile.

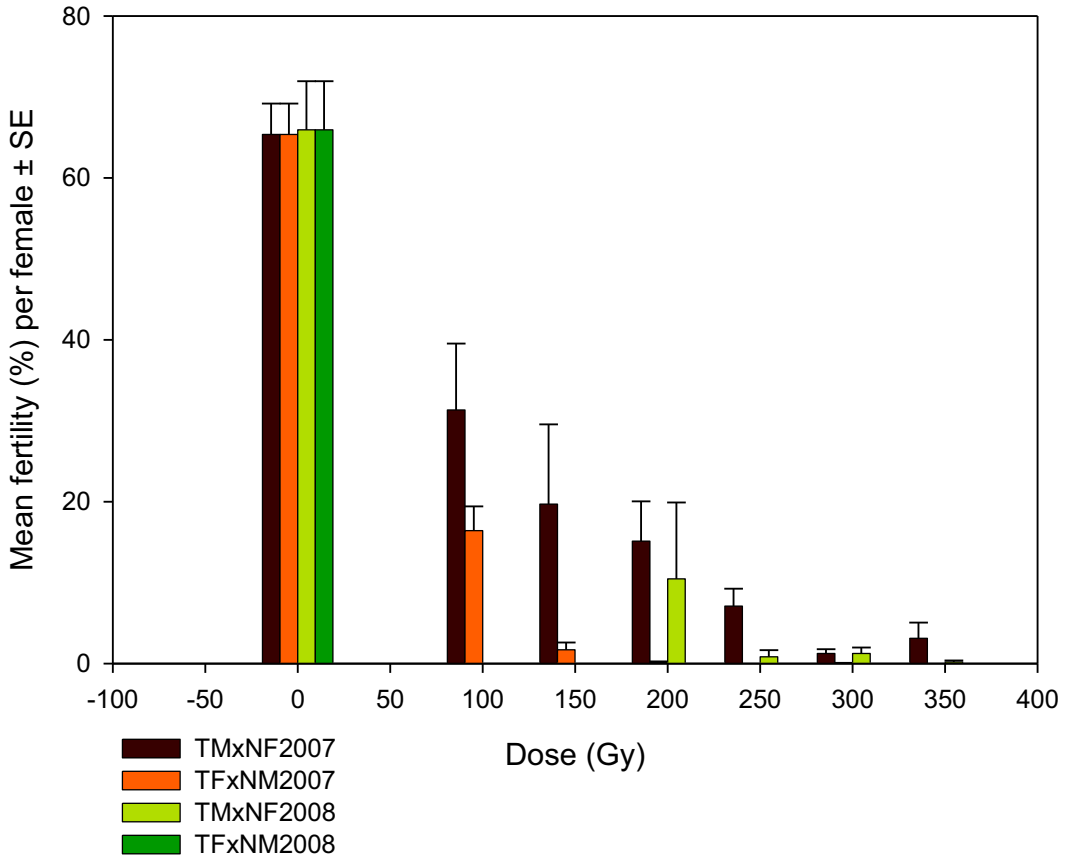

Figure 4.6.1. Comparison of mean fertility per *Eldana saccharina* female from treated x normal parental crosses made in 2007 and repeated in 2008 at radiation doses 200-350Gy (T= Treated, irradiated; N= Normal; M= Male; F= Female).

F_1 fecundity of adults irradiated at 200-350Gy

There was no significant interaction between doses of radiation and type of cross on mean F_1 fecundity (χ^2=0.94, df=2, P=0.629) from the Treated male x Normal female parental cross. Normal females x F_1 males mean fecundity ranged from 474 eggs at 200Gy to 535 eggs at 250Gy, similar to the control (513 eggs). F_1 females x Normal males laid a mean number of 413 eggs when their fathers were treated with 200Gy, similar to that of the control, (530 eggs) except at 250Gy, where the single treated F_1 female mated with a normal male, laid 297 eggs.

