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Effect of genetically modified sugarcane in combination with sterile insect releases to control *Eldana saccharina*: a shade house trial

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Effect of genetically modified sugarcane in combination with sterile insect releases to control *Eldana saccharina*: a shade house trial

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A thesis submitted to the School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, for the degree of Master of Science in Entomology

As the candidate's supervisor, I have approved this thesis for submission.

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ABSTRACT

This study investigated the synergistic effect of combining genetically modified (GM) sugarcane with the sterile insect technique (SIT) for controlling *Eldana saccharina*, a significant threat to sugarcane production in South Africa. The aim of the study was to evaluate the effectiveness of the *Bacillus thuringiensis* (Bt) toxin incorporated within the sugarcane and the impact of releasing sterile *E. saccharina* moths on sugarcane pest suppression. The study involved growing non-Bt (88H0019) and Bt sugarcane (GM CRY1A) in a treatment and control shade house. In the treatment shade house an overflooding ratio of 1 non-sterile: 10 sterile moths were released, while only non-sterile moths were released in the control. A survey on stalk damage focused on internode damage (%IND), stalk bored length (%SBL), stalk red length (%SRL) and infestation rates per 100 stalks were conducted once the sugarcane reached 12 months. The experiment resulted in only 2.02% of total stalks bored with 1.2 *E. saccharina* per 100 stalks (e/100) collected. The results indicated that GM CRY1A sugarcane had a significantly lower number of stalks damaged (0.12% stalks bored) compared to 88H0019 (1.09%). SIT releases failed to significantly reduce stalk damage, with the treatment having the most stalks bored (60%) overall. However, a shared trend of significantly low damage was found on GM CRY1A sugarcane in the treatment shade house, demonstrated by the %SBL (z-value = 6.70; p = 0.010), %IND (z-value = 7.75; p = 0.006), and %SRL (z-value = 26.48; p<0.001). The average *E. saccharina* infestation was low across both shade houses, but not significant (z-value = 2.96; p = 0.086). However, the treatment had a significant (z-value = 4.42; p = 0.036) difference in infestation, with 88H0019 sugarcane displaying higher infestation rates (3.686 e/100) than GM CRY1A (0.160 e/100). The current study yielded limited results due to the insufficient damage observed and the low number of offspring data collected during the surveys. These limitations lead to an insufficient conclusion to be drawn. Therefore, it is recommended that future studies should improve the execution of the study by introducing an initial infestation population, increase the frequency of SIT moth releases, and conduct surveys on more mature sugarcane. The latter could optimise SIT efficacy and further investigate GM crops long-lasting implications within integrated pest management frameworks. However, apart from the setbacks with the data obtained the findings suggest that while SIT may not directly mitigate stalk damage, integrating GM CRY1A sugarcane effectively suppresses *E. saccharina* populations and related damage. It highlights the importance of employing a multi-faceted approach to pest control, suggesting that integrating GM CRY1A sugarcane with SIT could provide a progressive solution for sustainable sugarcane production in South Africa.

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, **Vanessa Lauchande,** declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. their words have been re-written, but the general information attributed to them has been referenced and
   b. where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet unless specifically acknowledged, and the source being detailed in the thesis and in the References section/s.

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Name: Vanessa L. Lauchande

DECLARATION 2-PUBLICATIONS

Details of contribution to publications that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Details of publications:

Publication 1 (Submitted, International Society of Sugar Cane Technologists Congress)

Effect of genetically modified sugarcane in combination with sterile insect releases to control *Eldana saccharina*: a shade house trial

VL Lauchande, Dr LN Malinga & Dr TC Munyai

Author contributions:

From the above publication, VL Lauchande carried out all the experimental work and contributed to the writing of the publications under the supervision of Dr LN Malinga and Dr TC Munyai. The co-authors contribution was also that of an editorial nature, checking on the scientific content in their field, and correction of VL Lauchande's interpretation of the data in their field. Based on their expertise, they may have added minor parts to the manuscripts.

Signed: …[redacted]………
Name: Vanessa L. Lauchande

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Table of Contents

List of Figures

List of Tables

Chapter 1 General Introduction

1.1 Background

Sugarcane (*Saccharum officinarum* L.) is an industrial agricultural crop grown mostly in tropical and subtropical regions (Péné, 2018). Globally, the agricultural sector has faced problems with insect pests; among these, lepidopteran stem borers have been a key target pest for the sugarcane industry (Dessoky et al., 2021). Indigenous to Africa, *sa saccharina* Walker (Lepidoptera: Pyralidae: Gallerinae) is naturally found in indigenous grasses and wetland sedges located in tropical and subtropical regions (Potgieter, Van Vuuren & Conlong, 2012). The pest has an expansive host range of gramineous crops, *E. saccharina* has been reported to have spread throughout much of sub-Saharan Africa and is becoming a major concern in South Africa (Assefa, Conlong & Mitchell, 2006).

Initially identified in 1939 and resurfacing in 1970, *E. saccharina* has established itself as the most serious pest to sugarcane in South Africa (Botha & Stranack, 2017). Similar to other lepidopterans, *E. saccharina* undergoes complete metamorphosis, with the larvae stage being the most destructive to sugarcane (Walton & Conlong, 2016a). Once hatched, the larvae of *E. saccharina* bore into the sugarcane stalk, reducing the sucrose levels and increasing the sugarcane's susceptibility to the fungus species from the genus *Fusarium* (Barker, 2008; Mahlanza et al., 2014). An area-wide integrated pest management (AW-IPM) approach has been commonly used to mitigate the infestation of *E. saccharina* in South Africa (Conlong & Rutherford, 2009).

The AW-IPM control tactics currently used include cultural control, ecological-based approaches which involve habitat management strategies and chemical control through insecticidal spraying (Cockburn, 2013). However, in recent years, persistent outbreaks have been recorded along the sugarcane-growing regions in South Africa (Watt et al., 2010). The limited sugarcane production due to excessive misuse of cultivated land, reduced sucrose yield because of early harvesting of sugarcane and the inefficiency of insecticides due to the cryptic nature of *E. saccharina* have limited the effectiveness of these control methods (Barker, 2008; Cockburn et al., 2014). Additionally, the inability to identify the natural enemies of *E. saccharina* has limited the use of biological control measures (Horton et al., 2002). The limits experienced from the previous control measures, in congruence with the spread of outbreaks observed in recent years, have spurred researchers into considering new technologically advanced control measures. The South African Sugar Research Institute (SASRI) has been conducting intensive research programmes to find effective control measures for *E. saccharina* since it became a major pest in South Africa (Assefa, Conlong & Mitchell, 2006).

The sterile insect technique (SIT) is an AW-IPM approach used to control *E. saccharina,* which is currently being experimented at SASRI (Potgieter, Van Vuuren & Conlong, 2012). The sterile insect technique is a birth control measure used to suppress targeted pest populations. It involves the irradiation and mass rearing of sterile males that are released at an overflooding ratio into infested fields, where they mate with wild females by outcompeting wild males to produce infertile offspring (Potgieter, Van Vuuren & Conlong, 2013). For the past 60 years, numerous SIT programmes have been established to control insect species in orders such as Diptera, Lepidoptera, Hemiptera, and Coleoptera (Jena, Gena & Sahoo, 2022). However, the application of SIT is limited by several factors, such as the ability to mass-rear high-quality irradiated insects, the implication of handling and collecting insects which might impact their overall fitness, and the efficiency of these irradiated insects at outcompeting the wild population (Marc & Vreysen, 2019). Therefore, SIT is frequently used with additional pest mitigation methods to compensate for these limitations (Welburn & Maudlin, 2012).

Besides the research on SIT, SASRI is also exploring using genetically modified (GM) sugarcane to control *E. saccharina* (Watt et al., 2010). Genetically modified crops have become a driving force in the development of nutritional, herbicide-tolerant, stress-tolerant, and pest-resistant crops produced within the global agricultural sector (Huesing et al., 2016). Apart from tolerance, GM crops can assist in insect resistance, and one of the ways to achieve this is through the insertion of an endospore *Bacillus thuringiensis* (Bt) toxin into the genome of crops (Jain, Saharan & Pareek, 2016). The larvae feed on the Bt crop, and once ingested, the Cry toxin induces feeding inhibition, resulting in larvae mortality, which limits further damage to the Bt crop (Whalon & Wingerd, 2003). However, the major setback observed with Bt toxins comes with increased exposure to a large pest population for an extended period, which results in generational resistance to the Bt toxin (Tabashnik et al., 2021). Therefore, it is advised to integrate other control measures to limit exposure and lower the development of resistance (Kumar, Chandra & Pandey, 2008).

Researchers conjecture that since SIT does not target the larvae stage directly, the use of Bt toxins balances this shortcoming, while a reduced population caused by SIT limits the development of generational resistance (Suckling et al., 2014). Thus far, two agricultural programmes that successfully suppressed/ eradicated Lepidoptera pests were conducted on the painted apple moth, *Teia anartoides* Walker (Lepidoptera: Erebidae) in New Zealand and the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) in the southwestern United States (Suckling et al., 2007; Tabashnik et al., 2021). Therefore, further exploration needs to be conducted to investigate the efficiency of the AW- IPM approach that combines integrating SIT and Bt crops to suppress pest infestations (Tabashnik et al., 2021).

1.2 Problem statement

Current pest control methods have been associated with several instances of the *E. saccharina* population re-emerging. Therefore, exploring a new avenue of management is required. Based on the success of combining SIT and GM crops to control *P. gossypiella* on Bt cotton in the United States of America (Tabashnik et al., 2021). It could be beneficial to emulate this approach as a possibly innovative way to deal with the rapid outbreak of *E. saccharina* in South African sugarcane production. There has been no published experimental evidence exploring the effectiveness of combining SIT and Bt sugarcane. Researchers have postulated that shade house trial experiments on the ‘proof of concept’ for SIT releases have seen positive outcomes, and experiments utilising Bt sugarcane for a similar sugarcane borer pest have been favourable, integrating these two methods may result in long-term suppression of *E. saccharina* (Mudavanhu, Conlong & Addison, 2013; de Oliveira et al., 2022). However, because there are limited eradication programmes that incorporate SIT and Bt crops, no definite conclusion can be drawn on their effectiveness. Additionally, since the pink bollworm eradication programme published by Tabashnik et al. (2021) is currently the only successful programme incorporating Bt crop and SIT with various other control measures, it is difficult to justify the most effective method.

1.3 Rationale

The current study will produce an experiment-based proof of concept that endorses the efficiency of incorporating SIT and Bt sugarcane to suppress the infestation of *E. saccharina* in sugarcane. In addition, this study will innovate the agricultural industry by expanding the available AW-IPM approaches for farmers. Establishing a new pest control method is essential as it enhances sugarcane productivity, reduces crop losses and ensures the economic stability of the sugar industry. Additionally, pest control measures, which include insecticide usage, face issues with both the development of resistance by the target pest and their adverse environmental implication. Therefore, introducing new sustainable methods may delay the development of resistance and limit the usage of insecticides, which in turn promotes the use of sustainable agricultural practices. Apart from reducing chemical control, this approach can be used with the currently used push-pull and refuge technologies, providing support for the shortfalls that may have led to the increased outbreaks being observed. Furthermore, the outcomes of this study will be used to support sugarcane growers who may wish to deploy SIT on Bt sugarcane in the future. One of the main challenges with commercialising GM crops is meeting the regulatory frameworks needed to gain market acceptance. To achieve regulatory standards and address public concerns, continuous research needs to be conducted to demonstrate the efficiency, safety and environmental benefits of GM sugarcane. Therefore, if the current study proves successful, the SIT and Bt sugarcane control strategy may be used to promote the introduction of GM sugarcane in South Africa.

1.4 Research aim, objectives and hypothesis

1.4.1 Aim

The aim of this study is to develop a proof of concept that SIT combined with Bt sugarcane has a synergistic effect on the control of *E. saccharina* under a controlled environment.

1.4.2 Objectives

- To evaluate the effectiveness of *B. thuringiensis* (Bt) and sterile *E. saccharina* in mitigating sugarcane damage.
- To evaluate the synergistic effect of combining genetically modified sugarcane with sterile insect releases on suppressing *E. saccharina* populations.

1.4.3 Hypothesis

A pilot release study conducted by Mudavanhu (2012) and a reaction-diffusion model formulated by Potgieter et al. (2013) found that SIT can potentially reduce sugarcane stalk damage caused by *E. saccharina*. Additionally, it has been tested that Bt toxins are harmful to various lepidopteran species, including *E. saccharina* (Watt et al., 2010). Therefore, it is hypothesised that there will be little to no bored damage to the Bt sugarcane when sterile *E. saccharina* moths are released. It is further hypothesised that offspring suppression will be greater when *E. saccharina* is exposed to Bt sugarcane combined with SIT.

1.5 Thesis structure

The thesis follows a conventional Master's format. Chapter 1 consists of the general introduction, where background information is provided describing the importance of the thesis. It further states the aim, hypotheses, and objectives of the study. Chapter 2 is the literature review describing the importance of sugarcane, the impact that *E. saccharina* inflicts on sugarcane, previous management strategies used to control this pest, the use of SIT and GM crops in pest management, and the alternative use of SIT and Bt sugarcane as a combined control measure. Chapter 3 outlines the methods and materials used in the thesis. Chapter 4 reports the results obtained during the experiment, while Chapter 5 discusses the results. Chapter 6 summarises the outcomes of the study and provides recommendations for future research.

Chapter 2 Literature Review

2.1 Sugarcane production

2.1.1 Global production and use of sugarcane

For more than 75% of sugar produced globally, forecasts have estimated an increase of 2.5 million metric tons, amounting to approximately 186 million metric tons as of 2024/25 (USDA, 2024). As a good source of food and energy, sugarcane plays a key role in the Gross Domestic Product (GDP) by enhancing foreign exchange for many developing countries worldwide (Singh & Katiyar, 2016). Grown in about 26 million hectares of land in over 120 countries, the demand for sugarcane comes from its fibrous stalk rich with sucrose, commercially known as sugar (Mnisi & Dlamini, 2012; Voora et al., 2023).

The top 15 sugar produces globally include Brazil, India, European Union, China, Thailand, the United States, Pakistan, Russia, Mexico, Australia, Turkey, Egypt, Guatemala, Colombia, and South Africa (USDA, 2024). Driven by the recent increase in demand for Bioenergy production, Brazil has become the leading sugarcane producers globally, utilizing it primordially for first-generation ethanol and sugar (Coelho Junior et al., 2024). The successes of the country's bioethanol industry have resulted in majority of their vehicles being fueled using sugar converted ethanol (Coelho Junior et al., 2024). The expansion of Brazil's sugarcane industry has further influenced their impact on the sugar industry globally, as in 2024 they were recorded to have produced an impressive 43,000,000 metric tons of sugar, amounting to 23% of the sugar industry globally (USDA, 2024). In 2023, sugarcane dominated global crop production accounting for 22% of the crop industry which amounted to approximately 2 billion of the 9 billion tons of the total crops (FAO, 2023).

Besides sugarcane's primary process of producing sugar, the sugarcane production chain results in multiple economically beneficial by-products (Santos et al., 2019). These include the production of bioethanol from bagasse, molasses, and filter muds used for fertilizer and cow feed (Raza et al., 2010; Santos et al., 2019). About 29% of the sugarcane processed is transformed into biofuels (Voora et al., 2023). Sugarcane is important in energy production as it is the largest global source of molasses-based ethanol production (Rahman et al., 2019). As the world moves towards a more environmentally conscious generation, sugarcane and maize are the main raw materials used in ethanol production globally. Therefore, ensuring the quality and quantity of sugarcane is of high importance (Alonso-Gómez & Bello-Pérez, 2018).

