An agent-based simulation modelling approach to assessing various refuge strategies in Bt sugarcane

Dirk Johannes Human

Thesis presented in fulfilment of the requirements for the degree of
Master of Commerce
in the Faculty of Economic and Management Sciences at Stellenbosch University

Supervisor: Dr Linke Potgieter December 2019

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2019

Abstract

Crops expressing genes from the bacterium *Bacillus Thuringiensis* (Bt) produce a protein toxic to members of the order Lepidoptera and are a popular alternative to sprayed insecticides. Although these Bt crops are considered to be an effective pest control method, careless usage may add selective pressure on the pest population to develop resistance to the protein over time. One method of limiting the rate of resistance development is to keep small portions of the cultivated land planted with the non-GMO crop which then acts as a refuge area for susceptible pests, limiting its exposure to the protein. Varieties of Bt sugarcane for the South African market that should limit the damage caused by the stalk borer moth, *Eldana Saccharina* Walker, are being developed, and a prerequisite to releasing such a product is to manage resistance development. Refuge areas are the primary way of managing resistance, but a recommendation is required on the size and layout of the refuge areas to be planted, as an area too small may not curb the rate of resistance development enough, but an area too large may not be economically viable for the industry.

An agent-based simulation model is presented where individual moths are modelled as agents on an underlying sugarcane field that can either be Bt or refuge. The spatial aspect of the simulation model allows for testing the effectiveness of different shapes of refuge and not just the size. To reduce the complexity of the model, the field is divided into a series of smaller cells that can interact with each other, which allows us to model the impact of a severe infestation on an area the size of an average sugarcane farm. The simulation divides the simulated space into two grids representing the layers of the sugarcane that the different life stages of the insect move in. The first layer is the ground layer, representing the lower portions of the sugarcane where the eggs, larvae, pupae and egg-laying female moths are found. These cells are smaller as the immature life stages are nearly stationary and the female moth is assumed to have a much lower dispersal rate when searching for sights to lay eggs. The second layer is the sky layer with larger cells, where adult males and unmated females are able to move over larger distances to find mates. To further reduce complexity, every cluster of eggs laid by a female moth is modelled as a single agent that progresses as a unit through the immature life stages before being converted to many individual moth agents when they mature.

The model is then applied to three hypothetical case studies, each focussing on a specific aspect of refuge planning. The first case study focusses on the size and distribution of refuge, the second on the shape of the refuge, and the third assumes that the Bt sugarcane is introduced gradually and how this may affect the refuge strategy. The results from these case studies as well as those obtained from the sensitivity analysis are then summarised in the form of a decision support tool (DST), which provides some recommendations for refugia planning and highlights the risk factors for resistance development identified during the course of this study. Combining the recommendations with the risk factors that may be associated with a given scenario should provide the growers with a starting point of how to plan their refugia.

Uittreksel

Gewasse wat gene van die bakterie, *Bacillus thuringiensis*(Bt) uitdruk, produseer 'n proteïen wat giftig is vir lede van die orde Lepidoptera en is 'n gewilde alternatief vir chemiese insekdoders. Alhoewel hierdie Bt-gewasse as 'n effektiewe metode van plaagbeheer beskou word, kan die agterlosige toepassing daarvan seleksiedruk op die plaagpopulasie plaas wat kan lei tot die ontwikkeling van weerstand teen die proteïene. Een metode om die tempo van weerstandsontwikkeling te beperk, is om klein gedeeltes van die suikerrietlande te plant met die nie-GMO-gewas wat dan dien as 'n toevlugsoord vir die plaag, wat die blootstelling aan proteïene beperk en sodoende die vatbare gene kan onderhou. Bt suikerriet word ontwikkel vir die Suid-Afrikaanse mark wat die skade wat verrig word deur *Eldana saccharina* Walker kan beperk, en 'n voorvereiste om so 'n produk te bemark is om weerstand te beheer. Toevlugsoorde is hoe weerstand hoofsaaklik beperk word, maar 'n aanbeveling word benodig oor die grootte en uitleg van die toevlugsoorde wat geplant moet word, omdat 'n te klein gebied moontlik nie die tempo van weerstandsontwikkeling voldoende kan beperk nie, maar ook 'n te groot gebied is moontlik nie ekonomies vatbaar vir die bedryf nie.

'n Agent-gebaseerde simulasiemodel word aangebied waar individuele motte as agente gemodelleer word op 'n onderliggende suikerrietlande wat of Bt-suikerriet of nie-Bt-suikerriet kan wees. Die ruimtelike aspek van die simulasiemodel maak dit moontlik om die doeltreffendheid van verskillende vorme van toevlugsoorde asook die grootte daarvan te toets. Om die kompleksiteit van die model te verminder, word die veld in 'n reeks kleiner selle verdeel wat met mekaar kan omgaan, wat ons toelaat om die impak van 'n ernstige infestasie te modelleer op 'n gebied so groot soos 'n gemiddelde suikerrietboerdery. Die simulasie verdeel die gesimuleerde ruimte in twee roosters wat die twee vertikale lae van die suikerriet voorstel waarin die verskillende lewensfases van die insek kan beweeg en verkeer. Die eerste laag is die grondlaag, wat die laer gedeeltes van die suikerriet verteenwoordig waar die eiers, larwes, papies en eierlêende vroulike motte voorkom. Hierdie selle is kleiner omdat daar aanvaar word dat die onvolwasse lewensfases feitlik stilstaande is en die vroulike mot se verspreidingspatroon baie kleiner is terwyl sy soek vir toepaslike areas om eiers te lê. Die tweede laag is die luglaag met groter selle wat bo-op die grondlaag lê, waar volwasse manlike en ongepaarde vroulike motte voorkom wat in staat is om oor groter afstande na ander maats te soek. Om die kompleksiteit verder te verminder, word elke groep van eiers wat 'n vroulike mot lê gemodelleer as 'n enkele agent wat as 'n eenheid deur die onvolwasse lewensfases optree voordat hulle omgeskakel word na individuele motagente as hul volwassenheid bereik.

Die model word op drie hipotetiese gevallestudies toegepas wat elkeen op 'n spesifieke aspek van toevlugsoord-beplanning fokus. Die eerste gevallestudie fokus op die grootte en verspreiding van die toevlugsoorde, die tweede op die vorm en uitleg van die toevlugsoorde, en die derde veronderstel dat die Bt-suikerriet geleidelik in gebruik geneem word en hoe dit die toevlugsoord-strategie kan beïnvloed. Die resultate van hierdie gevallestudies sowel as dié wat uit die sensitiwiteitsanalise verkry is, word dan saamgevat in die vorm van 'n ondersteunende besluitnemingsinstrument, wat enkele aanbevelings vir toevlugsoord-beplanning bied en die risikofaktore vir weerstandontwikkeling wat tydens hierdie studie geïdentifiseer is beklemtoon. 'n Kombinasie

van die aanbevelings en risikofaktore wat met 'n gegewe scenario geassosieer word, behoort die produsente 'n goeie beginpunt te gee om hul toevlugsoord-beplanning aan te pak.

