

**Performance of sterilized *Eldana saccharina* Walker
(Lepidoptera: Pyralidae) adults in mating and cage trials:
Further steps towards its control using the Sterile Insect
Technique.**

by
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DECLARATION

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ABSTRACT

The sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is the most limiting factor in the South African sugar industry with losses to this insect pest estimated to be at least ZAR60 million per annum. Because of its cryptic nature as well as the fact that *E. saccharina* is both indigenous to Africa and occurs on several host plants, attempts to control or eradicate it using several available methods have not been very successful. However, the sterile insect technique (SIT) is one of the newer control methods that can be incorporated into an area-wide integrated pest management (AW-IPM) programme to achieve better control or eradication. The implementation of the SIT program needs to go through a series of well-researched phases in order to be successful. In the first of this multi-phase project, it was determined that *E. saccharina* is susceptible to ionizing radiation, and is thus a suitable candidate for the SIT development against it and that a sub-sterilizing dose of 200 Gy is sufficient to induce F₁(inherited) sterility in male and complete sterility in female moths respectively. The results presented here are discussed in the context of further development of the SIT as an addition to the arsenal of tactics in an AW-IPM programme against *E. saccharina*.

Based on these initial findings, the study examined the lek and mating behavior of male moths subjected to three radiation doses (150, 200, 250 Gy) against normal non-irradiated/fertile moths. Both mass-rearing and irradiation of *E. saccharina* led to a quantitative departure of male mating behavior away from that exhibited by their wild counterparts. However, treated males are still able to form leks and mate with wild females. Male *E. saccharina* irradiated at all three doses tested were found to be as active and competitive as wild males, but in some of the traits measured, performance diminished significantly with an increase in the radiation dosage. In general, the performance of moths treated at 200 Gy did not differ significantly from that of moths treated at 150 Gy and therefore the former dose is ideal for SIT development since it results in a lower residual F₁ fertility than the latter.

The level of mating competitiveness and compatibility was assessed under both laboratory and semi-field conditions in pairwise comparisons consisting of laboratory reared vs. wild (L-W), 200 Gy irradiated vs. wild (S-W) and laboratory reared vs. irradiated moths (L-S). Based on the results from the more robust field cage assays, the mating indices generated indicated that the mass-reared *E. saccharina* strain produced in South Africa has not yet evolved sexual behaviours suggestive of incipient pre-mating isolation barriers with local wild strains. Wild moths did not discriminate against either the partially sterile or laboratory reared moths and most importantly, the irradiated males mated significantly more than their wild counterparts regardless of the type of female. The irradiated insects could therefore achieve the purpose for which they are intended upon release into the field.

Third, the critical thermal limits (CTLs) to activity at high and low temperatures (i.e. critical thermal maxima “CT_{max}” and minima “CT_{min}”) of different *E. saccharina* strains/treatments were investigated under standard experimental conditions. The effect of laboratory rearing and increasing radiation dosage on thermal tolerance of the adult stage of *E. saccharina* was explored. There were highly significant differences between the laboratory-reared and wild strain and also between non-irradiated and irradiated strains in both CT_{max} and CT_{min}. Laboratory reared *E. saccharina* moths were more heat tolerant compared to wild moths for both genders while in the case of CT_{min}, the reverse was true. Irradiation had a negative effect on both CT_{max} and CT_{min}. Moths treated at the lowest radiation dose were more cold and heat tolerant than those treated at higher dosages thereby reinforcing the importance of lower dosages rather than those that induce full sterility against *E. saccharina*. In general, gender effects on the CTLs were non-significant.

Pilot sterile male releases in shade house trials to measure the impact of sustained releases of partially sterile adult males at an over-flooding moth ratio of 10T: 1U (treated to untreated), were conducted to measure their efficacy to stop *E. saccharina* incursions and suppress populations prior to testing in pilot studies under true season-long and area wide conditions. Results from the current study demonstrated that releasing partially irradiated (200 Gy) adult male moths at the aforementioned release rate significantly reduced sugarcane stalk damage as well as lowered the number of fertile progeny from F₁ to succeeding generations in a stable *E. saccharina* population initiated in a cage house. There were more damaged internodes per stalk in the control than in the sugarcane receiving regular releases of partially sterile male moths. Overall, there were significantly more undamaged stalks in the treated sugarcane than the untreated control. Furthermore, there were significantly more larvae per stalk retrieved from the control compared to the treated sugarcane suggesting that the sustained release of steriles was efficacious in reducing emergence of fertile larvae in the succeeding generations. The results of this study indicate that there is considerable scope for the SIT against *E. saccharina*.

OPSOMMING

Die suikerriet boorder, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is die mees beperkende faktor in die Suid-Afrikaanse suikerbedryf met verliese aan hierdie insekplaag na beraming minstens ZAR60 miljoen per jaar. As gevolg van sy kriptiese aard, sowel as die feit dat *E. saccharina* beide inheems aan Afrika is en op verskeie gasheerplante voorkom, was pogings om dit te beheer of uit te roei deur verskeie metodes wat tans beskikbaar is, nie baie suksesvol nie. Die steriele insek tegniek (SIT) is egter een van die nuwer beheermetodes wat in 'n area-wye geïntegreerde plaagbestuur (AW-GPB) program gebruik kan word om beter beheer of uitroeiing te verkry. Die implementering van die SIT-program moet deur 'n reeks van goed nagevorsde fases gaan om suksesvol te wees. In die eerste van hierdie multi-fase projek, is dit bepaal dat *E. saccharina* vatbaar vir ioniserende bestraling is, en dus 'n geskikte kandidaat vir die SIT ontwikkeling teen dit is en dat 'n sub-steriliseringsdosis van 200 Gy voldoende is om F1 (oorgeërfde) steriliteit in mannetjie en volledige steriliteit in wyfiemotte onderskeidelik te veroorsaak. Die resultate wat hier aangebied word, word bespreek in die konteks van die verdere ontwikkeling van die SIT as 'n toevoeging tot die arsenaal van taktieke in 'n AW-GPB program teen *E. saccharina*.

Gebaseer op hierdie aanvanklike bevindings, het die huidige studie die lek en paringsgedrag van mannetjie motte wat aan drie bestralingsdosisse (150, 200, 250 Gy) blootgestel is teenoor normale nie-bestraalde/vruggbare motte ondersoek. Beide massateel en bestraling van *E. saccharina* het gelei tot 'n kwantitatiewe afwyking van manlike paringsgedrag weg van dit wat deur hul wilde eweknieë getoon word. Behandelde mannetjies is egter steeds in staat om leks te vorm en met wilde wyfies te paar. *E. saccharina* mannetjies, bestraal by al drie dosisse wat getoets is, is gevind om net so aktief en mededingend soos wilde mannetjies te wees, maar in 'n paar van die eienskappe wat gemeet is, het prestasie aansienlik verminder met 'n toename in die bestralingsdosis. In die algemeen het die prestasie van motte wat by 200 Gy behandel is nie beduidend verskil van dié van motte wat by 150 Gy behandel is nie en dus is die eersgenoemde dosis ideaal vir SIT ontwikkeling, aangesien dit tot 'n laer oorblywende F1 vrugbaarheid as laasgenoemde lei.

Die vlak van mededingendheid en verenigbaarheid van paring is ondersoek onder beide laboratorium en semi-veld toestande in paarsgewyse vergelykings bestaande uit laboratorium-geteelde teenoor wilde (L-W), 200 Gy bestraalde teenoor wilde (S-W) en laboratorium-geteelde teenoor bestraalde motte (L-S). Gebaseer op die resultate van die meer robuuste veldhok proewe, dui die parings indekse wat verkry is dat die massa-geteelde *E. saccharina* stam wat in Suid-Afrika geproduseer is nog nie seksuele gedrag ontwikkel het wat dui op die aanvang van voor-parings isolasie hindernisse met plaaslike wilde stamme nie. Wilde motte het nie onderskei tussen die gedeeltelik steriele of

laboratorium-geteeldemotte nie en, die belangrikste, die bestraalde mannetjies het beduidend meer as hul wilde eweknieë gepaar, ongeag van die tipe wyfie. Die bestraalde insekte kan dus die doel waarvoor hulle bestem is met vrystelling in die veld bereik.

Derdens is die kritieke termiese beperkings (CTLs) tot aktiwiteit teen hoë en lae temperature (d.w.s. kritiese termiese maksima "CTmaks" en minima "CTmin") van verskillende *E. saccharina* stamme/behandelings onder standaard eksperimentele toestande ondersoek. Die effek van laboratorium teling en toenemende bestralingsdosis op termiese toleransie van die volwasse stadium van *E. saccharina* is ondersoek. Daar was hoogs beduidende verskille tussen die laboratorium-geteelde en wilde stamme en ook tussen nie-bestraalde en bestraalde stamme in beide CTmaks-en CTmin. Laboratorium-geteelde *E. saccharina* motte was meer hittebestand in vergelyking met wilde motte vir beide geslagte, terwyl in die geval van CTmin, die omgekeerde waar was. Bestraling het 'n negatiewe uitwerking op beide CTmaks en CTmin gehad. Motte wat by die laagste bestralingsdosis behandel is, was meer koue- en hittebestand as dié wat by hoër dosisse behandel is, wat sodoende die belangrikheid van laer dosisse eerder as daardie wat volle steriliteit teen *E. saccharina* veroorsaak, beklemtoon. In die algemeen was geslagseffekte op die CTLs nie-beduidend.

Loodsproewe van steriele man loslatings in skaduwee huise om die impak van volgehoue loslatings van gedeeltelik steriele volwasse mannetjies teen 'n oorlaaide behandelde tot onbehandelde mot verhouding van 10T: 1U te meet, is gedoen om hul doeltreffendheid om *E. saccharina* voorkomste te stop en die bevolking te onderdruk te meet voor die toets van die konsep in loodsprojekte onder ware seisoenlange en area-wye toestande. Resultate van die huidige studie het getoon dat die vrystelling van gedeeltelik bestraalde (200 Gy) volwasse mannetjie motte by die voormelde vrylatingkoers suikerriet stamskade aansienlik verminder het sowel as die aantal vrugbare nageslag van F1 tot daaropvolgende geslagte in 'n stabiele *E. saccharina* bevolking wat in 'n veld hok begin is, verlaag het. Daar was meer beskadigde litte per stam in die kontrole as in die suikerriet wat gereelde vrystellings van gedeeltelik steriele mannetjie motte ontvang het. In die geheel, was daar aansienlik meer onbeskadigde stamme in die behandelde suikerriet as die onbehandelde kontrole. Verder was daar aansienlik meer larwes per stam opgespoor van die kontrole in vergelyking met die behandelde suikerriet wat daarop dui dat die volgehoue vrylating van sterielele effektief was in die vermindering van die uitkoms van vrugbare larwes in die daaropvolgende geslagte. Die resultate van hierdie studie dui daarop dat daar heelwat ruimte vir die SIT teen *E. Saccharina* is.

DEDICATION

I dedicate this thesis to my wife Precious and daughter Makanaka Natasha. Your constant support, love and understanding during the course of this work continue to make me the proudest husband and father. To my parents Richard Austin and Raviro Audrey Mudavanhu you have always been instrumental in my life, you nurtured and groomed me well, I know this is what you have always desired.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Pest status

The sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is the most destructive pest and most limiting factor to sugar production in South Africa (Paxton, 1982; Anonymous, 2005; Webster *et al.*, 2005; Goebel & Way, 2007). This pyralid moth is indigenous to, and widely distributed throughout sub-Saharan Africa (Assefa *et al.*, 2006). It assumed pest status in South Africa in 1939 (Dick, 1945; Carnegie, 1974) and in many parts of East, West and South Africa it has been known to cause damage to sugarcane (Atkinson, 1980; Mutambara-Mabveni, 2007; Chinheya *et al.*, 2009), maize and other cereal crops (Girling, 1972; 1978; 1980; Carnegie, 1974; Sampson & Kumar, 1985;). It has also been recorded on a number of alternate host plants including millet, rice, sorghum, cassava, sedges, large grasses and pigweed (*Amaranthus dubius*) (Carnegie, 1974; Conlong, 1997). Of the four developmental stages of *E. saccharina*, the larval stage is the most destructive (Fig. 1.1) (Way & Goebel, 2003). The larvae bore and tunnel into the sugarcane stalk causing extensive tissue damage, loss of sucrose and secondary infections by microorganisms typified by a red coloration of the borings and surrounding cane tissue (Fig. 1.1) (Way & Goebel, 2003; Walton, 2011). The valuable sucrose is metabolized into glucose as a result of the activity of microorganisms, which consequently results in overall decline in sugarcane quality, as less sucrose is extracted at the sugar milling plant (Way & Goebel, 2003). Annual losses to the South African sugar industry due to *E. saccharina* damage are estimated to be in the region of ZAR 60-153 million (Goebel & Way, 2007; Conlong, pers. comm.¹).

¹Prof. D.E. Conlong (PhD), South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa.



Fig. 1.1 Typical damage on a sugarcane stalk caused by a larva of *Eldana saccharina* (A). Red coloration of tissue surrounding the tunnel created by the larval boring is indicative of secondary infection by microorganisms.

1.2 Management and control

In South Africa, the current preferred methods of controlling this pest include varietal resistance and cultural control (Keeping, 2006; Conlong & Rutherford, 2009). Extensive research has also shown that damage can be minimized if insecticide applications can be timed to coincide with adult moth peaks occurring in April/May and September/October (Leslie, 1997; Leslie 2003) where the newly hatched foraging and dispersing neonate larvae are targeted (Leslie, 1997). Research is ongoing with respect to possible deployment of biological control (BC) as an addition to the arsenal of tactics used against *E. saccharina* (Conlong, 1990; 1994a; 1994c; 1997; Kasl, 2004; Barker *et al.*, 2006). Cultural, habitat management and conservation practices (Conlong, 1990; 1994a; 1994c; Kasl, 2004, Barker *et al.*, 2006, Smith *et al.*, 2006) which make the habitat more favorable for the activity of natural enemies; thereby enhancing pest suppression (Landis *et al.* 2000) would be a good complement to BC. However this has been curtailed by the apparent lack of establishment of many BC agents tested against *E. saccharina* in sugarcane (Walton, 2011). To date *E. saccharina* still continues to defy and frustrate existing control strategies in sub-Saharan Africa as it is indigenous to the region, is well established on the wide array of host plants on which it occurs and is very cryptic in nature (Conlong, 1994a; Conlong 1994b).

1.3. The sterile insect technique

In the development of an area-wide integrated pest management (AW-IPM) strategy against *E. saccharina*, increasingly more environmentally-friendly pest control techniques are being sought, which are compatible with and sometimes increase efficacy of more conventional control measures. The use of the sterile insect technique (SIT) is one of such newer methodologies being developed for suppression, containment or eradication of this key pest. The SIT is a strategy that imposes birth control on the pest population to further reduce its numbers (Klassen & Curtis, 2005). The method involves rearing the target insect pest species *en mass*, exposing them to ionizing radiation in order to induce sterility and then releasing reproductively sterile males into a wild population of the same species so that they mate with and block the reproduction of wild females (Knippling, 1955; Robinson, 2005). Consequently a whole generation would never see the light of day.

The earliest most successful AW-IPM programme incorporating the SIT dates back to the 1950s, a period which saw the strategy deployed in the south-eastern USA to eradicate the deadly livestock parasite *Cochliomya hominivorax* (Coquerel) (Diptera: Calliphoridae) (Klassen & Curtis, 2005). To date the technique is widely applied against a number of tephritid fruit flies (Enkerlin, 2005; Dyck *et al.* 2005; Caceres *et al.*, 2007; Gavriel *et al.*, 2012). The SIT has also been successfully implemented for the eradication, of the tsetse fly *Glossina austeni* Newst (Diptera: Glossinidae) in Tanzania, a well-known vector of diseases that are a threat to both humans and livestock in sub-Saharan Africa (Vreysen *et al.*, 2000). The SIT is also recognized as an environmental-friendly, species-specific and effective addition to AW-IPM programmes against many lepidopteran pests (Bloem *et al.*, 2005; Klansmen, 2005; Vreysen *et al.*, 2007a). Examples of such successful SIT initiatives include the Okanagan-Kootenay SIT programme against *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) in British Columbia (Bloem & Bloem, 2000), the *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) SIT project in San Joaquin Valley, California (Staten *et al.*, 1993; Hanneberry, 1994), the *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) project in the USA and the *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Carpenter *et al.*, 2007) and *C. pomonella* SIT (Bloem *et al.*, 2010) programmes in South Africa. Research has also been conducted on the field application of SIT for the containment of many other economically important Lepidopteran pests, for example *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) (North & Holt, 1969), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Carpenter *et al.*, 1987; Carpenter & Gross, 1993); the light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Soopaya *et al.*, 2011) and *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Mastro, 1993).

According to Calkins and Parker (2005), induction of sterility is one of the most critical steps in the SIT. During the advent of the technique, it was achieved by means of chemical treatment, but this had several problems with dose uniformity, toxicity of the compounds used, environmental contamination and concerns over worker safety, which all led to the almost universal adoption of gamma irradiation (Calkins & Parker, 2005). However, it is also well known that irradiation results in loss or reduction of insect quality (Knipling, 1979; Lux *et al.*, 2002; Calkins & Parker, 2005). Some of the negative effects of irradiation include reduction in mating competitiveness, loss or alteration of behavioural and/or fitness traits that enable survival in the wild (Toolson & Hadley, 1974; Knipling, 1979; Lux *et al.*, 2002; Bakri *et al.*, 2005; Weldon, 2005). The direct effects of radiation on living organisms include damage or lesions in radiosensitive somatic and germ cells due to free radicals created by irradiation in normal atmosphere which leads to a reduction of locomotor activity, depression of male fertility and superficial mimicry of the aging process (Clark & Rockstein, 1964; Ducoff, 1972; Eischen *et al.*, 1984; Bakri *et al.*, 2005; Calkins & Parker, 2005). Reducing the negative effects of radiation in order to maintain or enhance insect quality has therefore been the focus of many researchers seeking to develop the SIT as an area-wide pest control strategy. Some of the ways to achieve this include adjusting the timing of irradiation (i.e. with respect to life stage of development) (Ruhm & Calkins, 1981; Bakri *et al.*, 2005; Calkins & Parker, 2005), reduction or exclusion of oxygen in the radiation chambers and insect containers to prevent creation of free radicals (Robinson, 1975; Calkins & Parker, 2005) and lowering the sterility dose (Toledo *et al.*, 2004; Parker & Mehta, 2007).

Mass-rearing and laboratory domestication are necessary to produce large quantities of males required for the SIT (Weldon, 2005). Several authors have discussed the adverse effects of this domestication on the overall quality of insects to be released in the field. The artificial rearing conditions, under which the insects are produced, result in alteration of essential behavioural and physiological traits (Calkins & Parker, 2005) through domestication, acclimatization and selection (Ochieng-Odero, 1994). These traits include fecundity, pre-oviposition period, oviposition, courtship, development rate, pheromone production and the response thereof (Miyatake & Yamagishi, 1999), eye morphology, visual sensitivity, metabolic rate and resistance to stress (Mangan, 1992). Since conditions in mass-rearing facilities are different from those in the wild, individuals are subjected to unnatural selection pressures which result in increased fitness and reproductive advantage for the new environment but potentially creating a genetic bottleneck and limited gene pool (Iwahashi, 1996; Matos *et al.*, 2000; Calkins & Parker, 2005). The changes resulting from this domestication process may be disadvantageous to the mass-reared individuals upon release into the field resulting in reduced mating competitiveness in relation to their wild counterparts (Calkins & Parker, 2005). Some of the traits that may be selected over time as a consequence of mass-rearing include rapid

larval development, short pupal period, early sexual maturity, reduced pheromone production, abbreviated courtship behaviour and early fecundity (Miyatake & Yamagishi, 1999; Cayol, 2000).

Research and development of the SIT is continually aimed at enhancing mating competitiveness, mating compatibility, adult emergence and mobility in order to locate food, shelter, mating arenas and wild females, successful sperm and accessory gland fluids transfer as well as good survival of the mass-reared sterilized males (Calkins & Parker, 2005). Quality control tests that are conducted regularly have and continue to be developed for the assessment and monitoring of parameters necessary for the efficacy of the SIT in any AW-IPM programme that employs it. This is important to ensure the rearing process maintains the quality of insects being released in order that they remain competitive and compatible with the wild target population as well as to detect and correct any undesirable changes that may arise during production (Calkins et al., 1988; FAO/IAEA/USDA, 2003; Calkins & Parker, 2005).

1.4 Milestones and prospects for *Eldana saccharina* SIT development

The successful deployment of the SIT against a number of economically important lepidopteran pest species (already discussed in 1.3) has motivated the development of the technique as an addition to the arsenal of tactics for use against *E. saccharina*. Pioneer studies by Walton (2011) on the general biology, parental and inherited (F_1) sterility revealed that *E. saccharina* is a suitable candidate for SIT development. It was determined that exposing adult females and males to gamma radiation doses of 200 and 250 Gy, respectively, is sufficient to induce F_1 sterility in *E. saccharina*. While lepidopteran species are known to be highly resistant to the sterilising effects of radiation, thereby necessitating the use of high doses to induce full sterility in males (Carpenter *et al.*, 2005; Robinson, 2005), this could affect their overall fitness and mating behaviour to the detriment of the technique (Suckling *et al.*, 2011). Because of the necessity to maintain insect quality of steriles, inherited sterility is therefore desirable for SIT development in Lepidoptera as the approach has been shown to produce offspring with higher sterility, an F_1 sex ratio skewed in favour of males, lower fecundity, longer larval development time and higher mortality than their parents which have been subjected to sub-sterilizing doses (North, 1975; LaChance, 1985; Carpenter *et al.*, 2001; Carpenter *et al.*, 2005). Additional benefits of partial parental sterility include better flight ability and field performance; hence better control compared to fully sterile yet poorly competitive and physically unfit counterparts (Kean *et al.*, 2008, Suckling *et al.*, 2011). The offspring of partially sterile males and fertile wild females have a greater population suppression potential courtesy of the radiation-induced deleterious effects of the development of dominant lethal genes (Bloem *et al.*, 1999; Soopaya *et al.*, 2011). However the impact of sub-sterilizing doses on male mating behaviour

characteristics, compatibility with wild females, competitiveness with respect to their wild counterparts, pheromone production and quality as well as sperm competition in *E. saccharina* has never been investigated.

Marking can be used as a tool to distinguish between laboratory reared sterile moths and fertile wild moths and thus mark and recapture techniques for population monitoring, determination of over-flooding ratios and measurement of the success of an SIT programme against the target pest become a reality (Southwood, 1966; Hagler & Jackson, 2001; Qureshi *et al.*, 2004). Furthermore it can be used to distinguish different moth strains in laboratory bench top or field cage mating trials. Walton (2011) found that *E. saccharina* can be marked without detriment to their biology, by the addition of Calco Red N1700 to their larval diet, which stains their fat bodies red.

Some progress in the development of a monitoring method using Delta traps with crude lures made from crushed male moths for cage trials (Rutherford *et al.*, 2009) has been attained, but considerable work is needed on this aspect. In addition, studies on the mating behaviour of *E. saccharina* (Atkinson, 1981; Zagatti, 1981) have revealed that the emission of pheromones by male moths is involved in the courtship process resulting in copulation between both sexes. Haracca *et al.* (2011) conducted a direct chemical analysis of the content of the male hair pencil gland and determined that other compounds including amines and not those with a structure related to vanillin (as previously reported by Burger *et al.* (1993) may be the critical cues for *E. saccharina* sexual attraction and behaviour. These latest findings will be a guide for future research on the development of a pheromone trap for monitoring *E. saccharina* in the field.

1.5 Mating system

Lance and McInnis (2005) reviewed the diversity of mating courtship systems which can be categorized according to factors such as their relationship to ecological resources (Hendrichs *et al.*, 2002), type or degree of aggregation, type or extent of male-male competition (Robacker *et al.*, 1991; Hendrich *et al.*, 2002), mode of mate selection by females (Eberhard, 1996) or the involvement and type of semiochemicals used in calling/attraction (Lance & McInnis, 2005). Insect mating systems can either be classified as simple or complex. In simple mating systems there is scramble competition for females (Lance & McInnis, 2005), very little information is collected about the male prior to copulation or a female may be abruptly seized and mated by a male she was previously unaware of (Eberhard, 1985; Alexander *et al.*, 1997). The gypsy moth, *L. dispar* is a typical example of an insect species exhibiting simple mating (Lance & McInnis, 2005). However, in complex mating systems, the males initiate complex courtship rituals near a group of other calling males in locations referred to as

“leks” (Lance & McInnis, 2005), while females may discriminate amongst these males on the basis of their pre-mating signals by means of non-genitalic cues before copulation begins and sometimes “may” or “may not” use additional genitalia criteria (Alexander *et al.*, 1997). In addition, species with complex male mating-related behaviours, as a general rule, present the greatest challenge in producing highly competitive sterile males (Lance & McInnis, 2005). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is an example of an insect species employing a complex mating system (Lance & McInnis, 2005). Table 1.1 summarizes the characteristics of insect mating systems that are either favourable or unfavourable for the development and operation of SIT programs according to Lance and McInnis (2005).

Table 1.1 Characteristics of insect mating systems that are favourable or unfavourable for the development and operation of SIT programmes (Rewritten from Lance & McInnis, 2005).

Characteristic of mating system	Favourable	Unfavourable
Behavioural role of male, including any courtship ritual.	Simple	Complex
Female choice of mates	Passive (accepts first male)	Active (chooses among males)
Sex pheromone	Female-produced, simple (1- or 2 component), long range	Male-produced, complex
Adult male characteristics	Long-lived, active disperser	Short-lived, sedentary
Male-male competition	Indirect (scramble for mates)	Contest for mates or resources
Mating in time and space	Distributed throughout habitat, asynchronous	Highly aggregated, e.g., termite swarms

The mating behaviour of *E. saccharina* has been studied and described by Atkinson (1981) and Zagatti (1981). The fact that males produce the pheromone which elicits attraction of females (Atkinson, 1981; Zagatti, 1981) renders *E. saccharina* unique amongst the Lepidoptera species targeted for SIT. The attraction of females to calling males is aided by the formation of a lek (male aggregations in mating arenas) (Hendrichs *et al.*, 2002). Lek-based mating systems are rather complex and characterized by low male potential to monopolize resources and females exert mate choice (Hendrichs *et al.*, 2002). This type of lek polygyny has also been observed in some tropical and subtropical tephritid fruit flies such as *C. capitata* as well as a number of *Anastrepha* and *Bactrocera* spp. (Diptera: Tephritidae) (Prokopy, 1980). So complex is this type of mating system that increasing over-flooding ratios is rendered less effective in overcoming reduced sterile male competitiveness compared to species employing other mating systems (Hendrichs *et al.*, 2002). This is because wild

females prefer males timely releasing pheromone (Heath *et al.*, 1994) and performing proper visual, sound and tactile courtship behaviours (Eberhard, 2000). Females may therefore still favour the courtship of wild males even though they may represent a minority within a mixed lek (Hendrichs *et al.*, 2002). The successful application of the SIT for a species with a lek polygyny such as *E. saccharina* thus requires a more detailed understanding of its mating system. This in turn necessitates the need for a sophisticated quality control system for measuring and assuring sterile male performance. Though the mating system of *E. saccharina* is complex, it may not preclude the use of SIT (Lance & McInnis, 2005), but may influence the efficiency and logistical difficulty of its implementation. The greater the levels of complexity in the role of the male in mating, the more the effort required in monitoring male behaviour as part of product quality control (Hendrichs *et al.*, 2002; Parker, 2005) which will diminish expectations of high mating competitiveness of the mass-produced steriles (Lance & McInnis, 2005).

1.6 Mating compatibility and competitiveness

Population suppression through the SIT is a function of successful matings between irradiated males and wild females (McInnis *et al.*, 1994). Therefore the ability of released sterile males to compete with their wild counterparts for mates as well as their compatibility with the target wild females in the field are very critical (Cayol *et al.*, 2002; Lance & McInnis, 2005). Lance and McInnis (2005) define mating competitiveness in the concept of SIT as a function of the mating propensity and compatibility of sterile males with respect to their wild counterparts. Ultimately sterile males must be competent in their ability to communicate with wild females, as receiver and/or sender of signals, in order to be fully competitive (Lance & McInnis, 2005). According to FAO/IEA/USDA (2003) mating compatibility is a relative measure of how readily two insect populations or strains are reproductively compatible, and in the context of the SIT, it specifically refers to matings of sterile males with fertile wild females. Though mating speed or virility is an indicator of fitness, rapid matings tend to be controlled by the male genotype, while the female genotype is important for slower matings (Parsons, 1974). In mating systems where females exert mate choice, mating speed is often misleading as it largely reflects that males selected under conditions of extremely high population density obtain rapid matings without going through the proper courtship sequence (Calkins & Parker, 2005). This is because most females become receptive at the same time as a result of the prevailing 1:1 operational sex ratio to courting males (Calkins & Parker, 2005). Under natural conditions these males are often rejected by the wild females they are attempting to court in leks and hence are out-competed by their wild counterparts to the detriment of the pest control programme where sterile insects are being released (Briceno & Eberhard, 2002; Hendrichs *et al.*, 2002).

Reduced competitiveness in mass-produced sterile insects does not only emanate from problems in rearing, irradiation or handling as earlier noted but also from inherent incompatibility between different strains of the same insect species (Calkins & Parker, 2005). For example, insect strains/populations of the same species from different origins may be sexually incompatible as was reported in some *Ceratitis* and *Bactrocera* species from different islands of the world (McInnis *et al.*, 1996; Miyatake, 1998) and in *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) from different regions of South America (Vera *et al.*, 2006). Geographical isolation of different populations of the same species may allow the accumulation of changes, which may eventually lead to reproductive isolation and incipient speciation (Briceño *et al.*, 2002; Calkins & Parker, 2005). However, the level of mating compatibility can be assessed cost effectively by observing the degree to which individuals from two different populations interbreed when confined together (Taret *et al.*, 2010). These tests when conducted under conditions that are as natural as possible, can produce data that is useful for generating simple, reproducible and meaningful indices that describe and quantify mating compatibility and overall mating performance, hence making it possible to track performance of individuals as well as compare strains from different geographical regions (Cayol *et al.*, 1999; Taret *et al.*, 2010).