2.1.2 South African production and use of the sugarcane

In South Africa, the first sugarcane cultivation dates back to 1852, with the industry rapidly expanding and further impacting the country's socio-economic development (Zhou, 2013). The establishment of the sugarcane industry has resulted in job creation, income provision, foreign exchange earnings, organization of resources, and the development of communication and transportation (Thibane et al., 2023). The sugar industry is estimated to account for 0,6% of the country’s GDP, with an estimated 435 000 direct and indirect employment (Department of Agriculture, Land Reform & Rural Development, 2024; Dlamini, 2021).

Since the early 2000s, South Africa’s long history in sugarcane production has maintained its standards of producing cost-competitive high-quality sugar, ranking it in the top 15 sugarcane producers globally (Baiyegunhi & Arnold, 2011). In 2024, South Africa has been estimated to produce over 2 111 metric tons and is the 8th highest exporter at 810 metric tons (USAD, 2024). The industry encompasses six milling companies that manufacture sugar, with 14 sugar mills operating mostly in the two main sugar-growing provinces, KwaZulu-Natal and Mpumalanga (Department of Agriculture, Land Reform & Rural Development, 2024). South Africa encompasses 14 sugarcane- producing areas, characterized by a subtropical climate, extending mostly from the Mpumalanga Lowvelds through KwaZulu-Natal coastal belts, with less production towards Northern Pondoland in the Eastern Cape (Mokonoto, 2018; Shikwambana et al., 2021).

As of 2023, sugarcane production in South Africa was estimated to cover 353 000 hectares of land, with over 21 926 registered sugarcane growers producing an average of 2.2 million tons per season (Department of Agriculture, Land Reform & Rural Development, 2024). About 80% of the country's sugarcane is produced by large-scale farmers, while the rest is supplied by smallholder farmers (Moobi & Woody, 2023). Additionally, by 2022, the sugarcane sector supported over 20 000 smallholder farmers, providing over 85 000 direct and 35 000 indirect jobs (Voora et al., 2023). From 2020 to 2021, South African sugarcane production dropped from 2,106 to 1,906 metric tons. Production then increased to 1,996 metric tons in 2022 and 2,075 metric tons by 2023 (Sugar: World Markets and Trade, 2024). From May 2024, South Africa’s sugar production increased to 2 111 metric tons, with approximately 810 metric tons being exported, making the country the 8th highest sugar exporter globally (Sugar: World Markets and Trade, 2024).

In South Africa, approximately 80-90% of the sugarcane produced is processed into sugar, with most of the waste from production being converted into molasses and bagasse for use in industrial ethanol production, electricity generation, animal feed, and paper manufacturing (Mohlala et al., 2016). The biofuel industry is still in its infancy, with South Africa's sugarcane processing industry typically generating 0.28 to 0.3 tons of bagasse per ton of sugarcane produced (Petersen et al., 2018). With majority of that bagasse being utilized for generating electricity used to heat up sugar milling operations (Petersen, Aneke & Görgens, 2014). Molasses account for less than 5% of South Africa's industrial revenue, which includes its use in alcohol fermentation, production of fertilizer and animal feed production, and as a raw material for other products such as through the manufacturing of paper (Mandergari et al., 2019).

2.2 *E. saccharina* as sugarcane pest

2.2.1 History of this pest in South Africa

Eldana saccharina also known as the African sugarcane borer, is the most prolific stem borer found in Southern Africa (Mulcahy, 2023). In South Africa, *E. saccharina* was recognized as a pest of sugarcane in 1913 and only became significant in 1939 when an outbreak was first recorded along the Umfolozi Flats in KwaZulu-Natal (Assefa et al., 2009). The pest went unnoticed as its population dwindled within sugarcane plantations until the 1970s, when it resurfaced in Hluhluwe, KwaZulu-Natal (Walton & Conlong, 2016a). A major reason why it "disappeared" from records was due to the expansion of its host range into various gramineous crops such as maize, sorghum, and saccharum species (Conlong, 1994; Conlong, 2001).

The pest was initially believed to be distributed along South Africa's coastal sugar belt due to the area's higher temperatures, the presence of their natural host plants, indigenous sedges and the commercial production of sugarcane (Atkinson, 1980; Conlong, 2001). However, by the 2000s, it was found that the *E. saccharina* outbreak had extended to the south and inland of South Africa, where colder temperatures were prevalent, and their host plants were less common (Assefa et al., 2008). It has been predicted that the short life cycle of *E. saccharina* compared to its host plants and the poor dispersal ability of the pest limited the gene flow within its metapopulations, which then induced natural selection, resulting in their shift in host plant preference (Serfontein, 2021).

2.2.2 The life cycle of E. saccharina

Since its discovery in 1865 in South Africa by the entomologist Francis Walker, *E. saccharina* has been a major pest of sugarcane in South Africa (Conlong, 2001; Li et al., 2024). However, the biology and significance of this pest were only entomologically researched in 1965 (Conlong, 1994). A vast number of studies were conducted on the biology of *E. saccharina* as a sugarcane pest (Atkinson, 1979; Atkinson, 1981; Atkinson, Carneige & Smaill, 1981; Girling, 1978; Sampson & Kumar, 1985). Under the common name, the African sugarcane borer, they are known to consume a variety of gramineous crops such as maize, sorghum, sugarcane, rice, sedges and grasses from wetland habitats (Girling, 1978; Li et al., 2024). However, because of the increased disturbances on wetlands due to afforestation and agricultural development, it has been hypothesised that in recent years, sugarcane has become the main crop host for *E. saccharina* in sub-Saharan Africa (Mulcahy, 2023). Additionally, it has been postulated that the adaptation of *E. saccharina* to a variety of host plants has led to its resurfacing and exponential outbreak observed in South Africa from 1970, with many researchers linking it to being either a widespread distribution of *E. saccharina* or widespread destruction of its natural hosts, such as wild grasses and other tropical grasses (Potgieter, 2013). However, evidence has suggested that *E. saccharina* prefer the cryptic egg-laying sites found along the dead sugarcane sheaths (Potgieter, Van Vuuren & Conlong, 2013).

The complete life cycle of *E. saccharina* lasts about two- three months, with the larval stage increasing from 20 to 30 days between summer and winter (Serfontein, 2021). As distinguished in all Lepidoptera, *E. saccharina* has four distinct stages, namely the egg, larval, pupa and moth (adult) (Horton, Hearne & Apaloo, 2000). During their adult stage, the moth has been estimated to survive for two weeks, with the first three days of emergence being their peak mating and oviposition period (Walton & Conlong, 2026a). A female is estimated to lay about 400-600 eggs during the oviposition period (Girling, 1978; Sampson & Kumar, 1985). In a laboratory-reared colony by Walton & Conlong (2016a), the mean fecundity of *E. saccharina* was recorded to be a maximum of almost 800 eggs per female. High egg production is pertinent, as 99.5% of the laid eggs are lost due to ants (Girling, 1978). The preference for female moth oviposition is dependent on various factors.

The female *E. saccharina* moth is known to oviposit eggs against the stalk and folds of dead sheaths (Barker, 2008). Research done by Atkinson (1979) showed that *E. saccharina* moths are lured by the dead leaf material encompassing sugarcane, which is deemed a satisfactory cryptic egg-laying site. The female moth is equipped with a prehensile ovipositor surrounded by a sensitive sensilla used to stimulate female egg laying between two narrow surfaces that touch at three points of the egg batches (Serfontein, 2021). The inner surface of the leaf sheath has regulated high humidity, lending to its preference (Sampson & Kumar, 1985).

Once laid, it has been estimated that the egg incubation period varies between 5-7 days, with this period decreasing with the increase in temperature (Sampson & Kumar, 1985). Within the larval stage, there are neonate larvae (including instars one to three) and large larvae (including instars four and above) (Horton, Hearne & Apaloo, 2000). Additionally, there are differences in instar number between male and female *E. saccharina* moths; males have been reported to have 5-6 instars, while females had 6-7 instars (Walton & Conlong, 2016a). The first and second instars usually feed on the leaf sheaths of the sugarcane and are the least active, causing low damage to the crop (Horton, Hearne & Apaloo, 2000). From the third instar onwards, they primarily feed and cause large damage to the stalk of the crop (Atkinson, 1980). This is noticeable by the increased amount of frass and silken material produced (Serfontein, 2021).

Of the four stages of their life cycle, the larval stage is their most destructive (Horton, Hearne & Apaloo, 2000). The eggs take 8-10 days to hatch, and the neonates immediately feed on the surrounding leaf sheaths until they become large larvae where they begin to enter the sugarcanes stalk (Sampson & Kumar, 1985; Karthikeyan et al., 2012; Serfontein, 2021). Once robust, they bore into the stalk, causing internal tissue damage by creating galleries in the sugarcane stalk, reducing sucrose capacity and biomass yield (Botha & Stranack, 2017; Lichakane & Zhou, 2017). For approximately 21 days, the large larvae bore into the sugarcane stalk, feeding veraciously on the sugarcane tissue (Walton & Conlong, 2016a). The larva at instar four to six generally undergo solitary tunnelling (Sampson & Kumar, 1985). The solitary tunnelling is not only done to obtain sufficient sustenance but also to form a protected area to allow pupation, as *E. saccharina* larvae are known to be cannibalistic (Girling, 1978).

Prior to pupation, the entrance point that is created by the larvae when they bore into the stalk is covered by frass to protect them against cannibalism (Serfontein, 2021). The larvae undergo the pre-pupal stage, where they enter an inactive state within a tough, silken cocoon (Sampson & Kumar, 1985). Once in pupation, the cocoon hardens to protect the pupa, which emerges into a moth between 7-13 days (Serfontein, 2021). At maturity, the male *E. saccharina* moth is identifiable as light brown with a distinct dark streak on both forewings (Atkinson, 1981). Compared to larger females, males are smaller and have a wingspan of approximately 30 mm (Carnegie, 1974). Females have a darker brown colour, distinctly characterised by one or two protruding larger labial palps and have an approximate wingspan of 39 mm (Carnegie, 1974).

2.2.3 The economic importance of E. saccharina

Over recent years, sugarcane production has decreased worldwide due to insect pest infestations (Dessoky et al., 2021). Most insects found in agroecosystems are regarded as key pests of different crops. *Eldana saccharina* is an African stalk borer known to be a damaging pest on various crops of the Poaceae family, such as sorghum, maize and most prevalent in South African sugarcane (Walton & Conlong, 2016a, 2016b). *Eldana saccharina* was initially recorded along the coastal regions of South Africa in 1939 among the sugarcane variety POJ2725 and was later identified as a pest in the sugarcane variety NCo376 in 1970 (Zhou, 2016). Since then, sugarcane farming has expanded to the south and inland areas of the Midlands, where it was believed that high altitudes and cooler temperatures would hinder *E. saccharina* infestation (Assefa et al., 2008).

The persistent infestation of *E. saccharina* has led to the destruction of entire sugarcane fields, causing economic injury levels that have influenced South Africa’s GDP (Pedigo & Higley, 1992; Berry et al., 2010). The South African Sugarcane Research Institute (SASRI) has since conducted industry-wide annual pest surveys to boost sugarcane production in South Africa (Singels et al., 2019). Continuous research has identified *E. saccharina* as a key pest in the industry, causing a reduction in both sugar quality and biomass quantity (Singels et al., 2019).

There are two notable impacts *E. saccharina* infestation inflicts on sugarcane that would cause economic injury to a country’s GDP (Goebel & Way, 2003; Leslie, 2009). Firstly, the larvae consume a significant amount of stalk tissue during their developmental stage, reducing sucrose levels and impacting glucose extraction during sugar production (Goebel & Way, 2003; Potgieter, Van Vuuren & Conlong, 2013). Additionally, the contamination of opportunist fungus, *Fusarium* spp. Link (Nectriaceae: Hypocreales) causes reddening associated with the breaking down of the stalk's sucrose. Generally, the damage is extended past the internodes bored to the rest of the stalk, causing further reduction in sucrose availability during sugar production (Mahlanza, 2015; Prinsloo, 2024). Farmers generally associate the dark-red discolouration as a sign of a loss of sucrose content, signifying a possible reduction in sugar production, leading to a reduction in the grower's profitability (Mahlanza et al., 2014; Dela Cueva et al., 2019).

Secondly, the indirect impact infestations have on sugarcane has been noted to cause more damage as farmers are advised to conduct early harvesting to control the infestation (Serfontein, 2021). Generally, growers tend to harvest 'stand-over' (older than 12 months) sugarcane due to the high sucrose levels they retain (Carnegie, 1974). However, extended harvesting time has led to large economic losses because of *E. saccharina* infestation (Horton et al., 2002). The prominence of *E. saccharina* infestation is linked to the ageing of the sugarcane, as the higher sugar levels found in 12–15-month sugarcane result in greater yield damage (Berry et al., 2010). Furthermore, younger larvae prefer softer sugarcane over harder varieties as it is easier to bore; however, softer varieties easily release juice during processing and, therefore, are preferable to growers (Potgieter et al., 2012). Therefore, the impact *E. saccharina* has on the sugarcane industry has warranted further research on ways to limit its ecological and economic impact (Malinga, 2024).

It has been estimated that there is an approximately 1% loss in recoverable sucrose for every 1% internode bored, resulting in an *E. saccharina* infestation incurring a significant loss in crop and sucrose levels (Potgieter, Van Vuuren & Conlong, 2013). Crop losses caused by *E. saccharina* infestation amount to 0.1% yield loss for every 1 % of damaged stalks (Li et al., 2024). Large infestations cause both direct and indirect losses amounting to over R1 billion per season (Botha & Stranack, 2017). A drop in yield for an essential agricultural crop such as sugarcane leads to drastic economic issues for South Africa (Berry et al., 2010). However, the resilience of this pest and its cryptic nature have resulted in various management attempts to effectively suppress their population.

2.2.4 Management strategies used to control E. saccharina

2.2.4.1 Cultural control

Common cultural methods used are pre-thrashing, cultivar resistance, fertilizing with silicon, nitrogen application, intercropping and early harvesting, which results in reduced sucrose production (Keeping, Rutherford & Conlong, 2007; Van Antwerpen, Conlong & Miles, 2011). The first major control measures occurred in the mid-1970s, and in the early 1980s, these methods were based on cultural control measures used as an accessible way to manage populations by reducing egg and pupa numbers (Conlong, 1994; Girling, 1978). These methods included the pre-thrashing of mature sugarcane to limit the availability for oviposition due to the reduced abundance of dry leaves; this includes the additional removal of any eggs present (Carnegie & Smaill, 1982). Cutting the infected ratoon crop as low to the ground as possible reduces the carryover of eggs and pupa to the next ratoon (Carnegie, 1974).