Acknowledgements

The author wishes to acknowledge the following people and institutions for their various contributions and support towards the completion of this thesis:

- Dr L Potgieter, who promised to take me on as a part-time and long-distance student after letting her know that I would be leaving Stellenbosch at the end of my Honours year. She pushed, guided and mentored me to be the best researcher I can be while still being cognizant and sympathetic with my part-time status, but never allowing me to become complacent either. I also thank her for being a friend in this time and being open to discussing any of my odd ideas over WhatsApp.
- Dr S Snyman and Prof DE Conlong, for their professionalism and patience with a student with no background in biology beyond the ninth grade. Their insights were immeasurable in value and their willingness to assist in any way made it clear that they were passionate about their respective fields and interested in the research. I would also like to thank Ms R Jacob for taking the time to get me up to speed with using the CHPC facilities.
- James Rhodes of Biosafety SA, for asking some seriously challenging questions about the model during its development. Many of the features in the final model were included as part of his queries, thoughts, and recommendations.
- My past and present managers at FNB: Carlien, Ryno, David and Marnitz, for understanding how time and energy intensive writing a thesis can be. I was never wanting for more leave and they helped me maintain a decent work-life balance. Mostly.
- All of my friends and family for the moral support and for keeping me distracted and laughing when I needed it most. I was fortunate enough to have two house-mates, Javis and Pieter, that were also busy with board exams or their own studies with whom I could vent when deadlines were approaching.
- My parents, Lizette and Martin, for allowing me to reach a point where I could even think of pursuing my postgraduate studies through their unwavering support, both financially and emotionally. Being able to graduate without debt has been a true blessing and something I would never be able to thank them enough for.
- My girlfriend, Lexi, who never actually knew me as anything but a postgraduate student. Her support, patience and endless supply of tea during late nights made even the worst moments tolerable. Thank you for sharing me with the moths for so long and sticking by me no matter what. It means more to me than I could ever express.

The financial support received from the South African Sugarcane Research Institute through Biosafety SA, Stellenbosch University and FirstRand Group Ltd. is hereby acknowledged and thanked for making this research possible. The Council for Scientific and Industrial Research (CSIR) is thanked for the usage of their Centre for High Performance Computing (CHPC), without which the simulations would not have reached the scales they were designed for.

Table of Contents

List of Figures xiii

List of Tables xv

List of Algorithms xvii

List of Symbols xix

1 Introduction 1

1.1 Problem Description 2
1.2 Scope and Objectives of this Thesis 3
1.3 Thesis Organisation 4

2 Survey of Biological Literature 5

2.1 Sugarcane and the South African sugar industry 5
2.2 *Eldana sacharina* Walker 6
2.2.1 General biology 7
2.2.2 Current and proposed control strategies 8
2.3 Transgenic crops 9
2.3.1 *Bacillus Thuringiensis* 10
2.3.2 Bt crops as a pest suppressant 11
2.4 Genetic flow in populations 14
2.5 Chapter Summary 16

3 Computer Simulation Modelling 17

3.1 Simulation modelling 17
3.1.1 The components of a simulation model 18
3.1.2 Different classes of simulation modelling 19
3.1.3 Simulation modelling paradigms 19
3.2 Steps in developing a simulation model 20
3.3 Advantages and disadvantages of simulation modelling 22

3.4 Simulation and mathematical modelling of ecological systems 24
3.5 Chapter summary 26

4 Simulation Specifications 27
4.1 Model paradigm 27
4.2 Modules 28
4.2.1 Field object 28
4.2.2 Egg and larval objects 30
4.2.3 Moth object 35
4.3 Simulation framework 36
4.3.1 Life stage management 37
4.3.2 Time step management 39
4.3.3 Female mating and oviposition 42
4.3.4 Moth movement between cells 43
4.3.5 Moth movement between layers 46
4.4 Simulation outputs 46
4.5 Model implementation 47
4.6 Chapter summary 47

5 Verification, validation and sensitivity analysis 49
5.1 Verification 49
5.1.1 Verification of cane growth 50
5.1.2 Verification of larval capacity in the stalk 50
5.1.3 Verification of immature life stage maturation and mortality 52
5.1.4 Verification of adult moth life cycle 53
5.2 Validation of immature life stage maturation and mortality 58
5.3 Face validation 60
5.4 Sensitivity analysis 62
5.4.1 General experimental set-up 63
5.4.2 Results 66
5.4.3 Discussion 70
5.5 Chapter summary 74

6 Case studies and a decision support tool 75
6.1 Case studies 75
6.1.1 Case study 1 – Refuge distribution 76
6.1.2 Case study 2 – Refuge shapes 81
6.1.3 Case study 3 – Gradually introducing Bt sugarcane 85

6.2 Decision support tool 90

6.2.1 Necessary assumptions 90

6.2.2 Risk factors and recommendations 91

6.3 Chapter summary 94

7 Conclusion **95**

7.1 Thesis summary 95

7.2 Contributions of this thesis 97

7.3 Proposals for future work 98

7.3.1 Recommendations for future entomological research 98

7.3.2 Proposals for future simulation work 99

Bibliography **103**

A Contents of the disc accompanying the thesis **113**

List of Figures

2.1 Sugarcane field . . . 6
2.2 Sugarcane stalks . . . 6
2.3 *E. saccharina* life cycle . . . 7
2.4 Bt gram stain . . . 10
2.5 Bt crystals . . . 11
2.6 Bt cotton with refuge border . . . 12
2.7 Refuge layouts . . . 13
2.8 DNA, genes and chromosomes . . . 14
2.9 Two blesbuck phenotypes . . . 15
2.10 Pea plant experiment Punnett square . . . 16

3.1 Steps in a simulation study . . . 23

4.1 Immature life stages maturation rates . . . 33
4.2 Cell structure of the simulation . . . 36
4.3 Flow of each object within a ground cell . . . 37
4.4 Flow of each object within a sky cell . . . 40
4.5 Percentage eggs laid per night . . . 42
4.6 Graphical representation of the female moth's biased flight . . . 45
4.7 Female moth dispersal radius . . . 46

5.1 Sugarcane stalk growth . . . 50
5.2 Sugarcane stalk growth . . . 50
5.3 Sugarcane stalk capacity . . . 51
5.4 Sugarcane stalk capacity with larvae . . . 51
5.5 Graphical representation of verification scenarios . . . 54
5.6 Total moth population for the one year population scenario . . . 56
5.7 Male moth dispersal over six days . . . 57
5.8 Female moth dispersal over six days . . . 57
5.9 Female moth descent pattern into older cane . . . 58

5.10 Female moth descent pattern into slightly older cane 59
5.11 Male moth biased dispersal over six days 60
5.12 The validation workshop attendees. 61
5.13 Field layout for sensitivity analysis 65
5.14 Time series plot of the baseline moth population 65
5.15 Time series plot of the moth population with resistance added after warm-up . . 66
5.16 Time series plots of the moth population for stable runs 72
5.18 Time series plots of the carrier moth population for runs developing resistance . 72
5.17 Time series plots of the moth population for runs developing resistance 73
5.19 Time series plots of the carrier moth population for a 10 month harvest cycle . . 74