It has also been shown that mass-reared males are generally less able than wild males to induce wild females to copulate (Briceño *et al.*, 2007). It is also known that there is a tendency to maintain the same strain for long periods of time in many mass-rearing facilities around the world (Roessler, 1975) which leads to deterioration of insect quality after a certain number of generations (Partridge 1996). As a consequence, several aspects of male courtship behaviour are modified or changed during the process (Zapien *et al.*, 1983; Calcagno *et al.*, 1999; Briceño *et al.*, 2001). However it is not clear whether this inferiority is produced by these or other male traits (Eberhard, 2000). Nonetheless, such deviations in laboratory male behaviour may in the medium term lead to strain incompatibility and sexual isolation (Lux *et al.* 2002b; Orozco *et al.*, 2007). To the contrary, extensive comparisons of different strains of *C. capitata* from around the world (Cayol *et al.*, 1999; 2002) or *Anastrepha ludens* (Loew) (Diptera: Tephritidae) (Orozco *et al.*, 2007), with their respective laboratory reared strains revealed no significant mating barriers, indicating that any mass-reared strain is compatible with any wild population in these species (Calkins & Parker, 2005).

A change in the temporal mating period of the laboratory reared population – usually a consequence of intense un-natural selection pressures in mass-rearing facilities (Calkins 1984; Boake *et al.*, 1996; Iwahashi 1996; Briceño & Eberhard 1998), may lead to assortative mating where mating commences significantly earlier when compared to the wild population (Calkins & Parker, 2005). In addition, colonization during establishment of a mass-reared strain can also alter the mating behaviour of

laboratory-reared organisms as well as promote selection for assortative mating traits (Huho *et al.*, 2007). The evolution of assortative mating preferences reduces ability of laboratory reared males to mate with wild-type female conspecifics, and can occur in as few as three generations of laboratory maintenance (Spates & Hightower, 1967; Reisen, 2003). Evidence of assortative mating between wild and laboratory reared strains has been reported in releases of *Culex tritaeniorhynchus* Giles (Diptera: Culicidae) (Reisen *et al.*, 1980) and *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) (Wong *et al.*, 1982). Another factor which has been shown to influence mating competitiveness in relation to SIT is size of males, where females prefer larger males over smaller ones because the former are more competitive against rival males (Calkins & Parker, 2005), disperse farther and live longer in field releases (Bloem *et al.*, 1994). Examples of species where size matters include *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) (Burk & Webb, 1983) and *C. capitata* (Economopoulos *et al.*, 1993; Bloem *et al.*, 1994). However in Lepidoptera, large adults from large pupae are less competitive and not amenable with field release due to reduced flight ability (Calkins & Parker, 2005).

Simple mating systems are highly desirable for the SIT and can lead to production of highly competitive sterile males but are often associated with short adult life spans and compressed mating periods (Lance & McInnis, 2005). Species with complex mating systems are such that slight variations between the wild and sterile strain can translate into poor competitiveness (Lance *et al.*, 2000). On the other hand natural selection may potentially favour wild females that are proficient at identifying and rejecting sterile males, resulting in wild populations that are behaviourally resistant to the SIT (Ito & Yamura, 2005; Lance & McInnis, 2005; Whitten & Mahon, 2005).

The effectiveness of mating between sterile males and wild females can be lost partially or entirely in the event that the targeted wild females re-mate with wild males and preferentially use sperm from the latter for fertilization (Lance & McInnis, 2005). Knipling (1955) asserts that it is more desirable for the SIT if females mate only once, but in the event that they mate more frequently, then the sperm from irradiated (sterile) males must be produced in essentially the same quantity and must be able to compete sufficiently with sperm from the fertile wild males. However it has been determined that the competitiveness of sterile males is greatly influenced by post-copulatory factors, including ability to induce mating refractoriness in females, sperm competition and/or sperm precedence regardless of the number of times a female normally mates (Lance & McInnis, 2005).

The transfer of a full complement of sperm is a critical factor that may turn off female receptiveness in some insect species and hence SIT programmes against such should ensure that sterility is based on dominant lethal mutations rather than elimination of sperm production in order that the released steriles remain competitive (LaChance, 1975). The offspring of sub-sterilized males may transfer less

than a full complement of sperm (Proshold *et al.*, 1993; Carpenter *et al.*, 2005) thus giving inherited (F_1) sterility the advantage over full sterility. Conversely, the radiation dose used to induce reproductive sterility in a given species can reduce the quantity and/or quality of a male's sperm (LaChance *et al.*, 1979; Proshold *et al.*, 1993). In addition, the sperm in irradiated males are often depleted faster than in non-irradiated males after just a few matings (Haynes & Mitchell, 1977). Sterilization-related reductions in the amount of sperm transferred to wild females can therefore reduce sterile male competitiveness by increasing the likelihood of remating among females that copulate with both sterile and wild males (Haynes & Mitchell, 1977; Carpenter *et al.*, 1987). Walton (2011) reported that *E. saccharina* females in the laboratory are capable of mating more than once. Where females mate frequently with both sterile and wild males the proportion of eggs fertilized by sperm of sterile males may be influenced by patterns of sperm precedence and/or competitiveness of male ejaculate (Lance & McInnis, 2005). However in several insect species, sperm from recent matings takes precedence over that from earlier matings (Brower, 1975; Saul & McCombs, 1993) and where this phenomenon is complete, specialized mechanisms exist to exclude sperm from previous matings (Waage, 1979). The main factors influencing ejaculate competitiveness – another determinant of the proportion of offspring fertilized by males of a given strain include, male age and quantity and quality of sperm transferred (Saul & McCombes, 1993; Alyokhin & Ferro, 1999; LaMunyon & Huffman, 2001). Sterilization procedures (LaMunyon, 2001), radiation dose (Carpenter *et al.*, 1997) and age at irradiation (Villavaso *et al.*, 1998) can reduce the proportion of eggs laid by females that are characterized by multiple matings.

Eldana saccharina is indigenous to Africa and at least three biotypes of this pest have been identified based on geographical isolation in Africa (Assefa *et al.*, 2006). It is therefore necessary that the existence of any compatibility issues be investigated, given the geographical distribution of this pest across the African continent. It can be envisioned that facilities in countries implementing sterile releases to manage a common pest problem but situated in different hemispheres could complement each other in that instead of scaling down operations (off-season), one country could supply sterile moths to the other experiencing a high demand and vice versa (Taret *et al.*, 2010). This would result in increased commercialization and hence optimization of investments in mass-rearing facilities (Bloem *et al.*, 2010). In addition, it would result in efficient and cost effective SIT programmes as nations collaborate and work in partnership on a regional and inter-regional basis to achieve a common goal.

1.7 Insect thermal biology

Temperature affects a range of biochemical and physiological processes in living organisms (Chown & Nicholson, 2004) and therefore this abiotic factor influences insect population dynamics (Nyamukondiwa & Terblanche, 2009). An insect's body temperature closely resembles ambient temperatures and hence extreme thermal conditions can be harmful to their fitness (Chown & Nicholson, 2004; Angilletta, 2009). While insects are also exposed to some form of thermal stress during their life-cycle due to environmental temperature fluctuation (Feder *et al.*, 2000; Chown & Terblanche, 2007; Nyamukondiwa & Terblanche, 2009), they respond by altering behaviour (over short time-scales), physiological compensation via acclimatization or diapause (short to intermediate time-scales within or between generations) or by physiological adaptations (evolved over longer timescales between generations) (Angilletta, 2009). Over longer time-scales such fluctuations affect seasonality and evolutionary responses (Chown & Nicholson, 2004; Lee & Denlinger, 2010). At sub-lethal temperatures, rate of resource acquisition and consumption are significantly affected and consequently also growth, development and reproduction (Stotter & Terblanche, 2009). Since temperature significantly influences likelihood of mortality, population decline is also inevitable at extremes (Hoffmann *et al.*, 2003; Chown & Terblanche, 2007). The ability to withstand thermal stress is therefore significant for the success of insect populations and evolutionary fitness in the wild (Toolson & Hadley, 1974; Loeschcke & Hoffmann, 2007; Sørensen *et al.*, 2009).

As earlier noted, minimizing sources of variation that may obscure detection of relationships between outcome variable and hypothetical causative or correlated factors of interest is a major objective borne in the mind of the researcher implementing any laboratory-based experimentation (Huho *et al.*, 2007). This can be achieved through studying organisms in simplified environments that simulate conditions in the field and any bias culminating from these simplifications is deemed acceptable when weighed against the powerful hypothesis testing permitted in such experimentation (Huho *et al.*, 2007). It is, therefore, critical that the physiology and behaviour of laboratory reared individuals closely represent those from the wild if estimation of parameters, in order to guide implementation of interventions targeted at natural populations, is the aim of such laboratory assays (Huho *et al.*, 2007).

Since mating ability, survival and fitness of mass-produced sterile males when released into the wild is critical to the success of the SIT, their field performance remains one of the greatest challenges to success of the SIT (Enserink, 2007; Terblanche & Chown, 2007; Simmons *et al.*, 2010; Chidawanyika, 2010). Sterile insect quality incorporates aspects of an insect's biology, physiology and behaviour. It is therefore critical that the released sterile males remain fit under thermal stress, respond to biotic

cues under field conditions and behave accordingly in order to achieve mating (Chidawanyika, 2010; Chidawanyika & Terblanche, 2011). While sterile insect quality can be improved by lowering radiation dose (Bloem *et al.*, 1999a; Judd & Gardiner, 2006), including diapause in the rearing regime (Bloem *et al.*, 1997; Judd *et al.*, 2006a), or exploiting the combination of both (Bloem *et al.*, 2004), thermal preconditioning is one such way that has never been established in *E. saccharina*. Typically, the mass-rearing and maintenance of *E. saccharina* employs only constant, optimal temperatures for the purpose of maximising rearing productivity (Graham & Conlong, 1988; Bloem *et al.*, 2004) despite the very different environmental conditions the steriles may be released into (Chidawanyika, 2011). This could have dire consequences with respect to competitiveness and fitness of sterile moths upon release into the field. For example, Fay and Meats (1987) made the assertion that thermal treatment of mass-reared steriles before releasing them into the field could enhance the performance of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) under conditions of low temperature.

Studies on the temperature biology of this insect pest have mainly been focussed on its reproduction (Way, 1994) and day-degree modelling (Way, 1995). According to a review by Way (1995) on the developmental biology of the immature stages of *E. saccharina* the lower development temperature thresholds were determined to be as follows: egg (5.3 °C), larvae (10.2 °C) and pupae (10.7 °C). His results confirmed earlier findings by Atkinson (1980). The total development time of *E. saccharina* from egg to adult was estimated to be 897.9 Day Degrees (DD) (Way, 1995). Day degrees are a measure of the number of heat units required over time to complete development of a given insect life stage above the lower/base development temperature threshold (Way, 1995). Atkinson (1980) also determined that the temperature below which males ceased to perform courtship displays was 15°C suggesting that mating also ceases to occur at about this temperature for *E. saccharina*. Thermal limits to activity in *E. saccharina* have not been explored, yet field performance, seasonality, evolutionary responses (Chown & Nicholson, 2004; Lee & Denlinger, 2010), geographical distribution (Bahrndorff *et al.*, 2009) and use of the SIT for control and eradication could be influenced by the insect's thermal tolerance. Huey and Stevenson (1979) illustrated how the relationship between body temperature and performance in ectothermic organisms is bounded by their critical thermal limits. The maximum performance (e.g. mating, locomotion, development and nutrient digestion; Chidawanyika & Terblanche, 2011) can be described by means of a performance curve (Fig. 1.2.; Angilletta *et al.*, 2002, Chown & Nicolson, 2004). This occurs at an optimal body temperature where the thermal performance breadth is the range of the body temperature that permits a certain level of performance (Fig. 1.2.; Huey & Stevenson, 1979).

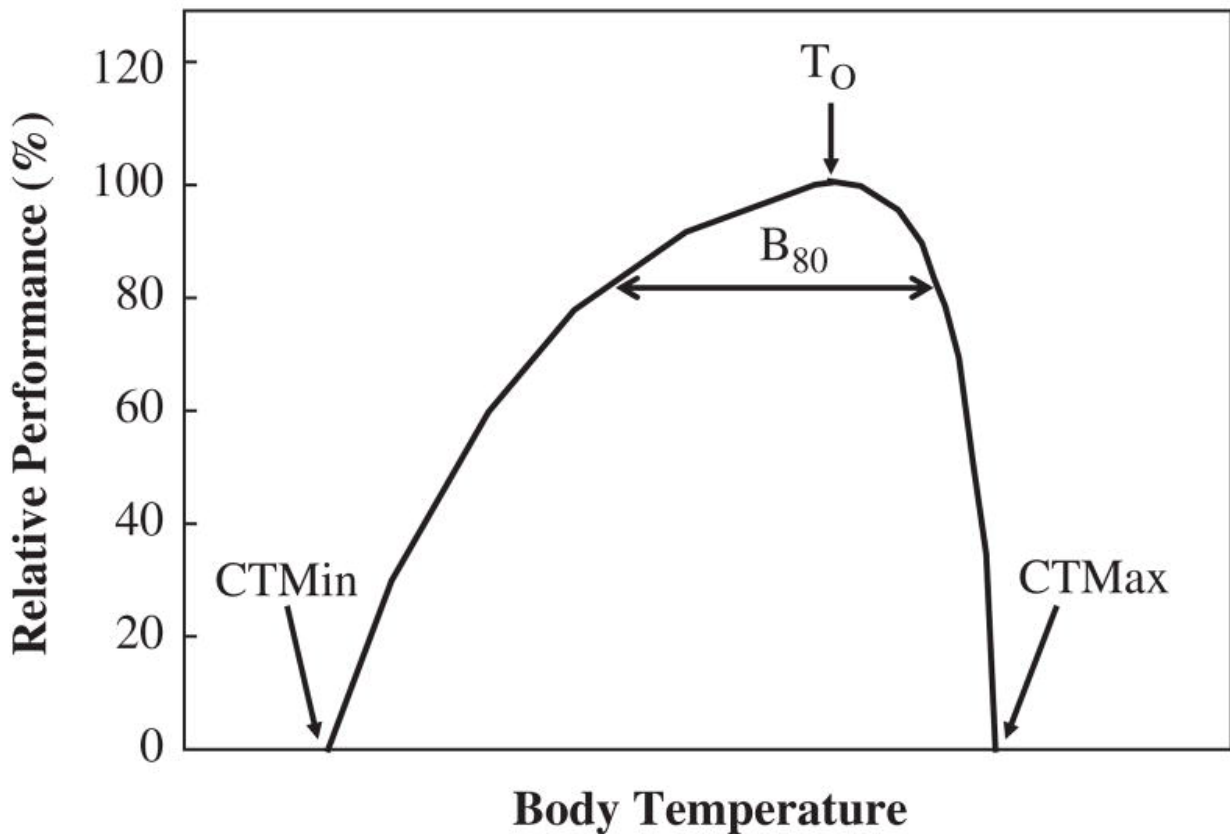


Fig. 1.2 The generalised thermal performance curve for insects showing the optimum temperature (T_o), performance breadth (B_o) and critical thermal maxima and minima (CTmax and CTmin) (Redrawn from Angilletta *et al.*, 2002).

Acclimation or evolutionary adaptation can however shift the performance curve (e.g. Gilchrist *et al.*, 1997; Deere & Chown, 2006; Angilletta *et al.*, 2002) resulting in changes in its position and shape. Insects can therefore be assisted by these thermal adaptations in achieving higher evolutionary fitness e.g. by either prolonging or improving flight in unfavourable or favourable conditions respectively (Chidawanyika, 2011; Chidawanyika & Terblanche, 2011). Extreme temperatures can influence physiological processes in living organisms and in the worst case scenario they can be lethal depending on the severity and duration of exposure (Cossins & Bowler, 1987). Every living organism has an optimal temperature range within which it is able to sustain life and biological functions, thus polar species are sensitive to warm temperatures but more tolerant to cold temperatures while the opposite is true for tropical species (Addo-Bediako *et al.*, 2000). The performance curve is therefore very convenient and useful for describing an insect's capacity response to temperature (Chown & Terblanche, 2007) and can be applied to any quantitative trait e.g. egg production, rate of development as well as metabolic efficiency (Denlinger & Yocum, 1998). Sub-lethal low temperatures are more important than sub-lethal high temperatures as insects tend to exhibit a wider range of responses to the former than in the case of the latter (Addo-Bediako *et al.*, 2000). In addition there is a steeper drop in performance at temperatures above the optimum compared to that at lower temperatures (Martin & Huey, 2008).

It is important to acknowledge that like any SIT enterprise where sterile insects are mass-produced, *E. saccharina* will typically be chilled for the purpose of irradiation, handling and sorting prior to and during field release (Carpenter *et al.*, 2007; Carpenter *et al.*, 2010; Simmons *et al.*, 2010). Stotter and Terblanche (2009) noted that the effect of such rapid chilling on subsequent extreme temperatures experienced by these laboratory-reared insects is poorly understood. This may compromise their field performance, survival and consequently the SIT programme (Terblanche *et al.*, 2008). For example in certain Diptera taxa, a rapid cold hardening response (Lee *et al.*, 1987; Nilson *et al.*, 2006), could result in recovery of the chilled individuals during handling and transport, which in turn could negatively affect the efficacy of laboratory work and SIT programmes (Terblanche *et al.*, 2008). There is also a risk of insects acquiring resistance during mass-rearing to these pre-release chill treatments, which would be detrimental to the SIT programme (Stotter & Terblanche, 2009). Furthermore the wide difference in physiology between mass-reared colonies and field populations (Terblanche *et al.*, 2006) could be a threat to mating compatibility and competitiveness of the former upon release into wild populations. It has also been found that physiological acclimation at high temperatures may accelerate age-dependant decline in heat resistance (Davison, 1971).

However there are merits to cold acclimation where in certain species, for example *Chymomyza costata* (Zetterstedt) (Diptera: Drosophilidae), it may contribute to freeze tolerance (Shimada & Riihimaa, 1988). Pre-exposure to sub-lethal environments (also referred to as rapid cold-hardening 'RCH' or rapid heat-hardening 'RHH') will therefore enable mass-reared strains of adult *E. saccharina* to survive and persist in otherwise lethal ambient temperatures (Slabber & Chown, 2005; Loeschcke & Hoffmann, 2007). This kind of manipulation will enhance temperature-dependent performance and survival in a variable thermal environment (Hochachka & Somero 2002; Angilletta 2009), to the benefit of such control tactics as the SIT (Bloem *et al.*, 2006; Chidawanyika & Terblanche, 2011). The costs and benefits of such thermal acclimation on field performance in relation to its importance to SIT have been shown in for example, *Drosophila* (Diptera: Drosophilidae), where recapture rates of cold-acclimated flies was higher than that of non-acclimated flies, suggesting strong benefits for acclimation in the field (Kristensen *et al.*, 2008). This was also true for warm-acclimated flies under warmer environmental conditions (Loeschcke & Hoffmann, 2007; Kristensen *et al.*, 2007) and similarly in the codling moth, *C. pomonella* (Chidawanyika & Terblanche, 2011). Phenotypic plasticity therefore plays an important role in altering behaviour and field performance (Kristensen *et al.*, 2008) which could be of practical value in manipulating field performance with potential improved efficacy in an SIT programme (Chidawanyika & Terblanche, 2011). No studies have been conducted on *E. saccharina* to demonstrate and resolve the above aspects and their implications on any future SIT initiative against this pest. The initial step towards achieving this would therefore be to determine

the upper and lower critical thermal limits (i.e. CT_{max} and CT_{min}, respectively) to activity of adult *E. saccharina* (as it is the life stage that is critical for the SIT). A further step would be to investigate how and to what extent these limits can be altered as a result of factors such as thermal history either within its own or parental lifetime (Crill *et al.*, 1996; Hoffmann *et al.*, 2003; Chown & Nicolson, 2004), age, body size, feeding status (Nyamukondiwa & Terblanche, 2009), development (life stage) and gender (Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009), origin and irradiation (Toolson & Hadley, 1974; Huho *et al.*, 2007).

1.8 Investigation protocols

1.8.1. Field cage trials

It has been shown that conducting competitiveness or compatibility tests in the laboratory may not reliably indicate the situation in the field environment where a given species is naturally adapted (Vreysen, 2005). However the use of field cages with host trees (Taret *et al.*, 2010), provides a much better measure of these parameters (Calkins & Webb, 1983; Chambers *et al.*, 1983) as they act as a good compromise between laboratory conditions and open field trials (Taret *et al.*, 2010). Field cage experiments are also useful for more detailed assessments of insect behaviour such as time of mating, mating duration, pheromone calling and the sequence and timing of specific behaviour components (Calkins & Parker, 2005). The downside of the field cage is that, although tests are conducted under natural conditions and host trees involved, the test individuals confined within, cannot freely fly away or “escape” from the cage and neither can newcomers mix with the caged individuals. The value of the test is thus reduced in this regard (Economopoulos & Mavrikakis, 2002).

1.8.2 Pilot sterile insect releases

Releases of irradiated moths in pilot studies to suppress wild population can be done using two approaches namely the conventional system approach (Kunz *et al.*, 1984; Bloem & Bloem, 2000; Walters *et al.*, 2000) or the green/shade house approach (Rosca & Barbulescu, 1993; Sutrisno & Hoedaya, 1993; Calvitti *et al.*, 1997; 1998; 2000; Hofmeyr *et al.*, 2005). In the conventional approach, study sites are carefully chosen, where pilot releases of the sterile insects are completed and populations monitored closely over a number of years to measure the impact of SIT releases. However this may be inappropriate if information on the basic biology of the species in question is unknown. The approach is also not feasible in the absence of a good and reliable field population monitoring method as is the current scenario with *E. saccharina*. In addition the current mass-rearing of *E. saccharina* still needs improvement and methodology changes, in order to provide the numbers

of irradiated moths need for pilot studies in South Africa (Conlong, pers. comm.²). Furthermore the lack of a radiation facility in or close to the geographical region targeted for pilot SIT releases raises logistical problems with regards to getting mass-reared males irradiated and released in the pilot sites on the night of irradiation. Therefore, researchers developing the SIT programme against a given pest species cannot proceed with the conventional system approach prior to solving the aforementioned aspects.

The shade house approach can provide valuable insights into the success/potential of the technique in both preventing spread and suppressing existing populations of a pest species targeted for control using the SIT (Calvitti *et al.*, 1997; 1998; 2000). The advantages of this approach is that it can provide an indication of the possibility of controlling the pest in actual field releases (Rosca & Barbulescu, 1993), it can be run concurrently while biology and behavior assays are being completed and can generate valuable data that can be used to evaluate the concept in the short or medium term.

1.9 Objectives of the study

The aim of the study was to improve SIT for *E. saccharina* as follows:

1. To investigate the effect of gamma irradiation on the mating propensity and behaviour characteristics of *E. saccharina* (Chapter 2).
2. To assess levels of mating competitiveness and compatibility between different strains of *E. saccharina* under laboratory and semi-field conditions (Chapter 3).
3. To investigate the thermal physiology of the adult stage of *E. saccharina* (Chapter 4).
4. To conduct a pilot study on efficacy of the SIT for future area-wide control, suppression or eradication of *E. saccharina* (Chapter 5).

In chapter 2, the hypothesis that laboratory domesticated and gamma-irradiated *E. saccharina* moths are as competitive in lek formation and mating as their 'wild' counterparts is specifically tested. The impact of gamma radiation on following mating behaviour parameters is investigated:

- onset time of male calling
- onset time of mating
- mating duration
- mating success (mating frequency)

In chapter 3, the specific objectives investigated include:

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- effect of laboratory rearing on mating competitiveness with wild moths
- effect of sub-sterilization (at 200Gy - the dose of choice for further development of *E. saccharina* SIT) on mating competitiveness between laboratory and irradiated moths as well as between wild and irradiated moths
- the possibility of additive or synergistic effects due to laboratory rearing and irradiation on mating competitiveness and isolation
- generating mating indices that quantify and describe mating compatibility and competitiveness of different strains or treatments of *E. saccharina*

In chapter 4, the following objectives are investigated:

- determining the critical thermal limits to activity at high and low temperatures (CT_{max} and CT_{min} respectively) of different strains of *E. saccharina*
- exploring the effect of laboratory rearing and increasing radiation dosage on thermal tolerance of the adult stage of *E. saccharina*

The objective of chapter 5 is to conduct pilot releases of irradiated *E. saccharina* moths in shade house trials with the aims of:

- suppressing an existing *E. saccharina* infestation on sugarcane
- reducing levels of damage in a sugarcane plot treated by periodic release of sterile moths

1.10 References

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CHAPTER 2

THE EFFECT OF GAMMA RADIATION ON THE MATING BEHAVIOUR CHARACTERISTICS OF *ELDANA SACCHARINA*

2.1 Introduction

The successful implementation and efficacy of any pest control method depends on sound knowledge and understanding of the biology, behaviour and ecology of the target pest (Liedo *et al.*, 2002). Since the advent of the Sterile Insect Technique (SIT), the mating competitiveness of the sterile insects was recognized as a critical aspect for the successful application of the technique (Knipling, 1955). Mass-rearing is necessary to produce large quantities of males required for the SIT, but the laboratory environment imposes unnatural selection pressures to which a population must adapt, resulting in increased fitness for the new environment and not the field environment, enabling the laboratory population to persist (Iwahashi, 1996; Matos *et al.*, 2000). The strong laboratory selection may result in the alteration or loss of ecological and behavioural traits that are necessary for mass-reared males to remain competitive when released in the field (Saul & McCombs, 1995; Iwahashi, 1996; Dalby-Ball & Meats, 2000; Briceno & Eberhard, 2002). While it has been demonstrated in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) that sterile males are still capable of locating hosts and females in mating arenas or leks, mixing and interacting with their wild counterparts, the intense selection occurring under mass-rearing conditions caused slight quantitative changes in the courtship displays of males which resulted in female rejection (Liedo *et al.*, 2002).

The induction of sterility, on the other hand, is an essential step in the SIT (Calkins & Parker, 2005). The most common and almost universally adopted method of sterilization is through gamma irradiation, primarily due to its safety and dose delivery uniformity when compared to other methods (Calkins & Parker, 2005; Blomefield *et al.*, 2009). However, Bakri *et al.* (2005) stated that in many insect groups, irradiation results in a reduction in competitiveness due to adverse somatic effects induced by radiation during the sterilization process. Irradiation in air also creates free radicals that are detrimental to quality of the insects being sterilized (Calkins & Parker, 2005). Several studies on fruit fly species SIT have demonstrated that the exposure to irradiation in order to induce sterility affects mating performance of exposed fruit flies (Holbrook & Fujimoto, 1970; Hooper 1971; Knipling 1979; Lux *et al.*, 2002). It has now been shown that the adverse effects of selection pressures associated with mass-rearing on mating competitiveness of steriles are further compounded by irradiation (Leppla *et al.*, 1983; Harris *et al.*, 1986; Weldon, 2005).

Studies on the radiation biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) have been conducted and this economically important pest of sugarcane (Anonymous, 2005; Webster *et al.*, 2005; Goebel & Way, 2007) has been considered a suitable candidate for Sterile Insect Technique (SIT) development. Walton *et al.* (2011) demonstrated that at low doses of radiation (150-250Gy) F₁ sterility of the sugarcane stalk borer *E. saccharina* can be attained. There is thus considerable scope for further development of an efficient SIT programme against *E. saccharina*. However, the effect of radiation on successful mating needs to be determined. A better understanding of the courtship behaviour and mating system of an insect species targeted for SIT and how it is influenced by mass-rearing and irradiation, may lead to improvements in sterile male performance (Hendrichs *et al.*, 2002; Liedo *et al.*, 2002). This would also reduce the high sterile: wild flooding ratios routinely applied to compensate for the low effectiveness of mass-produced steriles (Hendrichs *et al.*, 2002). The performance is estimated to be between a third to half as competitive as wild males (FAO/IAEA/USDA, 2003). Should mating competitiveness be enhanced, it would significantly lower the costs of SIT application.

In this study a series of tests was developed to confirm that behavioural traits leading to successful mating and insemination of the targeted wild females are not lost in the mass-reared and sterilized male insects. The objectives of this study were to investigate the impact of gamma radiation on the onset time of calling, onset time of mating, mating duration and mating success (mating frequency). The hypothesis that laboratory domesticated and gamma irradiated male *E. saccharina* adults are as competitive in lek formation and mating as 'wild' *E. saccharina* moths was tested. The general goal of this research project was to investigate the effect of gamma irradiation on the mating propensity of *E. saccharina* and to determine a dose that of radiation which least effects this propensity.

2.2 Material and methods

2.2.1 Study populations

The mating behaviour of wild, mass-reared (laboratory domesticated) and sterile (laboratory domesticated, gamma irradiated) male with wild female adult *E. saccharina* was studied. Wild *E. saccharina* were collected from various sugarcane plantations in the KwaZulu-Natal Province either as sixth instar larvae and reared to pupal stage on 8 ml of artificial *E. saccharina* diet (Walton, 2011) or as pupae and stored singly in transparent 32 cell multi-cell eclosion trays sealed with perforated cling wrap in incubators (Huber® CC 410 WL, Olfenburg, Germany) at 27 ± 2°C, 75 ± 5 % relative humidity (RH) and (10: 14) light: dark (L:D) cycle. Mass-reared *E. saccharina* were also obtained as

pupae from the mass-rearing facility at the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe, Durban. The rearing procedure at SASRI is such that neonate larvae are reared in 32-cavity plastic multi-cell trays containing artificial diet described by Gillespie (1993) and held in rearing rooms ($28 \pm 2^\circ\text{C}$; $75 \pm 5\%$ RH; 0: 24 L: D cycle) for approximately 619 day degrees (DD) (Walton, 2011). Pupae are then harvested from the artificial diet and transferred to an adult emergence room ($27 \pm 2^\circ\text{C}$; $75 \pm 5\%$ RH; 8: 16 L: D cycle) for adults emergence mating (Walton, 2011). Eggs are laid on paper towelling and placed into incubators ($24 \pm 2^\circ\text{C}$; $75 \pm 5\%$ RH; 0: 24 L: D) for 119 DD, until neonate larvae eclosed from the eggs (Way, 1995; Walton 2011). The mass-reared *E. saccharina* were marked by addition of Calco Red to their larval diet, a tool that distinguishes them from wild moths (Walton & Conlong, 2008). The live insects were couriered by air in the described containers as pupae to the Conservation Ecology and Entomology Department at Stellenbosch University in the Western Cape Province, where all the experiments were conducted. On arrival the pupae from the different populations were maintained under the same conditions as described above except that the L: D cycle was adjusted to 10: 14 in the Entomology Department at Stellenbosch University. The pupae were checked at 08H00 daily for eclosion. Newly emerged adults were separated by sex and strain type (in groups of ten individuals) into cylindrical transparent plastic containers (200mm X 100mm) with perforated lids for ventilation and prevention of escape. Adults were provided with paper towel to perch on as well as prevent damage to scales of the highly active moths. The sterile adults were obtained by irradiating zero-day old (freshly eclosed) laboratory reared adult males using gamma-radiation from a ^{60}Co source in normal atmosphere at the SIT Africa Pty (LTD) radiation facility in Stellenbosch³ ($33^\circ 55' 26''$ S, $18^\circ 52' 25''$ E). Irradiation treatments commenced at 08H00 and the adults were exposed to increasing doses delivered at a rate of 375.36 rads/min. Radiation doses to which the groups of adults were exposed were 150; 200 and 250 gray (Gy). The moths were transported to the Stellenbosch University Entomology Department (3 km distance) in the same containers in which they were irradiated. Experiments were conducted approximately seven hours after exposure to radiation.