To reduce borer infestations, early harvesting, 10-13 months, compared to the optimal 10-18 months of sugarcane, was commonly practised (Serfontein, 2021). Furthermore, hot water treatments at 50°C for 30 minutes on highly infested fields, as well as the burning and thrashing during harvesting, were encouraged (Carnegie et al., 1976). Serfontein (2021) reported that an increase in stress and toxicity increases the susceptibility of sugarcane to *E. saccharina* infestation. Therefore, many farmers are spurred to apply fertilisers containing soil nutrients suited to their specific field to lower sugarcane toxicity (Keeping et al., 2014). A few methods recommended to maintain soil quality are by reducing the application of nitrogen and supplementing sugarcane with silicon fertilizers, increasing their resistance to *E. saccharina* (Keeping, Miles & Sewpersad, 2014). However, these measures limit sugarcane production as they are expensive, labour-intensive, time-dependent and carry the risk of causing severe sugarcane quantity and quality reduction (Leslie, 1994; Péné et al., 2019).

Intercropping is a fundamental defense against weeds and pests in crop cultivation for many subsistence farmers in South Africa (Abate, 1991). The growth of various compatible dissimilar plant species increases the diversity of possible predators and competitors for the targeted pest, therefore ensuring reduced infestations (Barker, 2008). Intercropping with 'dead-end trap crop' species, such as Bt maize, which contains the toxin CRY1Ab harmful to *E. saccharina,* has led to an overall reduction in sugarcane damage (Li et al., 2024). Genetic resistance is a key cultural control used to manage pests (Keeping & Rutherford, 2004). South Africa’s favourable climate led to the mass importation and development of newer varieties, with their differing susceptibility to various diseases and pests indigenous to the area (Zhou, 2013).

Breeding for resistant sugarcane varieties commenced in South Africa in 1980, and since then, numerous resistant cultivars have been identified and commercialised (Zhou, 2015). The resistant varieties produced were based on the chemical and physiological reaction of the larval stage *E. saccharina* to the external and internal characteristics of the stalk (Keeping & Rutherford, 2004). Resistant varieties are generally integrated with other control measures, as they cause a reaction in the larval stage of *E. saccharina* (Zhou, 2015). Currently, there are no resistant varieties that contain ovipositional antixenosis, which is used to influence female oviposition preference for *E. saccharina* (Serfontein, 2021). Therefore, some effective AW-IPM programmes integrated resistant varieties with insecticidal spraying as it targets both the adult moths and the early instar neonates that are found dispersing outside the sugarcane, resulting in an increased efficiency of insecticide application due to increased exposure (Bessin, Moser & Reagan, 1990).

2.2.4.2 Chemical control

During the initial exploration of *E. saccharina* control, insecticides cypermethrin and permethrin were initially aerially sprayed; however, due to low *E. saccharina* mortality, they were sprayed directly to the sugarcane stem below the leaf canopy (Carnegie & Smaill, 1982; Heathcote, 1984). Once the cryptic larva of *E. saccharina* successfully bore into the stalk, they become inaccessible, making the use of insecticides ineffective (Barker et al., 2006). Additionally, due to the negative impact pesticides have on the natural enemies of *E, saccharina*, the practice was not unanimously adopted by sugarcane growers in South Africa (Serfontein, 2021). Extensive research was then conducted to determine the application times, which include when adult moth populations peak and hatched neonates disperse and forage; this was estimated to maximise the insecticide efficiency (Leslie, 1997). However, routine application may lead to loss in diversity, resistant pest populations and an increase in infestation due to loss in predation, parasitism and competition (Helps, Paveley & Van Den Bosch, 2017).

2.2.4.3 Biological control

Extensive research has been conducted to identify any efficient biological control methods; however, due to the cryptic nature of *E. saccharina*, the knowledge of important factors such as its method of colonisation and behaviour in the field is lacking (Péné et al., 2018). From the early 1980s to the mid-1990s, biological control methods were studied due to the resurfacing of *E. saccharina* in the 1970s (Conlong, 1994). Since 1981, extensive research has been conducted by the South African Sugar Association experiment station to determine an effective biological control method to suppress this indigenous pest (Conlong, 1997). However, from the biological control measures explored thus far, their success has been limited, and *E. saccharina* has extended to most of the sugarcane growing areas in South Africa (Mulcahy, 2023).

Two methods were included, the first being the new association approach, which was unsuccessful, and the classical control principle, which was not able to establish a long-term effect on the *E. saccharina* population (Conlong, 1997). However, any of the parasitoids used to control the *E. saccharina* population failed to be established successfully over an extended period in South African sugarcane-infested fields (Barker, Conlong & Byrne, 2006). It has been hypothesised that the cryptic nature of *E. saccharina* also lends itself to reducing the ability of any existing enemies to locate the pest in its various life stages, which further limits the use of methods such as predation to control the population (Horton et al., 2002).

Similar to intercropping, in the early 2000s, the push-pull method, a habitat management strategy which is part of the newer ecologically based approaches, was introduced (Cockburn et al., 2014). The push-pull method involves the manipulation of the distribution and abundance of the pest and its natural enemies by utilising behaviour-modifying stimuli in the form of attractant (pull) and repellent (push) plants (Cook, Khan & Pickett, 2007). The aim of using the push-pull method is to reduce field infestation by driving *E. saccharina* towards their natural habitat through the planting of attractant and repellent plant species in or at the borders of sugarcane fields (Barker., 2008). An example of a repellent plant species that can be used for *E. saccharina* is molasses grass (*Melinis minutiflora* P. Beauv (Cyperales: Poaceae)) and an attractant plant, the indigenous wetland sedges (*Cyperus papyrus* and *Cyperus dives*) or Bt maize (Cockburn et al., 2014). The push- pull strategy is an instrumental part of the AW-IPM strategy currently used in the Midlands North farms found in KwaZulu-Natal; however, similar to intercropping, the use of attractant and repellent plants reduces the overall sugarcane yields produced (Cockburn et al., 2014).

2.2.4.4 Intergrated Methods

Thereafter, by the mid-2000s to the late 2010s, research shifted towards combining the already developed and conventional control measures and integrating them into the AW-IPM strategy (Conlong & Rutherford, 2009). One of the critical reasons why combined methods were explored was due to the cryptic nature of *E. saccharina*; when boring into the stalk, the larvae build up frass along the tunnels formed, obstructing the access of various control measures

(Legaspi et al., 1999). These methods included insecticides alpha-cypermethrin (Fastac), reduced harvesting time from 18 to 12 months, trash burning, the push-pull method, cultivating resistant sugarcane varieties and biological control (Cockburn et al., 2014; Conlong & Rutherford, 2009; Leslie, 1994; Zhou, 2015). Furthermore, SASRI has been exploring sterile insect technique (SIT) and gram-positive, aerobic, spore-forming bacterium *B. thuringiensis* as tools in their AW-IPM approach to *E. saccharina* (Barnes et al., 2015; Downing, Leslie & Thomson, 2000).

2.3 Sterile insect technique

2.3.1 History of the sterile insect technique

The earliest known theory based on controlling pest populations through the release of sterilized insects into wild populations was formulated in the 1930s and 1940s (Dyck, Hendrichs & Robinsons, 2021; Robinson, 2021). In 1937, Knipling reported that releasing sterile male screwworms, Calitroga hominivorax, in the southeastern states could be used to control their population (Baumhover, 2002; Jena, Gena & Sahoo, 2022). Baumhover et al. (1955) underwent the first experimental evidence needed to consider it as a method for pest control. The research paper was focused on Knipling's proposed idea, which was conducted on the screwworm-infested island of Curacao, Netherlands, Antile (Baumhover et al., 1955). The operation to eradicate screwworms in Florida and the southeastern United States of America (USA) lasted from 1957 to 1959, proving to be the proof-of-concept for SIT (Skoda, Phillips & Welch, 2018).

The control method was coined the sterile insect technique (SIT) by Knipling in 1955. It aims at reducing the chances of wild, fertile insects mating by releasing sterile insects (Baumhover, 2002; Parker & Mehta, 2007). SIT involves sterilizing and mass-rearing the target pest, followed by conducting a high "overflooding" area-wide release of them into the wild population (Marec & Vreysen, 2019). The purpose is to increase the proportion of sterile to non-sterile eggs produced, thereby suppressing the pest's growing population (Barnes et al., 2015). Sterilization is done through ionizing radiation, preferably on the male target species. However, the implementation and success of SIT relies on understanding the target species' ecology and biology (Jena, Gena & Sahoo, 2022). This includes (1) the mating behaviours of an insect are not impacted by irradiation during sterilization, (2) a female insect only mates once in its lifetime, (3) sterile to wild insect release at a high "overflooding" ratio, (4) no larval development from sterile fertilized eggs (Baumhover, 2002; Jena, Gena & Sahoo, 2022).

2.3.2 The sterile insect technique as a management strategy

2.3.2.1 Global view

For decades, broad-spectrum pesticides have been the key method used globally to mitigate agricultural insect pests (Bourtzis & Vreysen, 2021; Tudi et al., 2021). However, due to the environmental implications that pesticides pose and the resistant properties attained by pests, the excessive use of pesticides has drastically reduced (Jena, Gena & Sahoo, 2022). SITs favourability in recent years is associated with it being species-specific and environmentally friendly (Bourtzis & Vreysen, 2021). Following the initial implementation of SIT, it has been successfully incorporated into the AW-IPM approach to minimize operational costs (Welburn & Maudlin, 2012).

Henceforth, SIT has been used to eradicate pest infestations of various insect orders, such as Diptera, Lepidoptera, Hemiptera, and Coleoptera (Jena, Gena & Sahoo, 2022). However, SIT has recently resorted to a more suppressive role in 'areas of low pest prevalence’ (ALPPs) as it may not be financially feasible and overall difficult to reach pest eradication (Barnes et al., 2015). The use of SIT is estimated by the impact the target pest species has on the environment’s ecosystem. SIT has successfully suppressed or eradicated over a dozen significant pests within the agricultural, livestock and medical industries. The first, and well-documented decade-long eradication campaign was initiated in 1957 on the New World screwworm *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) pest, known to have infested the USA and the greater part of Central America (Vargas-Terán et al., 2021). Following this success, a large SIT programme was established in 1977 in southern Mexico against the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), known to be a notorious pest on deciduous fruits (Potgieter, Van Vuuren & Conlong, 2013). The continuous suppression seen with this programme has served as a model for later initiatives in 12 different countries including South Africa. Since 1957, there have been various successful SIT eradication programmes initiated globally towards managing insect pests, and five of these notable programmes are summarised in Table 1.

Table 1. Pests that have been successfully eradicated/suppressed through the utilization of SIT across the world

Scientific name	Common name	Country	Eradication period	Reason for eradication	Reference
Cochliomyia hominivorax	New World screwworm	North America	1957-1966	Key agricultural pests that increase management costs	Vargas-Terán et al., 2021
Bactrocera cucurbitae	Melon fly	Southwestern Islands of Japan	1972-1993	To prevent the spreading of the invasive species to the mainland	Koyama, Kakinohana & Miyatake, 2004
Glossina austeni	Tsetse fly	Island of Unguja, Zanzibar	1994-1997	Acts as a vector for detrimental diseases	Vreysen et al., 2000
Cactoblastis cactorum (Berg)	Cactus moth	Isla Contoy, Mexico	2007-2009	To prevent its accidental introduction	Bello-Rivera et al., 2021
Cydia pomonella	Codling moth	Similkameen Valley, British Columbia, Canada	1994-present	Suppression of the pest as an alternative to the use of harmful pesticides and insecticides.	Thistlewood & Judd, 2019
Pectinophora gossypiella	Pink bollworm	Southwestern United States of America and Northern Mexico	2006-2014	Eradication of one of the world's most invasive insect pest	Tabashnik et al., 2021

2.3.2.2 South African view

In 1996, to establish fruit fly-free regions in Western Cape, South Africa, SIT was initiated to control the Mediterranean fruit fly and the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae) (Potgieter, Van Vuuren & Conlong, 2013; Venter, Baard & Barnes, 2021). Following the success of the fruit fly-free initiative in 2003, further SIT programmes were approved by the International Atomic Energy Agency (IAEA) as a control measure for the codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) and the false codling moth (FCM) *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) (Marec & Vreysen, 2019; Stotter, 2009). By 2007, the SIT programme against the FCM was initiated, and due to the success of the programme and the similarities between FCM and *E. saccharina,* in 2009, IAEA further approved four lepidopteran SIT programmes at SASRI, which included *E. saccharina* (Potgieter, Van Vuuren & Conlong, 2013). Currently, the SIT shade house trials are in the developmental stage, with previous trials demonstrating an overall reduction in stalk damage and the *E. saccharina* population (Mudavanhu, 2012).

2.3.3 The sterile insect technique in the sugarcane industry

Since the 1970s, extensive research has been conducted to investigate the effectiveness of suppressing *E. saccharina* populations in sugarcane (Potgieter, Van Vuuren & Conlong, 2012; Barnes et al., 2015). For *E. saccharina* control, the parent moths are partially sterilized at lower radiation doses of 200 Gy, which induces inherited sterility in the F1 progeny (Barnes et al., 2015; Mudavanhu et al., 2016). The high resistance of Lepidoptera to ionized radiation has previously limited the use of SIT. However, because of research done on the codling moth, *C. pomonella* (L.), it was discovered that they undergo inherited sterility (Marec & Vreysen, 2019). Furthermore, Walton & Conlong (2016b) found that *E. saccharina* fecundity was not affected when untreated females mate with irradiated males. Therefore, it is recommended that irradiated males should be released to ensure the efficiency of SIT.

Prior to initiating SIT release programmes, as the external manipulation through the release of sterile moths might have confounding impacts on the natural systems in the ecosystem, mathematical models and cage trials were conducted (Murray, 2002). Extensive research by

SASRI formulated informative mathematical models used to project the possible population dynamics and parameters needed to conduct effective research projects and trials needed for the management of *E. saccharina* using SIT (Potgieter, Van Vuuren & Conlong, 2016). The mathematical models provided parameters such as the approximate number, ratio, spatial distribution and frequency of releases, and the programme's economic viability (Potgieter, Van Vuuren & Conlong, 2013).

Irradiated *E. saccharina* moths have been released within shade houses in pilot projects, with results showing a drastic reduction in stalk damage, reduction in population, and overall fertility of subsequent progenies (Mudavanhu, 2012). Further shade house trial research demonstrated that irradiated males successfully competed with wild males for females, while reaction-diffusion models illustrated their relatively comparative fitness and population mortality rate (Potgieter, Van Vuuren & Conlong, 2013). Therefore, SIT has the capability of reducing *E. saccharina* infestations; however, its execution was previously hindered by the irradiation facilities available for use in field trials (Barnes et al., 2015). In South Africa, the X Sterile Insect Technique (Pty) Ltd (XSIT) in Citrusdal, Western Cape, commercially irradiates false codling moths to manage infestations in citrus. In partnership with SASRI, they have expanded to the irradiation of *E. saccharina* in efforts to control infestation occurring in sugarcane (Marec & Vreysen, 2019). As the irradiation of *E. saccharina* is conducted remotely, the programme is conducted using first-generation offspring for environmentally controlled and field-release trials (Malinga, 2024). However, SIT has limitations, including high irradiation costs (Welburn & Maudlin, 2012). The order lepidopteran has a high resistance to irradiation and high gamma dosages are generally applied, which compromises the quality or fitness of the reared target pest, limiting their ability to outcompete wild males (Marec & Vreysen, 2019).

Due to the cryptic nature of *E. saccharina* and the lack of pheromones needed to conduct release and recapture traps, it is difficult to estimate the possibility of overflooding releases occurring (Serfontein, 2021). Additionally, it is difficult to determine the dispersion, dispersal and survival, which could determine how far and for how long they are able to travel to mate with wild females (Lance & McInnis, 2021). Furthermore, SIT works to limit the production of future offspring. Therefore, it does not directly impact the larvae present; since the adult is not as damaging as the larvae, it is essential to integrate a control measure that targets the larval stage (Kebede, 2015).