6.1 Refugia layouts for case study 1 76
6.2 Dsitribution of moths in case study 1 77
6.3 First cases of resistance development for $h = 20\%$ 78
6.4 First cases of resistance development for $h = 30\%$ 79
6.5 Growth in proportion of resistant population 81
6.6 Layout of the linear block refuge 82
6.7 Layout of the border refuge 82
6.8 Layout of the border and block refuge 83
6.9 Resistance development in the border and block layout 84
6.10 Resistance development in the revised border and block layout 85
6.11 The field layout for case study 3 86
6.12 The moth population over time for the two year harvest cycle 87
6.13 The moth population over time for the one year harvest cycle 88
6.14 Resistant populations for the four replacement schedules 90
6.15 Infographic for the four basic assumptions 92
6.16 Infographic for the risk factors and recommendations for refugia planning 93

List of Tables

4.1 The attributes and methods contained within the Field object. 29

4.2 The attributes and methods contained within the Egg object. 30

4.3 The attributes and methods contained within the Larvae object 31

4.4 A punnett square of the possible genetic combinations of parental alleles for a single allele. 32

4.5 A punnett square of the possible genetic combinations of two separate alleles. . . 32

4.6 Immature life stage maturation rates 33

4.7 Life stage specific mortality rates in the model 34

4.8 Life stage specific mortality rates 34

4.9 Larval mortality rates on different sugarcanes 34

4.10 The attributes and methods contained within the Moth object 35

4.11 Egg batch sizes per day 43

5.1 Egg batch genetic makeup 52

5.2 Experimental design for egg and larva maturity 52

5.3 Experimental results for egg and larva maturity 52

5.4 Experimental results for egg mortality 53

5.5 Experimental results for larval mortality 53

5.6 Parameter values for adult moth verification 55

5.7 Experimental results for egg and larva maturity 59

5.8 Experimental results for egg mortality 59

5.9 Experimental results for larval mortality 60

5.10 Egg batch sizes per day, modified 62

5.11 Parameter values for the sensitivity analysis 64

5.12 Symbols used for summary statistics 67

5.13 Sensitivity of moth population to parameters 67

5.14 Sensitivity of moth population to parameters looking at resistance 68

5.15 Sensitivity of moth population to field parameters 69

5.16 Sensitivity of moth population to field parameters looking at resistance 70

6.1 Moth movement parameters for the case studies 76
6.2 ν value per h/layout combination 78
6.3 μ_r value per h/layout combination 78
6.4 ν value per h/movement combination 80
6.5 μ_r value per h/movement combination 80
6.6 ν value per layout/movement combination 80
6.7 μ_r value per layout/movement combination 80
6.8 ν value per layout/movement combination 83
6.9 μ_r value per layout/movement combination 83
6.10 ν value for the border and block layout per movement combination 84
6.11 μ_r value for the border and block layout per movement combination 84
6.12 ν and μ_r value for random male movement 85
6.13 The sugarcane replacement schedule for case study 3 86
6.14 ν value for case study 3 with two year harvests 87
6.15 μ_r value for case study 3 with two year harvests 87
6.16 ν value for case study 3 with one year harvests 87
6.17 μ_r value for case study 3 with one year harvests 88
6.18 ν and μ_r values for case study 3 89
6.19 The sugarcane replacement schedule for case study 3 89
6.20 The sugarcane replacement schedule for case study 3 89
6.21 ν and μ_r values for case study 3 for the extended experiments 89

List of Algorithms

4.1 Progression from egg to larvae. 38

4.2 Progression from larvae to moth. 38

4.3 Updating the field object (`Next_Field`) 39

4.4 Updating a moth in a sky cell during a time step 41

4.5 Updating a moth in a ground cell during a time step 44

List of Symbols

The symbols listed are reserved for specific use unless specified otherwise within the local context of its usage. This list is not exhaustive, as some symbols may be used without reservation throughout the thesis.

Symbol	**Meaning**
$l(t)$	Stalk growth at time t
$h(t)$	Stalk height at time t
$lc(t)$	Stalk larval capacity at time t
$ic(t)$	Incremental stalk larval capacity at time t
$r_e(t)$	Egg maturation rate at time t
$r_l(t)$	Larval maturation rate at time t
$r_p(t)$	Larval and pupal maturation rate at time t
n_e	Number of fertile eggs a female moth is carrying
m_m	Male moth movement likelihood
m_f	Female moth movement likelihood in the ground cells
m_s^1	Female moth movement likelihood in the sky cells to a cell in the radius 1 neighbourhood
m_s^2	Female moth movement likelihood in the sky cells to a cell in the radius 2 neighbourhood
π_i	Female likelihood of selecting a sky cell i
λ_i	Male likelihood of selecting a sky cell i
β_i	Female likelihood of selecting a ground cell i
ρ_{im}	Female likelihood of selecting a ground cell m from sky cell i
ν	Number of simulation runs that develop resistance
μ_r	Average size of the resistant adult moth population
h	Proportion of the total area set as refuge

CHAPTER 1

Introduction

Contents

1.1 Problem Description 2
1.2 Scope and Objectives of this Thesis 3
1.3 Thesis Organisation 4

Since the dawn of agriculture, man has had to manage pests in their crops. Early farmers could do little more than account for the pests, planting enough crops for themselves and for the pests that would inevitably feed on those crops. The earliest known form of pest control arose when the ancient Sumarians used sulphur compounds as a form of rudimentary insecticide [79], but it wouldn't be until four millennia later that systematic pest control would become a widespread practice. Modern organic pesticides only came into large scale use in the 1950s with the introduction of DDT, a wunderkind compound that was lauded as a 'bright new star, a nova' [133]. Unfortunately, there is significant evidence of it being incredibly toxic to humans and animals. This has led to a significant reduction in and even outright criminalisation of its application, leading to a renewed investment into sustainable and environmentally responsible pest management and the variety of available pest control techniques are at an all-time high [79].

A key crop in South African agriculture is sugarcane, where nearly half a million hectares is cultivated by more than 20 000 registered growers, most of them in the KwaZulu-Natal province. The history of South African sugarcane dates back to at least the early 17th century, as Portuguese explorers that were shipwrecked on the coast of modern day KwaZulu-Natal in 1635 found that the local inhabitants were growing the crop [107]. Today, sugarcane is a large industry that directly and indirectly employs nearly a million people and generates approximately R12 billion in revenues annually [102], with cultivation and research overseen by the South African Sugar Association (SASA), which includes the South African Sugarcane Research Institute (SASRI).

As with any crop, sugarcane has its share of pests. One such pest, *Eldana saccharina* Walker, a stalk borer moth species, was first recorded in the Umfolozi area of KwaZulu-Natal in 1939. It was not able to spread very far, and after being considered extinct in the area from 1950, a new outbreak was discovered in Hluhluwe in the 1970s. It has since been a significant pest to sugarcane farmers [23, 48]. The *E. saccharina* infestation of sugarcane has been attributed to the significant dead leaf material found in sugarcane plantations. Larval activity inside of the sugarcane stalk reduces sucrose yields, which consequently results in financial losses to farmers.