F_1 fertility of adults irradiated at 200-350Gy

Increasing doses of radiation applied to the parent males had a significant effect on F_1 male and F_1 female fertility (χ^2=44.69, df=2, P=0.001). There was no survival of Treated male progeny at 300Gy and 350Gy and so F_1 crosses could not be made (Table 4.5). F_1 males from the 200Gy parental Treated male x Normal female cross were 14.57% fertile and at 250Gy, fertility of offspring from the same parental cross was 0% in the F_1 male cross and the F_1 female cross. Because of too few data points, a linear regression analysis could not be performed. Figure 4.6.2 compares F_1 sterility of treated male progeny from the repeated experiment in 2008 with the experiment performed in 2007. Figure 4.6.2 confirms that F_1 progeny of treated males are sterile at the higher radiation doses, particularly 300Gy and 350Gy. There was no F_1 offspring available to pair at these higher radiation doses, which is in contrast to that found in the experiment conducted previously. As mentioned above, the treated females exposed to 200Gy and mated with normal males were sterile. Therefore there were no F_1 offspring from that cross to assess F_1 fertility of Treated females.

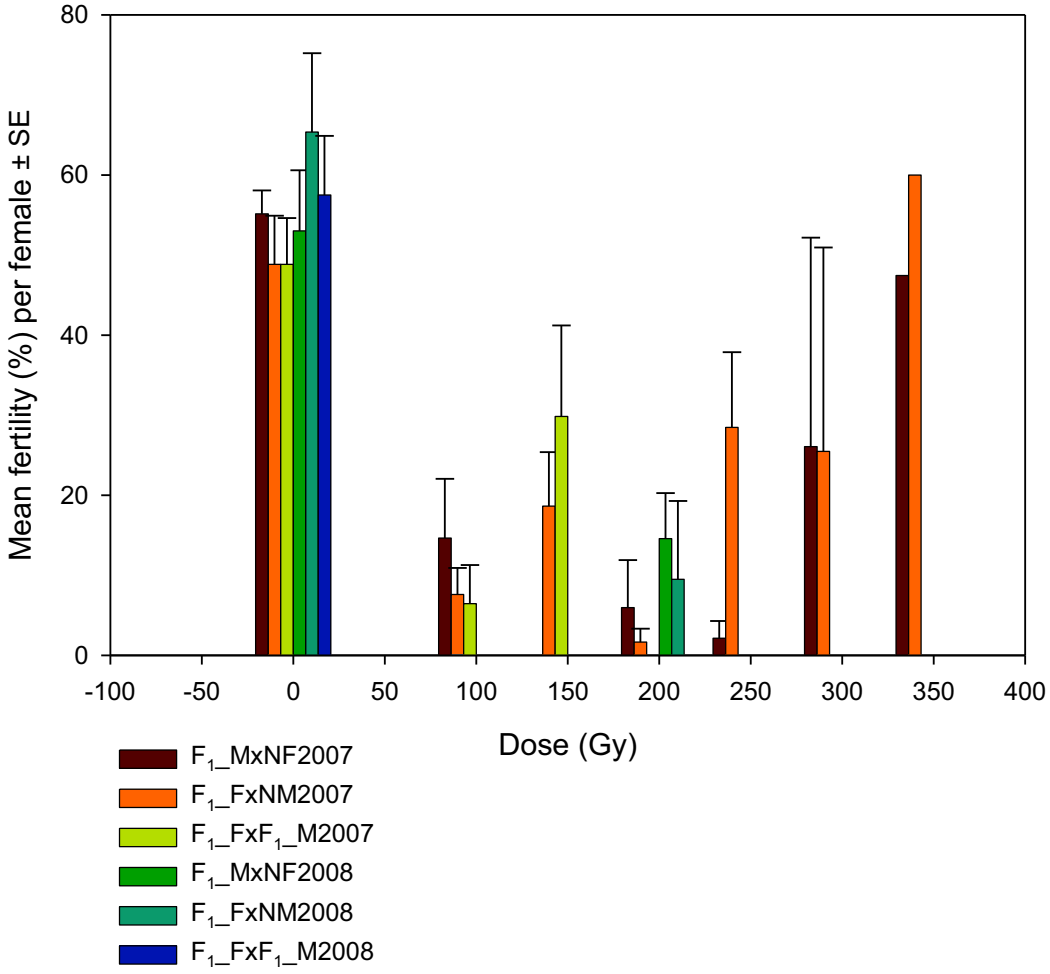

Figure 4.6.2. Comparison of mean fertility per *Eldana saccharina* female from F_1 crosses made from treated parent males in 2007 and repeated in 2008 at radiation doses 200-350Gy. (F_1= offspring from irradiated parent; N= Normal; M= Male; F= Female).