2.4 Genetically modified crops

2.4.1 History of genetically modified crop

The cornerstone of agriculture is based on improving crop varieties (Vitale & Greenplate, 2014). From the advent of the modern agricultural sector, various plant protection methods were established to ensure the quality, quantity, and sustainability of crops (Bruce, 2012). The global agricultural sector has shifted to a more selective breeding technique to meet the global demand for population growth, urbanisation, and the drop in agricultural quality (Lipton, 2007; Tester & Langbridge, 2010). Under these methods came genetic engineering through crop manipulation (Zadoks & Waibel, 2000). The development of crop breeding techniques has aimed at improving the global yield production of crops through new hybrid varieties to meet the higher demand for sugar by the world's population (Qaim & Kouser, 2013; Rumánková & Smutka, 2013). However, crop breeding has been problematic as it involves working with complex and restrictive genetic bases; it is time-consuming, costly, and easily susceptible to pests and diseases (Budeguer et al., 2021; Dessoky et al., 2021).

For the past 50 years, along with the advancements in technology, gene manipulation has led to genetic modification (GM), which is known to be a more selective breeding method (Raman, 2017). Genetic modification involves the modification of a plant's genome through the insertion of a desired additional genetic material (Karthikeyan et al., 2012). The manipulation of a living organism's genome enables an organism to perform a specific function (Zhang, Wohlhueter & Zhang, 2016). Through advancements in technology, gene identification and transfer have become more attainable, making it more feasible for the desired agricultural traits to be adapted into crops without hindering the crop's overall quality (Sanghera et al., 2011). Since 1994, a major jump to GM crops was through the insertion of insect-resistant genes, which were viewed as both cost-effective and less complex than the normally used crop breeding techniques (Dessoky et al., 2021). An advancement in GM was the introduction of the insect-resistant gene *Bacillus thuringiensis* (Bt) into the genome of crops (Karthikeyan et al., 2012).

The Bt gene is an insecticidal protein toxin found in the soil bacterium *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) discovered in 1901 by Shigetane Ishiwatari within a silkworm in Japan (Bravo et al., 2013). The bacterium was then isolated from a Mediterranean moth fly, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), by Ernest Berliner in 1911. Berliner then identified protein crystals along the endospores within the Bt spore in 1915 (Abbas, 2018). The insecticidal properties of these crystals were first identified when spores and crystals were found within dead flour moths (Roh et al., 2007). Initial experiments involved the direct feeding of the spores/crystals, which were proven to have no effect on the healthy caterpillars (Roh et al., 2007; Sanahuja et al., 2011). However, once the leaves used to feed the caterpillars were coated with the spores/crystals, it was found that they stopped feeding and eventually died (Roh et al., 2007; Sanahuja et al., 2011). The first field trials were conducted on the European corn borer (*Ostinia nubilalis,* Hübner) in 1928, where the isolated Bt strain was successfully utilized as an insecticide known as Sporine (Smith & Farhan, 2023). In 1938, France successfully produced Sporine, and it became the first commercialised Bt-sourced bioinsecticide that was used to mitigate flour moths (Sanahuja et al., 2011). By 1958, the USA manufactured Bt for commercialisation, and by 1961, it was officially registered by the US Environmental Protection Agency as a Bt-based bioinsecticide (Milner, 1994; Sanahuja et al., 2011). In 1979, Zakharyan isolated the plasmid needed to create the endospore and crystal. By the 1980s, insecticidal resistance by pests was observed globally, which spurred the agricultural sector to further explore Bt toxins as a control mechanism (Abbas, 2018).

2.4.2 Genetically modified crops as a pest management strategy

From the inception of genetically modified crops in the 1980s by the USA to their commercial use in 1994, GM crops have been a rapidly accepted crop technology for global agricultural production (Khush, 2012). The progressive use of GM crops over the years has been attributed to them being environmentally friendly. Nearly 40 years ago, the first GM plants were manipulated to attain tolerance traits to insect attacks and herbicides (Kamthan et al., 2016). In non-GM crops, between five and twelve pesticide sprays can be administered throughout the season, while in GM crops, one to three sprays could be administered to combat pest infestations (Qamar et al., 2021). In 2020 alone, pesticide use was estimated to be reduced by 748.6 million kg (−7.2%), leading to a drop in Environmental Impact Quotient of over 17.3% in insecticide and herbicide use on these crops from 1996 to 2020 (Brookes, 2022). Profit estimations were made from 1996 to 2020, with over US $261.3 billion increase in crop production from using GM crops (Brookes, 2022). The first GM crop to be widely planted and used in agriculture grew on 1.7 million hectares of land in the United States of America in 1996, and GM cotton since expanded in 2020 to 185.6 million hectares in 29 countries globally (Brookes, 2022). When the Food and Drug Administration (FDA) approved the industrialization of GM crops by the USA in 1996, several major crops genetically engineered to resist insect pests were introduced. The four noteworthy varieties are summarised in Table 2.

Table 2. Examples of the initial FDA-approved GM crops commercialized by the United States of America in 1996 for insect resistance

Crop	Genetic conferred trait	Pest mitigated	Reference
Soybean (*Glycine max*)	The gene EPSP synthase, derived from an agrobacterium species	Resistance to lepidopteran populations such as the African bollworm (*Helicoverpa armigera*), and tolerance to herbicide glyphosate	Shelke et al., 2023
Potato (*Solanum tuberosum*)	A modified *CRY3*Ab gene derived from the soil bacterium *Bacillus thuringiensis* induces insect resistance	Colorado potato beetle (*Leptinotarsa decemlineata*)	Cingel et al., 2016
Maize/corn (*Zea mays*)	A modified *CRY*1Ab gene derived from the soil bacterium *Bacillus thuringiensis* induces insect resistance	European corn borer (*Ostrinia nubilalis*)	Eizaguirre et al., 2006
Cotton (*Gossypium hirsutum*)	A modified *CRY*1Ac gene derived from the soil bacterium *Bacillus thuringiensis* induces insect	Tobacco budworm (*Chloridea virescens*) and Pink bollworm (*Pectinophora gossypiella*)	Kong-Ming, 2007

Known as the pioneers of GM research, the USA first explored genetically engineering the tobacco plant to enhance virus-resistant properties, with much of the work getting picked up by China by the 1990's to 2000's where it was advanced but not commercialized (Brookes, 2022; Ramen, 2017). The Flavr Savr^{TM} tomato, containing properties that delayed ripening and increased resistance to rot, was then commercialised by the USA in 1994, it was the first FDA- approved GM plant accepted for human consumption (Bawa & Anilakumar, 2013; Raman, 2017). In 1998, transgenic cultivation has proven to be successfully commercialized for the ringspot virus found in papaya (Gonsalves et al., 2000). Since 1996, canola, soybean, maize and cotton have been the main commercialised GM crops that have dominated the agricultural sector globally (Bawa & Anilakumar, 2013). Due to the extensive research on GM crops, over 166 Cry genes have been officially documented from over 30 groups and several subgroups of *Bacillus thuringiensis* (Li et al., 2024).

The control of insect pests using the Bt crop has shown great success. Therefore, the insertion of Cry genes has expanded to different varieties such as CRY1Ab, CRY1Ac, CRY1Ac+CRY1F, CRY2A, CRY1Ac+CRY2A (Qamar et al., 2021). In 2022, crop production containing the GM trait accounted for 47.7% of global agricultural production (Brookes, 2022). Currently, GM has expanded to include various fruits, vegetables and cereals, incorporating numerous traits to ensure their sustainability (Brookes & Barfoot, 2013). Due to the rapid impact of *E. saccharina* on annual sugar production in South Africa, SASRI has been investigating the use of GM sugarcane by introducing an insect-resistant gene (Dessoky et al., 2021).

2.4.3 Genetically modified technology in the sugarcane industry

Various lepidopteran larvae, such as *E. saccharina,* are inaccessible once they penetrate the stalk due to the production of frass, leading to the alternative use of GM sugarcane (Legaspi et al., 1999). Traditional sugarcane breeding techniques have been fruitless in improving its economic traits to defend against pests and diseases (Singh et al., 2013). Sugarcane's genomic complexity, its germplasm's lack of resistance, and the fact that traditional breeding is time-consuming have limited their use in recent years (Dessoky et al., 2021). Thus, extensive research has been conducted to produce persuasive evidence for the approval of Bt sugarcanes (Hoarau et al., 2007). Through the Bt gene, the modification of sugarcane has since become feasible (Huesing et al., 2016). Biotechnologists have found relative success by resorting to methods involving *Agrobacterium* and biolistic devices (Lakshmanan et al., 2005).

However, for the past 40 years, research on cell and tissue culturing of sugarcane has produced persuasive evidence to support the prospective use of plant genetic transformation as a viable method of genetic engineering (Singh et al., 2013). The first transgenic sugarcane was produced at the University of Queensland, Australia, by Bower and Birch in 1992 (Bower & Birch, 1992; Hotta et al., 2010). By bombarding the sugarcane embryonic callus with high-velocity DNA-coated microprojectiles, sugarcane transformation and regeneration were possible (Singh et al., 2013). Due to the increased infestation of stem borers on sugarcane fields in recent years, extensive investigation has been underway to determine the effectiveness of inserting the Bt CRY1A protein into the sugarcane genome (Dessoky et al., 2021). During sporulation, the Bt gene synthesizes highly insecticidal crystalline proteins at a very low concentration within the gut of diseased insects (Bravo et al., 2013; Karthikeyan et al., 2012).

Furthermore, the Bt strain has an expansive reservoir of genes encoded for insecticidal proteins that are crystallized in the bacterium’s inclusion bodies during sporulation (Deng et al., 2014). The genes that confer the formulation of proteinaceous crystals during sporulation are CRY/CYT proteins or are expressed during the bacterium’s growth, creating Vip protein (Federici, Park & Bideshi, 2010; Sanahuja et al., 2011). The Cry toxin is insect-specific and susceptible to species from the Coleoptera, Lepidoptera, and Hymenoptera orders (Ruiz de Escudero et al., 2006).

Therefore, the commercialization of the Bt strain as a bioinsecticide in 1996 was attributed to its ability to resist a variety of insect attacks, including those from problematic insects such as borers (Bravo et al., 2013). When the Bt crop is ingested, the crystals solubilize within their alkaline-coated midgut to form varying protoxins (Deng et al., 2014). The Cry toxin found in the Bt crop colonises and attacks the lining of the larvae’s alimentary tract, paralysing and inducing feeding inhibition, resulting in their mortality, which limits further damage to the Bt crop (Whalon & Wingerd, 2003).

There has been a lack of research on the durability of Bt sugarcane. Currently, transgenic sugarcane requires extensive time and is costly, and a lack of variety in their refuge strategy has hindered the ability to commercialize Bt sugarcane (Cristofoletti et al., 2018; Huesing et al., 2016). Since the late 1990s, intensive research has been conducted at SASRI in South Africa, with varying shade house trials being conducted to obtain regulation for field trials (Watt et al., 2010). In 2009, Australia approved biotechnology sugarcane field trials, while India followed suit and approved in 2011 (Srikanth, Subramonian & Premachandran, 2011). As Brazil is the world's largest sugarcane producer, in 2017, they were the first country to commercialise Bt sugarcane containing the Bt toxin CRY1Ab to manage the sugarcane borer, *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) (de Oliveira et al., 2022). In the following years, they had approval for three other Bt sugarcane varieties expressing a single gene CRY1Ac (de Oliveira et al., 2022). However, the major flaw with Bt crops is the potential for the target species' ability to generate resistance through generational consumption. Therefore, GM crops are generally utilised in conjunction with a variety of other control measures (Tabashnik et al., 2003). The use of refuges is often combined with the commercial use of Bt crops. Human & Potgieter (2023) estimated that approximately 10% to 30% of the Bt sugarcane growing fields should comprise conventional non-Bt sugarcane refuge fields to ensure efficiency. However, in terms of the sugarcane industry, the loss in yield numbers due to the reduced sugarcane fields is detrimental; therefore, including additional methods to reduce refuge sizes is essential (Potgieter, Human & Downing, 2024).

2.5 Integrating the sterile insect technique and genetically modified crop

When using Bt crops, it is essential to utilise an additional management method to curb resistance within the target pest (Tabashnik et al., 2010). A proposed addition, apart from the commonly used refuge method, is the use of SIT (Tabashnik et al., 2003). The sterile insect technique is a birth control measure that reduces the pest's reproductive rate, and over time, the overall genetic pool of the pest population may become genetically diverse (heterogeneous). This can slow the development of Bt crop resistance traits, making it less common in the population. (Potgieter, Human & Downing, 2024). Thus far, three major management programmes have combined SIT and Bt proteins; one focused on the biomedical industry, and the other two are used within the agricultural sector.

The majority of the IPM strategies that incorporate SIT and Bt protein were used against the Asian tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae), an invasive species originating from Southeast Asia (Becker et al., 2022). A vector for over 22 arboviruses, it has established populations in over 100 countries and is generally controlled using Bt-tablets (Culinex® Tab plus; Culinex Becker GmbH, Ludwigshafen am Rhein, Germany), door-to-door visits, public awareness of their breeding grounds and SIT (Becker et al., 2013; Becker et al., 2017). Since its establishment, SIT has become an integral component in the AW-IPM approach for management strategies utilised within the agricultural sector (Klassen and Vreysen, 2021). Through the integration of SIT and AW-IPM, the population of various insect pests has drastically been suppressed, prevented from being reintroduced, and eradicated globally (Klassen & Vreysen, 2021). However, the sole use of SIT has proven to be insufficient with two studies prior to 1996, demonstrating the lack of population control of pests such as the Painted Apple Moth *Teia anartoides* Walker (Lepidoptera: Erebidae) in New Zealand and the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) in the southwestern United States (Suckling et al., 2007; Tabashnik et al., 2021). The issue arose from the established population, as they outnumbered and outcompeted the sterile moths released (Henneberry & Naranjo, 1998).

Native to Asia, *P. gossypiella* first invaded the southern United States in 1917, possibly arriving naturally from northern Mexico due to increased cotton plantations or from international trade through infested shipments (Henneberry & Naranjo, 1998). Following this, *P. gossypiella* became the most destructive cotton pest in the southern United States (Tabashnik et al., 2010). Therefore, in 1996, Bt cotton was utilized to suppress the established *P. gossypiella* population using the insecticidal protein *B. thuringiensis* (Grefenstette, El-Lissy & Staten, 2009). There was a reduction in synthetic insecticidal use and damage expenditure following the use of Bt cotton (Tabashnik et al., 2021). However, cases within fields in India (Dhurua & Gujar, 2011) and experiments in Arizona found rapid evolution of resistance in the target species (Fabrick et al., 2015).

Therefore, it became mandatory in the USA to plant non-Bt cotton refuges along fields to allow for the mating of resistant and susceptible *P. gossypiella* to preserve superstability in their progeny (Tabashnik et al., 2010). Due to the loss in cotton production because of the unusable non-Bt cotton, research on methods to manage *P. gossypiella* was ongoing. In 2006, ventures into combining SIT and Bt cotton as a new multi-tactic area-wide release programme was initiated to eradicate the *P. gossypiella* infestation in Arizona (Tabashnik et al., 2010). By 2018, the USA Department of Agriculture (USDA) officially announced the complete eradication of the *P. gossypiella* in the greater USA and further recommended this method to countries such as India, which observed a resurgence of the *P. gossypiella* during the use of continuous use of Bt cotton alone (Naik et al., 2018; Tabashnik et al., 2021).