Due to the economic impact of severe *E. saccharina* infestations, pest control measures against it are still topical subjects within the wider sugarcane industry. SASRI has been in the front line of research efforts to combat the pest, with measures such as the development of resistant varieties [60], habitat management [25, 68] and biological control methods [28, 30] all having at least some success, but not providing a complete solution to the problem. Recent research into using more modern techniques such as Sterile Insect Technique (SIT) and genetically modified sugarcane has become more promising, as they could potentially provide a permanent control measure [86], with a combination of the two techniques being considered as a total control measure [112].

Genetically modified crops that expressed insecticidal properties were first publicly available in 1995 with the introduction of a variety of maize that contained genes from a bacterium known as *Bacillus thuringiensis* [3, 94]. This was a significant development in pest management, as it reduced the usage of sprayed pesticides that could have adverse effects on non-target organisms as well as the amount of effort required by the growers in controlling pest populations. In the years after the introduction it was found that some pests were displaying a level of resistance to the insecticidal crops [42, 70, 119]. To combat the development of resistance, a strategy that employs the use of high-dose GMO plants along with areas of non-GMO plants, or refuge areas, has been prescribed [81, 95]. The rationale being that refuge areas would house healthy populations of susceptible insects that could mate with resistant insects emerging from the GMO crops. The progeny of the resistant and susceptible insect would then thin out the resistance gene throughout the insect population, curbing resistance development. While the refuge areas will suffer heavy losses, it should theoretically make up the minority of the planted area, hence the majority of the planted area should suffer minimal losses, leading to a larger overall yield.

1.1 Problem Description

A prerequisite to launching a new pest resistant crop to the market is a study on what the refuge area requirements should be to successfully manage resistance development. SASRI is currently in the process of developing transgenic sugarcane varieties that will contain genes from *Bacillus thuringiensis*, allowing the plant to express the same insecticidal proteins that would be deadly to *E. saccharina*. SASRI, along with BioSafety SA, a government research body that primarily works in biotechnology, are required to provide the refuge requirements to the South African Department of Agriculture, Forestry and Fisheries before releasing the new varieties to the market.

A sufficient refuge requirement would have to provide enough refuge areas to house a large enough population of susceptible insects to curb the development of resistance in *E. saccharina* while still being small enough that the cost of yield loss in the refuge areas are offset by the yield gains in the GMO portion of the field. In this thesis, the problem of determining the best size and layout specifications of refuge areas within a sugarcane environment to combat resistance development in the target pest, *E. saccharina*, is considered using individual-based simulation modelling. The use of an individual-based simulation model aims to address weaknesses identified in population-based models developed by Tabashnik *et al.* [112] and Butterfield *et al.* [18], where these models made spatial assumptions that may not be applicable in the typical *E. saccharina* on sugarcane scenario. The model aims to capture genetic flow within a population more accurately by modelling individual insects with their own coded genome which can be passed from the parents to their offspring, but on a larger scale than the model developed by Garcia *et al.* [41].

The result is constrained by what is practical in current planting practices. For this reason the solution space should be limited to field layouts and best practice recommendations for planting that do not contradict what is assumed to be normal planting practices. For example, the strip layout refuge areas commonly seen in maize fields, which is all planted simultaneously, would not be suitable for sugarcane, as sugarcane is generally planted in blocks. The long timelines between the development of new varieties of sugarcane also requires the resulting solution to curb resistance development for several years, with Cristofoletti *et al.* [35] specifying at least ten years in their study.

1.2 Scope and Objectives of this Thesis

The scope of this thesis is limited to an *E. saccharina* on sugarcane scenario where a new sugarcane variety containing genes that allow for the expression of insecticidal proteins is employed as a pest control strategy. The thesis will not account for the effects of any other control strategies that may be employed as part of a wider integrated pest management scheme and assumes that Bt sugarcane is the only pest management strategy in effect. The main contributions include guidelines for refuge areas in Bt sugarcane, as well as a simulation model that can capture the population dynamics and genetic flow of the resistance gene within an existing population of *E. saccharina* on a commercial sugarcane plantation where the gene is already present in some small proportion. To aid in the Bt sugarcane development effort, the following objectives are pursued in this thesis:

1. To *perform a literature survey* on topics that are relevant to the study, including:
   (a) a study on the general biology of sugarcane and its cultivation in South Africa,
   (b) the biology and life cycle of *E. saccharina* as well as current pest control measures against it,
   (c) the history and use of insect resistant transgenic crops as a pesticide,
   (d) the models currently used most often in genetic flow modelling, and
   (e) the various techniques and approaches used in computer simulation modelling.
2. To *develop the general framework* of the individual-based simulation model, as it is not developed on an existing simulation software platform and does not use existing simulation libraries, which includes:
   (a) the objects that will represent the physical entities (eggs, larvae, moths etc.) within the model,
   (b) the support functions that can facilitate events and activities within the model, and
   (c) the framework that will facilitate the spatial representation and timed element of the simulation model.
3. Using information and data from Objective 1, determine suitable parameters for use in the entities specified in Objective 2 to *develop an individual-based simulation model* that approximates the behaviour of *E. saccharina* on sugarcane.
4. *Verify* and *validate* the output of the model developed in Objective 3 through results from previously developed models of gene flow and *E. saccharina*, feedback provided by subject matter experts and by performing a sensitivity analysis on parameters where there is high uncertainty.
5. Design a *decision support tool* based on the simulation results of the model developed in Objective 3 that can be used by industry professionals to aid in determining the refuge requirements that should be adhered to.
6. To *suggest additions or improvements* or *provide possible future directions* of research along which to proceed.

1.3 Thesis Organisation

This introductory chapter is followed by six additional chapters. The focus of Chapter 2 will be on the current available literature pertaining to the biological aspects and background required to complete this thesis. This includes background information regarding sugarcane as a plant, its cultivation in South Africa, the history and current state of transgenic crops, and combating resistance development in pests targeted by transgenic insecticidal crops. The chapter concludes with a brief overview on some key concepts in genetics that are relevant to this study.

In Chapter 3, the reader is introduced to the concept of computer simulation. An overview of the various simulation types, paradigms and components within a simulation model is presented, followed by a discussion on the steps required to develop a successful simulation model as well as the most commonly cited advantages and disadvantages of computer simulation modelling. The chapter concludes with an overview of ecological simulation modelling, focusing specifically on developed models that are closely related to *E. saccharina* and Bt crops.

In Chapter 4, the technical specifications of the developed computer simulation model for *E. saccharina* on Bt sugarcane, drawing from concepts discussed in Chapter 3 are discussed. This chapter focuses more on the technical implementation of the model, including the data structures, modules and algorithms that allow the model to function. Details of implementation such as programming language, editors and hardware used for the model are discussed in this chapter.

In Chapter 5, the individual modules of the model are tested to verify that they function as expected and controlled simulations are run to ensure that the entire model performs appropriately. Following on the verification process, the difficulties in performing validations due to limited experimental data is discussed, leading to the presentation of a brief face validation based on expert opinion and model performance compared to previously developed models. The parameters of the model are adjusted to observe their effects on the performance of the model as a whole. Results of these analyses assists in deciding on the core parameter variations that will be included in the final decision support tool, as some parameters have more significant influences on model behaviour and would be the most important to showcase to potential decision makers.