2.2.2 Experimental design and observations

A ventilated Perspex still-air cage (300 x 300 x 300 mm) with a transparent lid was used as a mating arena and installed in a humidity, temperature and light controlled room (720 m³). A Daikin® air conditioner model FTY-50 (Daikin Industries Ltd, Daikin, India) and a saturated sodium chloride (NaCl) solution were used to maintain temperature at $27 \pm 2^\circ\text{C}$ and relative humidity $75 \pm 5\%$ respectively. A (10: 14) light: dark (L: D) cycle was also maintained using six fluorescent lamps (Osram-L 65 W/25 S, Universal-White; photon fluence rate 30 to 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) controlled by an automatic time

³SIT Africa Pty (LTD), ARC-Infruitec/Nietvoorbij, Helshoogte Road, Private Bag X5026, Stellenbosch, 7599.

switch. These are the average photophase and scotophase durations for which pupae (for eclosion) and adults (for pairing) are maintained during mass-rearing (Walton, 2011). In addition, the L: D cycle generally resembles that of the KwaZulu-Natal Province (South African Weather Service, 2010) during April/May and September/October moth peak periods (Leslie, 1997; 2003). A Logitech® Pro 9000 webcam to record video footage of moth activity was placed 400 mm above the mating arena - the elevation at which all insects and activity in the arena could be captured as video footage. The mating arena was also illuminated by a 100 watt Phillips® IR95 infrared light bulb (Fig. 2.1), since *E. saccharina* moths mate during the dark (Atkinson, 1981; Zagatti, 1981). Virgin male and female moths designated as zero-day old on the day of emergence were used for the bioassays and were released simultaneously into the mating arena via the lid of the test cage approximately one hour before commencement of experiments (i.e. at lights off) to allow the test insects to calm down and get accustomed to the test environment. Since *E. saccharina* moths are sexually mature on their first night of eclosion (Atkinson, 1981) and their propensity to mate is highest on the first and second scotophase (Walton, 2011), mating behavior was observed continuously during the first and second scotophase after adult emergence. Mating behavior was observed and recorded the following morning by playing back the 14-hour video footage of adult behavior captured on camera. The terms scotophase and night refer to the same and hence are used interchangeably in the text. Video recording of moth activity in the mating arena during each scotophase commenced at 18H00 and lasted for 14 hours.

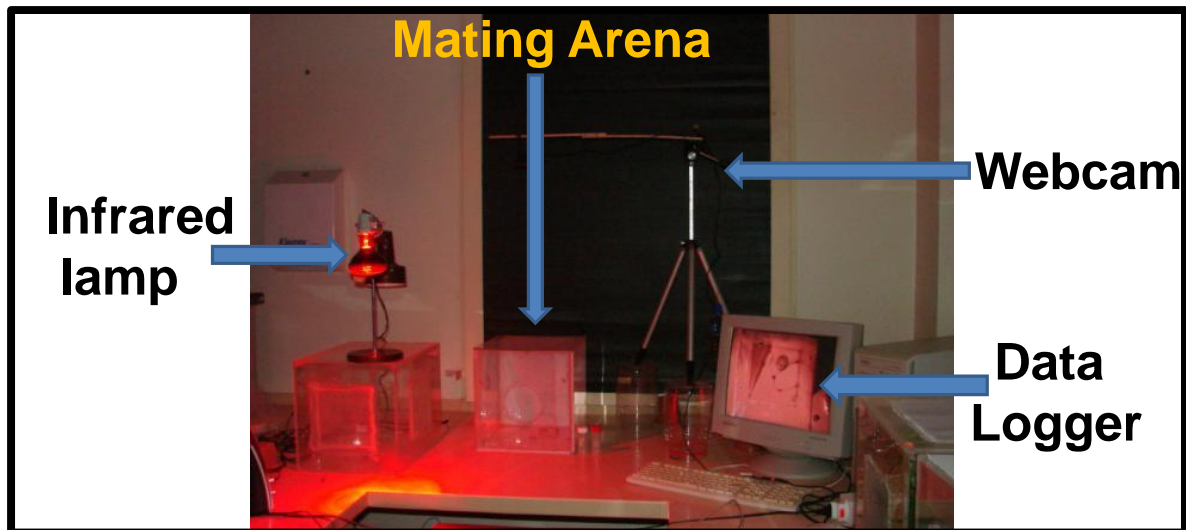


Fig. 2.1 Experimental set up for *Eldana saccharina* mating behavior study using video technology.

The moths were maintained and studied under the same conditions described in 2.2.1. The test insects were provided with water in a standard plastic petri-dish containing a piece of damp cotton wool placed on the floor of the mating arena. Imitation or natural foliage was not used in the mating arena since moths choose to perch on the walls, roof or base of the test cage (Mudavanhu unpublished data 2010).

Each treatment consisted of an equal sex ratio of 10 males and 10 females confined in one mating arena. The terms “mating type” and “male type” refer to treatment of adults depending on the context in which they were used and hence are used interchangeably with the term “treatment” in the text. Although laboratory reared and sterilized adults were pre-marked with Calco Red and an attempt made to individually label the test insects, it was not possible to see the marks or distinguish the individual moths in the arena under infrared illumination. For this reason all measurements are collective descriptors of a group of 10 males and 10 females. According to Weldon (2005), although this masks individual variation among males or females, it still enables an overall assessment of variation among the different strains or treatments.

The following mating treatments were used:

- i) wild males and wild females (Control) (W)
- ii) laboratory reared males and wild females (LW)
- iii) 150Gy sterile males and wild females (ST150MW)
- iv) 200Gy sterile males and wild females (ST200MW)
- v) 250Gy sterile males and wild females (ST250MW)

The above sterility doses were selected based on recommendations by Walton *et al.* (2011) who showed that ionizing radiation doses between 150Gy and 250Gy were suitable for further development of SIT against *E. saccharina*. Five replicates were performed for each treatment. The following measurements were recorded in order to assess moth quality:

- (i) onset of calling (time after lights were switched off (scotophase) when a male in a given treatment first adopts the calling posture continuously for at least five minutes, Fig. 2.2a). The five male treatments examined in this test were:
 - a) Wild or Control (W)
 - b) Non-irradiated laboratory reared (L)
 - c) 150 Gy irradiated (ST150M)
 - d) 200 Gy irradiated (ST200M)
 - e) 250 Gy irradiated (ST250M)
- (ii) onset of mating (the time when the first couple in a given treatment commenced copulation)
- (iii) mating duration
- (iv) mating frequency

The onset of calling was defined as the time after lights were switched off when a male in a given treatment first adopts the calling posture (i.e. extension of the abdominal hair pencils coupled with continuous or intermittent wing fanning; Atkinson, 1981; Zagatti, 1981; Fig. 2.2a) for at least five minutes. The onset of mating was defined as the time when the first couple in a given treatment commenced copulation. Male calling always precedes copulation (Atkinson, 1981; Zagatti, 1981). At the end of each replicate (a replicate is considered complete when test insects have been observed continuously during both scotophases), the abdomens of all females in each treatment were dissected under a light stereoscope to check for the presence and number of spermatophores contained within the bursa copulatrix (Walton, 2011) as confirmation of mating and to assess polyandry, respectively.

2.2.3 Statistical analysis

The data from each nightly observation period were checked for normality and homogeneity of variance using Shapiro-Wilk and Hartley-Bartlett tests, respectively. Data were analyzed by ANOVA and means were compared by Tukey test at 95% confidence interval. Data that did not conform to the assumptions of normality and/or homogeneity of variance even following transformation were analyzed using a non-parametric approach: Kruskal-Wallis test (Statistica 10.0; Statsoft Inc., Tulsa, Oklahoma, USA). However, results of the non-parametric analysis did not alter the conclusions drawn

from ANOVA. Therefore for the sake of brevity results of ANOVA are reported. Since the video assays were conducted over two successive nights, factorial analyses were conducted to test for interaction effects between treatment and scotophase. There were no significant interactions between type of mating pair and night in the case of onset time of mating and mating duration; therefore data for both nights were pooled together to test for significance differences between the mating types.

2.3 Results

2.3.1 General courtship and mating behaviour

As expected all mating behaviour and copulations occurred on the floor, walls and/or roof of the mating arena irrespective of treatment, ruling out the need for any artificial or natural foliage to simulate natural conditions.



Fig. 2.2.A: Calling posture of a male *Eldana saccharina* with recurved abdomen and extended abdominal hair pencils releasing sex pheromone compounds, B: male (right) and female (left) in copulation. The conjoined genitalia of the mating pair are covered by the wings of the male.

Male displaying either singly or in groups, which constituted calling and lek behaviour, was typified by rhythmic wing fanning, recurving of abdomen, extension of abdominal hair pencils (AHP) (Fig. 2.2a) and aggressive male-male interactions. In the case of the laboratory reared and sterile male strains, courtship behaviour occurred before and during the night and only during the later period in the case of wild males. In all treatments, extension of abdominal hair pencils by displaying males was characterised by emission of a strong pungent scent. Female behaviour consisted of wing flapping and visitation of leks (displaying males) where first contact with a potential mate is via the antenna. A courtship ritual between the male and female ensues where the couple rotates around each other followed by the former mounting the latter and gripping her thorax using his labial palps. The male then twists his abdomen in an attempt to attach his genitalia with those of the female following which he falls back to assume the end-to-end mating posture (Fig. 2.2b). Male-on-male mounting in leks or on non-calling males (males in non-territorial encounters) and males attempting to mount established mating pairs were not observed in the wild (control) strain but in treatments with non-irradiated laboratory reared and sterile male types. This was regarded as male mating confusion or distortion.

Since Walton (2011) reported that *E. saccharina* females are capable of mating more than once in the laboratory, an assessment of polyandry was done in the present study by counting the number of spermatophores dissected from the bursa copulatrix. Not more than one spermatophore was ever found in any of the females dissected (Fig. 2.3) indicating that none was mated more than once.



Fig. 2.3 Bursa copulatrix (circled) of a dissected mated female *Eldana saccharina* containing a single spermatophore.

The wild females remained motionless and unresponsive to male behaviour (i.e. either control or test males) during the photophase (before lights off). Copulation in all treatments occurred during the scotophase.

2.3.2 Onset of calling

The onset time of calling after lights were switched off was significantly influenced by an interaction between scotophase (night) and treatment ($F_{(4, 40)} = 20.608, P < 0.001$). This interaction was largely due to non-irradiated laboratory reared (L) and sterile males irradiated at 200 and 250Gy (ST200M, ST250M) which showed distinct differences in the onset time of calling between first and second scotophase (Fig. 2.4).

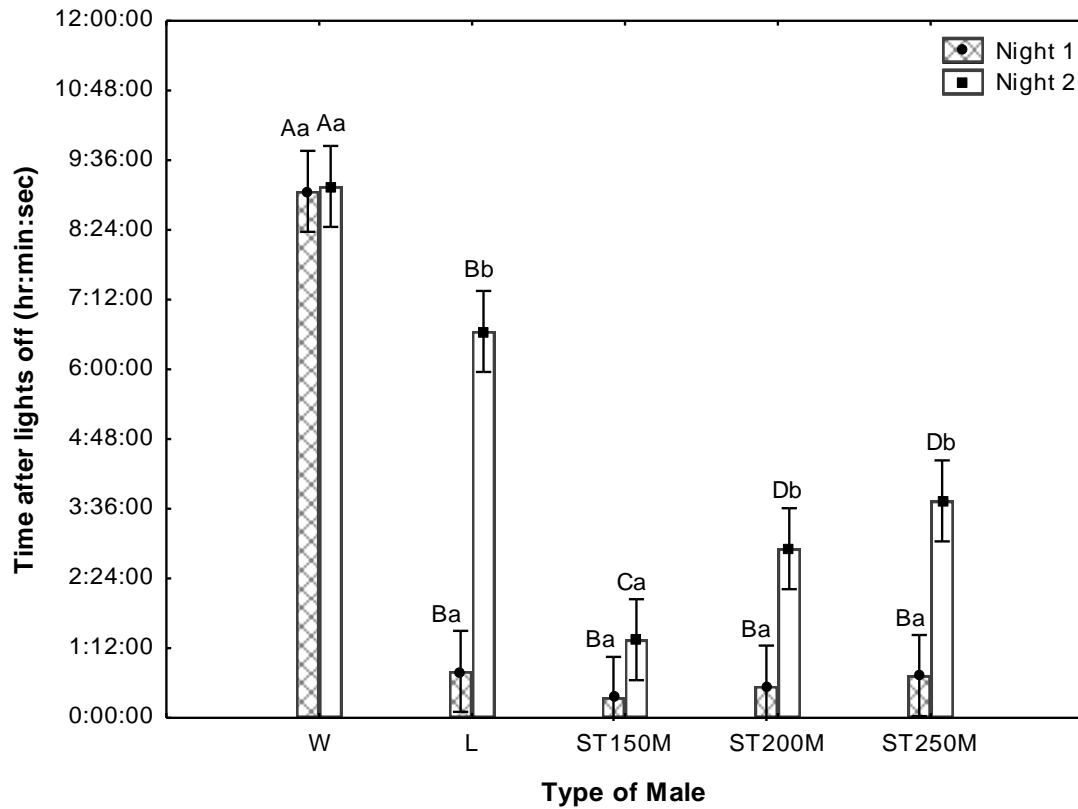


Fig. 2.4 The mean onset time of calling after lights off by males of five different treatments during two successive 14-hour scotophase periods. Means at each male type with the same lower case letter are not significantly different. For each night, means with the same upper case letter are not significantly different at 95% C.I.

In all treatments except in the control (W) the general trend was that males called earlier during the first scotophase compared to the second. Wild males (W) called significantly later than those in all other male treatments during both nights (Fig. 2.4). Males irradiated at 150Gy (ST150M) were the earliest to commence calling during both nights although this was non-significant when compared to laboratory reared males or the other two sterile male types during the first night. In the second scotophase, all sterile male types commenced calling significantly earlier than both the wild and laboratory reared males (Fig. 2.4). A summary of the mean onset times of calling is given in Table 2.1.

Table 2.1 Mean onset times of calling after lights were switched off for five male types of *Eldana saccharina* during two successive 14-hour scotophase periods.

Male Type*	Night 1: Time ± SE (hr:min:sec)	Night 2: Time ± SE (hr:min:sec)	No. replicates (n)
W	9:03:57 ± 0:45:08	9:08:55 ± 0:27:05	5
L	0:48:02 ± 0:13:37	6:39:14 ± 0:22:13	5
ST150M	0:21:07 ± 0:02:13	1:20:42 ± 0:07:24	5
ST200M	0:32:46 ± 0:07:19	2:54:44 ± 0:18:32	5
ST250M	0:43:41 ± 0:10:44	3:44:06 ± 0:16:16	5

*W: wild (control) males, L: non-irradiated laboratory reared males, ST150M: sterile males irradiated at 150Gy, ST200M: sterile males irradiated at 200Gy, ST250M: sterile males irradiated at 250Gy.

2.3.3 Onset time of mating

There were no significant interactions observed between the mating type and night ($F_{(4, 40)}=1.1959$, $P = 0.328$), therefore data for both scotophases were pooled together to obtain an average for each treatment.

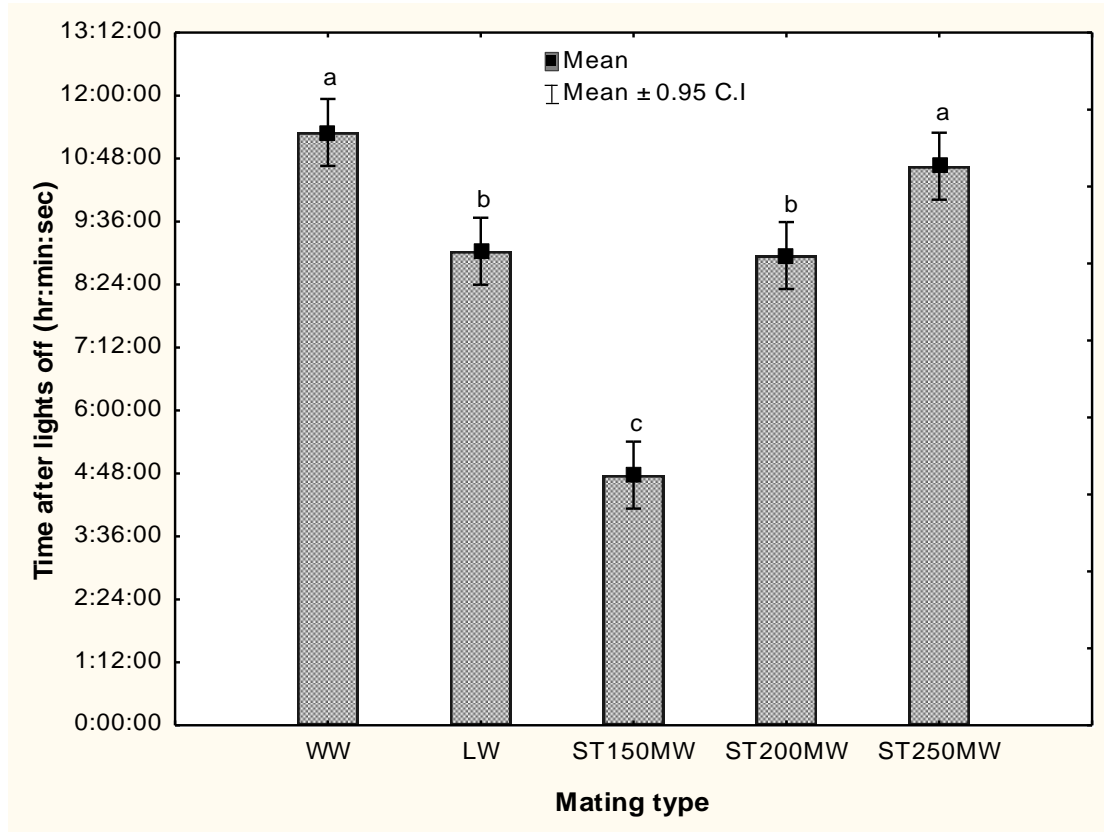


Fig. 2.5 The combined mean onset time of mating after lights were switched off for night 1 and night 2 in five *Eldana saccharina* mating types. Means at each mating type with the same lower case letter are not significantly different at 95% C.I.

There were significant differences in onset times of mating after lights off between the mating types ($F_{(4, 40)} = 65.108$, $P < 0.001$). Males irradiated at 150Gy (ST150MW) were the earliest to commence mating (Fig. 2.5) just as they were also the earliest to call (Fig. 2.4). Wild males (WW) which were the latest callers also registered the latest onset time of mating though this did not significantly differ from that of males irradiated at 250Gy (ST250MW)(Fig. 2.5). The onset time of mating in sterile male treatments increased with a corresponding increase in radiation dosage where those irradiated with the lowest dose (150Gy) were the earliest to commence mating while those treated with the highest dose (250Gy) were the latest (Fig. 2.5). A summary of the mean onset times of mating by the five mating types is given in Table 2.2.

2.3.4 Mating Duration

The interaction effect of night and treatment on mating duration was non-significant ($F_{(4, 100)} = 2.4117$, $P = 0.054$) in all five *E. saccharina* mating types. The mean mating durations of each mating type were therefore pooled together to obtain an average for both nights. There were significant differences in mating duration between the mating treatments ($F_{(4, 100)} = 5.8099$, $P = 0.001$). Males irradiated at 150Gy (ST150MW) mated with wild females for a significantly longer duration compared to those in other mating types (Fig. 2.6).

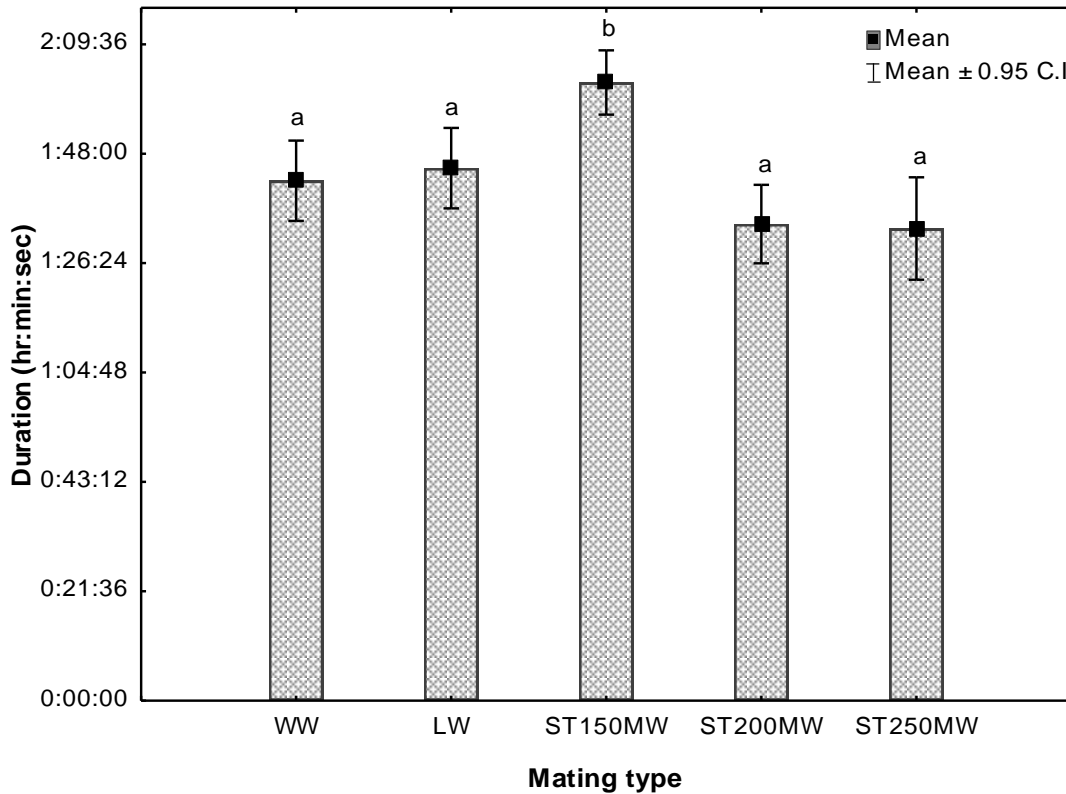


Fig. 2.6 The combined mean mating duration for night 1 and night 2 in five *Eldana saccharina* mating types. Means at each mating type with the same lower case letter are not significantly different at 95% C.I.

Table 2. Mean onset times of mating, mating duration and mating frequencies observed in five mating types of *Eldana saccharina* during two successive 14-hour scotophases.

Mating type	Onset of mating	Mating duration	No. of Matings		No. replicates (n)
	Time \pm SE (hr:min:sec)	Time \pm SE (hr:min:sec)	(mean \pm SE) Night 1	(mean \pm SE) Night 2	
WW	11:17:55 \pm 0:20:51	1:42:40 \pm 0:05:02	1.6 \pm 0.2	2.6 \pm 0.5	5
LW	9:02:05 \pm 0:13:17	1:45:09 \pm 0:04:01	3.6 \pm 0.2	1.0 \pm 0.4	5
ST150MW	4:46:02 \pm 0:20:59	2:05:21 \pm 0:04:51	5.8 \pm 0.5	1.2 \pm 0.4	5
ST200MW	8:57:07 \pm 0:22:50	1:34:07 \pm 0:02:23	3.4 \pm 0.2	1.4 \pm 0.2	5
ST250MW	10:39:16 \pm 0:16:26	1:33:14 \pm 0:02:42	1.6 \pm 0.2	1.0 \pm 0.0	5

*See 2.2.2 for keys to mating types.

The mating duration of all other mating types did not differ significantly (Fig. 2.6). A summary of the mean mating durations in each mating type is given in Table 2.2.

2.3.5 Number of matings

There was a highly significant interaction effect between night and mating type on the mating frequency of *E. saccharina* ($F_{(4,40)}=19.509$, $P = 0.001$). This effect was largely a consequence of distinct differences in number of matings between the two scotophases in the case of LW, ST150MW and ST200MW mating types (Fig. 2.7).

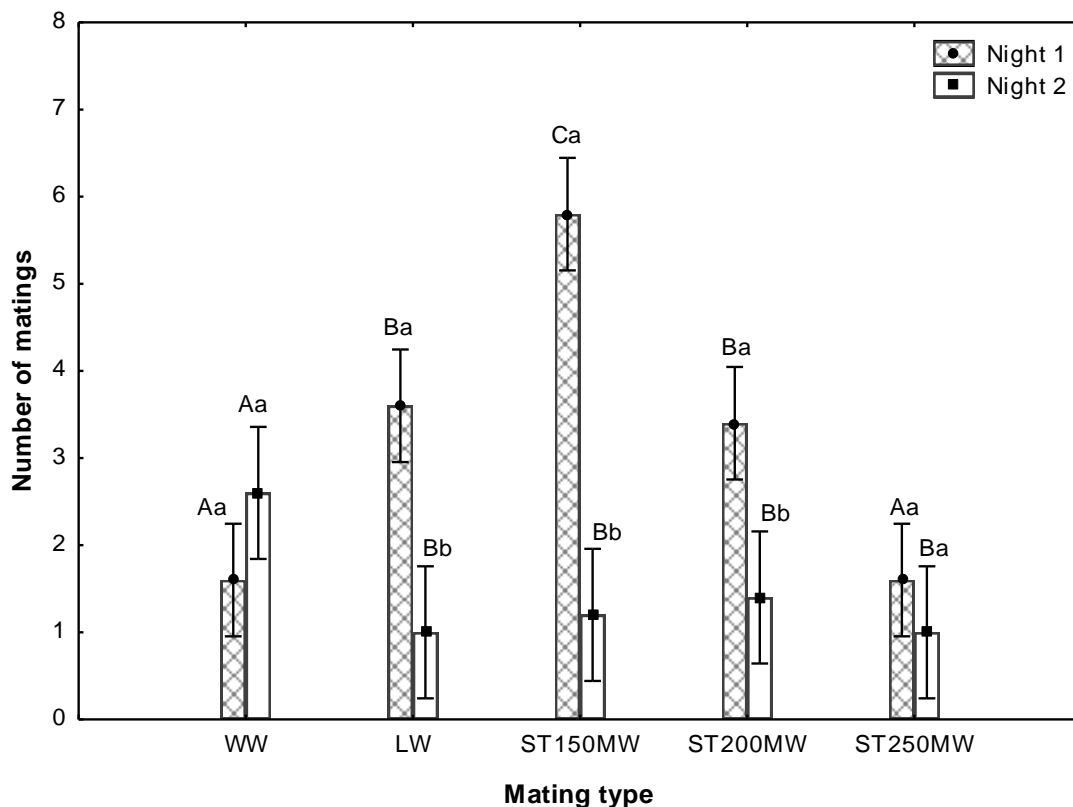


Fig. 2.7 The mean number of matings of five different mating types during two successive 14-hour scotophases. Means at each mating type with the same lower case letter are not significantly different. For each night, means with the same upper case letter are not significantly different at 95% C.I.

The highest number of matings in all treatments except in the control (WW) was recorded during the first scotophase (Fig. 2.7). During the first scotophase the ST150MW mating type registered the highest number of matings while both WW and ST250MW had the lowest counts, of which they did not differ significantly (Fig. 2.7). Number of matings of non-irradiated laboratory reared males with wild females (LW) were higher than the control and the ST250MW treatments but did not differ from that observed in the ST200MW type during the first night. The number of matings observed in the sterile male treatments (ST150MW, ST200MW and ST250MW) significantly declined with an increase in radiation dose during the first night but did not differ during the second night (Fig 2.7). In the

second scotophase, the WW mating type registered the highest number of matings compared to all other mating types whose number of matings did not differ significantly (Fig. 2.7). A summary of the mean number of mating in each mating type during the two scotophases is given in Table 2.2.

2.4 Discussion

2.4.1 General courtship and mating behavior

The general courtship behavior observed in these trials was consistent with observations by Atkinson (1981) and Zagatti (1981). Except for the two types of mating distortions observed in the test strains (i.e. sterile and non-irradiated), no major qualitative differences were observed in their courtship sequences when compared to the control. The test strains were able to perform courtship displays and subsequently mate with wild females. However, several consistent quantitative variations between the test strains were noticed.

The results from these bioassays indicate that both mass-rearing and sterilization of *E. saccharina* lead to a departure of male mating behavior away from that exhibited by their wild counterparts. Orozco *et al.* (2007) explain that, unlike the wild strain which is exposed to natural environmental conditions, laboratory reared and sterile strains, which are both mass-produced, are exposed to fairly controlled and stable conditions which may lead to a change in their adult behavior. This is primarily attributed to genetic differentiation in the mass-reared strain (Kuba & Koyama, 1982). Male mating confusion observed in test strains was not seen in the control. Male-on-male mounting has been reported in mass-reared strains of tephritid species such as *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), *B. cucurbitae* (Coquillet) (Diptera: Tephritidae), *B. dorsalis* (Hendel) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Suzuki & Koyama, 1981; Gaskin *et al.*, 2002; Weldon, 2005), where males emit sex pheromones to attract females. This has been attributed to olfactory confusion under mass-rearing conditions where males are stimulated to court and mount other males (Weldon, 2005) or selection of an alternative mating tactic under mass-rearing conditions. In addition, the process of irradiation may further compound the effects of mass-rearing (Bakri *et al.*, 2005; Weldon, 2005).

In all treatments a strong pungent scent could be detected in the mating arena. Zagatti (1981) described the odour emitted by the AHP of male *E. saccharina* as a strong vanillin smell while Atkinson (1981) described it as a sweet scent. Several authors have reported that the AHP when extruded could possibly be emitting aggregation and sex pheromones to elicit lek formation and attract female mates (Atkinson, 1981; Bennett *et al.*, 1991; Burger *et al.*, 1993; Harraca *et al.*, 2011).

However the chemical composition of this odour needs to be examined further by techniques such as gas chromatography to assess differences between males exposed to different treatments.

2.4.2 Impact of mass-rearing and radiation dose on male calling and mating success with wild females

Males in the test strains commenced calling and subsequently mated (generally in the order by which they called) significantly earlier than wild males during both scotophases. These observations confirm conclusions drawn by other authors regarding the effects of mass-rearing and sterilization on matings. For example, Briceno and Eberhard (1998) demonstrated that males from mass-reared populations of *C. capitata* court much earlier and for shorter periods in order to increase their likelihood of securing mates as well as to avoid frequent interruptions of courtships under conditions of overcrowding. Traits that favour simpler and earlier courtship sequences to ensure copulation success and changes in sexual competitiveness may be selected under mass-rearing conditions (Boake *et al.*, 1996). The high densities at which adult *E. saccharina* are reared and stored could thus be an explanation for the early initiation of calling and mating observed in the *E. saccharina* test strains, all of which were mass-produced in the laboratory. Briceno and Eberhard (2002) found that in the presence of intense competition from other males, it may be advantageous for males to commence calling earlier even though mating success with respect to this strategy would be governed by co-evolution in female choice criteria. Under field conditions, early mating behaviour by *E. saccharina* (as shown by the irradiated males in these trials) would be desirable for the SIT programme as many wild females would be mated by sterile males before wild males have the opportunity to do so, thereby achieving the targeted number of sterile matings that result in population reduction.