4.6.3 Discussion

Parental fecundity at 200-350 Gy

In the repeated experiment, there was no significant effect of increasing radiation dose applied to male and female *E. saccharina* on female fecundity. This is similar to that reported in the previous experiment and for *C. cactorum* (Carpenter et al., 2001), which confirms that fecundity in the family Pyralidae may not be affected by increasing doses

of radiation as are other species from the Tortricid and Crambid families (e.g. *C. pomonella* (Bloem et al., 1999; Blomefield et al., 2010); FCM (Bloem et al., 2003) and *D. saccharalis* (Sanford, 1976) respectively). However, fertility is a more important assessment than fecundity in order to determine an insects' suitability for SIT. Sterile eggs can enhance a SIT programme (section 4.3.3) by providing hosts and food sources for parasitoids and predators (Carpenter et al., 2005; Vreysen et al., 2006).

Parental fertility at 200-350 Gy

The repeated experiment shows that *E. saccharina* females treated at 200Gy and mated with normal males were sterile (Figure 4.6.1). This is in contrast to that found in the previous experiment, which in the same cross at the same radiation dose, 0.16% residual fertility was recorded. Bloem et al. (2003) and Carpenter et al. (2001) found 200Gy induced full sterility in FCM and *C. cactorum* females respectively. In the previous experiment, of the three neonate larvae that were inoculated onto diet, two females emerged (66% survival). In contrast, in the repeated experiment the eggs failed to hatch and thus survival was 0.

In the Treated male x Normal female cross, fertility declined significantly as radiation dose was increased (Figure 4.6.1). The level of fertility attained at 350Gy in this experiment was 0.19%, lower than the first experiment (3.11%) but not significantly so. These decreasing fertility with increasing radiation results agree with the findings in section 4.3.3 and of the authors work discussed in that section. They further agree that females are more sensitive to radiation than males.

F_1 Fecundity at 200-350 Gy

Fecundity of F_1 offspring of treated males mated with normal females was not significantly affected by radiation doses in this trial. This is in contrast to that found in the previous experiment where fecundity of F_1 offspring from treated males mated with normal females declined significantly at higher radiation doses. However, sample sizes in the first experiment were larger (Table 4.3) at the same radiation doses and more radiation doses were tested compared to the repeated experiment (Table 4.5).

F_1 Fertility at 200-350 Gy

In this repeated study full F_1 offspring sterility was attained from treated male parents irradiated at 250Gy and mated with normal females (Figure 4.6.2). F_1 male and F_1 female offspring had fertility of 14.57% and 9.50% respectively when their male parents were treated with 200Gy of radiation (Figure 4.6.2). In the previous experiment, F_1 male and F_1 female offspring of parent males radiated at 250Gy had fertilities of 2.14 and 28.46 respectively (Figure 4.4.2). In addition, in the previous experiment, the fertility of F_1 offspring crosses at higher radiation doses of 300 and 350Gy with untreated adults of the opposite sex were similar to the control and was in contrast to published literature on F_1 sterility in Lepidoptera (Figure 4.4.2) as discussed in section 4.4.3. The more carefully controlled repeated study confirmed that fertility of *E. saccharina* F_1 progeny declined significantly when parent males were treated with increasing doses of radiation. This decline in F_1 fertility is similar to that found for *P. interpunctella* (Brower, 1979), *O. nubilalis* (Nabors and Pless, 1982), *P. gossypiella* (LaChance et al., 1973; Henneberry and Clayton, 1988), C*. pomonella* (Bloem et al., 1999), *E. kuehniella* (Marec et al., 1999), *C. cactorum* (Carpenter et al., 2001), *S. litura* (Seth and Sharma, 2001) and FCM (Bloem et al., 2003) and for Lepidoptera in general (North, 1975; Carpenter et al., 2005).

The most appropriate dose selection for the development of a SIT programme is critical (Bloem et al., 2003) both in terms of maximising irradiated male fitness, and preventing further outbreaks from partially sterile females. To implement SIT, large numbers of adult insects are mass reared and released into the environment (Parker, 2005). It is thus not easy or practical to separate large quantities of insects by sex (Bloem et al., 2003). Therefore, to ensure that the released females in a SIT programme do not exacerbate crop infestations, they must be fully sterile (Robinson, 2005). The radiation dose where this was achieved for treated *E. saccharina* females was 200Gy. Futhermore, it is vital that irradiated males are able to compete readily with wild males for mates (North, 1975; Omar and Mansor, 1993; Carpenter et al., 2005) and therefore

the lowest possible radiation dose is preferred to ensure fitness of F_1 males so that they are able to mate with wild females (Bloem et al., 2001).