Results obtained from running a set of select case studies and how the model performs are presented in Chapter 6. Here, the final decision support tool is also showcased, including variations of the most influential parameters on various configurations of refuge areas. The contents of this chapter can be viewed as the final results that would be presented to key decision makers.

The thesis concludes with Chapter 7, which provides a general summary of the thesis along with detailing the contributions made to the body of knowledge as well as listing some potential future topics to build on the work in this study.

CHAPTER 2

Survey of Biological Literature

Contents

2.1 Sugarcane and the South African sugar industry 5
2.2 *Eldana sacharina* Walker 6
  2.2.1 General biology 7
  2.2.2 Current and proposed control strategies 8
2.3 Transgenic crops 9
  2.3.1 *Bacillus Thuringiensis* 10
  2.3.2 Bt crops as a pest suppressant 11
2.4 Genetic flow in populations 14
2.5 Chapter Summary 16

In this chapter, a review is given on the biological background of importance in this thesis. Background and literature on the sugarcane plant and sugarcane industry in South Africa are presented, followed by the biology and life cycle of *Eldana Saccharina* Walker, a background on transgenic crops, specifically crops with genes from *Bacillus thuringiensis*, and finally an overview of genetic flow in populations.

2.1 Sugarcane and the South African sugar industry

Sugarcane (*Saccharum officinarum* L.) cultivated in South Africa is a tall, perennial grass of the family *Gramineae*. The plant consists of a clump of unbranched stems that can reach a height of 5 meters and is jointed, with nodes situated at the bases of alternate leaves (see Figures 2.1 and 2.2). The space between nodes, or internodes, vary in length between 11 and 18 centimetres and have a diameter of up to 5 centimetres. The stalk contains a fibrous white pith immersed in a sugary sap. The plant is crowned with long, narrow leaves that can reach up to 150 centimetres in length with a width of only 6 centimetres [26]. Dead leaves remain on the lower portions of the stalk. *S. officinarum* originated in New Guinea and grows best in tropical climates.

In South Africa, sugarcane is cultivated in 14 areas stretching from the Eastern Cape, through coastal KwaZulu-Natal and the KwaZulu-Natal midlands and finally up into Mpumalanga. Approximately 430 000 hectares of sugarcane is planted in these regions, with 68% grown within 30 km of the coast [38]. These areas produce roughly 22 million tons of sugarcane a season, delivering 2,2 million tons of sugar. South Africa currently ranks as the 15th largest sugar producer in the world, equating to a R12 billion industry mainly situated within rural areas of

Figure 2.1: Immature sugarcane growing in KwaZulu-Natal [85].

the country [102]. As with all commercially grown crops, sugarcane has several associated pests that damage crops and reduce overall yields [29, 46].

Sugarcane is not traditionally grown from seed, but rather from stalk remnants from the previous growing season known as setts. The setts are cut into lengths of about 40 centimetres and dropped into furrows where they are covered in soil and fertilizers. As sugarcane is a tropical plant, it requires a very wet climate with rains of at least 1500 millimetres annually and plenty of sunlight. In coastal regions, sugarcane takes between 9 and 16 months to grow to maturation whereas it can take up to two years in cooler inland regions such as the Natal midlands. Cropping cycles of sugarcane usually consist of a planted crop and three or four regrowth crops, known as ratoon crops [20].

(a) *A section of the sugarcane stalk [71]* (b) *A close up view of the stalk [36].*

Figure 2.2: The grassy stalks of sugarcane are rich in sucrose.

2.2 *Eldana sacharina* Walker

Eldana saccharina Walker (Lepidoptera: Pyralidae) is a stalk borer moth indigenous to Africa and makes its habitat in grassy plants and sedges, where it lays its eggs in the dry leaf matter [6]. There is evidence that suggests that the movement of *E. saccharina* onto sugarcane is primarily linked to the abundance of dead leaf material on mature sugarcane and not necessarily because of its nutritional content [5]. It is one of the only consistent pests in cultivated sugarcane grown in Africa and has been identified as a pest as early as 1855 [8, 123]. Initial infestations of *E. saccharina* in South African sugarcane was reported in the Umfolozi area of KwaZulu-Natal from 1939 to approximately 1950, then again in the Hluhluwe area in 1970, where it spread to other areas of eastern South Africa and Swaziland [5, 6, 23].

2.2.1 General biology

E. saccharina follows a life cycle that is similar to other lepidopteran insects, consisting of eggs, larvae, pupae and finally the adult moth as may be seen in Figure 2.3 [6, 8]. The development of *E. saccharina* is heavily dependant on the temperatures experienced [8, 127], with higher temperatures generally leading to quicker development. On average the eggs will hatch in 8–10 days, larvae will pupate in 20–60 days depending on the season and adult moths will emerge from the pupae in approximately 10 days. The adult moth usually only lives between 5 and 7 days [23].

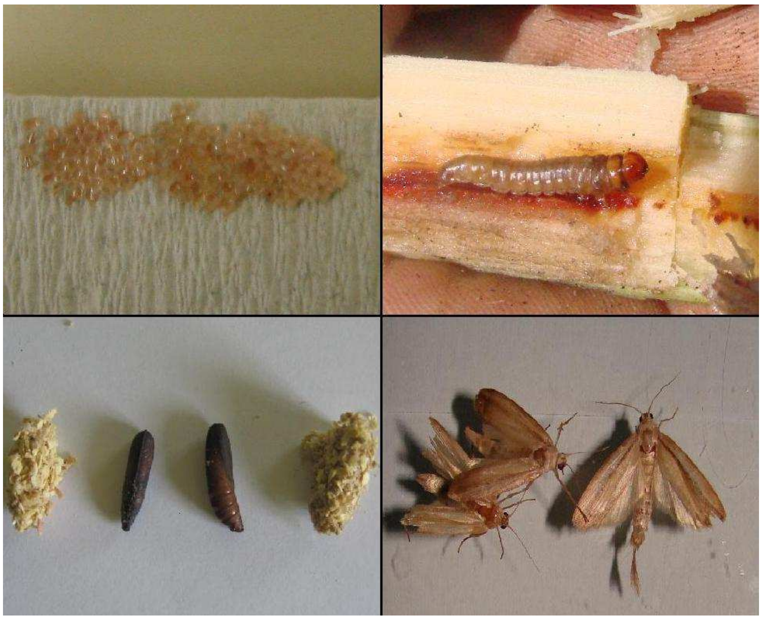


Figure 2.3: *The life cycle of* E. saccharina *showing, from top left to bottom right, the eggs, larvae, pupae and adult moths [86].*

The fertilised female will lay batches of approximately 20 eggs in the dry leaf material of the lower parts of the stalk [6, 23]. A female can lay about 500 eggs in her lifetime, with cases of more than 700 eggs being observed in laboratory conditions [125]. Once the eggs hatch, the young larvae will primarily feed on leaf and other organic material and can disperse to nearby plants by climbing to the canopy and using silk to ‘parachute’ to neighbouring plants [120]. The larvae will feed outside of the stalk until it is robust enough to bore into the stalk, where it consumes the internal tissues and, as these tissues are the sucrose-rich parts of the sugarcane plant, can cause significant yield losses in heavy infestations [5, 23]. The feeding larvae needs to consume between 2 and 8 centimetres of sugarcane to fully develop [5], with up to 12 larvae being able to feed on a single sugarcane internode as they prefer the mineral rich lower portion of the stalk, even boring into the underground parts of the stalk [23]. The feeding larvae produce a residue known as frass which can be pushed out through the holes they bored into the stalk. The frass serves as a visual indicator of larval activity within the stalk [100]. The mature larvae spin a protective cocoon around themselves and will attach themselves either to the inside of the hollowed out stalk, or under a leaf sheath close to their exit hole and will remain in this pupal stage until they emerge as adult moths [23, 100].