Differences in performance of the sterile strains with respect to onset time of calling, onset times of mating and number of matings are attributable to radiation dose. Males treated with a lower dose of radiation (150Gy) called and mated significantly earlier (i.e. during second scotophase in the former) and registered higher numbers of matings (i.e. during first scotophase where most activity was recorded) than those treated with higher dosages. This is desirable for successful SIT. Bloem *et al.* (2001; 2004) demonstrated that mass-reared codling moths, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) a key pest of apples and pears, treated with lower doses of radiation are more competitive than those treated with higher doses. Irradiation degrades insect quality (Calkins & Parker, 2005) and hence the higher the dosage the more deleterious its effects are on the target insect. Lux *et al.*, (2002) demonstrated that with increasing dose of radiation, male *C. capitata* (Diptera: Tephritidae) ability to participate in lek formation was severely reduced which is manifested

by passiveness or mating confusion/distortion. A high radiation dose, though guaranteeing total sterility of both sexes so as to ensure efficiency of SIT treatment, partially incapacitates the mass-reared males and diminishes sexual motivation, thereby substantially reducing their performance and increasing overall costs of the SIT operations (Lux *et al.*, 2002). Therefore, the use of low doses of radiation allows good mating performance, although not ensuring total male sterility (Lux *et al.*, 2002). However in the case of *E. saccharina* the use of a low sterility dosage bodes well with the end goal of F_1 (inherited) sterility since the major suppressive effect of the technique will be primarily due to enhanced fitness and mating competitiveness of sub-sterile parents and F_1 progeny.

Numbers of successful matings in the test strains were higher (i.e. LW, ST150, ST200MW) or similar (i.e. ST250MW) to the control during the first scotophase. However during the second scotophase there was a significant decline in the number of copulations in all test strains, to levels significantly less than those of the control. These observations suggest aging in both the non-treated mass-reared and the irradiated males may have reduced mating probability, competitiveness and performance given that *E. saccharina* has a short-lived adult stage (5-8 days) (Betbeder-Matibet *et al.*, 1977; Atkinson, 1981; Walton 2011). This theory is consistent with findings by other authors who reported that aging dramatically reduces mating propensity and competitiveness of males and is a key factor within the framework of SIT (Eischen *et al.*, 1984; Charlesworth, 2000; Papanastasiou *et al.*, 2011). It has been shown that irradiation does not only have adverse somatic effects on exposed individuals (Bakri *et al.*, 2005) but also depresses male fertility and causes lesions in radiosensitive germ cells which subsequently reduce locomotor activity (Eischen *et al.*, 1984). It also shortens male longevity through superficial mimicking of the aging process (Clark & Rockstein, 1964; Ducoff, 1972). Though the effects of age on mating competitiveness of irradiated *E. saccharina* were not investigated, several capabilities including sexual behaviour and mating may diminish with aging (Eischen *et al.*, 1984). Individuals may become less vigorous at calling and less successful in courting females, resulting in a significant decline in overall performance (Eischen *et al.*, 1984). Whether or not irradiation compounds the effects of age and mass-rearing in *E. saccharina*, resulting in reduced sterile male performance in successive nights following release would require further testing.

Wild males on the other hand commenced calling and mating significantly later than the other strains during both nights. This was not unexpected as it is well known that inducing wild insect populations to reproduce in the laboratory is difficult (Roessler, 1975; Rossler, 1975) because conditions significantly differ from those in the wild (Cayol, 2000) which inevitably influences their behavior. However, in the case of numbers of matings, a significant increase was observed during the second scotophase. Wild strains of adult *E. saccharina* males are known to mate more than once and their propensity to mate is highest during the first three days of their lifespan (Betbeder-Matibet *et al.*,

1977; Atkinson, 1981; Walton 2011). It is therefore likely that the poor performance of wild males during the first night may have been due to test conditions which are very different from those in the natural environment. The increased performance during the second night might reflect an adaption of wilds to the artificial conditions of the mating arena. Nevertheless, influence of aging on competitiveness of *E. saccharina* over a wider range (i.e. beyond sexual maturity attained in the newly eclosed adults) in order to assess middle aged and older males is still unclear and further study in this regard is recommended.

2.4.3 Impact of mass-rearing and radiation dose on mating duration

The similarity in mating duration between the control and test strains of *E. saccharina* is desirable for development of the SIT against this pest. Saul *et al.* (1988) showed that mating duration is a determinant of the refractory period and remating rate. Therefore, as mean copula duration increases, remating probability decreases (Vera *et al.*, 2002). It follows that females mated to sterile males are inseminated with inactive sperm and therefore have a higher likelihood of remating compared to those mated to normal males (Cavalloro & Delrio, 1970; Katiyar & Ramirez, 1970; Bloem *et al.*, 1993). According to Bloem *et al.* (1993) this increases the chances that the female will encounter and mate with at least one fertile male. *E. saccharina* females have been shown to mate more than once in the laboratory (Walton, 2011). Should this remating be as a result of inferior sperm quality, it is therefore a cause of concern for the SIT development and hence needs further investigation. In addition the mean mating durations recorded in this trial provide evidence that males from the colony at SASRI are regularly colonized or closely resemble wild ones since aging/long-established strains have shorter mating times (Vera *et al.*, 2002).

Further investigation using a two or more-choice experimental design is necessary to evaluate whether sterile males are as competitive as their wild counterparts when females have a choice between strains (Weldon, 2005). In addition, it is necessary that the results of these trials be verified in the field in order to confirm that sterile *E. saccharina* males are still capable of locating hosts, mating arenas (leks), and mixing and interacting with their wild counterparts under natural conditions (Zapien *et al.*, 1983; Shelley & Whittier 1996; Liedo *et al.*, 2007). While performance of the test strains exceeded my expectations, the experimental conditions may have influenced the outcomes of this experiment. The laboratory conditions under which the tests were conducted were typified by a mating arena extremely restricted in space. This may have encouraged frequent and random interactions between the test insects, resulting in successful copulations since females were forced to repeatedly interact with males of the same type even in cases where males are otherwise expected to be less sexually motivated or competitive (Lux *et al.*, 2002a; 2002b). Nonetheless, the

data are still valuable in overall assessment of variations in behavioral traits amongst different male strains. To ensure that released sterile males are of high quality, their competitiveness and compatibility with the targeted population must be periodically assessed under semi-controlled field conditions ((FAO/IAEA/USDA, 2003; Weldon, 2005). The next chapter therefore investigates whether irradiated males are more or less competitive than their wild counterparts when females are presented with a choice between strains using a two-choice (or more) experimental design. Since mass-rearing and irradiation can potentially alter mating behavior (Shelly & Whittier, 1996; Lux *et al.*, 2002), the next chapter thus explores how *E. saccharina* female choice in the field, may be influenced by these factors. According to Weldon (2005) the evolution of sterile male resistance could reduce the efficacy of SIT programmes despite female acceptance of both sterile and wild males being similar in a population naive to sterile male release. The long-term success of any SIT programme therefore requires a thorough understanding of female preference functions (Jennions & Petrie, 1997; Widemo & Saether, 1999), the cues used by females to choose males and the costs associated with female choice (Weldon, 2005).

2.5 Conclusion

The results of the study have shown that both non-irradiated and sterile males treated within the given range of radiation dosage are as active and competitive as their wild counterparts, capable of forming and locating leks, courting and subsequently copulating with wild females in a no-choice scenario. Encouragingly, the lower radiation doses (150 and 200 Gy) do not impact negatively on *E. saccharina* male mating behaviour as shown in the present study. In general, qualitative mating behavioural traits are not lost in the mass-produced sterile strains and hence there is scope for further development of the SIT against *E. saccharina*. This indicates that quality and competitiveness of *E. saccharina* was maintained in males treated with the sub-sterilizing doses of 150 and 200 Gy and hence the goal of implementing inherited (F_1) sterility for area-wide control of this pest is attainable. Male moths irradiated at sub-sterilizing doses are likely to have better fitness (e.g. survival, dispersal) in the wild and be more competitive (e.g. mating) thus resulting in better control than fully sterile but physically unfit and less competitive counterparts (Kean *et al.*, 2008, Suckling *et al.*, 2011). In addition surviving F_1 progeny will have a greater population suppression potential due to dominant lethal genes and radiation-induced chromosomal aberrations inherited from their partially sterile male parents (Bloem *et al.*, 1999; Soopaya *et al.*, 2011). Since there is currently no effective way of separating male and female *E. saccharina*, it is important that released females have no residual fertility, as this may increase the wild population (Walton, 2011). Therefore, further *E. saccharina* SIT development should focus on the sub-sterility dose of 200 Gy since it does not only

affect mating propensity of treated males but results in a lower male residual fertility and complete female sterility than the 150 Gy dosage level (Walton, 2011).

2.5 References

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CHAPTER 3

MATING COMPATIBILITY AND COMPETITIVENESS BETWEEN WILD AND LABORATORY STRAINS OF *ELDANA SACCHARINA* WALKER AFTER RADIATION TREATMENT

3.1 Introduction

In the last twenty years the Sterile Insect Technique (SIT) has gained recognition as an environmental-friendly and effective method of control for use in area wide integrated pest management (AW-IPM) programmes against many lepidopteran pests (Bloem *et al.*, 2005; Klaasen, 2005; Vreysen *et al.*, 2007a). Although the concept of SIT is simple, its implementation is very complex (Seawright, 1988). This is because there is a tendency for the sterile insects to lose their ability to survive and perform behaviours that allow them to successfully mate and block reproduction of wild females (Calkins & Parker, 2005), as they go through a behaviour trait-altering chain of processes from laboratory or facility production to final release into the field and thus wild population.

The effectiveness of the SIT when applied as part of AW-IPM strategies is based on the efficient transfer of sperm carrying dominant lethal mutations from sterile males to wild females (Knippling, 1955). Lepidopteran species are amongst the most radio-resistant insect orders (LaChance 1985, Bakri *et al.*, 2005) such that in order to achieve full sterility a large dose of radiation is required. However this may reduce their competitiveness and performance in the field (Suckling 2001). The use of inherited or F_1 sterility is one way to circumvent the negative effects associated with the high radio-resistance (Bloem & Carpenter, 2001; Suckling, 2003; Soopaya *et al.*, 2011). This method is a variation on the original approach and involves the release of partially sterile insects of superior fitness (Suckling, 2003) and more effective at overall population suppression due to the requirement for competitive mating (LaChance, 1985) and the resulting heterozygote offspring carrying dominant lethal genotypes which are passed into the wild population (Suckling, 2003; Soopaya *et al.*, 2011). F_1 progeny have a higher sterility than their parents, lower fecundity, longer larval development times, higher mortality, and a skewed sex ratio in favour of males (North, 1975; LaChance, 1985; Carpenter *et al.*, 2001). In addition, the females are more sensitive to radiation than are males of the same species (Bloem & Carpenter, 2001) thus allowing the dose of radiation to be adjusted in such a way that treated females are completely sterile while males are partially sterile (Bloem & Carpenter, 2001; Carpenter *et al.* 2001; Soopaya *et al.*, 2011). Since success of the SIT is dependent on the quality, fitness and ability of sterile males to locate and mate with wild females (Orozco *et al.*, 2007),

the phenomenon of inherited sterility is now the preferred approach for lepidopteran SIT (Suckling, 2003).

Because mass-rearing is necessary to produce the large number of males required for the SIT (Weldon, 2005), most rearing facilities maintain the same strain for long periods of time (Roessler, 1975). This consequently results in deterioration of insect quality after a number of generations (Partridge, 1996). The biotic and physical conditions in mass-rearing facilities are very different from those in the wild and can greatly affect the phenotype of the sterile strain by selecting for genetic differences between laboratory and wild populations (Lance & McInnis, 2005). Orozco *et al.* (2007) noted that one of the ways to counteract the un-natural selection pressures imposed by the laboratory environment which result in a reduction in the gene pool is by regularly replacing the colony with field collected material. However this presents a new set of challenges where during colonization of a new strain, a production bottleneck is encountered as only a fraction of individuals survive and reproduce during the initial phase of colonization (Leppla, 1989). This consequently causes a delay in attaining a sufficient colony size to sustain SIT operations, a reduction in the new strain's initial gene pool and ultimately, variations in behaviour of the mass-produced insects due to this reduction, manifested by strain incompatibility and sexual isolation with respect to the wild population (Orozco *et al.*, 2007).

On the other hand commercialization of sterile insect production has also greatly increased in countries where the SIT is used as part of the arsenal of control tactics in AW-IPM programmes against economic insect pests (Taret *et al.*, 2010). For this reason, one country could be contracted to supply sterile insects to a programme in another which is faced with the same pest problem or in the case of facilities located in two different hemispheres where production seasons alternate, a country in one hemisphere scaling down operations during winter could supply steriles to another that is experiencing a high demand in summer (or vice versa) (Taret *et al.*, 2010). It is therefore critical to monitor and assess the existence of mating barriers between released strains of insects and the target populations in different geographical localities. The fact that, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) has a wide geographical distribution across Africa (Assefa *et al.*, 2006) and that it has at least three confirmed biotypes (Sampson & Kumar, 1985; Assefa *et al.*, 2005; 2006), makes such quality control screening highly relevant in the context of implementing efficient, optimized and collaborative SIT on a regional or inter-regional basis (Taret *et al.*, 2010).

Levels of mating competitiveness and compatibility can be evaluated cost-effectively by observing the extent to which individuals from two populations interbreed when confined together (Taret *et al.*, 2010). The data from such tests can be used to generate simple, reproducible and meaningful

indices for tracking performance and making comparisons between strains (Cayol *et al.*, 1999; Taret *et al.*, 2010). As noted in 1.5 *E. saccharina* has a complex lek mating system (Atkinson, 1981; Zagatti, 1981) that is unique amongst Lepidoptera species targeted for the SIT. Therefore, where the wild strain is pitted against a laboratory reared or irradiated strain, it is critical that the afore-mentioned evaluations be done under conditions that closely resemble those of the wild strain's natural habitat given the complexity of the species mating system. These tests are best run under field cage conditions (Chambers *et al.*, 1983; Cayol *et al.*, 1999) where the presence of host plants would be a good compromise between the laboratory environment and natural open field trials (Cayol *et al.*, 2002; Allinghi *et al.*, 2007; Taret *et al.*, 2010).

The African sugarcane stalk borer, *E. saccharina*, is a key pest of sugarcane in East, West and South Africa (Atkinson, 1980; Chinheya *et al.*, 2009) and yet still continues to defy current control strategies (Conlong, 1994; Conlong & Rutherford, 2009). Since *E. saccharina* occurs in a variety of host plants and is very cryptic in nature (Conlong, 1994), it has frustrated and complicated several attempts to control it through conventional methods including cultural techniques, use of resistant varieties and insecticide applications (Walton, 2011). Focus is now given to Area-Wide Integrated Pest Control (AW-IPM), where newer and promising methods such as the Sterile Insect Technique (SIT) are incorporated into the existing conventional methods mentioned above (Conlong & Rutherford, 2009). According to Knippling (1955) the principle of the SIT is simple and basically involves releasing of a large number of reproductively sterile male insects into a wild population of the same species so that they mate with and block the reproduction of wild females.

Walton (2011) has demonstrated that F_1 sterility is attainable in *E. saccharina* at radiation doses comparable with other lepidopteran SIT programmes such as the false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) (Bloem *et al.*, 2003) and the cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) (Carpenter *et al.*, 2001; Bloem *et al.*, 2007). This is encouraging for the development of an F_1 male sterility SIT approach against this insect pest. Furthermore in Chapter 2 it was demonstrated that under laboratory, and non-competitive trials, male *E. saccharina* were as good as, and even better at calling and mating if irradiated at 150 and 200Gy, than their wild counterparts. The primary question of concern now is whether or not irradiated *E. saccharina* reared in the laboratory are competitive with respect to wild *E. saccharina*. The most straightforward approach to answer this question would seem to be to release irradiated laboratory reared adults into the field to compete with wild adults already present in the field. However, if the irradiated laboratory reared moths are not competitive, several reasons could explain this inferiority such as degradation of moth quality due to radiation (Calkins & Parker, 2005), handling (Terblanche *et al.*, 2008), laboratory rearing regime (which also disrupts

synchrony)(Weldon, 2005), the laboratory colony being lab-adapted in a way that promotes assortative mating (Calkins & Parker, 2005; Huho *et al.*, 2007), population size of *E. saccharina* in the wild being inaccurately estimated or all of the above.

Because of these complexities, the objectives of this study were to:

- (1) Examine the effect of laboratory rearing mating competitiveness and isolation
- (2) Examine the effect of radiation (at 200Gy- the dose of choice for further development of *E. saccharina* SIT) on mating competitiveness and isolation, and
- (3) Examine the possibility of additive or synergistic effects due to laboratory rearing and irradiation on mating competitiveness and isolation.

The data generated from this study will be useful for present and future assessment of strain compatibility and hence success of the SIT since ability of released mass-produced sterile individuals to mate with females of the target population forms the basis of the technique.

3.2 Material and methods

3.2.1 Study populations

The origin of the laboratory reared, sterile and wild strains used for this study as well as their rearing conditions and handling protocol are described in section 2.2.1. The laboratory reared colony was last changed completely in November 2009. However since then there has been daily infusion of wild moths into the colony which still constitutes less than 10 % of the total laboratory moths produced.

3.2.2 Experimental design and observations

The mating trials were conducted during the summer period of November-December 2011 and run concurrently in a small bench top cage (0.027m³) (Fig. 3.1a) under controlled laboratory conditions and in a large out-door walk-in field cage (3 m diameter, 2 m height) (Fig. 3.1b.). The bench-top cage was installed in a climate and photoperiod controlled room (27 ± 2°C, 75 ± 5 % relative humidity (RH); (10: 14) light: dark (L: D) cycle) in the Entomology Department at Stellenbosch University. Access into the bench top cage was by means of a transparent Perspex lid while ventilation was by means of white cotton mesh cloth on the side walls. The field cage was made of Lumite® (poly-monofilament) screen of mesh (thread count) size 32 x 32. Screening of the field cage was light brown in colour to allow good sun penetration. The bottom edges were reinforced with polypropylene tape for extra strength. Access into the field cage was by means of two zippers opened from both ends to form a

door and seal the cage in order to prevent escape of test insects or intrusion by foreign individuals. The field cage was attached to a wire frame for support, installed on an open area (Chambers *et al.*, 1983) in a cleared apple orchard surrounded by a pine tree wind break at Welgevalen Farm in Stellenbosch and provided with a single 22-months old potted sugarcane plant (variety NCo 376) (Cayol *et al.*, 1999).

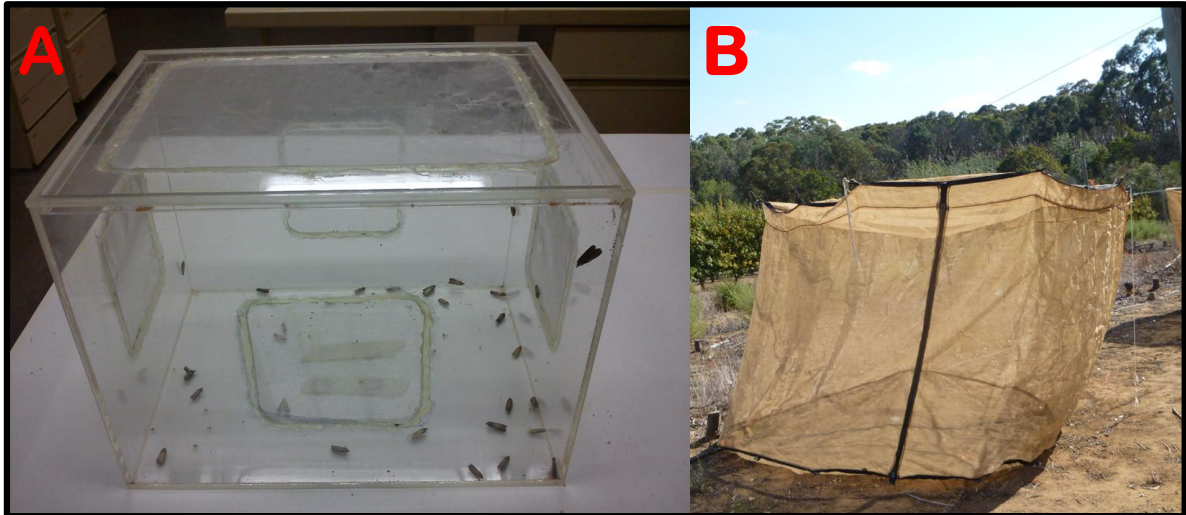


Fig 3.1 *Eldana saccharina* mating arenas: (A) small bench-top mating cage in the laboratory environment, (B) large walk-in field cage located in an open field environment.

The laboratory and field cage comparisons were run concurrently in order to examine for possible interactions between adult strain performance and type of cage (for example a particular adult strain may be competitive with another in the small bench-top cage but less competitive in the large walk-in cage with sugarcane). Both male and female adults separated into groups of 6 individuals were placed in standard petri-dishes sealed with masking tape and enclosed in transparent zip-lock polyethylene bags. The adults were then chilled at 6°C for five minutes in order to immobilize them and lightly marked on the dorsal part of the thorax using black and red Pentel® permanent markers in order to distinguish strain type by placing them into a programmable waterbath (Grant GP200-R4, Grant Instruments, UK). The waterbath was filled with a 1: 1 water: propylene glycol (Chown & Nicolson, 2004; Terblanche *et al.*, 2007) in order to accommodate sub-zero temperatures. After marking, adults were allowed to recover in a climate controlled room at 26±1°C, 60 ± 10 % relative humidity (RH) and maintained at those conditions for 3 hours prior to commencement of trials. Full recovery usually occurred within approximately seven to ten minutes following retrieval from water bath and marking. Sexually mature (zero-day old) virgin males were released into the cages approximately two hours before sunset. Male *E. saccharina* were given 30 minutes to disperse and become accustomed to the cage environment following which the zero-day old virgin females of each corresponding strain were then released into the cages. Each cage was stocked with an equal sex ratio of six females and six males for each treatment. Three types of pair-wise

comparison/competition trials were conducted, where each trial was identified by the respective *E. saccharina* adult strains constituting it as follows:

- (i) non-irradiated and wild adults (“L-W”);
- (ii) irradiated (200Gy) and non-irradiated adults (“L-S”); and
- (iii) irradiated (200Gy) and wild adults (“S-W”).

Trial 1 examined effect of laboratory rearing on mating competitiveness and isolation of male laboratory reared adults with respect to wild males. Trial 2 examined the effects of irradiation on mating competitiveness and isolation between non-irradiated laboratory-reared males and irradiated laboratory reared males. Trial 3 examined the possibility that there are additive or synergistic effects due to laboratory-rearing and irradiation on mating competitiveness and isolation by comparing mating competitiveness and isolation between irradiated laboratory reared males and wild males. Overall, each trial compared the performance of different moth treatments/strains in two different mating arenas.

Five replicates were performed for each trial. Due to limitation in adult emergence and availability of field cages replication was done by night where each trial consisted of one cage per location for each of five nights. All mating pairs were collected in perforated and lidded disposable foam cups at regular intervals (i.e. every hour) until all mating had ceased. Mating was considered to have ceased either when all females had been mated, since all mated individuals were removed from the cages for identification, or at the break of dawn. Since experiments were conducted during the night, a Bushnell® Night Vision 2 x 24 Night Watch Monocular was used to locate adults in the cages. In each cage, the type of moths engaged in mating was recorded (e.g. in trial 1, moths were expected to pair as follows: wild♀ × wild♂; lab♀ × lab♂; lab♀ × wild♂; or wild♀ × lab♂). Please note the convention is to list the type of female first in any mating pair combination and thus this system is adopted in the rest of the text unless otherwise mentioned. In summary, the following were recorded for each mating pair collected: type of female and male, time interval, cage type, cage replication number, and date. In the case of the large walk-in field cage temperature, relative humidity and light intensity (moon effect) were also noted every hour. The mated moths were neither replaced nor released back into the cages after separation (Chambers *et al.*, 1983). Tests were conducted over a 12-hour scotophase commencing at sunset and ending at dawn.

3.2.3 Statistical analysis

A factorial ANOVA was performed on the data with number of mating pairs as the dependent variable and with cage type (i.e. location), time of night mating occurred, and type of mating pair as

the independent variables, using Statistica 10; Statsoft Inc., Tulsa, Oklahoma, USA. The suitability of the moths and the cage environmental conditions for mating was determined by calculating the participation in mating (PM). The formula for calculating PM is as follows:

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

According to IAEA (1997) and FAO/IAEA/USDA (2003) a PM value of 0.2 is regarded the minimum proportion of mating for inclusion of data in the compatibility tests. Mating indices (McInnis *et al.*, 1996; Cayol *et al.*, 1999; Cayol *et al.*, 2002; Taret *et al.*, 2010) were used to quantify sexual compatibility, performance and isolation between the moth strains in each trial. The index of sexual isolation (ISI) accounts for the number of pairs obtained for each possible mating combination and is calculated using the following formula:

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

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

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

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

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

The values of these two indices also range from +1 (i.e. all matings done by males (MRPI) or females (FRPI) of one type [the first to be listed (A)]), through an equilibrium at 0 (i.e. equal participation in mating by males or females of both strains/types), to -1 (i.e. all matings achieved by males (MRPI) or

females (FRPI) of the other type [(B)]). The MRPI and FRPI explain the role of the males and females of the two strains compared in each trial, and thus complement the ISI very well (Taret *et al.*, 2010). In order to give a reliable illustration of mating performance of the different moth treatments, all three mating indices were considered together. A chi-square test of independence was used to test for significant departures of all the indices from zero. The number of homotypic (e.g. "AA") and heterotypic (e.g. "AB") couples within each cage and between both cage types were also compared using ANOVA, followed by Tukey's HSD *post hoc* tests to identify statistically homogenous groups at $P = 0.05$, in Statistica 10; Statsoft Inc., Tulsa, Oklahoma, USA.

3.3 Results

3.3.1 Participation in mating

The mean PM values obtained in all mating trials regardless of location (laboratory and field cage) confirmed that the cage environmental conditions were suitable for mating since 0.5 was the minimum proportion of matings recorded in the trials (Table 3.1). In all tests the PM was above 0.2 therefore none of the data were rejected. In all tests there were no significant interaction effect ($F_{(2, 30)} = 0.23985$, $P = 0.788$) between location and treatment with respect to participation in mating (Table 3.1). However, the PM value for trial 2 in the laboratory location ("L-S", i.e. non-irradiated versus sterile adults) differed significantly ($F_{(5, 30)} = 5.1993$, $P = 0.001$) from those for trial 1 in both locations ("L-W", i.e. non-irradiated versus wild adults) as well as that for trial 3 in the field location ("S-W", i.e. sterile versus wild adults) (Table 3.1). These results show that significantly more matings took place in the small bench-top cage between non-irradiated and sterile adults compared to tests involving other strains.

3.3.2 Effect of laboratory rearing on mating competitiveness

There was a highly significant three-way interaction ($F_{(18, 280)} = 4.5948$, $P = 0.001$) between time of night, type of cross and location of trials (Fig. 3.2).

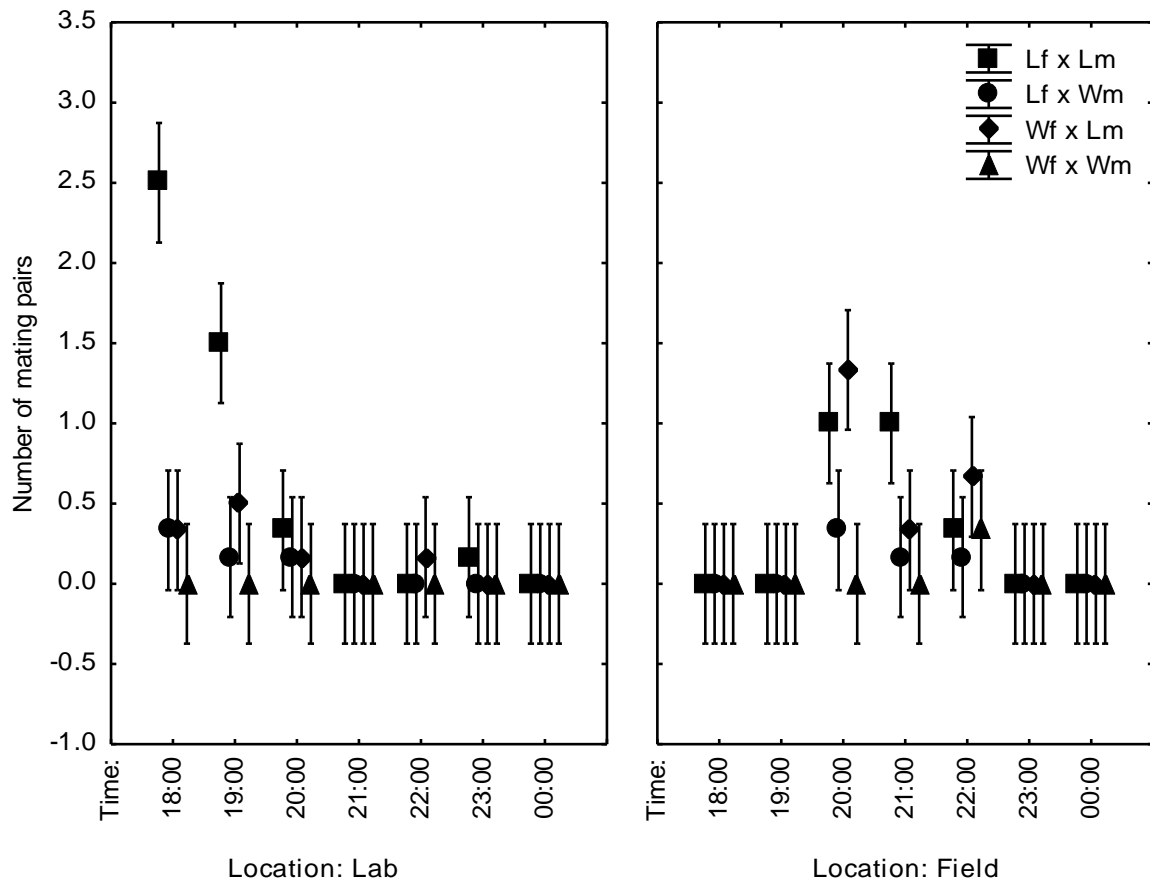


Fig. 3.2 The mean number of matings in a pair-wise comparison between non-irradiated and wild *Eldana saccharina* adults showing a significant three-way interaction between time of night, type of cross and location of trials. The expected possible mating combinations were: (i) non irradiated female and non-irradiated male (Lf x Lm); (ii) non-irradiated female and wild male (Lf x Wm); (iii) wild female and non-irradiated male (Wf x Lm); and/or (iv) wild female and wild male (Wf x Wm). Error bars denote 95% confidence limits.