A similar programme combining SIT and Bt application was done to eradicate the Australian painted apple moth *T. anartoides* Walker (Lymantriidae), a detrimental pest to the horticultural industry in New Zealand (Suckling et al., 2014). Apart from SIT and Bt spraying, the programme involved chlorpyrifos, deltamethrin insecticide application, host plant removal and trapping, and was held from 1999-2004 (Marec and Vreysen, 2019). Contrary to the use of Bt crop, the programme used the aerial application of the insecticidal pathogens from *B. thuringiensis* (Berliner), subsp. kurstaki (Btk) (Suckling et al., 2007). From 1999 to 2006, the management strategy involved the continuous release of an aerial applicant utilising proteins from *Bacillus thuringiensis* (Berliner), subsp. Kurstaki (Btk), SIT, host plant removal, insecticide applications and trapping (Suckling et al., 2007; Suckling et al., 2014). As the *T. anartoides* caterpillar caused severe damage to the leaves of garden plants, the aerial application of the Bt insecticidal pathogen proved to be effective when sprayed on the surface of the host plant (Suckling et al., 2014). Therefore, similar to *P. gossypiella*, the eradication of *T. anartoides* was only achieved once SIT and Bt proteins were integrated into the management strategies used (Suckling et al., 2014).

A review done by Marec & Vreysen (2019) highlighted the successful suppression of the codling moth, *Cydia pomonella* (L.) (Tortricidae), using the Okanagan Kootenay Sterile Insect Release (OKSIR) programme in Canada. For more than 20 years, this AW-IPM programme has incorporated SIT with surveillance, orchard sanitation, banding trees, and mating disruption using pheromones (Thistlewood & Judd, 2019). However, due to the unavailability of *E. saccharina* hormones at this immediate stage, the use of mating disruption is limited, therefore spurring the use of other methods of suppression (Mudavanhu, 2012). Based on the current study’s findings, the possibility of incorporating Bt sugarcane as an alternative seems favourable. Therefore, combining SIT and Bt sugarcane is possibly an innovative way to deal with the current rapid infestation of *E. saccharina* in South Africa’s sugarcane.

Chapter 3 Materials and Methods

3.1 Study site

The trial was conducted at the South African Sugarcane Research Institute (SASRI) Blackburn estate in Mount Edgecombe (29°42' 27" S; 31° 02' 50" E), located 19.3km north of Durban, KwaZulu-Natal. The study area is located at 91masl, and the overall area is characterised by a humid subtropical climate that generally undergoes warm temperatures with a hot and wet climate in summer and moderately mild and dry winters (Govender, Conlong & Smith, 2011; Jury, 2022). Durban receives annual precipitation of approximately 893mm and experiences a mean temperature of 22°C in summer and 14°C in winter (Mkungo et al., 2023). The subtropical and coastal climate has resulted in the are being dominated by various types of indigenous vegetation types such as, mangroves, coastal forests and grasslands (McLean et al., 2016). However, a large part of the area has been replaced with sugarcane plantations. The region's fertile soil, warm climate, and abundant rainfall made it ideal for this type of crop.

3.2 Trial layout

The trial was conducted in two shade houses (each 14m x 15m x 3.3m height), with walls made of 40 % green shade cloth, roofing made of transparent fiberglass and 19mm stone chip flooring to assist in drainage of irrigated water. One shade house (B1) was designated as a control, and the other (B2) as a treatment. Along each irrigation line, there were 26 drippers with two pipes, which divided the line into two rows. Each shade house had 16 rows, each consisting of 26 (25L PVC) pots, overall, 832 pots were used during the experiments with 416 pots per shade house. Each irrigation line then consisted of a row of 26 pots containing conventional (88H0019) sugarcane and 26 pots containing Bt (GM CRY1A) sugarcane. There were 2800 seedlings (1400 per sugarcane type) grown with only 2496 of them being transplanted (excess grown as a replacement for dead seedlings). A limited number of shade houses were available to conduct the experiment, therefore, to ensure that each shade house had two replicates, the sugarcane was grown in four different batches (repeated measure) and transplanted on two different irrigation lines (replicate). Each batch had a total of 624 seedlings that were grown in three-month intervals, November 2022, February 2023, May 2023 and August 2023. For each batch, depending on the seedlings condition after hardening, 350 seedlings were grown for each sugarcane type. From the seedlings grown, 156 seedlings were transplanted in each shade house. In each pot, three sugarcane seedlings were planted. To ensure that the experiment was unbiased, when designating the irrigation lines, Microsoft Excel was used to conduct a randomized design layout (Figure 1) (Microsoft Excel (Microsoft Corporation). Once each sugarcane batch reached 12 months, they were then harvested (four surveys in total dung sampling period).

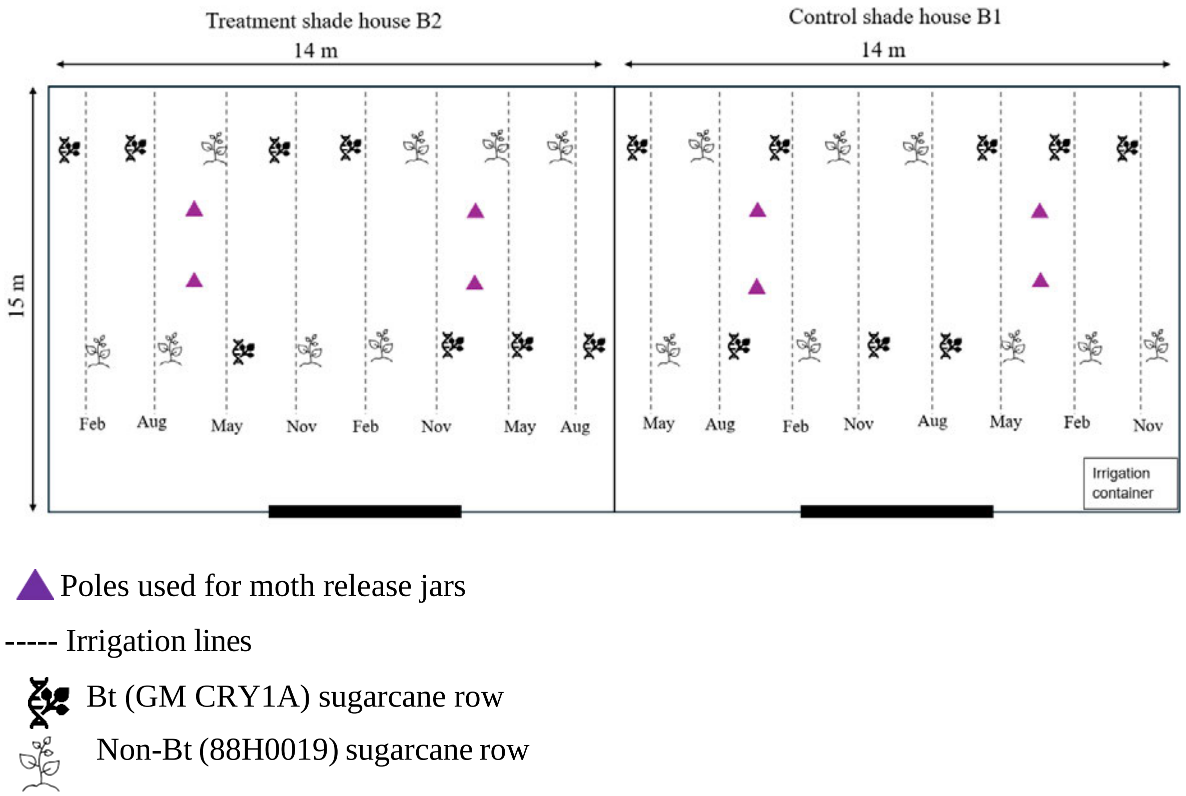

Figure 1. Illustration of the trial layout for shade house B1 (control) which has only non-sterile moth releases, and B2 (treatment) with both sterile and non-sterile moth releases.

3.3 Seedling preparation

Non-Bt sugarcane (88H0019) was obtained from the SASRI farm located on their Blackburn estate. Following the methods conducted by Ramgareeb et al. (2010), sample single-budded nodes (SBN) were extracted from the stalks and propagated at SASRI's Biotechnology laboratory. The SBN was incubated until node shoots formed, and meristems were then extracted from these nodes. Then, they underwent subculturing on charcoal media until callus developed. To formulate the GM CRY1A sugarcane, a portion of the sample explant calli was bombarded and transformed using CRY1A-trunc protein extracted from the bacterium *B. thuringiensis*. The 88h0019 and GM CRY1A calli were then placed in a photoperiod growth room (16 h light/8 h dark, 28 °C) to form 3 cm seedlings. For each batch, the 350 cultured seedlings were planted into 98 cells loose insert plastic seedling trays [capacity: 90cm^3, weight: 1.53 kg, propagation tubes: 9cm, dimensions- 69cm(L) x 38cm(W) x 32cm(H)], with substrate containing only peat moss, sand, and vermiculite and then hardened under ambient conditions in the glasshouse for at least three months. The glass house maintained a daytime temperature of 25-30°C and a nighttime temperature of 18-20°C. The humidity ranged between 60-80% and it maintained a natural sunlight condition. It used a sprinkling system which operated with 30-minute sessions every second day at 8am and 4pm.

The first batch of 88H0019 and GM CRY1A sugarcane was moved into the glasshouse in November 2022, and the other three batches of cultured seedlings were moved into the glasshouse in three-month intervals (February, May and August 2023). Once each batch reached three months of maturity, they were then transplanted into the shade houses. The shade houses were covered with knitted shade netting to emulate the surrounding conditions but within a closed environment, the shade house maintained a general temperature of 20-30°C, and a humidity of 80-90%. The first batch was transplanted in February 2023, while the others were done in three-month intervals (May, August, and November 2023). Each row was irrigated automatically with the following schedule: 1.2 litres per interval, with 18-minute watering times twice a day (08:00 and 15:00). Fertilisation involved the monthly application of 30g of Talborne Vita Green 5:1:5, 30g LAN and 300g ammonium sulphate + 0.5g Microplex® in 25 L water at 500 ml per pot (Keeping, Miles & Sewpersad, 2014).

3.4 Insect rearing

SASRI owns numerous sugarcane farms and have partnered with various South African sugarcane farmers to conduct research. Farm inspections are regularly conducted along 21 sugarcane farms, found in the Midlands North in Pietermaritzburg. From the numerous surveys conducted, *E. saccharina* pupae samples were collected for insect rearing to diversify the gene pool. These pupae were then brought to the Insect Rearing Unit at SASRI in Mount Edgecombe for mass rearing. The mass rearing procedure followed the work done by Walton and Conlong (2016), and the reared population was used to produce the mother colony (non-sterile) and F1 progeny (sterile). The pupae were put into individual plastic multicell trays (32-celled trays; Lovell Industries©) and were covered with perforated cling wrap (Handywrap®), which weas then placed in a moth emergence room with a temperature of 26-

30°C at a humidity of 60-80% for over two weeks. The day after the pupae were placed in the emergence room and the following two weeks they are monitored for emergence. Within one to two days of emergence the emerged moths were then mated in mating boxes made of ventilated transparent perspex (500mm x 500mm x 500mm) for two to five days depending on the moth's survival and egg laying ability. After mating, the eggs were then incubated for 7 days at (24 ± 2°C; 75 ± 5% relative humidity (RH); 0h:24h D: L photoperiod) to allow for hatching to occur (Serfontein, 2021). With a general hatching rate of 70-90%, once most of the eggs hatched, the neonates were inoculated onto 4L plastic trays with a 2L rabbit pellet diet sprinkled with sago (Ngomane et al., 2022). The trays were stored at (26 ± 2 °C, 72 ± 5% RH and 8-h L: 16-h D photo phase) for 28 days until they pupated. Generally, 90% pupation was expected for each tray and once confirmed they were then harvested. Harvesting involved sifting through diet-filled trays in search of pupae when found they were placed into multicell trays and covered with perforated clingwrap. Approximately 5000 pupae (157 multicell trays) of the initial pupae reared were kept in a moth emergence room and allowed to emerge into adults. The newly emerged male and female moths were paired and mated using the mating procedure described above to produce the eggs. These eggs were then further reared from an egg to an adult following the above-mentioned procedure to produce the mother colony population needed for release in the shade houses.

Ten thousand pupae (313 multicell trays) of the initial pupae reared were sent by courier to be irradiated at X- Sterile-Insect-Technique (XSIT) Pty (Ltd), where the sterile insect technique (SIT) programme is conducted. The irradiation and sterility of insect pests are conducted at XSIT, an initiative formed by Citrus Research International working to commercialise SIT. The irradiation procedure followed the methods described by Mudavanhu et al. (2016). Within one to two days of the pupae emerging, the male moths characterized as small and light brown were collected using plastic vials and the parentals were gamma irradiated for 20 minutes at 200 Gy to ensure sterility. Following the same mating methods mentioned above, the non-irradiated females were mated with the partially sterile irradiated males to produce the egg progeny with inherited sterility. The eggs were couriered to SASRI from XSIT where they underwent the same rearing procedure the mother colony population underwent. After pupating, both F1 and mother colony pupae were transplanted into multicell trays and relocated to the adult emergence room at (~24 °C with a 12h:12h Dark: Light (D:L) photoperiod) for 10 days, where they were monitored and collected once they emerged as moths.

3.5 Moth collections

The F1 and mother colony moth emergence was checked daily in the adult emergence room. For each release, shredded papers used as a perch for the collected moths were stuffed into 12 500 ml plastic jars with mesh-covered lids to allow for ventilation. Using a plastic vial, female and male mother colonies and only male F1 progeny moths were hand-collected from multicell trays to avoid collecting highly mutated moths. The 200 male F1 progeny moths collected were separated into four release jars. A maximum of 50 F1 progeny moths were collected in each jar in order to prevent overcrowding. The stress caused by the poor conditions could lead to an interference with the experiment. While the 20 male and female mother colony moths were separated into eight release jars prior to release. The number of release jars were dependent on the number of hanging poles available in the shade houses.

3.6 Insect release

One-to-three-day-old sterile F1 and mother colony (non-sterile) male and female moths were released in shade house B2, while, in shade house B1, only mother colony male and female moths were released. The release was done at an overflooding ratio of 10: 1. In B2, 200 F1 males and 20 mother colony moths (10 males and 10 females) were released, and in B1, only 20 mother colony moths (10 males and 10 females) were released. Moth releases commenced when the first batch of three months old sugarcane was transplanted into both shade houses. The moths were released twice weekly (Monday and Thursday) in both the shade houses. A total of 31 200 *E. saccharina* moths were released over the 130 releases that were conducted for 15 months from May 2023 to August 2024 (to allow the four batches to reach 12 months prior to harvesting). Out of the total releases 26 000 were the F1 male progeny, while 2600 were mother colony males and 2600 were mother colony females.