2.2.1.1 A summary of the mating process of *E. saccharina*

Once the adult moths emerge, it can take several hours before their wings set and they are able to fly. Once their wings set, the males will congregate in groups of 3 to 6 to display together as a *lek* in the early evening [7]. The display consists of vigorous flapping of wings, raising pencil hairs on their abdomen to form a brush and releasing pheromones into the air. The female will then approach a *lek* and will choose a single male to mate with. While the female has been observed to mate with several males in laboratory conditions [124], this is hardly observed in wild females. This is most likely due to their short lifespan and the durability of the male's spermatophores [23] and it can be assumed that the vast majority of females will only mate with a single male, but that a single male is able to mate with multiple different females. The female will start ovipositing in desirable areas such as dried leaf sheaths about 24 hours after mating.

2.2.1.2 A summary of the spatial movement of *E. saccharina*

Once larvae hatch from their eggs, the neonatal larva can disperse in nearby sugarcane by climbing to the top of the plant and 'parachuting' onto the leaves of nearby plants, granting them a degree of local movement before boring into the stalk and effectively remaining stationery until they emerge as adults [120]. The moth is considered a weak flier overall, but the larger and stronger female moth has been observed to disperse great distances if they remain unmated [86]. The female can also disperse in search of suitable oviposition sites, as they have a preference for older sugarcane stalks with more dead leaf material and a larger number of internodes [12, 33]. The female is believed to be sensitive to plant volatiles released when the cane is being fed upon, leading to dispersion of the pest away from highly infested areas [120]. Entomologists are still unsure exactly how male moths disperse, but in discussions with entomologists at SASRI, Van Vuuren *et al.* [120] deduced that the males would likely congregate around areas of stressed cane with other males where they would be able to form *leks*.

2.2.2 Current and proposed control strategies

The negative impact of chemical pesticides on the environment has led to a global movement to reduce dependence on sprayed insecticides and to invest in research pertaining to biological control methods. SASRI has been heavily involved in researching potential alternative control strategies, including biological control, varietal resistance, crop management and sterile insect techniques.

The use and efficacy of insecticides on *E. saccharina* was studied by Heathcote in 1984, where it was found that submerging infested sugarcane in solutions of insecticides in water was effective at killing the larvae in the stalk, although the bulk of the mortality could be attributed to the water. It was also observed that, while there was little effect on the adult moths where insecticides were sprayed on the cane canopy, in pre-trashed sugarcane treated with insecticides, a significant reduction in young larvae was observed [48]. A more natural approach to pest management is the use of biological control strategies where a natural predator or other antagonistic organism is introduced to reduce pest populations. There are several organisms that feed on *E. saccharina* in its various life stages including ants and spiders [31]. There have been several attempts to introduce parasitoids such as *Cyperus papyrus* L. as biological control agents, but due to sugarcane being harvested regularly, no populations have been able to establish themselves successfully [28, 30]. As a result, the feasibility of biological control strategies are still being studied.

An alternative approach to pest management is to make the habitat less attractive or even possibly hostile for the pest. Since the female moth prefers to lay eggs beneath dry leaf sheaths, it is possible to reduce the levels of infestation by pre-trashing the sugarcane, effectively reducing the number of potential egg-laying sites without loss in yield [25, 68]. Other methods of manipulating the habitat include removing old stalks and stubble from the field after harvesting, harvesting sugarcane earlier to limit dead leaf material, planting varieties that are in some way resistant to the pest and using less nitrogen rich fertilizers [24, 60, 128]. There are also some plants, such as *Melinis minutiflora*, that releases volatiles that repel *E. saccharina* and can be planted between sugarcane as a form of habitat management [93]. Apart from making the habitat more hostile, it is also possible to use pull plants to try and draw the pest away from valuable crops by planting crops that are more attractive to the target pest than the valuable crop [52].

A control strategy that is being piloted by SASRI is a method known as the *sterile insect technique*, or SIT [77]. This method requires the mass rearing and sterilisation of male insects that would then be released into the wild where they would mate with wild females, resulting in sterile offspring. Although still in early phases of implementation, significant research into the general biology of *E. saccharina* as a target for SIT [125] and modelling of such a program's efficacy and feasibility [86, 90] has been performed.

2.3 Transgenic crops

Although humanity has been selectively breeding crops, livestock and pets for millennia, it was not until the late 20th century that traits were selected on a genetic level [17]. In 1973, Cohen *et al.* first successfully introduced a piece of genetic material from one organism and implanted it into another. A gene conferring antibiotic resistance from a strain of *Escherichia coli* was transplanted into another bacterium of a susceptible strain, which successfully developed its own resistance [27]. The potential negative effects genetic engineering could have on the ecosystem was quickly realised and in 1975 the Asilomar Conference was held in which experts could debate the safety of genetic engineering experiments [94]. It was decided that the experiments should continue, but with stringent guidelines in place [11].

A key development in genetically engineered consumer products came in 1980, when the U.S. Supreme Court ruled that General Electric could patent a genetically engineered bacteria that could help break down crude oil [1]. This provided an incentive for companies and research institutions to focus on developing useful genetically modified organisms, or GMOs[1], for profit. The first product to be marketed for human use was insulin produced by a genetically modified bacterium, known as Humulin [59]. The first food product to be made commercially available was the FLAVR SAVR tomato, developed by Calgene, Incorporated [16]. This tomato was specifically developed to stay firmer for longer, which would allow the fruit to be ripened on the vine and then transported to the market instead of being picked while still green and being ripened by ethylene treatment [64].

Crops that were specifically designed to be easier to cultivate first came to market in 1995 [94] in the form of maize that has pesticidal properties derived from a bacterium, *Bacillus thuringiensis* [3].

---

[1] A GMO is an organism or microorganism that, through genetic engineering, has had its genetic material modified.

2.3.1 *Bacillus Thuringiensis*

Bacillus thuringiensis, also known as Bt, is a gram-positive[2], soil-dwelling bacterium (see Figure 2.4). Its use as an insecticide was first explored in 1901 when a Japanese scientist, Shigetane Ishiwata, isolated the bacterium from silkworm moths that have perished from 'sotto disease' or sudden-collapse disease [57]. Then, the bacterium was known as *Bacillus sotto*, until the bacterium was again isolated by a German scientist, Ernst Berliner, in 1911 in the larva of a flour moth. As the discovery occurred in the German state of Thuringia, it was renamed *Bacillus thuringiensis* in 1915 [75].