4.7 Conclusion

It was vital that neonate behaviour was taken into account when this experiment was designed and that extra care should have been taken to ensure cross contamination of oviposition substrates did not occur.

The repeated sections of this study were worthwhile as they confirmed that the levels of fertility attained at higher radiation doses in the first experiment were erroneous as suspected. Taking these new results into account, this study therefore confirmed that *E. saccharina* was sensitive to the sterilising effects of radiation, as reported for many other Lepidoptera. *Eldana saccharina* is a suitable candidate for the further development of a SIT programme using F_1 sterility against it. Treated *E. saccharina* female parents were sterile at 200Gy and F_1 male and female progeny from male parents treated at 250 Gy were fully sterile. The doses found in this study to induce sterility in treated females and the F_1 progeny of *E. saccharina* are similar to those used in a SIT programme targeting FCM in Citrusdal, Western Cape South Africa. These results are encouraging as it implies that a similar SIT programme will be successful for *E. saccharina* as part of AW-IPM against this pest in the South African sugar industry. This study was the first step in investigating the use of SIT against *E. saccharina.*

4.8 References

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1 Introduction

The most recent study on crop loss in the South African sugar industry estimated that *Eldana saccharina* Walker (Lepidoptera: Pyralidae) caused a R153 million loss in the 2003/2004 milling season (Way and Goebel, 2003). It has been the most economically important pest in the South African sugar industry since the 1970's (Carnegie, 1974) and has been the subject of much research at the South African Sugar Association Research Institute (SASRI). Despite many attempts to control *E. saccharina*, using methods such as good crop husbandry and hygiene, planting resistant varieties, insecticides, biological control and habitat management, *E. saccharina* remains an important pest in the industry (Anonymous, 2005). The use of Sterile Insect Technique (SIT) is an area-wide approach, environmentally friendly and species specific (Vreysen et al., 2006). Insects do not recognise borders or farm boundaries and it is therefore vital that an area-wide approach is taken. Modern Integrated Pest Management (IPM) is about holistic agro-ecosystem management, with knowledge about the complete ecology of the target pest and its interactions with environmental factors around it (Conlong and Rutherford, 2009). The SIT is based on the mass rearing and release of quality sterilised males of the target insect, which are released into the wild in large enough numbers to outnumber wild males and mate with the available wild females (Klassen, 2005). This induces sterility in the wild population and reduces the pest population.

The objectives of this study were threefold:

a) to review the general biology of *Eldana saccharina* and to assess adult mating frequency

b) to assess whether two oil soluble dyes incorporated into the routine artificial diet marked adult *E. saccharina* and if they were detrimental to *E. saccharina*'s biology

c) to assess the effects of radiation on newly emerged adult *E. saccharina* and the induction of sterility in the F_1 generation.

This chapter summarises the findings obtained from Chapter 2, 3 and 4 and provides recommendations for future studies towards developing an Area-Wide pest management programme, incorporating SIT, against *E. saccharina*.

5.2 *Eldana saccharina* Walker (Lepidoptera: Pyralidae) general biology

Because successful mating of irradiated males with wild females of the target insect in the field forms the basis of SIT, the biology, fecundity and mating behaviour of *E. saccharina* was reviewed and the mating frequency of both sexes studied. *Eldana saccharina* is indigenous to Africa. It has recently been found that there are at least three biotypes of *E. saccharina* in Africa based on geographical isolation (Assefa et al., 2006). Fecundity between these biotypes was found to be variable and was based largely on the population's nutritional status, and temperature at which they were reared. *Eldana saccharina* mean fecundity was 518 eggs per female and was similar (432 eggs per female) to that reported by Way (1994), who reared his specimens on the same artificial diet used for the current study. When compared to other Lepidopteran pests, which are the subject of area-wide SIT programmes against them, *E. saccharina*'s fecundity is much higher. For example the cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) has a mean fecundity of 119.8 ± 68.9 (mean ± SD) (Carpenter et al., 2001). The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), has a mean fecundity of 200 eggs per female (Bloem et al., 1999), and the false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), has a mean fecundity of 400 eggs per female (Bloem et al., 2003). *Eldana saccharina*'s high fecundity of more than 500 eggs per female therefore confirmed its potential as a crop pest.