In the outdoor walk-in field cage mating only commenced and peaked between 20H00 and 22H00 pm. Comparing the laboratory and field localities, there also were variations in frequency of matings between the crosses at the above times. In the laboratory there was a high frequency of “L♀ x L♂” and “W♀ x L♂” type crosses early in the night (18H00 – 20H00) and some mating of the same crosses later in the night (22H00 – 23H00). However, in the field, a high number of matings of “WL” and “LL” crosses occurred between 8 and 10 pm and some mating by “W♀ x W♂” and “L♀ x W♂” crosses at 22H00 (Fig. 3.2). In the laboratory, non-irradiated males mated more than wild males and generally matings occurred earlier than was the case in the field. In the field, wild females were more responsive to non-irradiated laboratory males than to their wild counterparts (Fig. 3.2).

3.3.3 Effect of irradiation (200Gy) on mating competitiveness

The three-way interaction effect between time of night, type of cross and location of trials on the pair-wise comparison of mating propensity between non-irradiated and irradiated *E. saccharina* moths was statistically non-significant ($F_{(27, 280)} = 1.1342$, $P = 0.296$) (Fig. 3.3.). There was also no significant time x cross type effect ($F_{(27, 280)} = 0.8914$, $P = 0.634$) nor location x cross type effect ($F_{(3, 280)} = 1.5014$, $P = 0.215$). Under laboratory conditions the early (between 18H00 and 20H00) most successful matings consisted of the “S♀ x S♂”, “L♀ x S♂” and “S♀ x L♂” mating combinations (Fig. 3.3). However there were no significant differences in mating success between the crosses. Steriles showed no ill-effects in their propensity to mate compared to the non-irradiated moths. There was a slight mating peak in the “L♀ x L♂” cross type at 01H00 but it was not significant and could be a laboratory environment artefact since this was not picked up in the field locality.

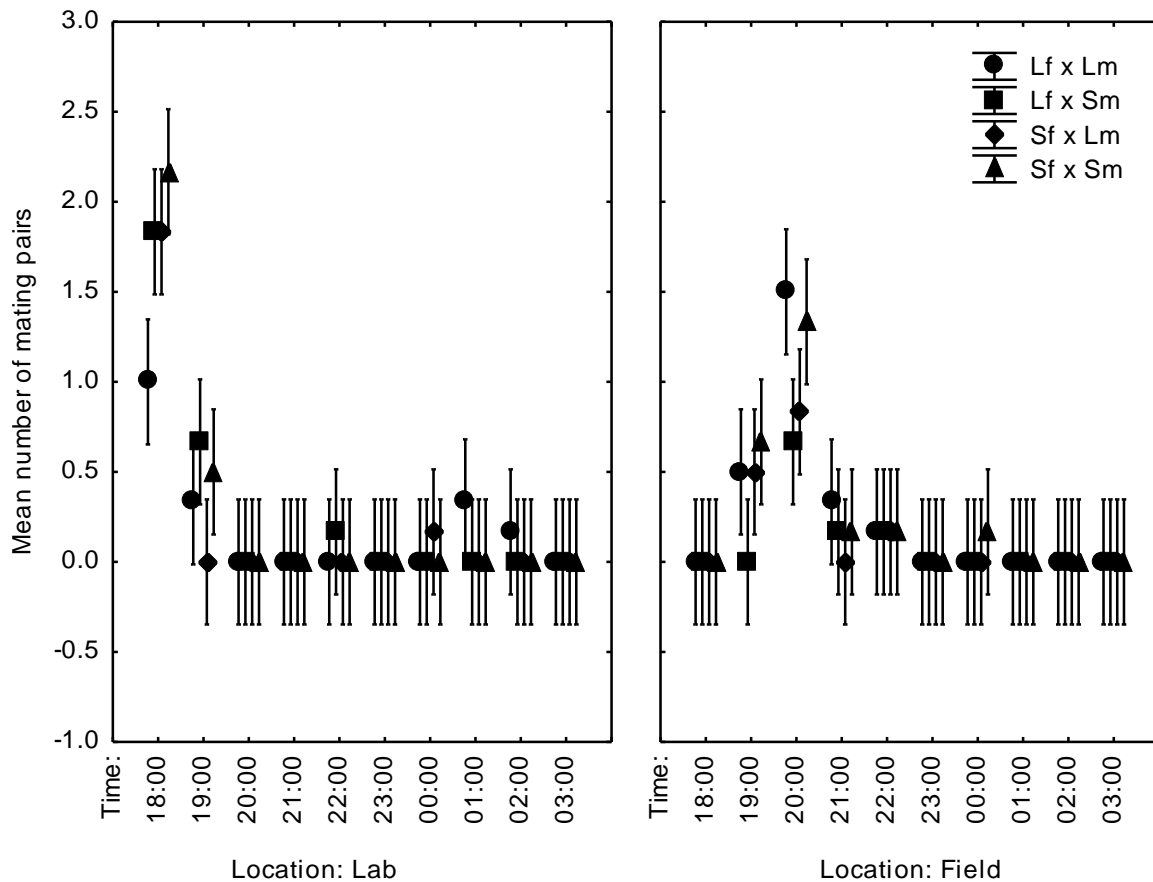


Fig. 3.3. The mean number of matings in a pair-wise comparison between non-irradiated and irradiated laboratory reared *Eldana saccharina* adults showing a non-significant three-way interaction between time of night, type of cross and location of trials. The expected possible mating combinations were: (i) non irradiated female and non-irradiated male (Lf x Lm); (ii) non-irradiated female and irradiated male (Lf x Sm); (iii) irradiated female and non-irradiated male (Sf x Lm); and/or (iv) irradiated female and irradiated male (Sf x Sm). Error bars denote 95% confidence limits.

Under Field conditions the most successful matings were observed between 19H00 and 22H00 in the “L♀ x L♂” and “S♀ x S♂” crosses but were not significantly different from the other crosses (Fig. 3.3). As observed in the laboratory trial, there were also no ill-effects due to irradiation in the field trial (Fig. 3.3.). However, there was a highly significant ($F_{(9, 280)} = 29.3624$, $P = 0.001$) two-way interaction effect between time of mating and location on mean number of mating pairs (Fig. 3.4.). In the laboratory location, most matings occurred between 18H00 and 20H00, with a significant peak at 18H00 as well some mating between midnight and 02H00, while in the field location, matings occurred between 19H00 and 23H00 with a peak at 20H00 (Fig. 3.4).

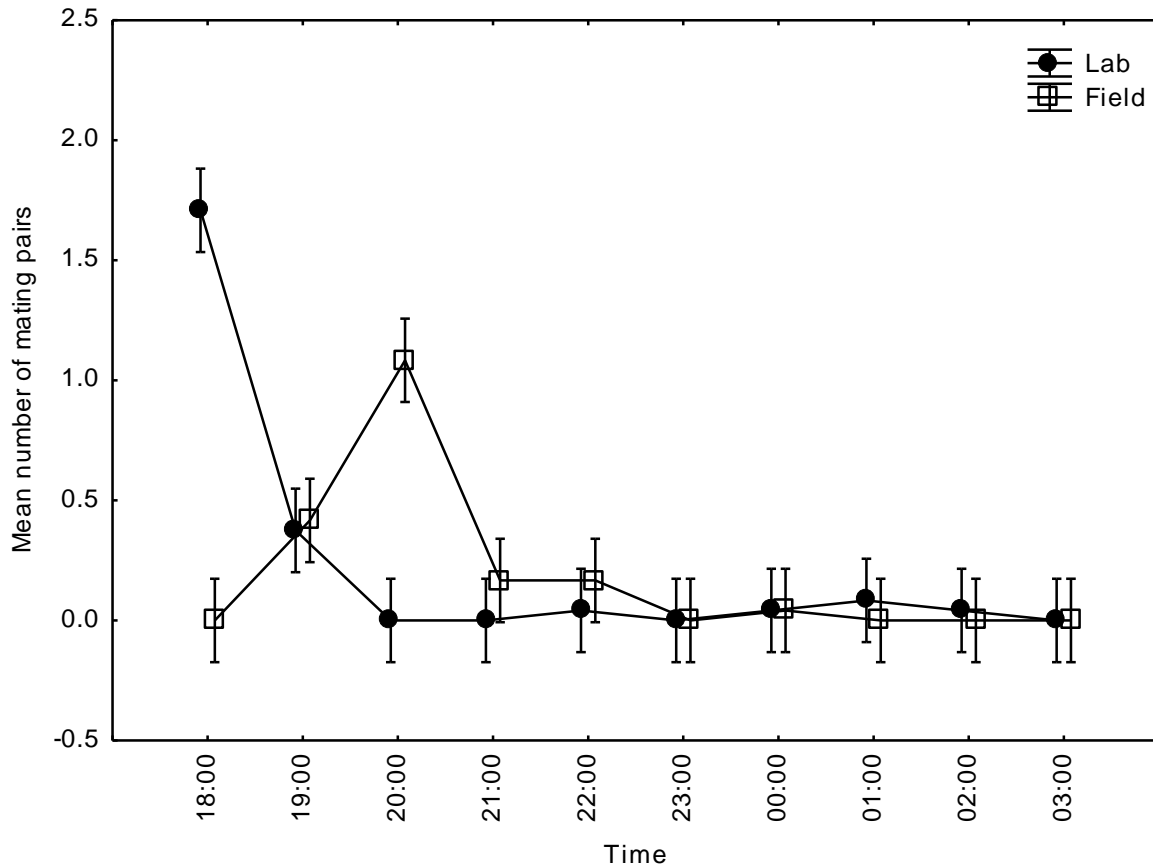


Fig. 3.4 The mean number of matings in a pair-wise comparison between non-irradiated and irradiated *Eldana saccharina* adults showing a significant two-way interaction between time of night and location of trials. Data were pooled across the entire observation period to obtain total matings irrespective of cross type. Error bars denote 95% confidence limits.

3.3.4 Additive or synergistic effects due to laboratory-rearing and irradiation on mating competitiveness.

There was a highly significant three-way interaction effect ($F_{(33, 280)} = 5.9327$, $P = 0.001$) between time of night, type of cross and location of trials on the number of matings in the pair-wise comparison of irradiated *E. saccharina* adults tested against their wild counterparts. In the laboratory, there was a peak in mating activity at 6 pm and mating continued until 22H00 (Fig. 3.5).

The observed matings were exclusive to “S♀ x S♂” mating combination. A second peak of mating activity was also observed between 01H00 and 05H00 but this was exclusive to “W♀ x W♂” combination (Fig. 3.5). The results show that in the laboratory setting “S♀ x S♂” matings were more prevalent than other mating combinations during the early part of the evening while the “W♀ x W♂” type was dominant in the early morning (Fig. 3.5).

In the field there was a significantly high number of matings by the “S♀ x S♂” cross type at 8 pm (Fig. 3.5). Between 20H00 and 23H00, equal mating was observed in all the mating combinations except the “W♀ x W♂” cross. There also was a significantly high mating peak exhibited by the “S♀ x S♂” type at 20H00 while between 21H00 and 23H00 there was equal mating success between all the crosses except the “W♀ x W♂” combination. No homotypic matings of wild moths (i.e. “W♀ x W♂”) were ever observed in the field location (Fig. 3.5).

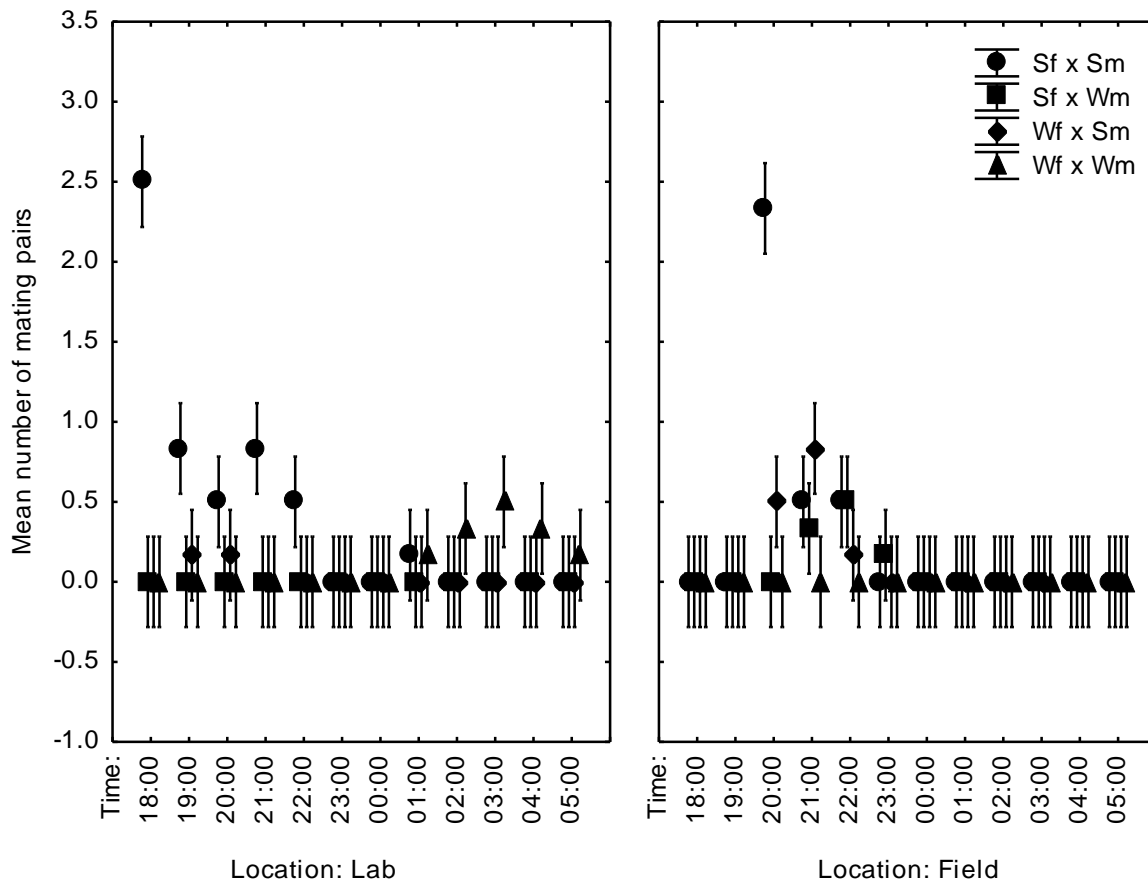


Fig. 3.5 The mean number of matings in a pair-wise comparison between irradiated laboratory reared and wild *Eldana saccharina* adults showing a significant three-way interaction between time of night, type of cross and location of trials. The expected possible mating combinations/cross types are: (i) irradiated female and irradiated male (Sf x Sm); (ii) irradiated female and wild male (Sf x Wm); (iii) wild female and irradiated male (Wf x Sm); and/or (iv) wild female and wild male (Wf x Wm). Error bars denote 95% confidence limits.

The detailed summary of the mean matings observed in the various pair-wise comparisons discussed above is given in Table 3.1.

3.3.5 Compatibility tests

Mating was observed in all four possible combinations in the following pair-wise comparisons and locations: “L-W” (field) and “L-S” (laboratory and field) (Table 3.1). Likewise, the absence of mating barriers in the afore mentioned was confirmed by the chi-square test of independence which showed that the mean ISI values of all mating combinations in the respective comparisons were not significantly different from zero (Table 3.1). However, there were no wild homotypic matings in the “L-W” (laboratory) and the “S-W” (field) and also no sterile ♀ × wild ♂ heterotypic matings in the “S-W” (laboratory) pair-wise comparisons (Table 3.1). This was reflected in the mean ISI values of these comparisons which were shown to significantly differ from zero. There was a significantly higher participation of irradiated and non-irradiated adults compared to wilds in the “L-W” (laboratory) and the “S-W” (laboratory and field) tests as reflected in the relatively high MRPI (0.6, 0.5 and 0.5 respectively) and FRPI (0.8, 0.6 and 0.7) values.

Where wild adults were involved (“L-W” and “S-W”), there were significantly more non-irradiated and irradiated homotypic pairs formed in laboratory tests compared to heterotypic matings indicating a laboratory environment artefact rather than inferiority in quality of wild moths (Table 3.1). On the contrary, the number of heterotypic pairs formed did not differ significantly from that of the homotypic pairs in the field location indicating equal participation between test adults and their wild counterparts and ruling out the possibility of adults from either strain being sub-optimal or incompatible.

As expected in the “L-S” comparison for both locations, irradiated and non-irradiated adults showed equal participation in mating by both genders as reflected in the MRPI and FRPI values which did not differ significantly from zero (Table 3.1). The number of heterotypic and homotypic couples was also similar, indicating that there were no mating barriers between fertile laboratory and irradiated laboratory adults. The chi-square test of independence also showed that ISI values for the “L-S” comparison in both locations did not depart significantly from zero confirming random mating between the strains.

Table 3.1 Mating compatibility and performance of lab-reared, wild and sterile (200Gy) *Eldana saccharina* in pair-wise combinations under laboratory and semi-field conditions.

Trial No.	Strains tested	Number of couples (avg ± SD)								No. Replicates	Chi Square test of Independence	
		AA	AB	BA	BB	PM	MRPI	FRPI	ISI		χ^2 (ISI)	P
1	L-W(Lab)	4.5Aa±0.8	0.7BCa±0.8	1.2Ba±0.8	0.0Ca	0.5a±0.1	0.6±0.2*	0.8±0.2*	0.44±0.10*	6	15.37	0.009*
	L-W(Field)	2.3Ab±0.8	1.7ABb±1.0	2.3Ab±1.4	0.3Ba±0.5	0.5a±0.1	0.1±0.3	0.7±0.3*	-0.02±0.17	6	10.92	0.053
2	L-S(Lab)	1.8Aa±0.8	2.7Aa±0.5	2.0Aa±1.1	2.7Aa±0.5	0.7b ±0.2	-0.0±0.2	-0.2±0.2	-0.02±0.05	6	1.06	0.958
	L-S(Field)	2.7Aa±1.4	1.0Ab±0.9	1.5Aa±0.8	2.5Aa±1.0	0.6ab±0.1	-0.1±0.2	0.1±0.2	0.23±0.10	6	9.988	0.076
3	S-W(Lab)	5.3Aa±0.5	0.0Ba	0.2BCa±0.5	1.5Ca±0.8	0.6ab±0.1	0.5±0.3*	0.6±0.2*	0.9±0.05*	6	51.67	0.000*
	S-W(Field)	3.3Ab±1.4	1.0Bb±0.9	1.5ABb±0.8	0.0Cb	0.5a ±0.1	0.5±0.3*	0.7±0.3*	0.16±0.17*	6	12.20	0.032*

*Observed index significantly departs from random mating (ISI) or equal performance of each sex (MRPI and FRPI).

For number of couples, means followed by the same Upper case letter in each row and means followed by the same lower case letter in each sub-column are not significantly different at the $P = 0.05$ level. "L-W": adult treatment = non-irradiated vs. wild adults, "L-S": adult treatment = non-irradiated vs. sterile adults, "SW": adult treatment = sterile vs. wild adults.

"AA" denotes mean number of matings of "A" females with "A" males for homotypic couples in the respective adult treatment.

"BB" denotes mean number of matings of "B" females with "B" males for homotypic couples in the respective adult treatment.

"AB" denotes mean number of matings of "A" females with "B" males for heterotypic couples in the respective adult treatment.

"BA" denotes mean number of matings of "B" females with "A" males for heterotypic couples in the respective adult treatment.

3.4. Discussion

3.4.1. Effect of laboratory rearing on mating competitiveness with wilds

The success of the SIT in any AW-IPM operational programme is reliant on adequate mating competitiveness and compatibility between the strain being released and that of the target wild population (Cayol *et al.*, 2002; Orozco *et al.*, 2007; Bloem *et al.*, 2010; Taret *et al.*, 2010). The current study is the first of its kind to report on mating competitiveness and compatibility between mass-reared, sterile and wild populations of *E. saccharina* in laboratory and field cage situations. There was an interaction between time of mating, location of trials and type of mating combination in the pairwise trial of non-irradiated laboratory reared and wild adults (L-W). This was largely a consequent of the origin of the respective strains, which had a significant influence on how they behaved outside the natural environment in which they were reared. According to Rossler (1975) it is generally difficult to induce wild insect populations to mate and reproduce in the laboratory since conditions significantly differ from those in the wild which inevitably influences their behavior. Males of the laboratory reared strain called and mated significantly earlier in the laboratory environment than they did in the field cage, while wild males called and mated much earlier in the field location than they did in the laboratory environment. This could be attributed to the fact that under mass-rearing conditions the requirements for appropriate mating “behavior” are removed as cost effective production processes demand that important compromises be made in the environmental arena presented to the moths for mating (Robinson *et al.*, 2002). First, the adult density in production cages leads to a degeneration in most aspects of the normal mating behavior such as early initiation of calling and matings (Cayol, 2000) and hence distortions in the field. Second, the abiotic conditions under mass-rearing conditions such as constant light and temperature regimes and artificial larval diet that is much richer in proteins compared to diets in nature significantly differ from those in the field (Robinson *et al.*, 2002). These changes therefore impact directly on mating behavior of the different strains in the laboratory viz field cage conditions. In addition space is drastically restricted under laboratory conditions such that frequent and random interactions result successful mating by the laboratory adapted test strains compared to the control, even in the case of males which are less sexually motivated or less competitive (Lux *et al.*, 2002).

More importantly, even though the laboratory reared males commenced calling and mating slightly latter in the field cage than they did in the laboratory location, times and number of matings did not differ from those exhibited by the wild strain regardless of the females involved. Mass-rearing conditions have been reported to increase male aggressiveness, favour fast mating and shorten courtship (Calcagno *et al.*, 1999; 2002), traits that may have evolved for the avoidance of

interruptions during mating and to increase likelihood of securing mates under conditions of overcrowding (Briceno & Eberhard, 1998; 2002). It may therefore be advantageous for males to begin calling earlier in the presence of intense competition from other males, however success of this mating strategy would depend on co-evolution in female choice criteria within the laboratory population (Briceño & Eberhard, 2002). Therefore under field conditions, early mating behaviour by the mass-reared sterile *E. saccharina* would be desirable for the SIT programme as many wild females would be mated by sterile males before wild males have the opportunity to do so, thereby achieving the targeted number of sterile matings that result in population reduction.

Another observation noted in the pair-wise trials between wild vs. laboratory reared (L-W) was that test females were more prone to mate than wild females regardless of the male type involved in both localities. This is a common characteristic of mass-reared individuals when they are confined together in competition tests with wild strains (Calkins, 1984; Harris *et al.*, 1986). According to Cayol *et al.* (2002) laboratory females tend to be less “choosy” than their wild counterparts. This is because traits that favor simpler, less discrimination and earlier courtship sequences to ensure copulation success and changes in sexual competitiveness may be selected under mass-rearing conditions in mass-reared females (Boake *et al.*, 1996; Cayol *et al.*, 2002). Rearing conditions may also represent a different environment where lek formation might not be as important as in nature (Rodríguez *et al.*, 2002). Since *E. saccharina* is a species with a complex lek polygyny, wild females may actively select and discriminate in favor of males releasing timely pheromone of the adequate profile (Heath *et al.*, 1994) and performing properly visual, sound and tactile courtship behaviors (Eberhard 2000). Wild females may therefore still favor the courtship of a wild male even though he may represent a minority within a mixed lek (Hendrichs *et al.*, 2002). It was nevertheless encouraging to note that in the field locality, both males and females of the test strains mated with both members of their own population and those of the wild strain. However, it is concerning that the number of homotypic matings of the test strains was significantly higher than that of heterotypic combinations in the laboratory location. This can be attributed to fact that where mass-produced males and females are released together they tend to mate amongst themselves before having an opportunity to mate with their wild counterparts (Moreno *et al.*, 1991; Hernandez *et al.*, 2003). This can also be linked to laboratory adaptation, mass-rearing and irradiation all of which produce genetic and physiological effects in conventional strains (Shelly *et al.*, 1994; Benedict & Robinson, 2003) thereby influencing their behavior as earlier noted. In fruit flies laboratory-reared strains tend to have a greater mating propensity and a significantly earlier age of maximum mating activity than wild strains (Liedo *et al.*, 2002).

3.4.3. Effect of radiation treatment on mating competitiveness

In the case of the non-irradiated laboratory reared and sterile comparison (L-S), the mean number of couples/matings was similar regardless of location. No ill-effects on the mass-reared strain due to irradiation were detected. Both strains are from the same origin and hence the traits selected in the laboratory environment were also expressed in the irradiated strain. There was no evidence of variations in times of mating or discrimination amongst the strains in each of the trial locations. These results indicate that the radiation dose of 200Gy is still within the range that will not compromise the performance of the treated moths. Any ill-effects due to radiation would be a result of increasing the sterility dose since the use of high ionizing radiation reduces sexual motivation and overall mating performance (Weldon, 2005). For example, Lux *et al.* (2002) reported that irradiated male Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae), were more passive, less vigorous and less sexually motivated than non-irradiated, mass-reared males.

Nevertheless, the only significant interaction in this pair-wise comparison was between location and type of cross which can be attributed to significant differences in peak mating times and mean number of matings achieved in the laboratory, viz field locations. In the former, most matings occurred during the early part of the night (18H00) while in the later peak mating was attained only two hours later (20:00) and the mean number of matings was significantly less. Mating behaviour characteristics of mass-reared and sterile insects in a natural environment may be influenced by acclimatization to outdoor conditions (Pereira *et al.*, 2007). For example, Judd and Gardiner (2006) state that temperature and light transitions are common in the field as the night progresses and hence may be responsible for differences in response of mass-reared and sterile strains to these changes when released into the field. Judd and Gardiner (2006) further state that mass-rearing could possibly affect temperature thresholds for general activity or dispersal from release locations. Pereira *et al.* (2007) reported that intense selection pressures imposed by the rearing conditions leads to a shift in the sequence and timing of mating away from that which is normally exhibited in the wild. The significantly early mating and high number of matings for both laboratory and irradiated strains observed in the laboratory viz field location are indicative of adaptation to laboratory conditions where emphasis is on high reproduction rate, earlier and shorter mating (Iwahashi, 1996; Matos *et al.*, 2000). According to Simmons *et al.* (2010) a high quality and productive insect in the mass-rearing facility is not necessarily a good performer in the field since the production facility results in the selection of traits that enable an organism to adapt and persist in the artificial environment in which it is produced.

3.4.4. Additive or synergistic effects due to laboratory rearing and irradiation on mating competitiveness

In the case of the pairwise comparison between irradiated and wild strain (S-W), it was encouraging to note that irradiation at the sub-sterilizing dose of 200 Gy did not affect the performance of *E. saccharina*. This differed from observations in similar studies on *C. capitata* where genetic changes due to selection under artificial mass-rearing, negative somatic effects of irradiation and handling procedures were shown to negatively affect vigor, behaviour of sterile males and reduce their mating performance with the wild population in the field (Economopoulos, 1996). Since laboratory domestication and mass-rearing are necessary to produce the large quantity of males required for SIT (Weldon, 2005), the intense unnatural selection pressures may result in loss of important ecological and behavioural traits necessary for survival and persistence in the wild. These laboratory-induced genetic and behaviour changes are further compounded by the adverse somatic effects of irradiation in air such as degradation of insect quality, infliction of lesions and damage of cuticle (Shelly & Whittier, 1996; Lux *et al.*, 2002; Bakri *et al.*, 2005). However, results from the current study were not so obvious and therefore differed from the observations by the afore-mentioned authors. Both irradiated male and female individuals did not discriminate against their wild counterparts in both the laboratory and field location. Weldon (2005) observed a similar effect in *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) and attributed it to the fact that irradiation of mass-reared males could counter any irregular mating behaviour selected in the laboratory such that mating behaviour of steriles is similar to that of wild strain. The mechanisms involved in such an effect are unclear but could be attributed to the radiation dosage which is low enough to ensure that treated moths remain competitive and their quality uncompromised yet sufficient to induce partial sterility.

The results from this study show that *E. saccharina* irradiated at 200 Gy are as competitive as the wild strain and therefore support the use of a sub-sterilizing dose rather than higher dosages to achieve full sterility. This bodes well with the end goal of implementing F₁ (inherited) sterility, the benefits of which include better mating competitiveness, flight ability and field performance and hence better control compared to fully sterile yet poorly competitive and physically unfit counterparts (Kean *et al.*, 2008, Suckling *et al.*, 2011). For example, Bloem *et al.* (1999) reported that male codling moths treated with lower (sub-sterilizing) doses of radiation (100 Gy) are more competitive than moths treated with higher doses (>150 Gy). While irradiation of adults did not negatively impact the mating competitiveness of *E. saccharina* there was no evidence of additive or synergistic effects due to laboratory rearing and/or irradiation. Results from the pairwise comparisons of the “L-W” and “S-W” showed no significant differences in mating competitiveness between the non-irradiated and sterile strains. The mating times and mating frequencies of both test

strains in comparison to the wild strain did not differ from each other. Furthermore the similarity in the mean number of matings regardless of location in the “L-S” comparison (see section 3.4.3) indicated that there were no ill-effects or enhancement in mating performance or behaviour of the mass-reared strain after irradiation. There also was no evidence of variations in times of mating or discrimination amongst the strains in each of the trial locations.

3.4.5 Compatibility tests

The mating indices generated from these data demonstrate that mass-reared *E. saccharina* in South Africa have not yet evolved sexual behaviours suggestive of incipient pre-mating isolation barriers with respect to the local wild strain under natural conditions. While the more controlled laboratory tests show a greater propensity of the test strains to mate with members of their own population, the more robust field tests have shown no evidence of sexual incompatibility between test strains and their wild counterparts. The combined data of the different indices (ISI, MRP and FRPI) complement each other very well and illustrate the sexual competitiveness and compatibility between the test strains and the wild *E. saccharina* population. The high positive MRPI values for tests in which the wild strain was involved indicate that for both locations test males (i.e. non-irradiated and irradiated) were more effective than wild males in copulating with either test or wild females. In the “L-W” field comparison the MRPI values did not differ significantly from zero indicating that laboratory males were as effective as wild males in copulating with wild females hence ruling out adverse effects due to mass-rearing.