3.7 Sugarcane harvesting and assessment of damage

The surveying of first batch commenced in November 2023 with the following surveys occurring in February, May and the last survey in August 2024. Once each sugarcane batch reached 12 months, the survey followed the standard procedures described by Goebel, Way & Gossard (2005) conducted on the stalk. The stalks were cut at the stalks root zone to ensure that the entire stalk length was assessed for stalk damage and larvae /pupae presence. The survey commenced with initial measurements of each stalk's length. The stalk length (SL) was defined by measuring the height of the stalk from the root zone to the green top (crown) where the new leaves emerge (Molijn et al., 2018). The number of stalks bored was recorded, while the borer damage was determined by splitting each stalk longitudinally to collect data on the number of internodes damaged (IND), stalk red length (SRL) (cm), stalk bored length (SBL) (cm), number of dead plants, size of larval instars, number of pupae, larvae and pupae cases.

3.8 Discarding of sugarcane

After each batch of sugarcane was surveyed the stalks from the 88H0019 sugarcane lines were transported to a compost waste field. The surveyed pots were then removed from the shade houses and emptied into a pile for two months to allow for the drying out of any residual rooting. The dried sand was then sieved to remove dead plant material and reused for future transplants. Since GM CRY1A sugarcane has not yet been permitted for commercial use, researchers need to undergo strict regulations for handling, treating and discarding any GM product used.

All the surveyed GM CRY1A sugarcane stalks were placed on a tarp in the shade house and left to dry for two months. For two months, the pots were left in the shade house to observe if there were any surviving plants grown from the residual roots in the pots. Glyphosate was then sprayed on any surviving plants. Once completely dried, the GM CRY1A sugarcane stalks and roots were then moved to a permitted GM crop discarding field. The GM material was placed in a pile, where an inspection of plant growth was conducted, and a certificate of treatment was issued.

3.9 Data analysis

All statistical analysis performed in this study was conducted on GenStat 22nd edition, and graphs were created using Microsoft Excel (Microsoft Corporation, 2018; VSN International, 2022). The average percentage of stalks bored was calculated using Microsoft Excel. After sampling it was found that there were limited data points available for analysis, the sample size was then reduced to enhance the overall value of the data points. The three individual stalks present in a pot and the data related to that stalk was then averaged in order to allow the single pot (containing three stalks) to be represented as a single stalk. Therefore, converting the pots into the data points instead of the individual stalks. The measurements used to assess damage were converted into percentages and were calculated using the following formula:

$$\%\text{IND} = \frac{\text{Number of internodes damaged}}{\text{The number of internodes of the sugarcane stalk}}\ x\ 100$$

$$\%\text{SBL} = \frac{\text{Stalk bored length}}{\text{The stalk length}}\ x\ 100$$

$$\%\text{SRL} = \frac{\text{Stalk red length}}{\text{The stalk length}}\ x\ 100$$

A Shapiro- Wilk test for normality was conducted using the collected measures of damage, which were not normally distributed. However, since the data was based on percentages and contained numerous zeros, a Generalised Linear Model (GLM) was used to analyse the percentages for IND, SBL, and SRL in GenStat. For each response variable (dependent variable) IND, SBL and SRL, the fixed model (factors) used an interaction between sugarcane type and shade house, with a random model that included the replicates survey date and pot number.

To perform the GLM, the data followed a binomial distribution with a logit link function. A Least Significant Difference (LSD) post hoc test was conducted on the %IND, %SBL and %SRL as the interaction between the sugarcane type and shade house was significant. Based on the LSD conducted the letters ranging from a to d were used to indicate the significant differences found when the variables interacted. Based on the LSD conducted means having different letters were significantly different. In Excel, the relative difference involved dividing the mean of the 88H0019 sugarcane by that of the GM CRY1A sugarcane. In GenStat, a Linear Mixed Model (LMM) under the restricted maximum likelihood (REML) was used to analyse the SL, *E. saccharina* population per survey because it was based on count data rather than percentages. To adhere to previously approved procedures, the recorded larvae and pupae numbers were converted to numbers in 100 stalks and abbreviated as e/100. The stalk length and e/100 stalks were used as the y-variant in the LMM, with the fixed model of sugarcane type and shade house and a random model of replicate, survey date and pot number.

Chapter 4 Results

4.1 The percentage stalks bored by *E. saccharina*

A total of 2467 sugarcane stalks were harvested of the 2496 sugarcane stalks (29 dead stalks) that were transplanted over the 15-month experimental period. Of the total harvested stalks, only 50 (i.e., 2.02%) were bored in both shade houses. The treatment shade house had 1227 stalks sampled with 30 (60%) stalks bored, while the control had 1240 stalks sampled with 20 (40%) stalks bored. The 88H0019 sugarcane in the treatment shade house had the highest stalks bored (1.09%), while the GM CRY1A sugarcane had the lowest (0.12%) (Table 3).

Table 3. The total number and mean percentage of GM CRY1A and 88H0019 sugarcane stalks bored in the shade houses that had SIT moth releases (treatment) and no SIT moth releases (control).

Table 3. The total number and mean percentage of GM CRY1A and 88H0019 sugarcane stalks bored in the shade houses that had SIT moth releases (treatment) and no SIT moth releases (control).

Shade house	Sugarcane type	Number of bored sugarcane stalks	Percentage (%) bored sugarcane stalks
Treatment	88H0019	27	1.09
	GM CRY1A	3	0.12
Control	88H0019	14	0.57
	GM CRY1A	6	0.24

4.2 Percentage stalks bored length and internodes bored

The release of sterile *E. saccharina* did not effectively reduce stalk damage. The treatment shade house (0.15 ± 0.03), which had sterile moth releases, had the most significant stalk bored length (z-value = 30.74; $p < 0.001$) compared to the control (0.09 ± 0.03), as shown in Figure 2. In both shade houses, the 88H0019 sugarcane (0.22 ± 0.05) had a significantly higher stalk bored length (z-value = 95.95; $p < 0.001$) than the GM CRY1A sugarcane (0.03 ± 0.01), depicted in Figure 2. Overall, there was a significant (z-value = 6.70; $p = 0.010$) interaction between the treatment and the sugarcane type, with the highest stalk bored length being on the

88H0019 sugarcane in the treatment (0.28 ± 0.08) and the lowest on the GM CRY1A sugarcane (0.02 ± 0.02) in the same shade house (Table 4).

Following the same trend mentioned above, the treatment shade house (0.19 ± 0.05) had the highest significant (z-value = 23.50; $p < 0.001$) internodes bored compared to the control (0.13 ± 0.04), depicted in Figure 2. The GM CRY1A sugarcane (0.04 ± 0.02) had significantly low (z-value = 92.28; $p < 0.001$) internode bored compared to the 88H0019 sugarcane (0.28 ± 0.06) in both shade houses (Figure 2). Similarly, significantly low (z-value = 7.75; $p = 0.006$) stalk bored length was observed with the GM CRY1A sugarcane (0.03 ± 0.02) in the treatment shade house compared to the 88H0019 sugarcane (0.35 ± 0.09) (Table 4). The GM CRY1A sugarcane was more effective in lowering stalk bored and internode damage, irrespective of sterile releases.

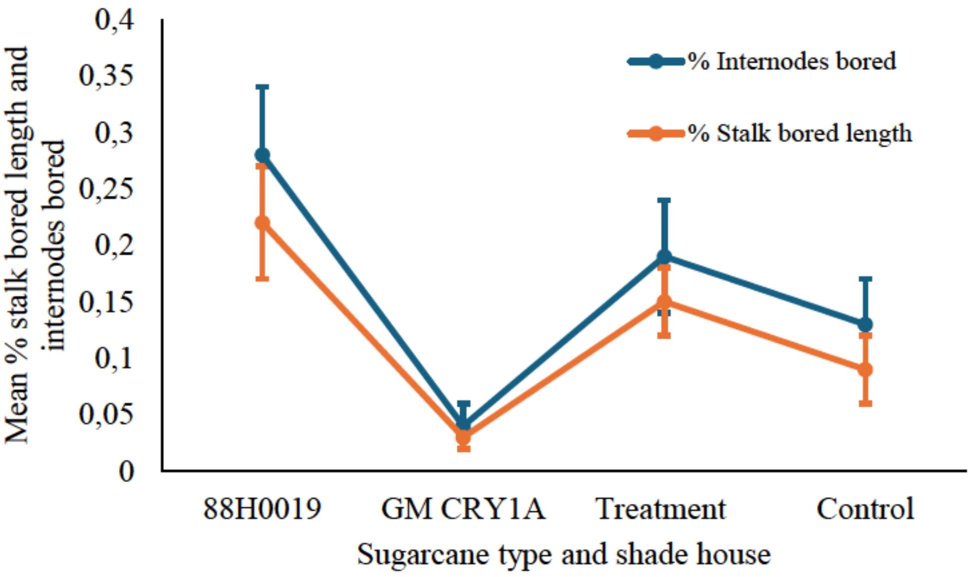

Figure 2. GLM results for the mean percentage and ± SE of stalks bored length and internodes bored observed in the variables sugarcane type (88H0019 and GM CRY1A) and shade houses (control and treatment).

Table 4. Summarised GLM results of percentage stalk bored length and internode bored damage observed on GM CRY1A and 88H0019 sugarcane in the control (non-SIT moth release) and treatment (SIT moth release) shade house.

Shade house	Sugarcane type	% Stalk length bored		% Internode bored	
		Mean	S.E.*	Mean	S.E.*
Control	88H0019	0.15	± 0.05 b	0.20	± 0.07 b
	GM CRY1A	0.03	± 0.02 a	0.06	± 0.03 a
Treatment	88H0019	0.28	± 0.08 c	0.35	± 0.09 c
	GM CRY1A	0.02	± 0.02 a	0.03	± 0.02 a

*An LSD post hoc test represented by a to b indicates the level of significance between the shade houses and sugarcane types.

4.3 Percentage of stalk red length damage

Overall, 16 stalks were infected with stalk red, with the total stalk red length amounting to 0.40%. The incidence of stalk red length was significantly higher (z-value = 61.84; $p < 0.001$) in the treatment shade house (0.42 ± 0.13) compared to the control (0.21 ± 0.06), shown in Figure 3. The GM CRY1A sugarcane (0.03 ± 0.01) was significantly more effective at lowering stalk red length (z-value = 28.92; $p < 0.001$) than the 88H0019 sugarcane (0,22 ± 0.05) in both shade houses (Figure 3). The GM CRY1A sugarcane (0.81 ± 0.26) in the treatment shade house had the highest damage, while the 88H0019 (0.03 ± 0.03) had the lowest (z-value = 26.48; $p < 0.001$) (Figure 4).

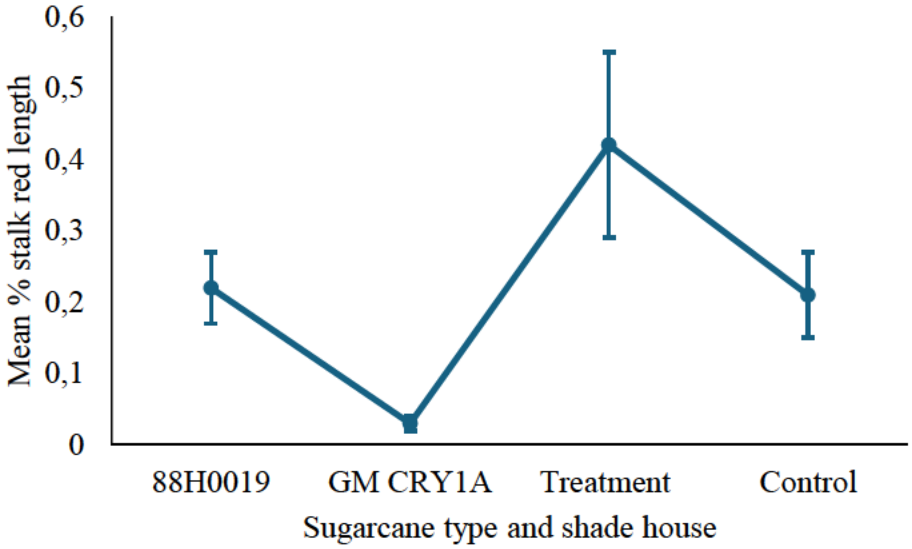

Figure 3. GLM results for the mean percentage and ± SE of stalk red length observed in the variables sugarcane type (88H0019 and GM CRY1A) and shade houses (control and treatment).

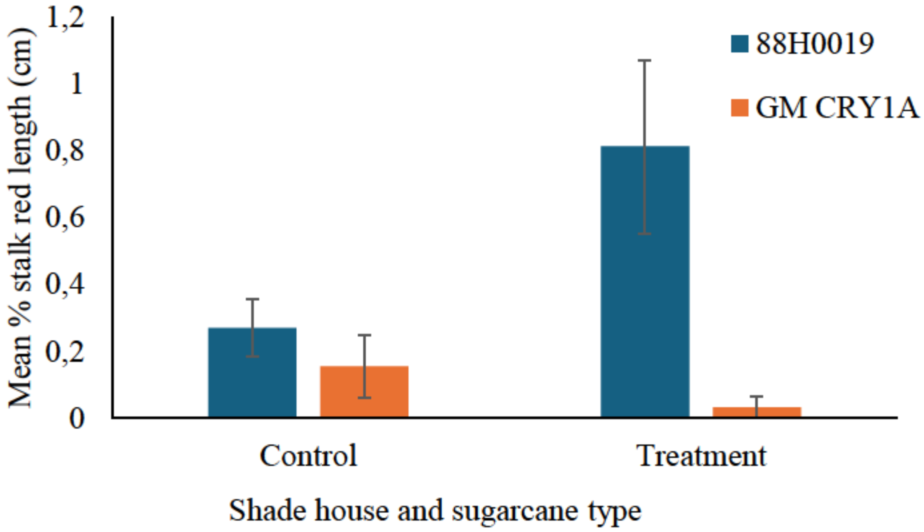

Figure 4. Summary of GLM results for the mean percentage and ± SE of stalk red length present on the GM CRY1A and 88H0019 sugarcane grown in the shade house that had SIT moth releases (treatment) and no SIT moth releases (control). An LSD post hoc test represented by a to d indicates the level of significance between the shade houses and sugarcane types.

4.1 Relative difference in GM CRY1A sugarcane stalk damage

The GM CRY1A sugarcane found in the treatment shade house had the least relative stalk damage compared to the control shade house (Table 5). For all damage measurements, the GM CRY1A sugarcane had over 10 times less damage in the treatment shade house, while the control had below 5 times less damage.

Table 5. The relative difference in damage represented by percentage stalk bored length, internodes bored and stalk red length between 88H0019 and GM CRY1A sugarcane found in the control and treatment shade houses.

Measured damage	Shade house	Relative difference in damage (n times less damage in GM CRY1A sugarcane)
% Stalk bored length	Control	4.4
	Treatment	12.22
% Internodes bored	Control	3.75
	Treatment	10.44
% Stalk red length	Control	1.74
	Treatment	25.29

4.2 Comparing the average stalk length of the sugarcane present in the shade houses

The treatment shade house (76.87 ± 1.62) had significantly higher stalk length (z-value = 61.87; $p < 0.001$) compared to the control (64.60 ± 1.25) (Figure 5). While the 88H0019 sugarcane (82.17 ± 1.42) grew to a significantly taller stalk length (z-value = 214.88; $p < 0.001$) than the GM CRY1A sugarcane (59.30 ± 1.31) in both shade houses (Figure 5). The interaction between shade houses and sugarcane type was insignificant (z-value = 2.60; $p = 0.107$), depicted in Figure 6.