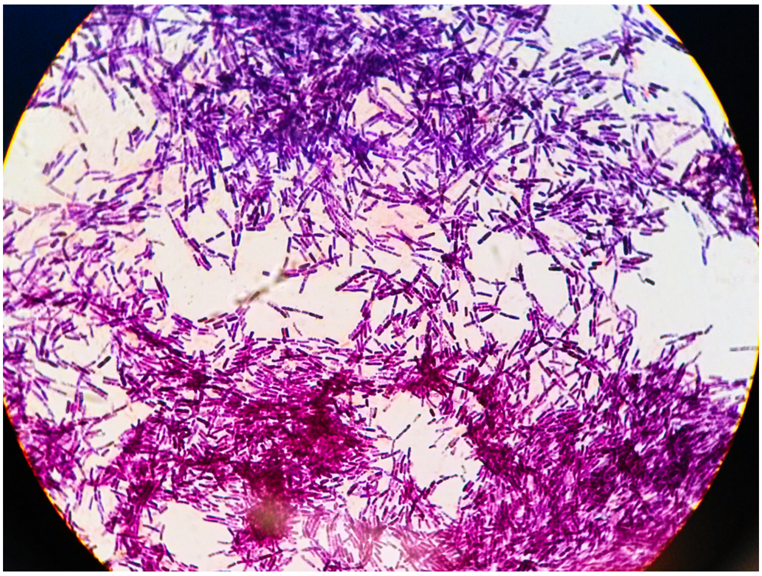


Figure 2.4: *A microscopic view of Bt after being gram stained [2].*

2.3.1.1 Insecticidal properties

Bt can reproduce by binary fission, where the bacterial cell splits into two daughter cells, or by producing single endospores in a process known as sporulation, during which crystalline inclusions can form (as seen in Figure 2.5) [51]. The crystals contain proteins, known as δ-endotoxins (delta endotoxins), that display strong insecticidal properties when ingested by species that are members of the *Lepidoptera* family [129] and also some members from other insect orders such as *Diptera* [4].

When the crystals, known as Cry proteins [57], are ingested by a species susceptible to its effects, it dissolves in the midgut and the proteins are released and are broken down by proteolysis into smaller, toxic polypeptides. These polypeptides can interact with the cells inside the insect's midgut, making them porous and resulting in the death of the cells [51]. With sufficient cell death, the insect will stop eating and eventually die. There are many subgroups and members of Cry proteins, and some of them are currently used commercially as insecticides, including Cry1Ab [40] and Cry1F [37]. It is also common for pesticides to include more than one Cry protein that targets a specific pest, referred to as multiple mode of action (MOA) pesticide.

---

[2] Gram-positive bacteria are bacteria that give a positive result in the Gram stain test. These bacteria have no outer membrane and as a result are more susceptible to antibiotics.

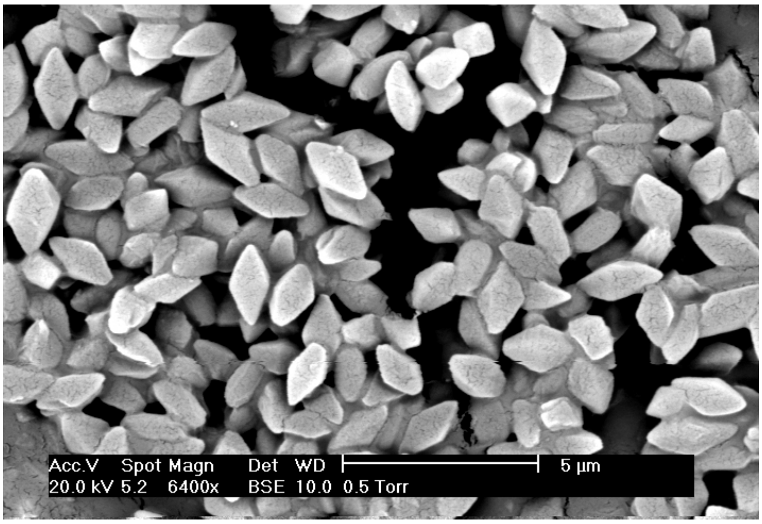


Figure 2.5: *A microscopic view of the crystals of the Bt toxin [130].*

2.3.1.2 Use in modern pesticides

Since the rediscovery of Bt in 1915, its potential as a commercial pesticide was quickly realised. The first commercial product based on Bt spores was developed in France in 1938 and was used to combat flour moth infestations [3]. In 1955, Hannay & Fitz-James discovered that the insecticidal properties of Bt was due to its formation of parasporal crystals, which led to increased interest and research efforts into its modes of action. In the United States of America, pesticides based on Bt proteins were first registered in 1961 with the Environmental Protection Agency (EPA) and there are currently approximately 180 Bt products registered with them [115].

Due to widespread use of synthetic pesticides, use of Bt pesticides as a natural alternative saw a significant increase, leading to several reported cases of resistance in target insects [51, 73, 111]. Although there has been some resistance development reported, the potential of Bt as a natural pesticide continued to draw research funding. Resistance to a Cry protein such as Cry1Ab does not necessarily mean that resistance has developed to all Cry proteins and the cumulative effect of using several Cry proteins would also delay resistance development. The first instances of genetically modified organisms (GMOs) containing Bt genes were tested in the early 1990s [63] and Bt maize, the first commercially produced Bt GMO, was first registered with the EPA in 1995 [3].

2.3.2 Bt crops as a pest suppressant

Bt crops were widely embraced and readily planted. In the United States, where the majority of maize planted is of a Bt variety, significant pest suppression of *Ostrinia nubilalis* (European corn borer) was observed, with a cumulative $3.2 billion benefit realised over 14 years [55]. In South Africa, Bt white maize was first planted in 1998, becoming the first developing nation to plant a genetically modified version of a staple food [44]. In China, Bt cotton was introduced to combat infestations of cotton bollworm and a significant decrease in pesticide usage without any reduction in yield was observed [54, 91]. To manage the efficacy of the Bt crops, regulatory bodies from every country where GMO crops are planted require that measures to curb insect resistance development are put in place [3].

2.3.2.1 Resistance management strategies

The primary mitigation measure is the use of a High-Dose/Refuge strategy. This approach uses very high doses of the pesticidal proteins combined with refuge areas, which are areas usually planted with non-Bt version of the crops that can maintain large populations of susceptible insects that will counteract any resistant insects [81, 95]. An example of a refuge area may be seen in Figure 2.6.

Figure 2.6: *A field of Bt cotton with a visible (darker) refuge border [3].*

Ostlie, Hutchison & Hellmich [81] describes three assumptions that need to hold for the strategy to be successful:

1. Major resistance genes must be sufficiently rare, with a gene frequency of less than one in 1000 recommended.
2. Resistance genes must be nearly recessive, meaning that heterozygote[3] individuals should have a similar mortality rate to the Cry proteins as a susceptible individual. There are documented cases of some species showing resistance traits that are not recessive [53], which would significantly undermine the efficacy of the strategy.
3. Refuge areas should be able to maintain populations of susceptible individuals and these individuals must be able to come into contact with potential resistant individuals within their typical dispersal area.