The positive and highly significant FRPI values obtained in the comparisons between non-irradiated or irradiated with the wild strain reflect a tendency for test females to copulate in greater proportion than the wild females. However the lower participation of sterile females in heterotypic matings (i.e. $S_{\text{♀}} \times W_{\text{♂}}$) generally suggests that sterile males have a greater opportunity to mate with wild females. The high participation of sterile females in homotypic matings (i.e. $S_{\text{♀}} \times S_{\text{♂}}$) on the other hand indicates the importance of being cautious not to release both sterile males and females together as they may mate amongst themselves before having the opportunity to mate with wild counterparts (Moreno *et al.*, 1991). Since copulating pairs were removed from the mating arena and not replaced after separation this could be a logical explanation for absence of wild homotypic matings in the “L-W” (laboratory) and “S-W” (field) comparisons. This also supports the suggestion that wild females respond much earlier and have a greater opportunity to mate with sterile rather than wild males. The indices in “L-S” comparison confirm random mating and equal performance between genders and strains thereby ruling out incompatibility issues resulting from effects of radiation treatment.

3.5 Conclusion

In general, despite differences in peak times of mating between the respective treatments, the data presented indicate that there is no evidence of any incompatibility between mass-reared, irradiated and wild *E. saccharina* strains. The results of the present study are consistent with observations reported from similar studies. Cayol *et al.* (2002) reported absence of pre-mating isolation barriers among local strains of *C. capitata* with those originating from at least nine countries in different continents. Taret *et al.* (2010) also showed that no mating incompatibility issues or incipient pre-mating isolation mechanisms had yet evolved in eleven populations of *C. pomonella* from different countries in both the northern and southern hemispheres. In addition, no evidence of mating incompatibility was found between mass-reared sterile and wild populations of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) from different regions in Mexico (Orozco *et al.*, 2007).

The results of the present study thus provide the necessary evidence and confidence that the mass-reared *E. saccharina* strain currently produced at the SASRI insect rearing unit is suitable for use in SIT-based projects. These data will be useful for strain management purposes as well as improvement of colonization procedures, thereby increasing efficiency of operational *E. saccharina* SIT programmes and reducing costs. Further field tests are recommended, to investigate the effects of climatic conditions, wild *E. saccharina* strains from different origins as well as age of wild strains since wild insects are known to attain the age of peak sexual activity much later than their mass-reared counterparts (Liedo *et al.*, 2002). In addition compatibility tests between the local mass-reared strains of *E. saccharina* with strains from other geographical regions should be done in order to optimize and expand the SIT on a global and inter-regional basis. According to Robinson *et al.* (2002) the lack of any mating barriers between the tested populations has major implications for the SIT in that a particular mass-reared strain can be used at any location to deal with outbreaks where the target population to be eradicated is of unknown origin. This has already enabled the large medfly rearing facility in El Pino, Guatemala to provide sterile male medflies, from a genetic sexing strain, to California, Florida, South Africa and Israel (Robinson *et al.* 2002).

3.6 References

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CHAPTER 4

IMPACT OF MASS-REARING AND GAMMA RADIATION ON THERMAL TOLERANCE OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

4.1 Introduction

Several biochemical and physiological processes in living organisms are directly affected by temperature (Chown & Nicholson, 2004) and therefore this abiotic factor influences insect population dynamics (Nyamukondiwa & Terblanche, 2009). In the long term, temperature affects seasonality and evolutionary responses in arthropods (Chown & Nicholson, 2004; Lee & Denlinger, 2010). Several authors also concur that insects are exposed to some form of thermal stress during their life-cycle as a result of temporal and spatial environmental temperature variations (Feder *et al.*, 2000; Gibbs *et al.*, 2003; Huey & Pascual, 2009; Chown & Terblanche, 2007; Nyamukondiwa & Terblanche, 2009). At sub-lethal temperatures, rate of resource acquisition and consumption are significantly affected and consequently so are growth, development and reproduction (Stotter & Terblanche, 2009). Since temperature significantly influences likelihood of mortality, population decline is also inevitable at extremes (Hoffmann *et al.*, 2003; Chown & Terblanche, 2007). The ability to withstand thermal stress is therefore significant for the success of insect populations and evolutionary fitness in the wild (Toolson & Hadley, 1974; Loeschcke & Hoffmann, 2007; Sørensen *et al.*, 2009).

Bahrndorff *et al.*, (2009) state that environmental temperatures and insect thermal tolerance may be significantly correlated, and thus thermal tolerance could be involved in limiting a species' potential geographic distribution. Huey and Stevenson (1979) also illustrated how the relationship between body temperature and performance in ectothermic organisms is bounded by their critical thermal limits. The maximum performance occurs at an optimal body temperature and the thermal performance breadth is the range of the body temperature that permits a certain level of performance (Huey & Stevenson, 1979). While feeding rate and digestive efficiencies are amongst the first functional aspects to change in response to shifts in temperature and water availability (Crafford, 1990), the physiological tolerances of an insect species are more important as they are likely to contribute considerably to its performance, distribution and continued survival (Davidson, 1990; Tenow & Nilsson, 1990; Kimura *et al.*, 1994; Strathdee & Bale, 1995). However, the temperature tolerance of an insect is not a fixed characteristic (Nyamukondiwa & Terblanche, 2009; Stotter & Terblanche, 2009) but rather it is influenced by a number of factors such as thermal history either within its own or parental lifetime (Crill *et al.*, 1996; Hoffmann *et al.*, 2003; Chown & Nicolson,

2004), age, body size, feeding status (Nyamukondiwa & Terblanche, 2009), development (life stage) and/or gender (Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009). Ultimately an insect's thermal tolerance is determined by a combination of the afore-mentioned factors as well as the complex interactions between duration and severity of exposure (Chown & Nicolson, 2004; Rako & Hoffmann, 2006; Marais *et al.*, 2009). Longer or more severe exposures typically result in lower survival (Stotter & Terblanche, 2009). However the degree to which these and other intrinsic factors influence thermal tolerance is still not well established for many insect species (Nyamukondiwa & Terblanche, 2009).

Chilling in order to facilitate handling and sorting prior to field release is one of the fundamental pre-treatments effected on mass-reared insects during implementation of the sterile insect technique (SIT) (Carpenter *et al.*, 2007; Carpenter *et al.*, 2010; Simmons *et al.*, 2010). Stotter and Terblanche (2009) noted that the effect of such rapid chilling on subsequent extreme temperatures experienced by these laboratory-reared insects is poorly understood. This may compromise their field performance, and consequently the SIT programme (Terblanche *et al.*, 2008). In addition, there could be dire consequences for the SIT programme in the event that the insects acquire resistance to such treatments during mass-rearing (Stotter and Terblanche, 2009). Since the ability to maintain biological functions at temperature extremes is very important for living organisms in any ecosystem, knowledge of an insect species' thermal relations is therefore critical to investigators who are attempting to control populations of economic pests through release of sterile insects (Toolson and Hadley, 1974). For example both genders of the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), have been known to exhibit reduced flight ability following heat stress with increase in age (Toolson & Hadley, 1974). However flight ability was not reduced in the absence of heat stress despite age of moths. Physiological acclimation at high temperatures may also accelerate age-dependant decline in heat resistance (Davison, 1971) while cold acclimation may contribute to freeze tolerance in certain species (Shimada & Riihimaa, 1988) thereby enhancing temperature-dependent performance and survival to the benefit of control programmes such as the SIT (Bloem *et al.*, 2006; Chidawanyika & Terblanche, 2011). However in some insect species, age differences may compound the effectiveness of acclimation (Toolson & Hadley, 1974).

Tolerance to thermal stress by irradiated insects is essential and knowledge of their physiological competitiveness relative to wild counterparts will determine success of SIT programmes developed for area-wide containment of economic pest populations. Toolson and Hadley (1974) demonstrated that increasing the radiation dose significantly decreased the thermal tolerance of *S. exigua*, exhibited by reduced flight capability following exposure to higher temperature. The thermal tolerance of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is poorly explored and studies on the

temperature biology of this insect pest have focussed on its reproduction (Way, 1994) and day-degree modelling (Way, 1995). The thermal tolerance of irradiated *E. saccharina* has never been studied. This study therefore investigates the effect of laboratory rearing and increasing radiation dosage on thermal tolerance of the adult stage of *E. saccharina*.

4.2 Material & Methods

4.2.1 Study populations

The origin of the laboratory reared, sterile and wild strains used for this study as well as their handling protocol are described in Chapter 2, section 2.2.1. The conditions under which the laboratory strain was reared are given in 2.2.1.

4.2.2 Critical thermal limits

The method described in Nyamukondiwa and Terblanche (2009) was used for measuring the critical thermal limits (CTLs) for both genders of *E. saccharina* from five different treatments (wild, lab-reared, steriles: ST150, ST200 & ST250, where ST150, 200 and 250 denote the dose (gray) of ionizing radiation used in the three sterile treatments respectively). The types of insights gained as well as their ecological relevance can be significantly influenced by the protocol used to carry out such measurements (Chown & Nicholson, 2004; Terblanche *et al.* 2007; Nyamukondiwa & Terblanche, 2009) therefore thermal tolerance was regarded as a measure of acute temperature tolerance under relatively standard conditions. *E. saccharina* moths were individually placed into an insulated double jacketed chamber ('organ pipes') connected to a programmable water bath (Grant GP200-R4, Grant Instruments, UK) (Fig. 4.1). In order to accommodate sub-zero temperatures the water bath was filled with 1: 1 water: propylene glycol mix (Chown & Nicolson, 2004; Terblanche *et al.*, 2007). Temperature in the control chamber was recorded via a copper-constantan thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05°C). It was assumed that body temperature of individual *E. saccharina* moths was in equilibrium with the chamber temperature under the experimental conditions employed (Terblanche *et al.*, 2007). Insects were thus allowed to equilibrate for approximately 10 minutes to a set point chamber temperature of 25°C from which temperature increased for critical thermal maximum (CT_{max}) or decreased for critical thermal minimum (CT_{min}). A ramping rate of 0.25°C/min was used in both experiments until all the insects reached their CT_{max}/CT_{min}. While this ramping rate is likely to be faster than actual heating or cooling rates in the wild, the impact of such variation on interpretation of acute temperature differences on insect population dynamics is largely unclear (Nyamukondiwa &

Terblanche, 2009; Nyamukondiwa & Terblanche, 2010; Mitchell & Hoffmann, 2010). Since the main aim of this study was to investigate effects of irradiation and mass-rearing, results were unlikely to be confounded by rate effects (Nyamukondiwa & Terblanche, 2009) as these were kept constant during all experiments. Chidawanyika and Terblanche (2011) also conducted a similar study on *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) and stated that since the body temperature is in equilibrium with chamber temperatures during ramping protocols, thermal inertia effects are therefore limited.

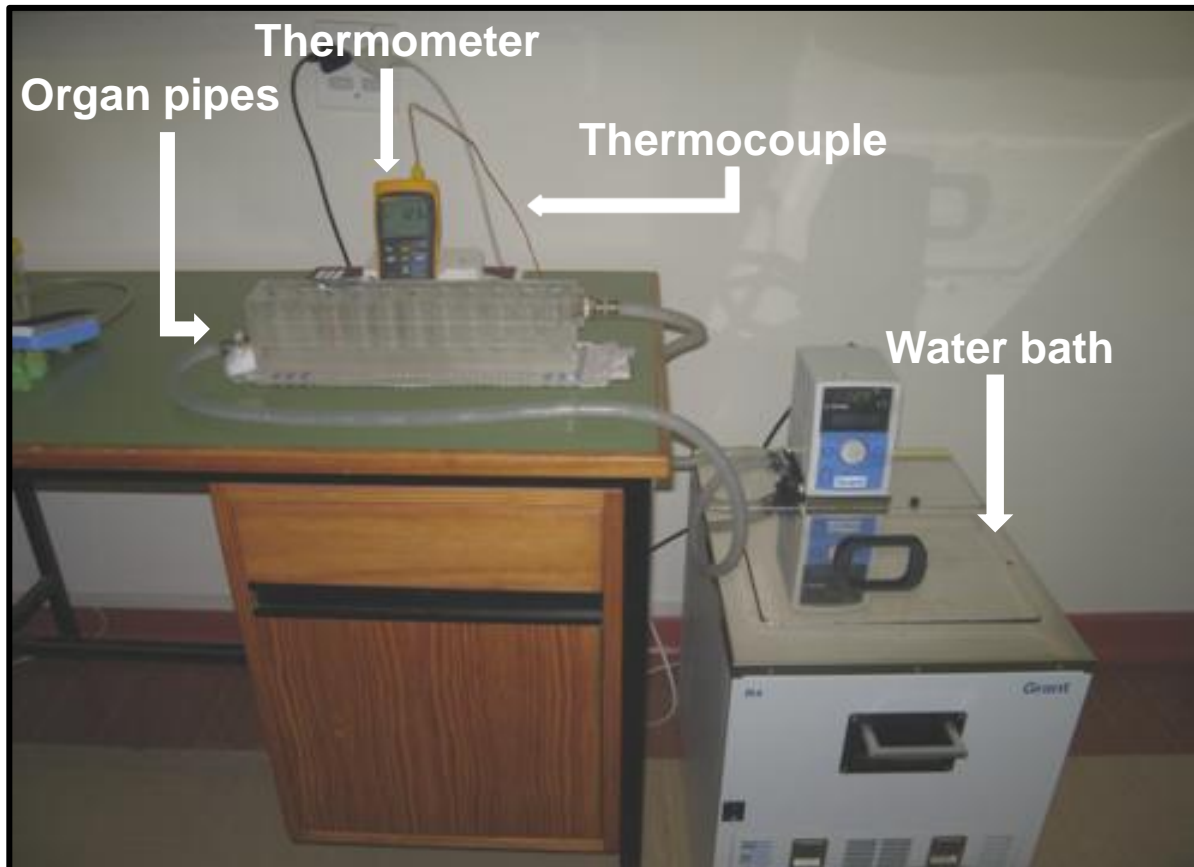


Fig 4.1 Experimental set up for *Eldana saccharina* critical thermal limits (CTLs) assays.

Critical thermal limits were defined as the temperature at which each individual moth lost coordinated muscle function (in the case of CTmin) or experienced onset of muscle spasms (CTmax), subsequently losing the ability to respond to mild stimuli such as prodding (Nyamukondiwa & Terblanche, 2009; Chidawanyika & Terblanche, 2011). For CTmax experiments, onset of muscle spasms coincided with death, thus recovery of moths was not possible, while in the case of CTmin, recovery occurred, and therefore was not immediately lethal. Age was strictly controlled in all assays as it has been shown to have a major effect on insect thermal tolerance (Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009). Freshly eclosed (0-day old) virgin moths were used in all experiments. Each individual moth was regarded a replicate and therefore ten individuals were used for all CTmin and CTmax assays. As a further quality control measure, individual moths were not

removed from the organ pipes to assess behaviour and those used in CTmin assays were never re-used for CTmax assays or vice versa but were immediately discarded after taking measurements.

4.2.3 Statistical analyses

The tests for normality and equality of variance were performed on the data using the Shapiro–Wilk and Hartley–Bartlett tests, respectively. In all cases, key assumptions of ANOVA were met. A factorial ANOVA in Statistica 10.0 (Statsoft Inc., Tulsa, Oklahoma, USA) was done on both CTmax and CTmin in order to determine the effect of mass-rearing and irradiation on the thermal tolerance of adult *E. saccharina*. The CTmin or CTmax were treated as the dependent variables, while gender and/or treatment were the independent variables in these analyses. Statistically homogenous groups were identified using Tukey–Kramer’s post-hoc tests.

4.3 Results

There were significant differences in critical thermal maxima between laboratory reared and wild *E. saccharina* moths ($F_{(1, 36)} = 8.45$, $p = 0.006$; Fig 4.2). Laboratory reared moths were more heat tolerant compared to wild moths for both genders. In the case of critical thermal minima, wild moths were significantly more cold tolerant than laboratory reared moths ($F_{(1, 36)} = 135.10$, $p < 0.001$; Fig 4.3). However there was no gender x treatment interaction effect on either CTmax or CTmin ($p > 0.05$ in both cases; Table 4.1).

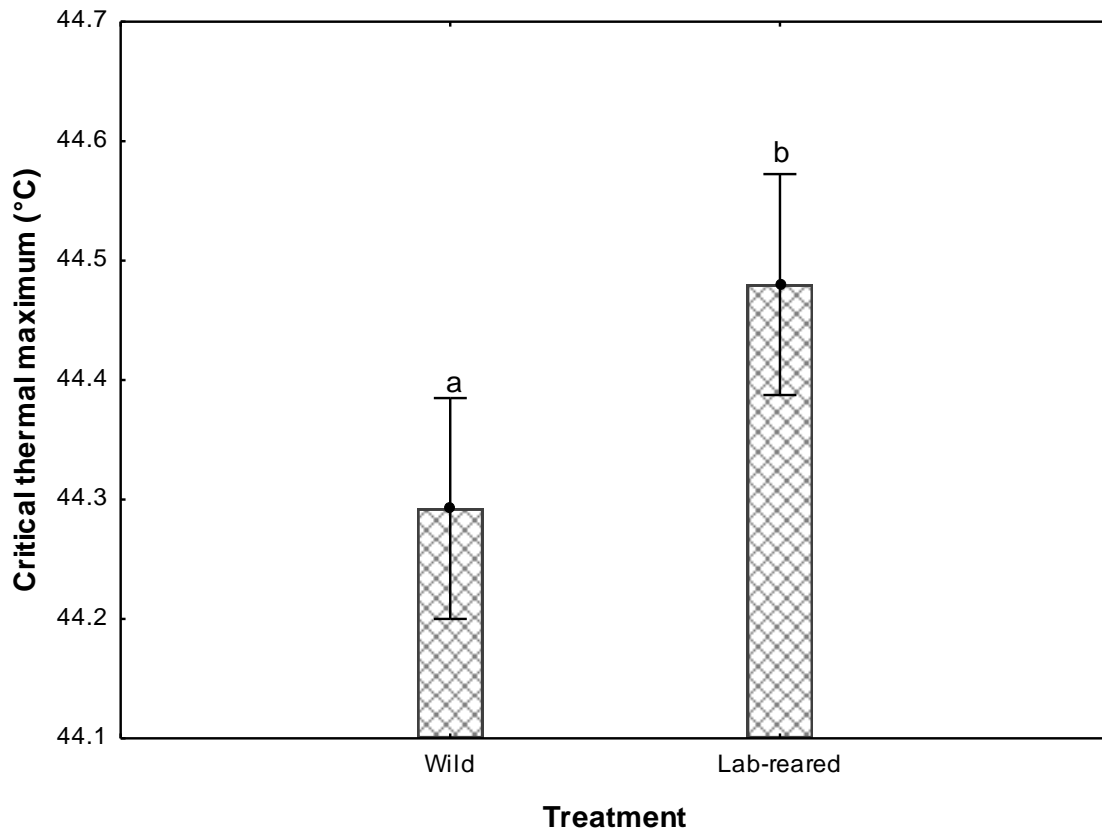


Fig. 4.2 Effect of laboratory rearing on adult *Eldana saccharina* CTmax, (Means \pm 95% C.I). $N = 10$ per group.

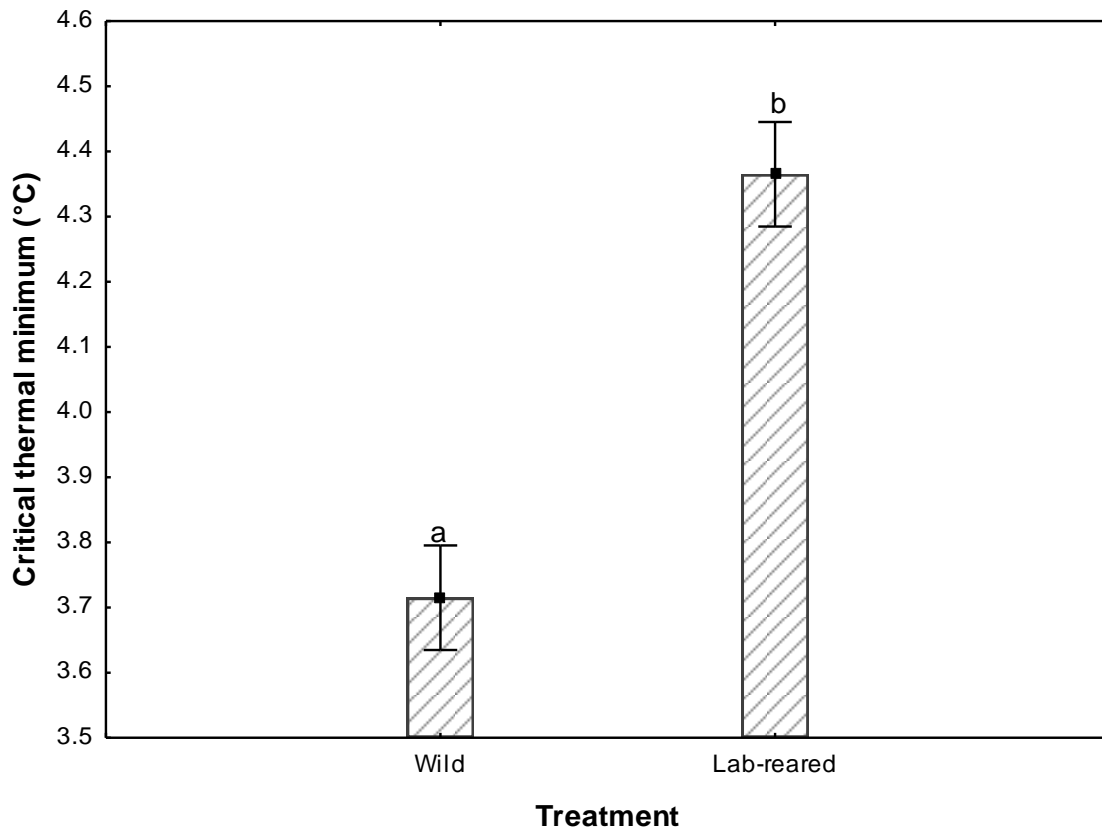


Fig. 4.3 Effect of laboratory rearing on adult *Eldana saccharina* CTmin, (Means \pm 95% C.I). $N = 10$ per group.

There was a significant interaction effect between gender and treatment on both CTmax ($F_{(3, 72)} = 4.82, p = 0.004$; Fig. 4.4) and CTmin ($F_{(3, 72)} = 3.40, p = 0.021$; Fig. 4.5) of *E. saccharina* moths assayed in the radiation treatment.

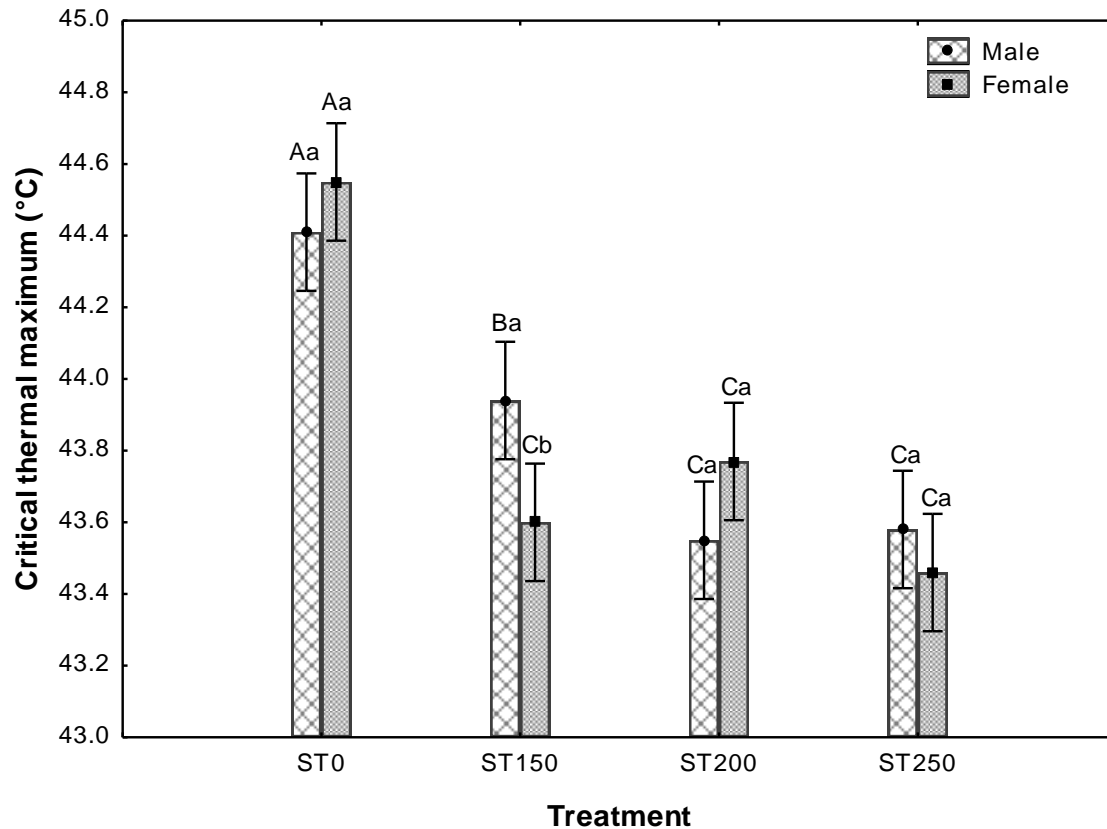


Fig. 4.4 Effect of increasing radiation dosage on adult *Eldana saccharina* CTmax, (Means \pm 95% C.I). $N = 10$ per group. Means at each radiation dosage with the same lower case letter are not significantly different. For each gender, means with the same upper case letter are not significantly different.

The interaction effect was a result of moths irradiated at 150Gy, which showed significant differences between males and females in both CTmax and CTmin. There were no significant gender differences in CTmax between all radiation treatments (Table 4.1). However in the case of CTmin, the difference between males and females was marginally significant ($p = 0.013$; Table 4.1). The data from both assays showed that CTmax and CTmin declined with an increase in radiation dosage. The non-irradiated moths (ST0) were significantly more heat or cold temperature tolerant compared to those that were treated at any of the three levels of gamma radiation (Fig. 4.4 and Fig. 4.5 respectively). For example in the case of CTmin, moths that were exposed to the highest radiation dosage were the least tolerant to low temperatures (Fig. 4.5).

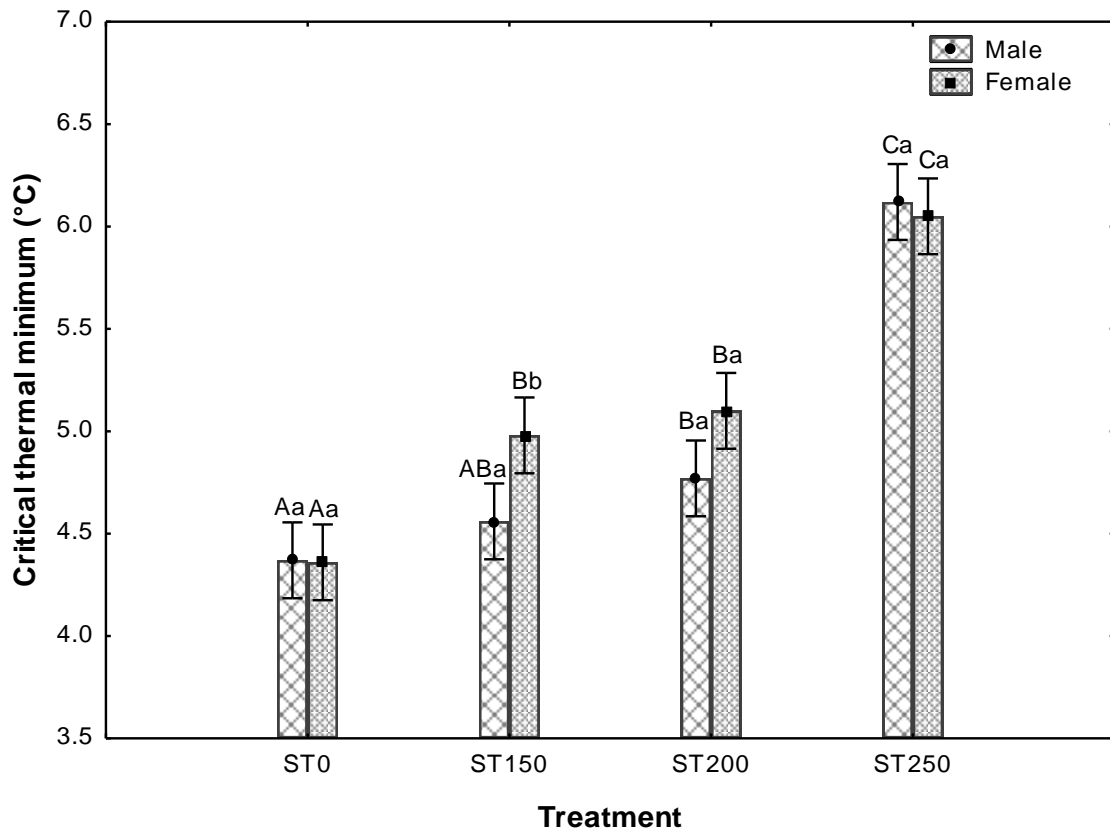


Fig. 4.5 Effects of increasing radiation dosage on adult *E. saccharina* CTmin, (Means \pm 95% C.I). $N = 10$ per group. Means at each radiation dosage with the same lower case letter are not significantly different. For each gender, means with the same upper case letter are not significantly different.

Table 4.1 Summary results from factorial ANOVAs showing effects of gender and treatment and their interactions on *Eldana saccharina* critical thermal maxima and minima (CTmax & CTmin, resp.)

Strains Compared	Trait	Effect	SS	d.f.	F-value	p-value
<i>Wild</i> vs. <i>Lab-reared moths</i>	CTmax	Gender	0.068	1	1.64	0.209
		Treatment	0.352	1	8.45	0.006*
		Gender*Treatment	0.033	1	0.80	0.379
	CTmin	Gender	0.016	1	0.50	0.479
		Treatment	4.225	1	135.10	0.000*
		Gender*Treatment	0.009	1	0.30	0.595
<i>Non-irradiated</i> vs. <i>Irradiated moths</i>	CTmax	Gender	0.010	1	0.19	0.668
		Treatment	10.960	3	54.09	0.000*
		Gender*Treatment	0.980	3	4.82	0.004*
	CTmin	Gender	0.560	1	6.50	0.013*
		Treatment	32.630	3	126.20	0.000*
		Gender*Treatment	0.890	3	3.40	0.021*

*Denotes statistically significant effect.

SS = sum of squares; d.f. = degrees of freedom.

4.4 Discussion

Adult thermal tolerance at daily scales is critical for success of the SIT, for example with respect to minimum temperatures required for sustaining flight, as well as high temperatures that limit both flight and mating (Chidawanyika & Terblanche, 2011). In light of this, thermal tolerance is an important aspect with respect to quality of laboratory-reared moths (Bloem *et al.*, 2006; Stotter & Terblanche, 2009; Chidawanyika & Terblanche, 2011) which may determine their field performance and survival in a sterile insect release programme.