Eldana saccharina fertility was also found to be variable ranging from 19.9% to 100% (Average 63.2 ± 4.2%). Studies by other authors also recorded such variability in fertility (Dick, 1945; Betbeder-Matibet, 1977; 1981; Way, 1994).

The current study showed for the first time that *E. saccharina* females are able mate more than once and confirmed the male's ability to mate more than once as reported by Dick (1945) and Betbeder-Matibet et al. (1977). Males mated up to six times, although the mean number of females mated per male was 3.3 ± 0.72. Females mated up to three times, although the majority of females only mated once (56.7%). A number of reasons have been proposed for females accepting another mate, one of them being sperm from previous matings was inadequate or of poor quality (Byers et al. 1982 cited by Gomez et al., 2000). The low fertility found in the current study of 19.9% was for one female out of 20. It was possible that the sperm she received was not adequate which contributed to poor fertility.

Future research

Because it has been found by Flint and Merkle (1983) that *Galleria mellonella* L. (Lepidoptera: Pyralidae) females had sperm present in their bursa copulatrix without the presence of a spermatophore, further investigation is needed to check for the presence of sperm in apparently unmated *E. saccharina*. Furthermore, future research should include assessment of sperm competition between sperm from radiated and unirradiated males, mating frequency in the field and whether mating frequency is influenced by the start of oviposition. This will assist in developing overflooding ratio's of mass reared, released and sterilised *E. saccharina* for SIT.

For mating, *E. saccharina* males form leks and call the females (Atkinson, 1981). Pheromones released by the males and ultrasound play important roles (Atkinson, 1982; Bennett et al., 1991). The pheromone components produced by male *E. saccharina* are complex. Burger et al. (1993) lists previously identified pheromone components (and their authors) as *trans*-3.7-dimethyl-6-ocent-4-olide (also known as

eldanolide), vanillin and 4-hydroxybenzaldehyde. In addition, Burger et al. (1993) identified further compounds: (Z)-3,7-dimethylocta-2,6-dienoic acid; 1-octadecane thiol; 16-hexadecanolide; 18-octadecaholide; (Z)-9-hexadecanenal and *cis*-3,7-dimethyl-6-octen-4-olide (*cis*-eldanolide). The effect of radiation on pheromone production needs to be assessed to ensure that released irradiated males are able to attract females.

5.3 The effect of oil soluble dyes on the biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

The use of oil soluble dyes incorporated into the artificial diet of insects have commonly been used to mark adults of lepidopteran pests, especially those reared and released for SIT programmes (Hagler and Jackson, 2001; Qureshi et al., 2004; Parker, 2005). These dyes are incorporated into the fat bodies of insects which make them easy to identify (Parker, 2005). Mark-release-recapture techniques are commonly used for behaviour, population, ecology and monitoring studies (Southwood, 1966; Hagler and Jackson, 2001). Calco Red N1700 is predominantly used as a marker for SIT programmes against insect pests and was not found to affect *E. saccharina*'s biology. In contrast Sudan Red was found to be detrimental to *E. saccharina*'s biology, as it prolonged development time and significantly reduced female fecundity and fertility. Marking studies on *E. saccharina* have not been investigated before and results reported in chapter 3 confirmed that Calco Red can be used for any population, ecology or behaviour field studies on *E. saccharina* that require mark-release-recapture techniques.

It is vital, especially for the use of SIT that the marking dye does not compromise fitness as SIT is based on the male's ability to compete with wild males for mates in order to induce sterility into the wild population. The findings from the current study are particularly advantageous for the development of an SIT programme, as these mark-release-recapture techniques are required to calculate overflooding ratios and to measure the success of an SIT programme against the target pest. If only marked

adults are caught in traps in the field, this implies that the wild population has been significantly reduced.

Future research

A successful monitoring method for *E. saccharina* using Delta traps with crude lures made from abdomens of fresh male *E. saccharina* moths was developed for cage trials (Rutherford et al., 2009). However, a successful monitoring technique in sugarcane fields using pheromone traps has not been developed as the blend of synthetic pheromone components reported by Burger et al. (1993) has not been optimised to attract females (or males) in the field. Future research on the development of a pheromone trap in order to successfully trap *E. saccharina* in the field is essential.