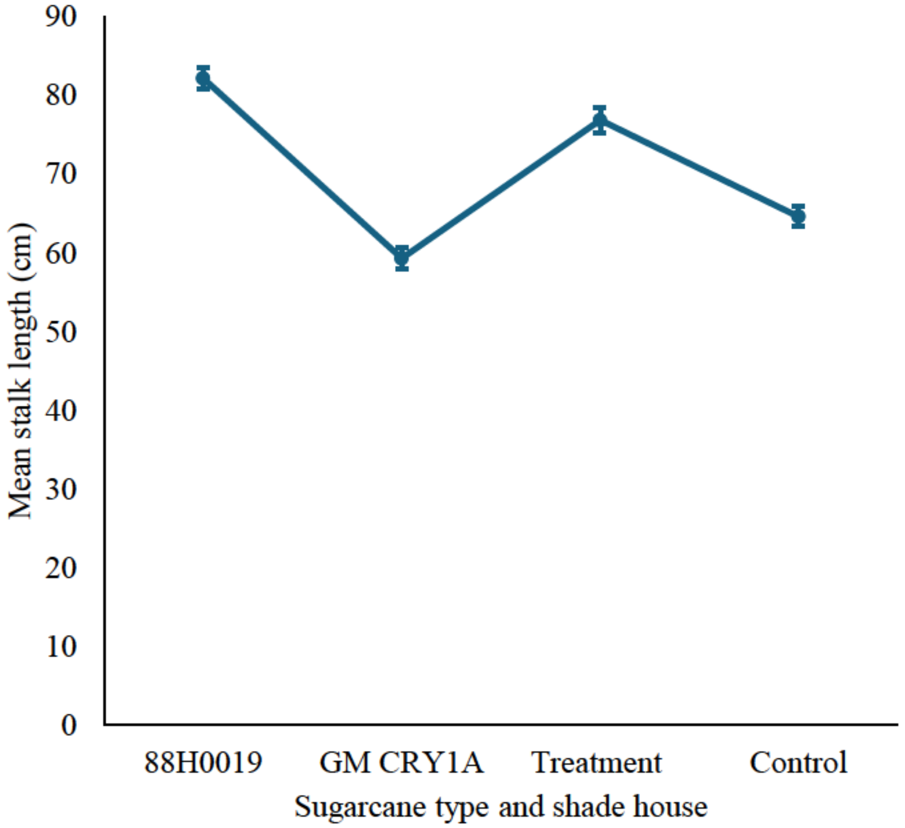

Figure 5. Summary of REML results for the mean percentage and ± SE for stalk length observed across sugarcane types (88H0019 and GM CRY1A) and shade house conditions (control and treatment).

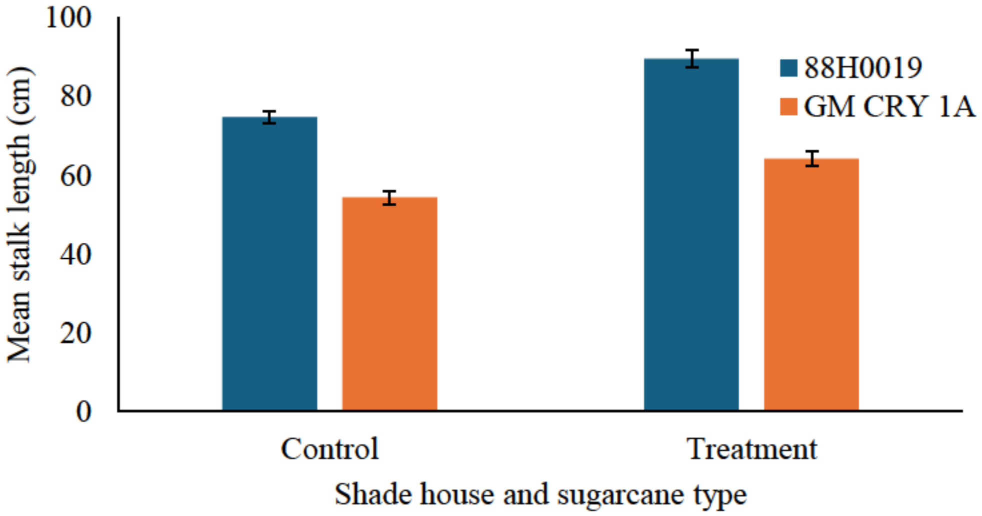

Figure 6 Summarised LMM results showing the mean and ± SE for stalk length of GM CRY1A and 88H0019 sugarcane in the control and treatment shade houses.

4.3 *E. saccharina* larvae infestation

The total larvae collected from both shade houses in the trial had a low average of 1.2 e/100. The release of sterile *E. saccharina* males had no significant effect in the e/100 found (z-value = 2.96; p = 0.086) (Figure 7). However, from both shade houses, the 88H0019 sugarcane (2.083 ± 0.835) had a significantly high e/100 (z-value = 4.42; p = 0.036) compared to the GM CRY1A sugarcane (0.321 ± 0.159). Furthermore, the treatment shade house had the highest significant e/100 (z-value = 4.42; p = 0.036) on the 88H0019 sugarcane (3.686 ± 1.642) and the lowest on the GM CRY1A sugarcane (0.160 ± 0.1603), as demonstrated in Figure 8.

The highest significant number of e/100 (z-value = 9.24; p = 0.027) was recorded from the first (November) survey (13.462 ± 6.293), while there was no larva or pupa present during the August (last) survey (Figure 9).

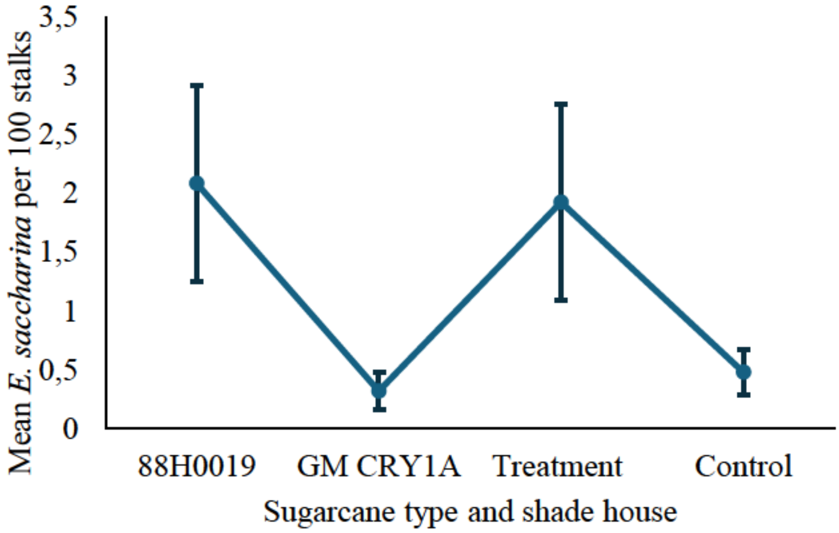

Figure 7 Summary of GLM results for the mean and ± SE of *E. saccharina* infestation (e/100) observed in the variables sugarcane type (88H0019 and GM CRY1A) and shade houses (control and treatment).

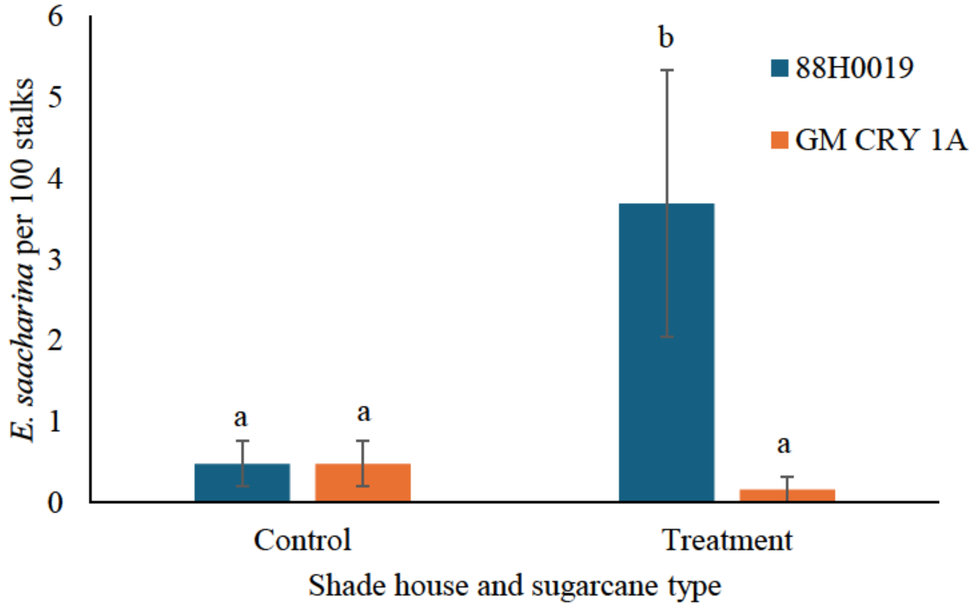

Figure 8. The summarised LMM results comparing the mean and ± SE of *E. saccharina* infestation (e/100) from GM CRY1A and 88H0019 sugarcane in the treatment and the control. An LSD post hoc test represented by a to b indicates the level of significance between the shade houses and sugarcane types.

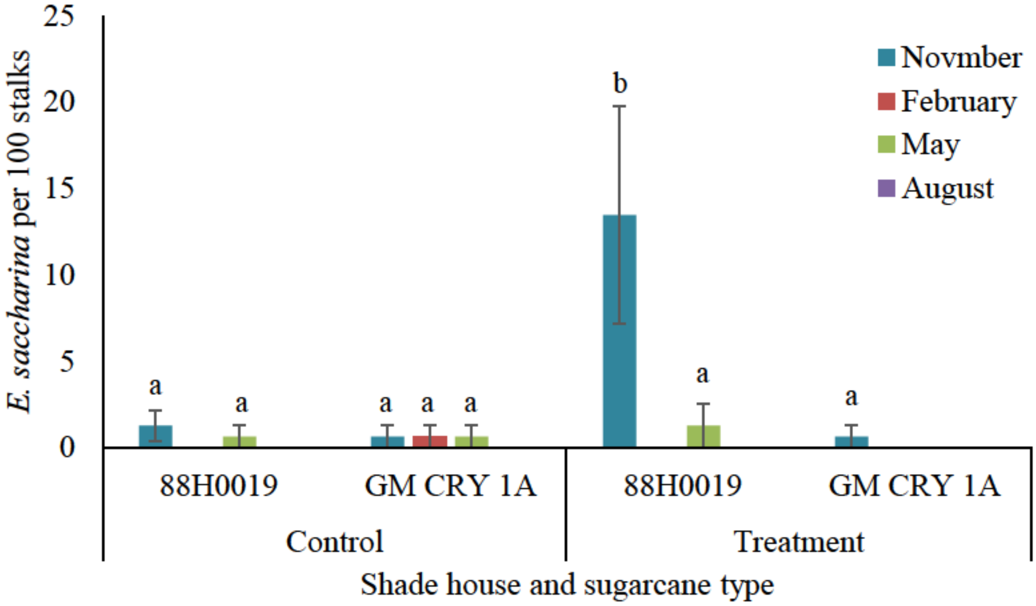

Figure 9. The summarised results from an LMM performed to compare the mean and ± SE of *E. saccharina* infestation (e/100) from four surveys conducted over 12 months (November 2023, February 2024, May 2024 and August 2024) from GM CRY1A and 88H0019 sugarcane in the treatment and control shade houses). An LSD post hoc test represented by a to b indicates the level of significance between the shade houses and sugarcane types.

Chapter 5 Discussion

Due to the indigenous status of *E. saccharina* in South Africa (Assefa, Conlong & Mitchell, 2006), the work done in this study is to potentially suppress rather than eradicate the pest. The current study aimed to develop a proof of concept of SIT combined with Bt sugarcane and investigated a synergistic effect on the control of *E. saccharina* under a controlled environment. As SIT and Bt sugarcane are newer interventions under consideration for suppressing *E. saccharina*, they are still in the research phase (Potgieter, Van Vuuren & Conlong, 2016). SASRI is currently conducting pilot projects utilising SIT and Bt sugarcane; therefore, limited cage/shade house trial studies are available (Human & Potgieter, 2024). However, a prerequisite to the launch of any new pest control measure is the use of a model to support decision-making in determining the feasibility and efficiency of the programme (Human, 2019).

The models developed by Potgieter (2013) and Van Vuuren, Potgieter & Van Vuuren (2018) found that the sterile insect technique demonstrated higher efficiency when combined with other pest control methods to lower the *E. saccharina* population. Additionally, models produced by other studies involved adding refuges into Bt sugarcane programmes to limit resistance to the pest (Butterfield, Brokensha & Wood 2002; Human 2019). However, due to variable farmer compliance, this approach has some limitations (Tabashnik et al., 2010). Computer simulations by Tabashnik et al. (2010) demonstrated the efficiency of using SIT as an alternative mechanism against Bt resistance in targeted pests. This resulted in large-scale deployment of this strategy on infested fields to reduce the pink bollworms (*P. gossypiella*) resistance to Bt cotton. Furthermore, an agent-based model formulated by Potgieter, Human & Downing (2024) compared the efficiency of using SIT and refuges to combat the resistance of *E. saccharina* to Bt sugarcane. This demonstrates that sterile insects need to be released in a targeted manner in order to effectively reduce Bt resistance (Potgieter, Human & Downing, 2024). Therefore, utilising these models, the current study tested the potential efficiency of Bt sugarcane and SIT within a controlled environment.

5.1 Stalk bored length and internodes bored

Contrary to what was expected, the release of sterile *E. saccharina* moths could not effectively reduce the sugarcane stalk damage. For stalks bored, percentage stalk length bored and percentage internodes bored damage measurements, the treatment shade house had the highest bored damage and stalk red length. The results of the current study contradicted the findings reported by Mudavanhu, Conlong & Addison (2013), who found significantly more undamaged sugarcane and the least internode damage in the sterile release shade house compared to the non-sterile release cage. The contradicting findings could be related to the dissimilarities in the methods conducted. In this study, releases were conducted twice a week when the sugarcane was 3 months old and harvested at 12 months old, while in the other study, only 15 releases (beginning every second month starting from January 2012 to March 2012, 750 sterile male moths were released with 150 moths being released every 2nd day for 5 days) were conducted when the sugarcane was 21 months old and harvested at 31 months old. Additionally, as all the sugarcane in Mudavanhu's study were of the same age and were harvested once, the initial infestation could equally impact all the sugarcane, ensuring the studies' integrity. However, if the same method had been used in the current study, the first sugarcane batch would have been primarily targeted by the initial pair releases, resulting in the inability to adequately compare the batches. Furthermore, research worked to determine the direct impact SIT releases may have on stalk damage and the establishment of offspring.

The current study focused on determining the efficiency of Bt and SIT when working together to control *E. saccharina*; however, from the experiment, SIT had no significant influence compared to the GM CRY1A sugarcane. However, it was found that GM CRY1A sugarcane significantly reduced damage in both shade houses. After conducting the surveys, the larvae found in the GM CRY1A sugarcane stalks later died because of the Cry toxins in the GM CRY1A sugarcane. This is explained by the work done by Whalon & Wingerd (2003) and Li et al. (2024), who found that the Cry toxins in the GM crop cause feeding inhibition, which results in larvae mortality and further reduces damage to the crop. Furthermore, the lack of significance does not totally discredit the effectiveness of SIT, as the addition of SIT moth releases could have contributed to the GM CRY1A sugarcane in the treatment having the lowest damage. The lower damage further displayed the need for combining a control method with SIT. As stated by Barclay (2021), who highlighted the various mathematical models formulated for SIT usage, it is common practice to reduce the target pest population by combining SIT with other control measures.