The results from the critical thermal limits (CTL) assays showed that laboratory rearing had a negative impact on low temperature tolerance of adult *E. saccharina* (i.e. laboratory reared strain had an inferior CT_{min} than the wild strain). Desirable behavioural characteristics in mass-reared insects may be compromised by rearing under artificial conditions, e.g. constant temperature, storage conditions and/or storage duration prior to field release, as a consequence of laboratory adaptation (Bloem *et al.*, 1998; Steinberg *et al.*, 1999). For example, in *Drosophila* (Diptera: Drosophilidae), laboratory adaptation has been shown to compromise or result in loss of various fitness traits such as egg to adult viability (Hercus & Hoffmann, 1999), fecundity (Simoes *et al.*, 2007) and thermal tolerance (Jensen *et al.*, 2010). While mass-rearing is necessary to produce the large quantities of insects required for SIT operations, several aspects of the laboratory rearing environment affect insect quality. These include the artificial diets themselves, nutritive elements, contaminants, moisture, texture and/or pH which can influence body size, survival, longevity, flight and mating ability and responsiveness to abiotic factors (Lance & McInnis, 2005).

Mass-reared insects are also subject to intense selection pressure that can result in rapid genetic drift, loss of heterozygosity and ultimately loss of ecological and behavioural traits that are necessary for these insects to remain fit and comparable to their wild counterparts (Dalby-Ball & Meats, 2000; Briceno & Eberhard, 2002; Parker, 2005). For example, some arthropod species produce heat-shock proteins (Hsps) under both high (McMillan *et al.*, 2005) and low temperature stress (Rinehart *et al.*, 2007) which act as molecular chaperones protecting other cellular proteins, thereby conserving key enzyme function (Chidawanyika, 2010). These are some of the important fitness traits that may be lost during the process of intense selection pressure under mass-rearing conditions in favour of other traits e.g. those that favour earlier and simpler courtship systems, shorter mating duration or even less discriminating females. Insect laboratory populations constantly face harsh/extreme climatic conditions due to sudden change from standard controlled conditions to a highly variable natural environment (Chidawanyika, 2010), which may result in survival failure or poor behavioural performance where their wild counterparts would otherwise be able thrive. Fitness trade-offs across

a range of environments have been reported in mass-reared populations exposed to various thermal stresses and regimes because they perform relatively better in the environment where they evolved (Lenski & Bennet, 1993; Partridge *et al.*, 1995). Genetic differences of a laboratory strain, therefore are not reflective of those in nature (Harshman & Hoffmann, 2000; Hoffmann *et al.*, 2001) which result in them exhibiting biological or behavioural traits (Huettel, 1976) which are different from their wild counterparts (Chidawanyika, 2010).

However in the case of *E. saccharina*, the CTmax results from the assays were not as obvious as those observed in CTmin assays. In the current study, results obtained in the wild vs. laboratory reared strain differed from observations by the afore-mentioned authors. Laboratory rearing did not have a negative impact on high temperature tolerance of adult *E. saccharina* (i.e. the laboratory reared strain had better tolerance to heat stress than the wild strain). A possible explanation for this is that laboratory adaptation does not necessarily affect biological and physiological traits in a negative way in some arthropod species (Nyamukondiwa *et al.*, 2011). For example, body mass and high-temperature tolerance in *Glossina* (Diptera: Glossinidae) (Terblanche *et al.*, 2006), cold tolerance (Strachan *et al.*, 2011), heat tolerance and expression of the 'heat shock protein' Hsp70 in *Drosophila* (Krebs *et al.*, 2001) have all been reported as not affected by laboratory adaptation. Nyamukondiwa *et al.* (2011) also state that where cross-tolerance mechanisms exist, that directly link upper and lower thermal limits to one another, laboratory adaptation may not always alter patterns typical of the wild despite relaxation of selection for high- or low-temperature tolerance. Heat shock proteins (Hsps) and other mechanisms which are normally activated for high-temperature protection may also be availed for low temperature protection (Rajamohan & Sinclair, 2008; Nyamukondiwa & Terblanche, 2010). In addition the higher continuous laboratory temperatures (26 -28 °C) to which the mass-reared moths are exposed could make them more heat tolerant and less cold tolerant compared to the wild strain (West-Eberhard, 2003). There is considerable evidence that rearing temperature and temperature pre-treatments have an impact on thermal tolerance of insects, and this has significant implications for SIT. Laboratory adaptation produces genetic and physiological effects in conventional strains (Shelly *et al.*, 1994; Benedict & Robinson, 2003) thereby influencing their behaviour. In addition the effects of various temperature pre-treatments e.g. rapid chilling to facilitate sorting and handling of laboratory individuals on subsequent extreme temperatures experienced in the field is poorly understood (Stotter & Terblanche, 2009). Consequently their field performance, survival and the SIT programme may be compromised (Terblanche *et al.*, 2008).

The nutritional status (i.e. energy reserves and diet quality) of insects has been found to directly or indirectly influence thermal physiology (Hoffmann *et al.*, 2005; Colinet *et al.*, 2006; Shreve *et al.*, 2007; Nyamukondiwa & Terblanche, 2009). In *E. saccharina* the adult stage is non-feeding but pre-

adult nutritional status can be carried over into the adult stage (Nyamukondiwa pers.comm). It is likely that at the time of these experiments the moth strain obtained from the wild may have had a poor nutritional history compared to that of the laboratory reared strain which is produced under constant standard conditions. Experiments were conducted during the post winter period of September/October 2011, where the nutritional quality of the sugarcane crop from which the wild strain was obtained, may have been low given that the year 2011 was declared a drought season (Singels *et al.*, 2012). However data on the nutritional quality of the sugarcane growing at that time of year was unavailable and hence it could not be concluded whether the nutritional history of the wild strain was inferior to that of the laboratory reared strain. Nyamukondiwa and Terblanche (2009) state that resources in the wild are not always abundant or immediately available both spatially and temporally. As such, lower thermo-tolerance of fasted fruit flies *Ceratitis rosa* (Karsch) (Diptera: Terphritidae) and *C. capitata* was attributed to lower energy reserves thereby rendering nutrition a critical aspect. They showed that both CT_{max} and CT_{min} of *C. rosa* and *C. capitata* (known to be chill-susceptible), were significantly enhanced with feeding by approximately 1-2°C. Although actual quantities of body lipid, water content and dietary cholesterol were not assayed in the present study, it is likely that variation of these components in the nutritional status of wild viz mass-reared moths will not only influence their critical thermal limits but also survival of extreme temperatures (Colinet *et al.*, 2006), and aspects of reproduction (Shelly & Kennelly, 2003). Terblanche *et al.* (2008) state that there is a link between insect thermal biology and energy reserves stored up in its body. For example in the wasp *Aphidius colemani* (Hymenoptera: Aphidiinae) there is substantial consumption of energy reserves as a counter response to temperature extremes (Colinet *et al.*, 2006), the survival of which would be compromised when energy sources are depleted. In *Drosophila melanogaster* (Diptera: Drosophilidae), an increase in dietary cholesterol enhances cold survival as a consequence of improved membrane fluidity (Shreve *et al.*, 2007). The extremely low chill coma temperatures characteristic of *Glossina pallidipes* (Diptera: Glossinidae) is also a consequence of high levels of body lipid and water content (Terblanche *et al.*, 2008).

Gender is one of the factors that can be attributed to variations in a population's thermal tolerance (Bowler & Terblanche, 2008; Stotter & Terblanche, 2009; Nyamukondiwa & Terblanche, 2009). However in this study we found almost no gender differences in all the assays conducted with the exception of CT_{min} in the radiation treatment where males were significantly more cold tolerant compared to females at the radiation dose of 150 Gy. Gender-related differences have been demonstrated in high temperature tolerance (Folk *et al.*, 2006; Pappas *et al.*, 2007) and low temperature tolerance (David *et al.*, 1998; Renault *et al.*, 2003), but gender is more critical for other physiological traits such as metabolic rate, where size is a correlated factor (Chown & Nicolson, 2004). However the results of the present study conform to those from other researchers who found

no gender differences in thermal tolerance (Stratman & Markow, 1998; Terblanche *et al.*, 2007; Jensen *et al.*, 2007; Stotter & Terblanche, 2009; Nyamukondiwa & Terblanche, 2009). These results therefore suggest that it may not be necessary to control for gender in thermal tolerance assays and that both genders of *E. saccharina* originate from similar thermal environments either in the wild or in mass-rearing facilities. However, there was a significant interaction between gender and treatment in the case of the irradiated *E. saccharina* in both CTmax and CTmin assays. This interaction was a result of the moths irradiated at 150Gy where differences in thermal tolerance between males and females were statistically significant. The reasons for this phenomenon are unclear since poor cold tolerance in females is normally attributed to oviposition behaviour which is demanding on body lipid reserves (Warburg & Yuval, 1996; Nyamukondiwa & Terblanche, 2009).

Nevertheless, the results of the present study show that, in the case of sterile treatments, irradiation had a significant effect on thermal tolerance of *E. saccharina*. This has implications for the implementation of the sterile insect technique (SIT) as an area wide integrated management strategy, as it is clear that there are distinct differences between irradiated and non-irradiated moths. Radiation degrades insect quality (Calkins & Parker, 2005) and the physical damage inflicted on an insect's body tissue and membrane chemical and physical structures as well as possible changes in gene expression may have a direct or indirect effect on thermal tolerance as well. It follows that the higher the dosage the more deleterious its effects are on the target insect. Typical damage induced by gamma irradiation includes lesions in radiosensitive germ cells which reduce locomotor activity (Eischen *et al.*, 1984) and shorten male longevity through superficial mimicking of the aging process (Clark & Rockstein, 1964; Ducoff, 1972). It has also been reported that when irradiated individuals are heat stressed, flight capability in beet armyworm, *S. exigua* declined significantly as dosages were increased (Toolson & Hadley, 1974). It is however unclear whether this reduction in temperature resistance is typical of the response of irradiated individuals over a wide range of temperatures approaching lethal levels (Toolson & Hadley, 1974).

Since radiation doses greatly affect the quality of reared insects (Chidawanyika, 2010), dose optimization (Judd & Gardiner, 2006) is critical in order to minimise somatic effects induced by radiation (Bakri *et al.*, 2005) yet maintaining competitiveness (Toledo *et al.*, 2004). More importantly, the results of this study showed that irradiated strains had a narrower thermal tolerance compared the control (ST0), while increasing the radiation dose significantly reduced both CTmin and CTmax of sterilized moths (i.e. decline in cold or heat tolerance). However *E. saccharina* is unlikely to be exposed to the levels of temperature extremes tested in these assays. In light of this therefore, temperature dependent performance and survival of the released strain can be enhanced by using various phenotypic plasticity manipulation techniques such as rapid heat hardening (RHH) and rapid

cold hardening (RCH) (Slabber & Chown, 2005; Loeschcke & Hoffmann, 2007) (see discussions in literature review, section 1.7). Rapid cold hardening may be a treatment option for enhancing performance of sterile moths released in the cooler midlands and higher lying regions of the South African Sugarcane belt.

4.5 Conclusion

The absolute temperature tolerances determined in CTL experiments, while raising concerns regarding their ecological significance (Chidawanyika, 2010) are useful in guiding research on enhancement of insect quality and future mass-releases. The microclimate data typical of the areas targeted for SIT deployment for containment of *E. saccharina* can be combined with the thermal tolerance estimates obtained from the laboratory assays performed in this study. This is critical for determining and predicting success and feasibility of such programmes if it is known whether there is a possibility of temperatures approaching lethal levels, by what frequency and duration as well as the periods of the year when likelihood of such events is high. Acclimation in order to enhance performance in variable thermal environments (Hochachka & Somero 2002; Angilletta 2009) is significant for survival and persistence of mass-reared sterile insects. Pre-exposure to sub-lethal environments (also referred to as rapid cold-hardening 'RCH' or rapid heat-hardening 'RHH') will enable mass-reared strains of adult *E. saccharina* to survive otherwise lethal ambient temperatures (Powell & Bale, 2005; Loeschcke & Hoffmann, 2007; Slabber & Chown, 2005) in the event that they are exposed to temperature extremes. Since insects are capable of adjusting their thermal tolerance - a phenomenon called phenotypic plasticity (West-Eberhard, 2003), I therefore recommend further investigation of this phenomenon in *E. saccharina*. This kind of manipulation will enhance temperature-dependent performance and survival to the benefit of control programmes such as the SIT (Bloem *et al.*, 2006; Chidawanyika & Terblanche, 2011).

4.6 References

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CHAPTER 5

RELEASES OF IRRADIATED *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) MOTHS IN PILOT STUDIES TO SUPPRESS WILD POPULATIONS.

5.1. INTRODUCTION

The sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is a major insect pest species in the South African sugarcane industry. Though several tactics are available for its management, *E. saccharina* continues to defy any efforts to eradicate it (Conlong, 1994a; Conlong 1994b). In the development of an area-wide integrated pest management (AW-IPM) strategy, increasingly more environmentally friendly pest control techniques are being developed, which are compatible with and sometimes increase the efficacy of more conventional measures. The sterile insect technique (SIT) is one of several such methodologies that meet the above criteria in addition to being species-specific (Suckling, 2003) and opening up new opportunities for IPM not only under open field but also green/cage-house conditions (Calvitti *et al.*, 2000; Kaspi & Parrella, 2003).

The SIT has been successfully implemented against a number of economically important lepidopteran pest species (see discussions in 1.3.; see also Bloem & Carpenter, 2001), which have motivated the possible extension of the technique towards *E. saccharina* control or eradication. Walton (2011) conducted pioneering studies on the radiation biology of *E. saccharina* in order to assess the suitability of SIT for control as part of an area wide integrated pest management programme (AW-IPM). She determined that irradiation of females and males at doses of 200Gy and 250Gy respectively resulted in complete sterility but high mortality of F₁ progeny. However, reducing the radiation dosage to 200Gy for treating male moths resulted in residual F₁ fertility of 14.6% and 9.5% in males and females respectively. Since it is critical that irradiated males are able to compete readily with their wild counterparts for mates (North, 1975; Omar & Mansor, 1993; Carpenter *et al.*, 2005), the lowest possible radiation dose is desirable to ensure fitness and competitiveness of F₁ males in order that they mate with wild females (Bloem *et al.*, 2001) as well as survive the stress they are subjected to upon release into the wild. Nonetheless, *E. saccharina* was found to be susceptible to ionizing radiation and therefore a suitable candidate for the further development of a SIT programme against it, using radiation doses of 200Gy and 250Gy (Walton, 2011).

Releases of irradiated moths in pilot studies to suppress wild populations can be done by either of two approaches, namely the conventional system approach (Kunz *et al.*, 1984; Bloem & Bloem, 2000;

Walters *et al.*, 2000) or the green/shade house approach (Rosca & Barbulescu, 1993; Sutrisno & Hoedaya, 1993; Calvitti *et al.*, 1997; 1998; 2000; Hofmeyr *et al.*, 2005). In the conventional approach, study sites are carefully chosen, where there is a general infestation by the native population of a target pest species, the season and host plant conditions are optimum and the sites are preferably isolated from other areas which can be potential sources of external re-infestation (DeBiasio, 1988; Bloem & Bloem, 2000; Calkins *et al.*, 2000; Walters *et al.*, 2000). Pilot releases of the sterile insects are conducted and native populations monitored closely over a defined period depending on the phenology of the pest species in order to measure the impact of the initial SIT releases. Different release rates are tested where the optimum or appropriate rate is determined by the risk - as calculated from native and sterile insect trap catch data and limited by availability of the steriles (Walters *et al.*, 2000). Theoretically, an extensive three year survey following programme completion should either reveal eradication (zero detection) or suppression (control) of the pest in the study area (Walters *et al.*, 2000). The approach is however not feasible where essential information such as basic biology of the pest species is scanty or unknown, a reliable field population monitoring method is non-existent, mass-rearing facility lacks the capacity to provide adequate numbers of sterile individuals for release in the pilot study on a regular basis and/or where the location of the radiation facility does not permit release of the mass-reared irradiated insects on the night of irradiation. Nevertheless, Carpenter and Gross (1993) successfully completed a conventional pilot study in small mountain valleys in North Carolina to assess the influence of released, sub-sterilized males on wild populations of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) as well as measure the infusion rate of F₁ sterility into the wild population.

The shade house approach can provide valuable insights into the success/potential of the technique in both preventing spread and suppressing existing populations of a pest species targeted for control via the SIT (Calvitti *et al.*, 1997; 1998; 2000). The idea to extend the evaluation or use of the SIT in a confined environment has been successfully implemented against *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) (greenhouse whitefly) (Calvitti *et al.*, 2000), *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (Hight *et al.*, 2005) and *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) (Hofmeyr *et al.*, 2005). However in the case of *E. saccharina* the idea is novel given that it is an outdoor pest species occurring and thriving under natural conditions where emigration or immigration from/to host and mixing of populations are freely possible. Nevertheless, *E. saccharina* is a multivoltine pest species occurring on sugarcane with a mosaic of ages. This mosaic of sugarcane age makes the timing and placement of sterile moth releases important so that numbers of sterile individuals needed to achieve suppression of the native field population can be drastically reduced thus reducing the costs of the technique. *E. saccharina* attacks the sugarcane plant from when it is 4 months old. The shade house approach provides the opportunity to test this

hypothesis considering that the insect rearing unit at the South African Sugar Research Institute (SASRI) currently lacks the capacity to reliably supply the quantities of irradiated moths that can be assured for field releases. In addition the approach is capable of providing a theoretical indication of the suppressive capacity of irradiated *E. saccharina* if releases of treated moths at the given dose and ratio were maintained on a semi-commercial scale or in field (Rosca & Barbulescu, 1993; Hofmeyr *et al.*, 2005). Shade house pilot trials can also be run concurrently while biology and behavior assays are being completed thus allowing the generation of valuable data that can be used to evaluate the concept in the short or medium term.

Based on the results and recommendations by Walton (2011), I tested the capacity of *E. saccharina* male moths sub-sterilized at 200Gy to suppress an existing stable “wild” population under cage-house conditions. Results from this pilot trial will be useful for guiding future deployment of the SIT in open field releases for management or eradication of *E. saccharina*. The objective of this study was to assess the efficacy of sub-sterilized male *E. saccharina* moths, to suppress or eradicate target/pre-established existing populations in a large cage-house. We also test the hypothesis that continuous releases of sterile moths into an existing stable population will reduce the level of crop damage (indicating pest control) compared to a scenario where a natural *E. saccharina* population is allowed to build up in the absence of any control intervention.

5.2 Material & methods

5.2.1 Trial location and cage-house design

The caged suppression of a stable laboratory population allowed to naturally infest potted sugarcane was conducted at the Agriculture Research Council Plant Protection Research Institute (ARC-PPRI) Vredenburg Quarantine Station (33.94 S, 18.84 E; Altitude: 153m) in Stellenbosch, Western Cape Province. Three hundred (300), 48-day old sugarcane seedlings (variety NCo 376, susceptibility to *E. saccharina*: moderate) sourced from SASRI were transplanted into black polythene 20 Litre planting bags (225mm x 200mm x 475mm) filled with locally obtained top soil on 13 March 2010. The potted sugarcane were placed into a large greenhouse (25m X 10m X 7m) fitted with an extractor fan and wet-wall system (Envirowatch®, Multigrow™, Durbanville, Cape Town, South Africa) pre-set to reduce greenhouse temperature to 26±1°C on reaching a critical threshold of 28°C as well as a drip irrigation system which fed water into each planting bag containing sugarcane. There was no heating mechanism yet low temperatures during winter did not affect the condition of the sugarcane. The greenhouse was partitioned into three equal blocks/cages (7m x 7m x 4m), each containing 100 potted sugarcane plants and sealed off with 70% green shade netting. There were 10 rows per cage

each consisting of 10 potted plants with a spacing of 600mm x 450mm. The three cages were identified by the treatment administered as follows: 1 (CONTROL: un-interrupted *E. saccharina* population build-up), 2 (BUFFER ZONE: to isolate or prevent cross infestation between the control and test cages) and 3 (SIT: test cage for measuring the impact of steriles on an existing stable *E. saccharina* population). The sugarcane in the buffer zone was regularly inspected to check for *E. saccharina* damage and evidence of cross infestation between the control and the test cages.

5.2.2 Study populations

Due to the difficulty in sourcing sufficient quantities as well as high variability in development and adult emergence of wild strains, laboratory reared *E. saccharina* supplied by SASRI were used for this study. A comprehensive description of the origin of the insects as well as their handling protocol is given in section 2.2.1.

5.2.3. Moth releases

This pilot cage/greenhouse release trial was based on protocols of similar trials by other researchers (Calvitti *et al.*, 1998; 2000; Nguyen Thi & Nguyen Thanh, 2001; Hight *et al.*, 2005; Hofmeyr *et al.*, 2005; Wise de Valdez *et al.*, 2010; Ant *et al.*, 2012). Modifications were done where necessary. In order to establish the initial stable infestation of *E. saccharina* in the control and the test cages, two identical laboratory reared populations comprising equal numbers of freshly eclosed male and female moths (i.e. 100 moth pairs), were allowed to mate in ventilated transparent perspex breeding boxes (500mm x 500mm x 500mm) in the laboratory at $27 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity (RH) and (10: 14) light: dark (L: D) cycle. This was done as a precautionary measure to ensure that the females had been mated prior to release into semi-natural cage house conditions, since mass-reared populations are more likely to mate under controlled laboratory conditions in which they are produced (Roessler, 1975; Rossler, 1975). The 100 pairs of moths were released into each of the cages in batches of 25 pairs over four consecutive days to establish the first (initial) infestation. The sugarcane crop was aged 21 months when this initial *E. saccharina* infestation was introduced. To ensure an even distribution of the infestation within each cage, moths of both sexes were placed in large petri-dishes at the base of the host plants in the center and at the four corners of each cage and allowed to disperse freely.

Since there is no reliable pheromone trap currently available for monitoring *E. saccharina* populations, 100 sugarcane stalks in each of both plots were inspected for signs of damage (i.e. exit holes) after 898 DD° (the estimated number of day degrees required for complete development from

egg to adult emergence in *E. saccharina*, Way, 1994; 1995) following initial introduction to initiate an infestation. This was done in order to provide an estimate of the population size in each plot and determine the rates and quantity of sterile moths to be released. Based on the moth production index for estimating *Buseola fusca* Fuller (Lepidoptera: Noctuidae) field population numbers, (van den Berg, 1997) it was assumed that *E. saccharina* behaves the same and therefore the index was adopted for estimating population numbers in the cages. The field sex ratio of *E. saccharina* is approximately 1: 1 (Conlong, pers. comm.⁴). In addition 10 damaged stalks with signs of frass were selected from each of the cages and slit longitudinally to assess the *E. saccharina* life stages present. This was done in order to check for synchrony between the number of day degrees (DD°) accumulated and the developmental stage of the pest at the time of sampling (i.e. at the end of the first 898 DD° following introduction and establishment of the first infestation).

The day degree model by Way (1994; 1995) states that, in the field, 898 DD° are required for the completion of *E. saccharina* development from egg stage to adult emergence. Therefore the DD° model is a useful tool for predicting and monitoring insect developmental life stages and synchronizing adult emergence with sterile male releases which coincides with the end of each generation. Three sterile male moth releases were conducted in the test cage every 898 DD° following the first infestation establishment. The sterile males were never chilled and releases were always conducted at 15H00 to allow time for adjustment to local (greenhouse) conditions as well as in the same manner as described above for the moths that established the initial infestation. The sterile moth quantity and release rate was fixed in all three releases and was based on a treated (T) to untreated (U) adult over-flooding ratio of 10T: 1U (Nguyen Thi & Nguyen Thanh, 2001; Hight *et al.*, 2005; Hofmeyr *et al.*, 2005) which is also similar to the ratio currently implemented at X-Sterile Insect Technique (XSIT) False Codling Moth Sterile Insect Release programme at Citrusdal, Western Cape Province, South Africa (Stotter, pers. comm.⁵). A summary and time-line of the releases, moth quantities and action steps taken during the pilot release study is given in table 5.1.

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Table 5.1 Timeline of action steps taken in the sterile *Eldana saccharina* cage house pilot release trial

Date	Action Steps Taken		
	Cage 1 (Control)	Cage 2 (Buffer)	Cage 3 (Treatment)
13 March 2010	Seedlings transplanted <ul style="list-style-type: none"> • Cane age: 48 days 	Seedlings transplanted Cane age: 48 days	Seedlings transplanted Cane age: 48 days
30 October 2011	Establishment of infestation <ul style="list-style-type: none"> • 100 moth pairs released (25 pairs/day x 4 days) 	Nil	Establishment of infestation <ul style="list-style-type: none"> • 100 moth pairs released (25 pairs/day x 4 days)
2 January 2012	Population determination & life stage-DD° synchrony check. (sampling at ≈898 DD°) <ul style="list-style-type: none"> • 69 <i>E. saccharina</i> borings detected. • Pupae and final instar larvae found. 	Stalk inspection for cross infestation (inspection at ≈898 DD°) <ul style="list-style-type: none"> • No damage detected. • No <i>E. saccharina</i> found. 	Population determination & life stage-DD° synchrony check. (sampling at ≈898 DD°) <ul style="list-style-type: none"> • 75 <i>E. saccharina</i> borings detected • Pupae and final instar larvae found.
3 January 2012	Nil	Nil	First sterile male release <ul style="list-style-type: none"> • 750 sterile moths (150 moths every 2nd day for 5 days)
2 March 2012	Nil	Stalk inspection for cross infestation (inspection at ≈898 DD°) <ul style="list-style-type: none"> • No damage detected. • No <i>E. saccharina</i> found. 	Second sterile male release <ul style="list-style-type: none"> • 750 sterile moths (150 moths every 2nd day for 5 days)
21 May 2012	Nil	Nil	Third sterile male release <ul style="list-style-type: none"> • 750 sterile moths (150 moths every 2nd day for 5 days)
13 July 2012	Cane harvest & final stalk damage sampling	Stalk inspection for cross infestation <ul style="list-style-type: none"> • No damage detected. • No <i>E. saccharina</i> found 	Nil
14 July 2012	Nil	Nil	Cane harvest & final stalk damage sampling

5.2.4 Evaluation of the impact of sterile male releases on an existing stable *E. saccharina* population and crop damage

The sugarcane crop was harvested at age 31 months to assess the impact of the three sterile male *E. saccharina* moth releases on the existing stable population initially established on the crop. At this stage the crop was fully mature and having well developed tillers (side shoots). In both the control and the test cages, 100 mature sugarcane stalks (i.e. randomly selected parent stalks or tillers) from each of the cages were cut down from the root zone to assess damage and presence of F₃ *E. saccharina* progeny. Damage on sugarcane associated with *E. saccharina* is manifested by red coloured borings caused by the larvae tunnelling into the sugarcane stalk resulting in extensive tissue damage, loss of sucrose and secondary infections by microorganisms on the borings and surrounding tissue (Way & Goebel, 2003; see Fig. 1.1. in literature review section). The total number of internodes and damaged internodes (expressed as a percentage) on each stalk and the overall total number of damaged stalks were also recorded. This was done by slitting the stalks (see Fig. 1.1 in literature review section), checking for damage and collecting and recording the different life stages of *E. saccharina* found inside the borings.

5.3 Data analyses

The data for percent internodes damaged was tested for normality and homogeneity of variance using the Shapiro-Wilk and the Hartley-Bartlett tests, respectively, and it did not satisfy the conditions of normality despite transformation. Therefore, the non-parametric Mann-Whitney U test for comparing two independent groups was performed in Statistica 10.0 (Statsoft Inc., Tulsa, Oklahoma, USA) to test the hypothesis that the median amount of crop damage (i.e. percent internodes damaged) in the control is not significantly different from that in the test cage.

To test the hypothesis that the number of damaged sugarcane stalks in both cages is independent of treatment administered (i.e. there are no differences in number of damaged stalks between the control and the treatment), the empirical logistic transform for comparing two samples was used (Cox, 1970). The logit transform of the number of damaged stalks is given by

$$Z_j = \ln \left(\frac{R_j - 1/2}{n_j - R_j + 1/2} \right),$$

and the variance is given by

$$V_j = \frac{(n_j + 1)(n_j + 2)}{n_j (R + 1)(n_j - R_j + 1)},$$

where, j =treatment 1 or treatment 2, R is the number of damaged stalks and n is the total number of stalks.

The transformed difference between the two treatments, Δ , is $Z_1 - Z_2$, and the standard error approximation of the difference is given by $\sqrt{V_1 + V_2}$. Then the standard normal deviate can be

estimated using $\frac{\Delta}{\sqrt{V_1 + V_2}}$. This can be used to test the hypothesis that the two treatments are the

same, using the two-tailed normal distribution.

5.4 Results

5.4.1 Cross infestation between cages

There was neither damage nor were there any *E. saccharina* eggs, larvae, pupae or moths on any of the sugarcane stalks in the buffer cage.

5.4.2 Synchrony of *E. saccharina* life stages with day degrees

Following the end of the first 898 DD°, seven pupae (4 empty/eclosed, 3 intact) and six large (6th instar) larvae were retrieved from the 10 sugarcane stalks sampled from the control group. Nine pupae (2 empty, 7 intact) and 5 large larvae were retrieved from the sugarcane stalks split open from the treatment group.

5.4.3 Efficacy of sterile male releases in suppressing population growth and crop damage

There were significantly less F_3 larvae per sugarcane stalk recovered from the treated cage (SIT) compared to those from the untreated (control) cage (Mann-Whitney U Test: $U = 4139.5$; $P = 0.036$) after the final sampling. With regards to effect of sterile male *E. saccharina* releases on the incidence of internode damage, the differences in median percent damaged internodes per sugarcane stalk between the control and treatment were highly significant (Mann-Whitney U Test: $U = 3999.5$; $P = 0.015$; Fig. 5.1). There were significantly more damaged internodes per stalk in the control than in the treatment group. In both cases, damage was mostly concentrated on the bottom third of the

sugarcane stalk. The average number of internodes per stalk did not differ significantly (Kruskal Wallis Test: $H_{(1, 200)} = 0.402$, $P = 0.526$) indicating that at the time of harvest, the crop age and condition was similar for both treatment groups. The mean numbers of internodes per stalk (\pm SE) for the sugarcane in the treatment and control group were 17.80 ± 0.63 and 17.64 ± 0.68 respectively.