5.4 Parental and F_1 sterility of *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

Lepidoptera are resistant to the sterilising effects of radiation as used in SIT programmes against dipteran pests, and high doses are required to induce sterility in males (Carpenter et al., 2005; Robinson, 2005). This could affect their overall fitness and mating behaviour so that they are unable to compete with wild males for mates after release. However, inherited sterility is well documented in Lepidoptera (North, 1975; Carpenter et al., 2005). Unfortunately, in the present study on *E. saccharina*, the first experiment conducted to assess parental and F_1 fertility produced erroneous results. There was high survival of female offspring when the parent female was treated with 200Gy. In addition, F_1 offspring of treated males at higher radiation doses of 300 and 350Gy mated with normal females had levels of fertility similar to that of the control. These results contradicted the literature published on F_1 sterility in Lepidoptera. It was suspected that neonate larvae from the controls and lower radiation doses contaminated the oviposition substrates from the higher radiation doses causing the erroneous results. Because of this, selected radiation doses for Treated females mated with Normal males and Treated males mated with normal females were repeated. Extra careful precautions were taken to ensure the oviposition substrates for each radiation

dose and cross were kept separate. The repeated study found that *E. saccharina* males are resistant to high doses of radiation as males were 0.19% fertile when treated with 350Gy. This is similar to what was found in the first study. However, when males were treated with 250Gy their F_1 offspring were sterile. There was no survival of F_1 larvae when their fathers were treated with 300 and 350Gy radiation levels. These results were in contrast to that found in the first study and agreed with published literature on other Lepidoptera that are the subjects of SIT programmes (LaChance et al., 1973; Henneberry and Clayton, 1988; Bloem et al., 1999; 2003; Carpenter et al., 2001).

Eldana saccharina females were found to be more sensitive to radiation and were sterile when treated with 200Gy, also in contrast to that found in the first study. The results obtained in the repeated experiment indicate that *E. saccharina* is a suitable candidate for further research towards a pest management programme incorporating SIT.

Future research

Because there was some residual fertility in F_1 male and female offspring, where parental males were irradiated at 200Gy and mated with normal females, it is recommended that fitness of irradiated males be assessed at 200Gy and 250Gy (where F_1 offspring were found to be sterile). Because the F_1 progeny have longer development times (North, 1975; Carpenter et al., 2005) and because biotic and abiotic factors are more variable in the field compared to the laboratory, it is recommended that F_1 fertility field studies for *E. saccharina* are conducted where parent males are irradiated at 200Gy and 250Gy. The hatched neonate larvae attained in this experiment at 200Gy may die in the field as a result of biotic and abiotic factors, making 200Gy a more suitable dose for the development of a SIT programme against *E. saccharina.* Released males irradiated at 200Gy will be fitter than those radiated at 250Gy, as lower doses are preferred (Bloem et al., 2001; 2003; Robinson, 2005) and is vital that released males are competitive with wild males (North, 1975; Omar and Mansor, 1993; Carpenter et al., 2005).

Limitations to the current study

Prolonged development time and a male biased sex ratio at increasing doses of radiation were not observed due to poor survival of F_1 offspring. As *E. saccharina* fecundity is in excess of 500 eggs per female, it was not possible to complete more replicates at the time of radiation due to the quantity of eggs and emerging F_1 neonate larvae that required processing, especially for the controls and lower radiation doses. It was very difficult to ensure that all pairs were mated following irradiation to the adults as dissections were done after the females completed oviposition (i.e. five days after radiation). As the radiation facility was based in the Western Cape, much planning was required to do the experiment, including flight and accommodation bookings as well as ensuring enough insect material was transported to the Western Cape. Repeat irradiation could not be completed in the time that was available for the first radiation experiment, which is why the repeated experiment was planned a year later. The absence of an irradiation facility close by to the rearing facility in Mount Edgecombe, KZN, is a large limiting factor to the further development of this technique as a method of pest control against *E. saccharina*.

5.5 Concluding remarks

Eldana saccharina is a suitable candidate for the development of a SIT programme against it.

The effect of radiation on male mating behaviour, pheromone production and sperm competition on *E. saccharina* and their performance in the field or cage trials now needs to be completed, using the radiation doses of 200Gy and 250Gy. The development of a trap is vital in order to be able to trap *E. saccharina* in the field effectively, to calculate overflooding ratios and monitor the success of the SIT programme.

Because large quantities of quality moths need to be mass reared in a cost effective manner for release into the field in an SIT programme (Parker, 2005) further

development of mass rearing methodology for *E. saccharina* is necessary in order to produce the numbers required for field studies.

5.6 References

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