The idea of combining SIT and Bt crop has been explored before and was proven to be successful in the eradication of the *P. gossypiella,* in the USA (Tabashnik et al., 2019). In 1968, a containment programme involving the release of sterile moths began; however, over 20 years of successive releases, a high number of *P. gossypiella* was still observed along the infected cotton fields (Marec & Vreysen, 2019). Therefore, in 1996, Bt cotton was introduced, and following this in 2002, the AW-IPM approach commenced, which integrated Bt cotton, SIT, mating disruption, and extensive surveys (Henneberry, 2007; Tabashnik et al., 2021). By 2018, *P. gossypiella* was completely eradicated in the Arizona region of the USA (Tabashnik et al., 2021). Therefore, similarities could be drawn from the eradication of *P. gossypiella,* and it can be inferred that when only SIT was used in the absence of Bt cotton, there were relatively high infestations, which correlates with the high stalk damage observed on the 88H0019 sugarcane in the treatment shade house. However, within this same shade house, there was a notable damage reduction once larvae attacked the Bt sugarcane, similar to when Bt cotton was added to the *P. gossypiella* infested cotton fields.

5.2 Stalk red length damage

From the controlled shade house experiment conducted, a significantly lower incidence of stalk red damage was observed in the GM CRY1A sugarcane compared to its 88H0019 counterpart. The reduction observed can be attributed to the presence of the Cry toxins in the genetically modified Bt (*Bacillus thuringiensis*) strain of sugarcane (Cristofoletti et al., 2018). Cry toxins produce crystalline proteins that act as an insecticide used to defend the GM CRY1A sugarcane against specific insect larvae (Qamar et al., 2021). These toxins are lethal to specific insect larvae, effectively curtailing their survival rates and thus preventing extensive tissue damage that typically renders the stalks more vulnerable to fungal infections, particularly from species such as *Fusarium* (Mahlanza et al., 2014).

Apart from the physical damage caused by the larvae boring into the stalk, the entry points they create become openings for pathogenic fungi, for example, *Fusarium* spp., to enter the stalk

(Serfontein, 2021). The extensive damage inflicted by the larvae compromises the integrity of the sugarcane stalk, making it increasingly susceptible to infections by *Fusarium* spp., which can exacerbate the overall health decline of the plant (Mahlanza et al., 2014). Moreover, research indicates that the presence of Bt toxins not only contributes to larval mortality but also plays a role in mitigating the spread of *Fusarium* in infected crops (Rocha et al., 2014). Consequently, these factors culminate in a significantly lower incidence of stalk red damage in GM CRY1A sugarcane relative to 88H0019 sugarcane, as observed in both shade house settings.

Contrary to post-hoc tests' findings regarding stalk bore length and internode bore damage in the current study, the GM CRY1A sugarcane exhibited notably higher stalk red length damage in control environments than treated ones. This paradox can be unravelled in light of existing studies (examples of such studies: Ako et al., 2003; Govender, McFarlane & Rutherford, 2010; McFarlane, Govender & Rutherford, 2009; McFarlane & Rutherford, 2005) that suggest a unique interaction between *Fusarium* spp. and the behaviour of larvae. McFarlane, Govender & Rutherford (2009) found that larvae are particularly attracted to volatile compounds emitted by Fusarium-infected plants, which may result in a higher aggregation of larvae at these infected sites along the sugarcane stalk. The isolates of *Fusarium* spp. not only serve as a food source but may also enhance the developmental success of the larvae, making these infected stalks more appealing for oviposition (McFarlane & Rutherford, 2005).

Furthermore, Ako et al. (2003) observed an increase in oviposition rates of female *E. saccharina* on maize stalks infected by *Fusarium* in West Africa. Govender, McFarlane & Rutherford (2010) further indorsed Ako et al. (2003) findings, and implied a plausible hypothesis that a similar assemblage of larvae could occur at previously infected sugarcane stalks, thereby contributing to the increased stalk red length damage noted in the current study observations.

In contrast, the presence of Cry toxins in GM CRY1A sugarcane creates a hostile environment for any larvae that penetrate the sugarcane stalk, leading to their mortality and consequently reducing the prevalence of stalk red damage in GM CRY1A sugarcane across both shade house environments (Barclay, 2021). The diminished stalk red length found in GM CRY1A sugarcane may further deter *E. saccharina* from favouring these plants for oviposition, resulting in a decreased larval population on the GM CRY1A sugarcane compared to 88H0019 varieties (Govender, McFarlane & Rutherford, 2010).

5.3 Comparing relative damage and stalk length

Overall, the GM CRY1A sugarcane in the treatment shade house had approximately 3 times less stalk length and internode damage and 25 times less stalk red damage than the control. This could be attributed to the benefits of combining SIT and GM CRY1A sugarcane to control *E. saccharina*. The results from the current study show that the treatment had the tallest sugarcane and the most stalk damage, with the 88H0019 sugarcane in both shade houses having the highest stalk length. It is still unclear as to why the treatment had the tallest sugarcane, a possible reason could have been related to the location of the shade houses as the two shade houses could have developed different microclimates, such as the variation in shade or ventilation. However, there is not enough data to draw a conclusion. Berry et al. (2010) conducted a study to characterize the damage caused by *E. saccharina*. They identified that the larvae tend to infest taller sugarcane with high internode numbers (more than 16 internodes) as they have high growth potential and contain higher nitrogen levels and nutrients (Berry et al., 2010). The latter study concluded that the female moth and its progeny either by chance or may already possess the ability to identify tall sugarcane or sugarcane that has the potential to grow taller, as the survival and emergence of progeny are greater on taller, healthier, and more nutritionally rich stalks. Therefore, the shorter height observed in the current study on the GM CRY1A sugarcane could have led to its lack of favourability, as the reduction in height could signify the lack of nutritional quality present in the stalk. However, because there was no difference between the interaction of shade house and sugarcane type in the current study, there can be no definite conclusion on whether stalk length influenced the study’s findings.

5.4 *E. saccharina* population

Since no reliable pheromone is available to monitor the *E. saccharina* population (Mudavanhu, 2012), all larvae and pupae were recorded as a reference for the pest’s population. Zhou (2015) noted that sugarcane harvested at 12 months have lower levels of *E. saccharina* populations because of the limited time available for natural infestation to occur. Previous studies, which focused on testing sugarcane pest control, generally recommended harvesting 18 - 31-month-old sugarcane as adequate time is given for the *E. saccharina* population to stabilize and for damage to be observed (Mudavanhu, 2012; Rhodes, Miles & Keeping, 2014; Serfontain, 2021: and Zhou, 2015). *Eldana saccharina* infestation is positively associated with increased sugarcane age, and the low nutritional content in immature sugarcane decreases the number of *E. saccharina* offspring (Carnegie & Smaill, 1982). Therefore, since the releases in the current study commenced when the sugarcane was 3 months old and the harvest occurred once they reached 12 months when nutritional content was not satisfactory, the number of offspring collected was limited. Additionally, the lack of an established infestation population limited the number of offspring observed, irrespective of the constant releases recommended by Mudavanhu (2012) and Potgieter, Van Vuuren & Conlong (2012).

Contrary to the findings by Mudavanhu, Conlong & Addison (2013), the number of *E. saccharina* offspring found in the treated shade house in the current study was greater than in the control. However, since the current study included both GM CRY1A and 88H0019 sugarcane, and the GM CRY1A sugarcane had the least e/100 present, combining the methods indicated a positive decline in the fertility of future populations. This was further emphasised by the decline in overall larvae collected during surveying over the progression of releases. From the work done by Klassen & Vreysen (2021), it can be inferred that the high moth density found in the treatment shade house increased the potential for stalk damage. However, based on a study by Cristofoletti et al. (2018), who estimated the feasibility of using GM CRY1A sugarcane varieties against sugarcane borers, the Cry toxins present in the GM CRY1A sugarcane limited any further stalk damage due to the increased mortality in the larvae.

The first survey had the highest e/100 compared to the other three surveys. This could be due to the quality and development of the rest of the sugarcane and that during the experiment the sugarcane was not pre-thrashed. Apart from the first batch, the following batches had various confounding factors that could have left them quite immature for their age. The renovations that were conducted on the glasshouse used and the change in the substrate used for seedling hardening led to early transplanting of the second and third batches, which could have put them under stress. Additionally, stress was put on the newly transplanted batches due to the presence of sugarcane scale (*Aulacaspis tegalensis*) and leafhoppers (*Perkinsiella* *saccharicida*) generally observed in closed environments such as shade houses, where natural enemies are absent (Keeping, Miles & Sewpersad, 2014). Even with continuous manual cleaning and spraying of the insecticide Bandit® (chlorpyriphos 2mL L^{-1} water), the damage was persistent throughout the three batches until they were harvested. Therefore, the first batch harvested was the tallest, had the most dead leaf material due to the lack of pre-thrashing and was believed to be the healthiest. From the investigations done by Carnegie & Smaill (1982), who tested the effectiveness of sugarcane pre-thrashing on *E. saccharina* management, it can be inferred that the previously mentioned factors made the first batch a more favourable oviposition site.

When selecting fields for carrying over to the next season, surveys are conducted to determine the presence of *E. saccharina*. Thus far, 10 e/100 to 20 e/100 is the accepted infestation level (Way & Goebel, 2007). When including both shade houses, the current study had an infestation of 1 e/100, which is lower than the recommended threshold for rating *E. saccharina* hazard in potential carryover cane (Webster, Brenchley & Conlong, 2009). However, when looking at the objective of the study, the treatment shade house had the highest *E. saccharina* per stalk on the 88H0019 sugarcane (3.7 e/100) and the lowest on the GM CRY1A sugarcane (0.16 e/100), still within the low infestation level.

It is expected that *E. saccharina* populations increase as sugarcane fields experience water stresses, such as through drought (Prinsloo, 2024). However, a review conducted by Conlong, Webster & Wilkinson (2016) discussed the innovations observed in the AW-IPM strategies used to control *E. saccharina* by comparing 73 farms over a 10-year period. Surprisingly, the study reported a low incidence of *E. saccharina* in a drought-prone area. As the current study has had incidents oof water stress, and low *E. saccharina* numbers there could be a correlation. However, since it was not recorded and further investigated it cannot be determined whether it truly had an impact on the results found.

Another aspect that needed to be considered was the possible impact of nitrogen on the fertiliser application. Goebel, Way & Gossard (2005) have previously reported that increased nitrogen application is associated with higher levels of stalk damage caused by *E. saccharina*. Nitrogen increases host plant nutritional quality and growth and promotes larval survival, especially through crop stress (Rhodes, Miles & Keeping, 2014). It has been recommended that growers with *E. saccharina*-prone fields lower their nitrogen levels to 10-30kg/ha

(Keeping, Miles & Sewpersad, 2014). In comparison, through conversions from the current studies methods, 1.2 kg/ha of fertiliser was used monthly, which could have reduced the quality of the sugarcane, making it less favourable for infestation and hindering the data available for collection.

Chapter 6 Conclusion and Recommendations

6.1 Overall remarks and hypotheses

The limited control over the execution of the experiment has resulted in the collection of inadequate data for a definite conclusion to be drawn from the study's results. However, based on the available data, some reasonable assumptions and inferences can still be drawn, although these conclusions should be considered with caution. The findings from this study supported the hypothesis that the lowest stalk damage and *E. saccharina* offspring occurred on the GM CRY1A sugarcane when sterile *E. saccharina* moths were released. Therefore, supporting the idea that *E. saccharina* populations could potentially be managed by combining GM sugarcane and SIT. However, the highest damage was unexpectedly observed on the 88H0019 sugarcane in the same shade house with sterile moth releases. This showed a possible indication that SIT had no significant impact on the damage caused by *E. saccharina* infestation on the sugarcane. GM CRY1A sugarcane successfully reduced the stalk damage and further lowered *E. saccharina* offspring in both shade houses with and without sterile moth releases. However, the lowest damage was identified in the sterile moth release shade house.

The contrasting lack of significant impact SIT has does not dispute the efficiency of SIT, as SIT might have led to a slight but not significant effect on the damage observed. Therefore, the possibility of combining SIT and GM CRY1A sugarcane is possible; however, further work needs to be done to adjust the efficiency of SIT. As GM CRY1A sugarcane played a major role in managing *E. saccharina* infestation, it can be summarised that the interplay between GM CRY1A sugarcane, Cry toxins, larval behaviour, and potential *Fusarium* infection presents a compelling narrative about pest management in agricultural practices.

Overall, the study is based on a novel concept as most of the published work on combining SIT and Bt crop has focused on simulation papers and a single experimental research paper. There were various unexpected setbacks which could act as a learning curve for future research. Even with the relatively low levels of damage seen the reduced stalk damage in GM CRY1A sugarcane not only highlights the effectiveness of genetic engineering in crop protection but also underscores the complex ecological interactions that influence agricultural productivity and pest dynamics. This multifaceted understanding is essential for developing effective pest management strategies to sustain crop health while minimizing reliance on chemical pesticides.

6.2 Limitations of the study

The study had various methodological limitations, contributing to the conflicting overall results. One crucial issue was the low data obtained during each harvest, which could have been caused by the lack of an initial release of non-sterile *E. saccharina* moths. Furthermore, a low number of *E. saccharina* sterile moths were available for release, possibly reducing the overall mating potential between the male sterile moths and non-sterile females in the treatment shade house. As it was opted to do continuous weekly releases, it was expected that a sufficient infestation would have been established, allowing for an adequate data set to be obtained. However, this was not achieved, and therefore, the interpretation, especially regarding the e/100 data, was limited.

Additional contributors to the limited data set collected could have been attributed to the hampered condition of the sugarcane, as various issues with early transplanting, water stress, lack of nitrogen application and issues with untargeted pests resulted in the delayed maturity of the sugarcane. The lack of maturity then curbed the sugarcane’s favourability for oviposition and feeding by *E. saccharina*, reducing the overall damage observed. Overall the insufficient data may have hindered the studies ability to estimate the potential effectiveness of the methods used, making it difficult to draw definitive conclusions about the effectiveness of integrating Bt and SIT.

6.3 Recommendations for future studies

The primary challenge encountered in the current study was the insufficient data on stalk damage and offspring collected during the surveys. Based on the results of this study, the following recommendations are made:

I. It would be beneficial to introduce the non-sterile moth pairs into the shade houses to establish an initial infestation. However, this can only be conducted if all the sugarcanes are of the same age within the controlled environment, or periodic mass infestation releases should occur when planting new sugarcane so as to expose all the samples to the same condition. By establishing a stable population similar to the one conducted by Mudavanhu (2012), the data might increase substantially, improving the comparability of the data once analysed.

II. SASRI is still conducting research to improve the efficiency of irradiating, mass rearing and handling sterile moths to ensure an increase in SIT moth's fitness and overall production. Therefore, increasing the SIT release numbers to meet the overflooding ratio needed to control a stable infestation would be beneficial.

III. Since the current study was restricted to the time schedule indicative of a Master's thesis, the sugarcane growth time was limited to 12 months. Therefore, it would also be important to harvest the sugarcane after 12 months, possibly once they have reached optimum maturity, which is recommended by Serfontein (2021) to be above 18 months, as the infestation is given an adequate time to establish as *E. saccharina* are more prevalent on older sugarcane.

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