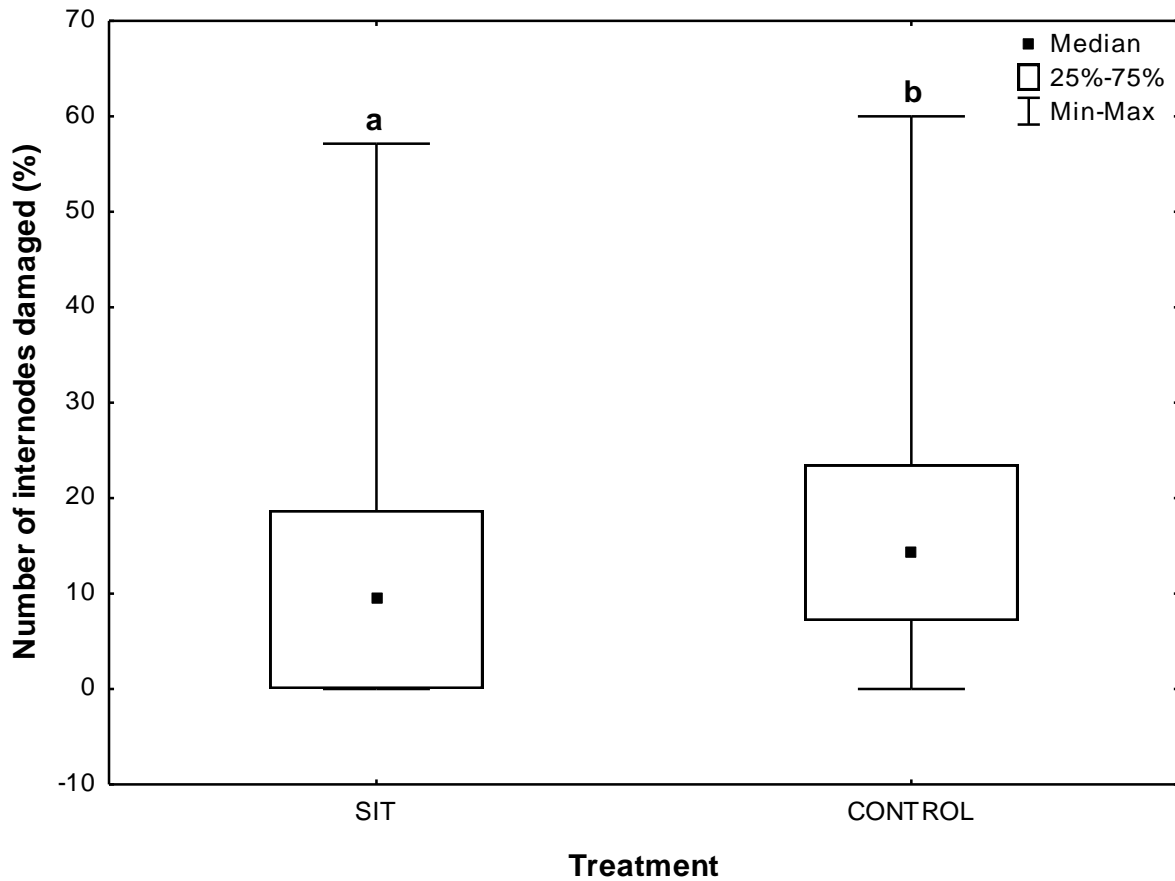


Fig. 5.1 Efficacy of three sterile male releases maintained at a sterile (T) to untreated (U) adult *Eldana saccharina* over-flooding ratio of (10T: 1U) in lowering levels of stalk damage (% internodes damaged). The % damaged internodes per stalk is less in the sugarcane treated with sterile moth releases (SIT) compared to the CONTROL which did not receive any treatment to suppress pest population.

Analysis of differences in the overall total number of damaged sugarcane stalks between the control and the treatment groups using the empirical logistic transform confirmed and complemented the results of the Mann-Whitney U test by showing that the amount of damage in a given plot is dependent on the treatment. The test showed that there was a significant difference in the total number of damaged sugarcane stalks between the control and the SIT treatment ($Z = 2.494$; $P = 0.013$; Fig 5.2). From the 100 stalks sampled in each of the cages, 30 and 15 of them were undamaged in the treated (SIT) and untreated (CONTROL) sugarcane respectively (Fig. 5.2).

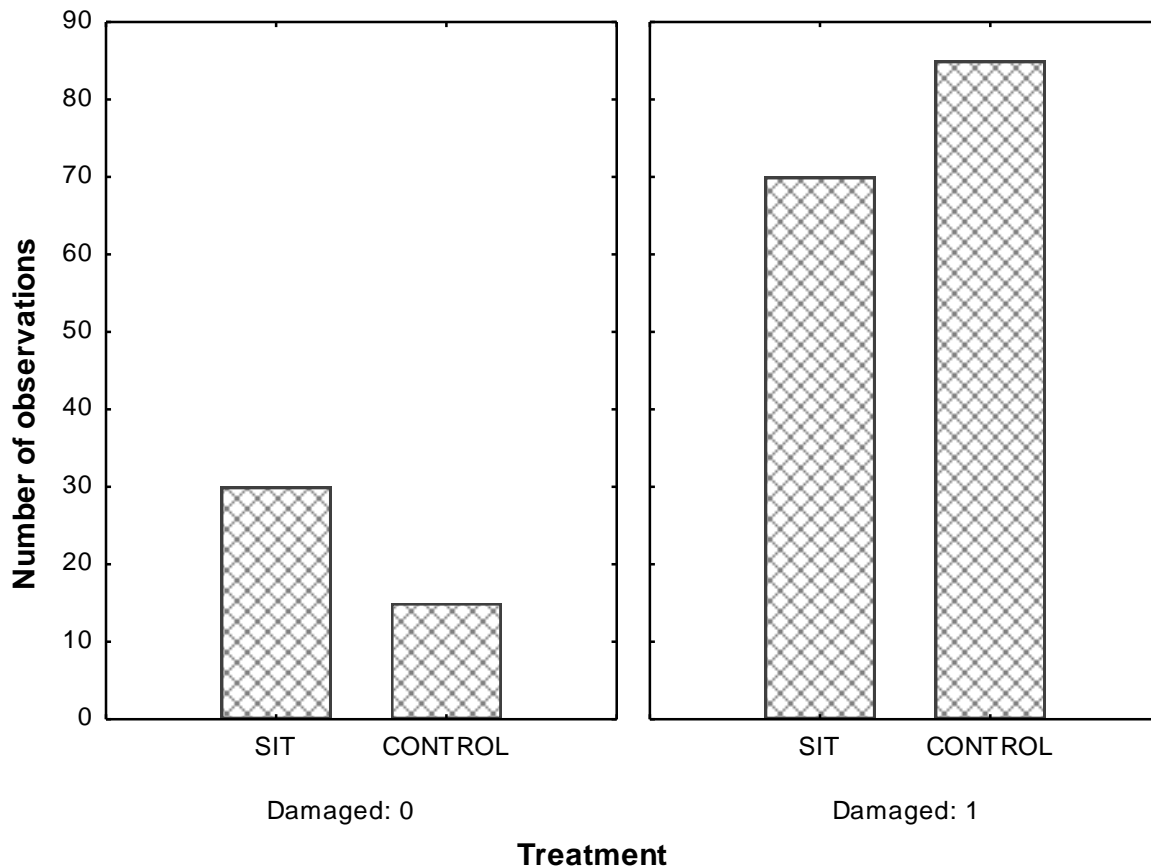


Fig. 5.2 Histograms of overall total number of undamaged (0) and damaged (1) sugarcane stalks in the untreated (CONTROL) and the treatment (SIT) cages, where the sugarcane was aged 31 months at the time of final sampling and total number of stalks sampled (n) = 100. In the treatment cage three releases of irradiated male *E. saccharina* adults at a release ratio of 10T: 1U (treated: untreated) were carried out to investigate their efficacy in suppressing an existing pest population and crop damage. There were no sterile releases or pest control intervention done in the control cage.

5.5 Discussion

The absence of damage or any live or dead *E. saccharina* in the buffer zone indicated that there was no cross infestation between the test and the control cages. Therefore the observations and results from the treatment and control groups were independent of each other.

The *E. saccharina* life stages (i.e. pupae and final instar larvae) retrieved from both cages following the expiry of the first 898 DD° after establishment of the initial infestation indicated a positive synchrony with the day degree model. These observations reflect the findings by Way (1994; 1995) who accurately calculated the lower developmental temperature thresholds for the different life stages and the DD° required for the completion of *E. saccharina* development. The presence of pupae and empty cocoons at the time of sampling provides evidence of the reliability of the model as well

as the confidence that released sterile individuals encountered moths emerging from the native population.

Evaluating the results of F_1 (inherited) sterility as a method for population suppression or eradication in a SIT programme is particularly complex (Nguyen Thi & Nguyen Thanh, 2001). The offspring of the released partially-sterile males and the native fertile females typically continue to mate in the same manner as their parents. During the process inherited radiation-induced deleterious effects are passed on to succeeding generations resulting in population reduction due to aspects such as reduced egg-hatch, increased sterility and predominantly male sex ratio (North, 1975). Release programmes continue until these effects are ascertained. The ability to track the descendants of released individuals through subsequent generations is the most reliable way of evaluating the success of any SIR programme which can be achieved by exploiting dominant and co-dominant mutations as biological markers (Bartlett, 1967). While Walton (2011) showed that *E. saccharina* can be marked by addition of Calco Red into larval diet which stains their fat bodies without any known detriment to the insects' biology, the technique unfortunately does not allow tracking descendants of released individuals through subsequent generations (Nguyen Thi & Nguyen Thanh, 2001). No studies have explicitly examined the use of biological markers in *E. saccharina*. However comparing incidence of crop damage (Hofmeyr *et al.*, 2005) and/or population growth (e.g. larval emergence and fertile offspring in succeeding generations) (Nguyen Thi & Nguyen Thanh, 2001; Hight *et al.*, 2005; Hofmeyr *et al.*, 2005) between a treated and untreated plot are some of the ways to circumvent these limitations.

The data collected from the current pilot field cage release study demonstrated that releasing partially sterile (200Gy) *E. saccharina* males into an existing stable population and sustaining the release ratio of 10T: 1U in as few as three releases over a period of 6 months was capable of significantly reducing sugarcane stalk damage. Compared with sugarcane in the control group, the cage receiving treated male *E. saccharina* adults had significantly less damaged sugarcane stalks, suggesting that the fertility of the F_1 adults was significantly reduced. This also indicated that the treated males had competed successfully with their untreated counterpart males for untreated females and hence pest population growth could be significantly impacted by the release of irradiated conspecifics (Hofmeyr *et al.*, 2005).

While data on sizes of the F_1 and F_2 populations were not collected in order to accurately calculate the rates of population increase or decrease, the fewer F_3 larvae per stalk obtained from the treated sugarcane (SIT) compared to the control indicates that the fertile population was in decline from P_1 to the succeeding generations. It is important to note that the statistical difference in number of F_3

larvae recovered per stalk between the treatment and control groups was marginal ($P = 0.036$). Several authors have reported that releasing both genders together in a SIT programme is more effective than releasing males only (North & Holt, 1971; Nguyen Thi & Nguyen Thanh, 2001; Hight *et al.*, 2005). It has been hypothesised that releasing partially sterile females could positively contribute a significant fraction of offspring in the target population to the benefit of the SIT program if the released females carry dominant lethal genes that will be passed on to the target population (Whitten & Taylor, 1970; Allam & Galun, 1976; Nguyen Thi & Nguyen Thanh, 2001). In addition females act as a sperm sink in that as they compete with fertile females for mates, limited fertile sperm is removed from the system (Hight *et al.*, 2005).

A repeat of this pilot study where both sexes of sub-sterile moths are released simultaneously could therefore result in a greater degree of population and crop damage suppression since the maximum economic efficiency in the exploitation of (F_1) inherited sterility for lepidopteran pest population suppression is influenced by this aspect (North & Holt, 1971). This has been demonstrated in similar field cage trials with several Lepidoptera e.g. navel orange worm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Husseiny & Madsen, 1964), tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Guerra *et al.*, 1974) and pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (van Steenwyk *et al.*, 1979). In F_1 sterility, the major suppression of the target population is greatly enhanced in the second filial generation (North & Holt, 1971). Nevertheless the results of the current study support findings reported by Hight *et al.* (2005) who showed that sustaining an over-flooding ratio as low as (5T: 1U) or the optimum (10T: 1U) was adequate enough to bring about a significant reduction in the “wild” population under field cage conditions, irrespective of whether treated males only or both sexes were used in the release.

In other programmes using the SIT to control Diptera, the release of males only is very important in view of the fact that sterile females can have serious repercussions because they still puncture fruit to lay sterile eggs thereby scaring fruit and facilitating secondary infection by pathogens (Franz & Kerremans, 1993). For example in *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) the use of genetic sexing strains has made it possible to use male-only releases in routine area-wide suppression programmes (Franz & Kerremans, 1993; Rendon *et al.*, 2000; 2004). No efficient technique currently exists for separating males and females in Lepidoptera despite results showing that male only releases had a significant impact on the existing population and reducing crop damage. Consequently, simultaneous release of treated male and female *E. saccharina* moths is unavoidable at this stage. Nevertheless experimental evidence also demonstrates the role of sterile females and benefits of releasing mixed genders in SIT enterprises.

5.6 Conclusion

Results from the current study demonstrated the efficacy of irradiated and released *E. saccharina* adults in reducing stalk damage as well as in lowering the number of fertile progeny under controlled cage house conditions. The results corroborate the findings reported in similar studies for example on *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Bloem *et al.*, 1999), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Nguyen Thi & Nguyen Thanh, 2001) and on *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (Hight *et al.*, 2005). It is essential that the efficacy demonstrated herein be projected for several generations in order to envision the potential impact of releasing partially sterile *E. saccharina* against a fertile population in the field and on an area-wide basis (Hofmeyr *et al.*, 2005). The data presented suggests that there is therefore great scope and motivation for the continued development and assessment of inherited sterility as a SIT control tactic for incorporation into AW-IPM against *E. saccharina*. It is therefore recommended that future research on further development of the SIT for control or eradication of *E. saccharina* should review the over-flooding ratios and aim for one between 5T: 1U and 20T: 1U as well releasing both genders and ultimately testing the results presented here under true season-long and area wide conditions. If both genders are to be released for the benefit of bringing about control and population reduction sooner than in the case of male only releases (Hight *et al.*, 2005), it is essential to evaluate whether releasing mixed genders will increase the incidence of crop damage, in which case it can be a potential disadvantage as reported by Nguyen Thi and Nguyen Thanh (2001).

5.7 References

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The results of the present study contribute to understanding the technical and operational feasibility of implementing the sterile insect technique (SIT) as a major component of tactics for use against the sugarcane stalk borer, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) in an area wide integrated pest management approach (AW-IPM). Based on pioneer studies by Walton (2011) on the radiation biology, parental and F₁ sterility of *E. saccharina*, three doses of radiation namely, 150, 200 and 250 Gy were selected for further investigation in the present study.

A series of video assays were conducted (Chapter 2) to confirm that behavioural traits leading to successful mating and insemination of wild females are not lost in the mass-reared and sterilized insects. In general, qualitative mating behavioural traits such as ability to form and locate mating arenas or leks, courtship and subsequent copulation with wild females are retained in the treated strains and hence there is considerable scope for further development of the SIT against *E. saccharina*. Despite both mass-rearing and irradiation of *E. saccharina* leading to a quantitative departure of male mating behaviour away from that exhibited by their wild counterparts, treated males were still as active and competitive. Encouragingly, the lower radiation doses (150 and 200 Gy) did not impact negatively on *E. saccharina* male mating behaviour, which bodes well with the end goal of deploying F₁ sterility against *E. saccharina*. Inherited or F₁ sterility is a variation of the conventional SIT (Hight *et al.*, 2005) that offers great potential for managing the spread of *E. saccharina* on an area-wide basis. The ability of irradiated males to mate successfully with wild females forms the basis of the SIT (Calkins & Parker, 2005). Using a lower radiation dose (200 Gy) will be of great benefit to the technique in that time and costs of irradiation will be lowered, while quality and competitiveness of the irradiated moths is maintained or improved (North, 1975). Furthermore, a dose that is lower than would be necessary to induce full sterility in parental males would also ensure a high turnover/emergence of fully sterile F₁ progeny fit enough to mate with wild females and pass on dominant lethal mutations to succeeding generations. This will result in population suppression due to reduced emergence of fertile offspring (Nguyen Thi & Nguyen Thanh, 2001; Carpenter *et al.*, 2005; Hight *et al.*, 2005).

In order to fully ascertain the effects of inherited sterility, it is recommended that the F₁ progeny be subjected to a series of similar assays to confirm that mating behaviour characteristics are not lost as demonstrated in the parental moths. According to Nguyen Thi and Nguyen Thanh (2001) major suppression of the target population is deferred to (but greatly enhanced in) the second generation

hence the necessity to track the performance of F_1 progeny and individuals of succeeding generations. LaChance (1985) states that greater suppressive potential is offered by the field-produced fully sterile F_1 progeny originating from matings between released partially sterile males and wild females. Even greater results will be achieved when this is combined with continued regular releases of sub-sterile males and fully sterile females. It has also been hypothesised that the maximum economic efficiency of F_1 sterility for the suppression of lepidopteran populations is realized when both sexes of partially sterile moths are released (North & Holt, 1971). The current study focussed on male *E. saccharina* only and therefore the ability of treated females to mate with wild males upon release should be evaluated in the same manner. The costs and benefits of using either fully or partially sterile females will also need to be validated. Nevertheless experimental evidence strongly suggests that sterile females play an important role in the reproduction dynamics of a wild population under the SIT (Nguyen Thi & Nguyen Thanh, 2001; Hight *et al.*, 2005).

Second, the level of mating competitiveness and compatibility between different strains of *E. saccharina* (i.e. laboratory reared, partially sterile “irradiated at 200Gy” and wild moths) was assessed under laboratory and semi-field conditions (Chapter 3). The adaptation of insects to laboratory conditions and irradiation produces genetic and physiological effects in conventional strains (Shelly *et al.*, 1994; Benedict & Robinson, 2003). This influences their behaviour and may lead to sexual discrimination of the released strain as well as assortative mating problems upon release into the field. In the present study the mass-produced irradiated insects commenced sexual activity significantly earlier than the wild strains resulting in a significantly high number of homologous/homotypic pairings as was also reported by Moreno *et al.* (1991) and Hernandez *et al.* (2003) for different fruit fly species. Since population suppression by the SIT is a function of successful matings between the released irradiated males and wild females (McInnis *et al.*, 1994), the ability of the former to compete for mates with their wild counterparts, as well as their compatibility with the target wild females in the field are critical (Cayol *et al.*, 2002; Lance & McInnis, 2005).

The mating indices generated from these data demonstrated that the mass-reared *E. saccharina* strain produced in South Africa has not yet evolved sexual behaviours suggestive of incipient pre-mating isolation barriers with local wild strains under field cage conditions. The laboratory strain reared at the South African Sugar Research Institute’s mass production facility is supplemented with wild strains every four years. There was a high number of homotypic matings involving members of the test strains (i.e. Lab ♀ x Lab ♂ and Sterile ♀ x Sterile ♂) in the pairwise test in which they were pitted against the wild strain under the controlled artificial laboratory location. To the contrary, there was a significantly higher number of heterotypic matings obtained in the more robust field cage

tests, which were run concurrently with the laboratory location tests. Both non-irradiated and irradiated male *E. saccharina* were equally attractive to wild females and vice versa, thereby ruling out any concerns about quality, mating competitiveness or compatibility due to irradiation treatment and mass-rearing. Different mating indices, namely, index of sexual isolation (ISI), male and female relative performance index (MRPI and FRPI) were used to describe mating compatibility and mating performance between the mass-reared, irradiated and wild strains. Even though the mean ISI values of the pairwise comparisons between laboratory reared and wild moths (L-W) and also the sterile and wild moths (S-W) showed a tendency for matings between males and females of the same origin, there was certainly no evidence of sexual isolation. Most importantly, the results presented show that wild moths did not discriminate against either the partially sterile or laboratory reared moths. There was also a tendency for test females to copulate in greater proportion than wild females as reflected by positive and highly significant FRPI values obtained in the pair-wise comparisons between either non-irradiated or irradiated with the wild strain. Encouragingly, sterile males mated significantly more than their wild counterparts regardless of the type of female. In the field, the mating combinations obtained, all took place well before homotypic matings comprising members of the wild strain. However the high number homotypic matings consisting of members of the released strain is troublesome and necessitates caution where both sterile males and females are released together as they may mate amongst themselves before having the opportunity to mate with wild counterparts (Moreno *et al.*, 1991). It is therefore essential that adequate numbers of sterile insects be released to ensure the target population is sufficiently flooded with treated individuals and that insect quality enables sufficient dispersal and distribution of the released steriles across the entire environment.

While the results of the present study corroborate findings on mating competitiveness and sexual compatibility by several authors e.g. Cayol *et al.* (2002), Pereira *et al.* (2007), Orozco *et al.* (2007), Bloem *et al.* (2010) and Taret *et al.* (2010) and support the implementation of the SIT against *E. saccharina*, further research should examine several other factors such as the impact of strain (laboratory reared and irradiated) on longevity, survival and sperm competitiveness (Bloem *et al.*, 2010). There is also a need to optimize investments in mass-rearing facilities that serve AW-IPM programmes in which the SIT is a component (Bloem *et al.*, 2010) due to increased commercialization and rapid expansion of this species-specific and biologically based control technique. It can therefore be envisioned that facilities in countries implementing sterile releases to manage a common pest problem but situated in different hemispheres could complement each other in that instead of scaling down operations (off-season), one country could supply sterile moths to the other experiencing a high demand and vice versa (Taret *et al.*, 2010). Since *E. saccharina* is distributed across Africa (Assefa *et al.*, 2006) and at least three biotypes having been confirmed (Sampson &

Kumar, 1985; Assefa *et al.*, 2005; 2006), it is crucial to assess whether mating barriers exist between released *E. saccharina* moth strains and the target populations in different geographical localities. Because only wild *E. saccharina* moths collected from a single location in KwaZulu-Natal Province of South Africa were tested, other wild moth strains may show different behavioural characteristics. Therefore competitiveness and compatibility testing should continue to be a part of quality assurance in future AW-IPM programmes involving the SIT for control or eradication of *E. saccharina*. Furthermore regular testing will be useful in determining whether a given mass-reared strain needs replacement since maintaining a colony for an extended duration eventually and adversely affects performance of sterile insects (McInnis *et al.*, 1996).

Third, the critical thermal limits to activity at high and low temperatures (CT_{max} and CT_{min} respectively) of different *E. saccharina* strains/treatments were investigated (Chapter 4). The effect of laboratory rearing and increasing radiation dosage on thermal tolerance of the adult stage of *E. saccharina* was explored. The major highlights of this trial include the significant differences between the laboratory-reared and wild strain and also between non-irradiated and irradiated strains in both critical thermal maxima and minima. There were also no gender effects on the critical thermal limits (CTLs), a result similar to what was reported in desert *Drosophila* (Diptera: Drosophilidae) (Stratman & Markow, 1998), tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae) (Terblanche *et al.*, 2007), in false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Stotter & Terblanche, 2009) and in fruit flies, *Ceratitis capitata* and *C. rosa* (Diptera: Tephritidae) (Nyamukondiwa & Terblanche, 2009). Laboratory reared *E. saccharina* moths were more heat tolerant compared to wild moths for both genders while in the case of critical thermal minima, the reverse was true. However, CT_{min} is more important than CT_{max} in the context of *E. saccharina* population dynamics, ecology and survival since in reality the latter may never be attained in the field and hence further research should be directed towards enhancing or improving CT_{min} of treated moths.

Irradiation had a negative effect on both CT_{max} and CT_{min} while mass-rearing had a negative effect on CT_{min}. Since the wild strain used in these trials was obtained from Tinley Manor Sugarcane Estate – an area that is situated on the warmer north eastern coast of Durban, KwaZulu-Natal Province, there may have been an acclimation effect due to highly variable climatic conditions which could have influenced the result. It is recommended that trials be repeated using wild *E. saccharina* strains collected from the cooler inland growing regions such as Eston in the midlands region of KwaZulu-Natal or from different wild hosts such as *Cyperus papyrus* (L.). Moths treated at the lowest radiation dose (150 Gy) were more cold and heat tolerant than those treated at higher dosages. The importance of using lower dosages (150 & 200 Gy) to improve sterile moth quality was also reflected

in the moth's capacity to tolerate temperature extremes much greater than in cases where a higher radiation dosage (250 Gy) was used. This is therefore an additional justification of the goal of implementing inherited rather than full sterility against *E. saccharina*. Mass-rearing on the other hand results in selection of biological or behavioural traits which are at variance with those exhibited by wild strains due to intense unnatural selection pressures imposed by the rearing environment (Iwahashi, 1996; Matos *et al.*, 2000). However acclimation in order to enhance performance in variable thermal environments (Hochachka & Somero 2002; Angilletta 2009) is significant for survival and persistence of mass-reared sterile insects. Future research should therefore be directed towards improving quality of mass-reared *E. saccharina* through pre-exposure to sub-lethal temperatures (rapid cold-hardening 'RCH' or rapid heat-hardening 'RHH') (West-Eberhard, 2003), inclusion of diapause in rearing (Bloem *et al.*, 1997; Judd *et al.*, 2006) combined with using a lower dosage of gamma radiation (Bloem *et al.*, 2004) to minimize radiation induced somatic effects (Bakri *et al.*, 2005). It is unlikely that sterile releases will ever be done in lethal temperature conditions in the South African sugar belt. However survival and temperature-dependent performance can be enhanced for released mass-reared sterile *E. saccharina* in cooler or hotter conditions by using RCH and RHH manipulation techniques, to the benefit of AW-IPM programmes incorporating the SIT (Bloem *et al.*, 2006; Loeschcke & Hoffmann, 2007; Chidawanyika & Terblanche, 2011). Other factors which can be manipulated to improve sterile insect quality include nutrition, for example, possible use of probiotic diets (Niyazi *et al.*, 2004; Yuval *et al.*, 2007) or protein and carbohydrate rich diets (Barry *et al.*, 2007) which enhance stored energy reserves that play a critical role in an insects thermal biology (Terblanche *et al.*, 2008). In addition, it is recommended that a validation of the costs and benefits of manipulating thermal environments or incorporating acclimation into current rearing protocols on field performance for pest control, be done for *E. saccharina*, as reported by Kristensen *et al.* (2008) for *Drosophila* (Diptera: Drosophilidae) and by Chidawanyika and Terblanche (2011) for *C. pomonella* (Wiedemann).

Lastly, a pilot study on efficacy of the SIT for future area-wide control of *E. saccharina* was conducted in a cage scenario (Chapter 5). Results from the study demonstrated the efficacy of partially irradiated (200 Gy) and released adult male moths in reducing crop damage as well as lowering the number of fertile progeny from F_1 to succeeding generations in a stable *E. saccharina* population under controlled cage house conditions. The results corroborate findings reported in similar studies on *C. pomonella* (Bloem *et al.*, 1999), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Nguyen Thi & Nguyen Thanh, 2001) and on *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (Hight *et al.*, 2005). It is encouraging to note that the effects of inherited sterility were demonstrated using a treated to untreated adult *E. saccharina* over-flooding ration of 10T: 1U and male only releases.

Moving forward with the development and implementation of the SIT as a component of AW-IPM programmes against *E. saccharina*, a review of the over-flooding ratio is necessary in order to determine the most economic release rate that will ensure sufficient flooding of females of a target population. Further study in this respect should typically aim for release ratios between 5T: 1U and 20T: 1U similar to those reported in other lepidopteran species targeted for the SIT. These include *C. cactorum* (Hight *et al.*, 2005) and *C. leucotreta* (Hofmeyr *et al.*, 2005). The degree of sterility introduced into a wild population in order to overcome the rate of increase (reproductive success) of wild females (Klassen, 2005) is dependent on the over-flooding ratio which must be sufficiently high to ensure that the goal of overall population reduction in all parts of the release area (Knipling, 1968) is realised. Experimental evidence has demonstrated the role that sterile females can play when released together with partially sterile males. Releasing both genders together in a SIT programme is more effective than releasing males only (North & Holt, 1971; NguyenThi & Nguyen Thanh, 2001; Hight *et al.*, 2005) as these females act as a sperm sink that effectively removes fertile sperm from the system as they compete with fertile females (Hight *et al.*, 2005) and also contribute a significant proportion of progeny carrying dominant lethal chromosomes, that can be passed on to succeeding generations resulting in further population reduction (Nguyen Thi & Nguyen Thanh, 2001). It is recommended that an investigation into releasing mixed genders will further enhance population suppression levels presented in this pilot study.

In addition, it is essential to determine if the release of mixed genders will further reduce the levels of stalk damage presented herein or rather increase the incidence of crop damage as reported by NguyenThi and Nguyen Thanh (2001) in the case of *P. xylostella*. It is also critical that the efficacy demonstrated herein be projected for several generations in order to estimate the potential impact of releasing partially sterile *E. saccharina* against a fertile population in the field and on an area-wide basis (Hofmeyr *et al.*, 2005). Finally further studies are essential to evaluate the possibility of combining other pest control techniques with sterile *E. saccharina* release to achieve better control. For example Knipling (1979) and Carpenter (1993) suggested that combining parasitoid releases with sterile insect release might yield both additive and synergistic effects. Nguyen Thi and Nguyen Thanh (2001) showed that the use of F₁ sterility in combination with releases of a specific larval parasitoid *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), in field-cages resulted in a 40% decrease in the diamond back moth population in the F₁ and more than 90% in the F₂ generation. The use of F₁ sterility in combination with releases of *Goniozus natalensis* (Hymenoptera: Bethyridae) an indigenous parasitoid of *E. saccharina* (Graham & Conlong, 1988) should therefore be investigated.

In conclusion, the findings of the present study have answered the key research questions asked. First, in spite of quantitative differences in mating behaviour between the wild strain and the mass-

reared sterile strains tested, the critical behaviour characteristics leading to successful copulation with wild females are not lost in the latter. Second, the mass-reared *E. saccharina* strain produced by the insect rearing unit at the South African Sugarcane Research Institute has not yet evolved sexual behaviours suggestive of incipient pre-mating isolation barriers with respect to the local wild strain under natural conditions. Adult *E. saccharina* moths irradiated at the sub-sterilizing dose of 200Gy are as competitive in lek formation, mating and attractiveness to wild females as are their wild counterparts and therefore will be able to fulfil the purpose for which they are intended upon release into the field. Third, while mass-rearing and irradiation negatively affect thermal tolerance of *E. saccharina*, sterile moth quality can be enhanced via a number of ways recommended in various literature on this subject. In light of temperatures the moths may be exposed to in the South African sugar belt, cold hardening may be a treatment option for moths released in the cooler midlands and higher lying region in the SA cane belt. Lastly, sustained releases of partially sterile males into an existing stable population can significantly reduce incidences of crop damage and achieve a significant level of pest suppression under a confined environment such as the greenhouse/cage-house. Therefore there is great scope and motivation for the continued development and assessment of inherited sterility as a SIT control tactic for incorporation into current AW-IPM against *E. saccharina*.

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