An added assumption that is popular amongst model developers in resistance management is that the pest population exhibits random mating behaviour [81]. This assumption will ensure that resistant individuals that come in contact with susceptible individuals have an equal likelihood of mating, leading to a large population of susceptible heterozygote individuals. Wright (1965) describes a $\boldsymbol{Fis}$ measure that describes the randomness of the mating process, with $\boldsymbol{Fis} = 0$ indicating that mating is completely random and $\boldsymbol{Fis} > 0$ indicating that there is some preference to mating with resistant individuals. In some species, such as the European corn borer, experimental studies have shown that the $\boldsymbol{Fis}$ measure is not 0 and this assumption would not hold [78].

General recommendations for how refuge should be planted varies according to the crop and pest biology, although the regulatory body of the country it is used in as well as the producer of the crop would set recommendations according to their interpretation of these principles. In the United States, for example, the EPA has varying requirements for the refuge depending on how many Cry proteins the crop contains and the pest it is targeting [95]. An example of such a requirement is 20% of the planted crop to be structured refuge for Bt maize that produces a single Cry protein, grown in the American Corn Belt[4] with Lepidoptera as the target pest.

---

[3] A heterozygote is an individual that only has a single copy of a given gene.

[4] The Corn Belt is a region in the Midwestern United States that produces the majority of maize in the United States.

This requirement would jump to 50% if it was in the American cotton regions. The EPA lists 3 different approaches to refuge areas [95], namely:

- **Structured refuge** – where a dedicated portion of land is planted with non-Bt versions of the crops in close proximity to the Bt crops. Refuge layout will vary according to how the crop is planted, eg. sowed versus planted, and the mobility of the target pest. Some examples of how structured refuges can be planted in maize are illustrated in Figure 2.7.
- **Seed blends** – where bags of Bt seed are mixed with non-Bt seeds, leading to automatic compliance as no separate refuge is planted.
- **Natural refuge** – in cases where target pests feed on multiple plant hosts, having wild hosts in close proximity to the Bt crop can act as refuge. This strategy's effectiveness depends on the specific pest, as there has been little development of resistance in *Helicoverpa armigera* in Chinese cotton farms, which employs a zero-refuge policy [92]. The same result could not be replicated in African stalk borer populations, with wild refuge areas not providing adequate protection against resistance development [118].

Figure 2.7: *Different possible structured refuge layouts. From top left: linear block, bracket, border, block, and strips. Recreated from an illustration by Aroian [3].*

2.3.2.2 Development of resistance

Since the development of Bt crops, researchers and seed producers have been tracking the potential for resistance to develop. Studies on two moths, *Heliothis virescens* and *Ostrinia nubilalis* Hübner, showed that the frequency of resistant alleles to be between 0.01% and 0.1% before the introduction of Bt crops [15, 43]. An analysis performed by Tabashnik *et al.* in 2003 [110] confirmed that, in several instances in both the United States of America and China, the frequency of resistant alleles did not increase since the introduction of Bt crops, indicating that the prescribed High-Dose/Refuge strategy was possibly successful. However, there are several cases where field resistance has been reported in many different insect species on several different varieties of Bt crop. Three such examples are *Busseola fusca* (Fuller) larvae with resistance to Cry1Ab proteins in Bt maize being found in South Africa [119], larvae of *Helicoverpa zea* found in Bt cotton in Arkansas [70] and instances of resistant *Diabrotica virgifera virgifera* being found in Bt maize fields in Iowa [42].

The development of resistance can be curbed by developing varieties of plants that produce multiple Cry proteins, as it has been found that cross-resistance between various Cry proteins is rare [62]. Most Bt crops currently available on the market and under development contain multiple Cry proteins, ensuring that each plant has more than one ‘modes of action’ to kill the target pest [35]. This could reduce refuge requirements as the proportion of insects that display resistance to all modes of action would be significantly less than those that are resistant to an individual mode of action. As an example, if a variety has two modes of action, and $R_1 = 0.1\%$ of the target pest population are resistant to one mode of action and $R_2 = 0.01\%$ are resistant to the second mode of action, then theoretically only $R_{1+2} = 0.001\%$ should be resistant to both. Some varieties of Bt maize, for example the Genuity® SmartStax® variety marketed by Monsanto, contain 6 Cry proteins and require as little as 5% refuge to curb resistance development [76].

2.4 Genetic flow in populations

Every living (and some non-living) organism carries a genetic code in its cells. This code consists of strings of deoxyribonucleic acid (DNA) that form genes and these genes can combine to form a chromosome (see Figure 2.8). Each gene contains a single set of instructions, some of which will code for protein synthesis and other (non-coding DNA) that assist in gene expression. Genes are the basic unit of heredity and most multicellular organisms have two copies of the same gene, known as a diploid, which is inherited from its parents. Genes are always located on the same place in a chromosome, known as a locus, and alleles are variants of genes that are different between the two copies on the same locus. The entire genetic code of an organism is known as the genotype, and the differences in alleles that result in a difference in the outward appearances and behaviours of an organism is known as the phenotype. An example of a phenotypic variation can be seen in Figure 2.9, where the antelope on the right displays a rare “masked” facial marking pattern.

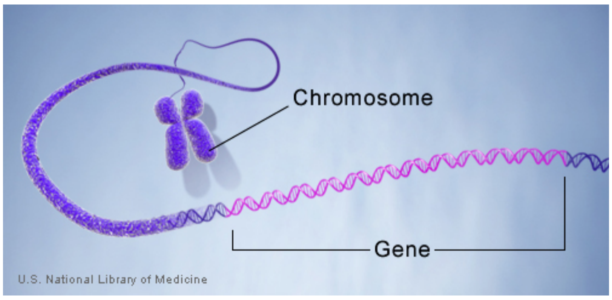


Figure 2.8: *Genes are located on strands of DNA which form chromosomes [116].*

The relationship between alleles of a gene and their associated phenotypes is known as *dominance*. A *dominant* allele only requires a single copy in a given locus to result in the associated phenotypic variation, as its effect on the phenotype masks the effect of the *recessive* allele. Gregor Mendel first observed and documented the effects of dominance in pea plants in the 19th century [74]. He observed that when crossing two purple pea flowers, on average, three of the offspring would bear purple flowers and one would bear white flowers. He hypothesised that there existed hereditary units, now known to be genes, that would influence what characteristics an organism would exhibit. A *Punnett square* for the pea plant may be seen in Figure 2.10.

In living organisms, genes that allow individuals of some organism to better adapt to its local environment, or *local adaptation*, are more likely to be passed to future generations in a process that is known as *natural selection* [67], forming populations of locally optimum phenotypes. How these genes then migrate between different populations of the same organism can then be defined as *gene flow*. Slatkin [105] defines several key terms that are prerequisite to understanding gene flow and natural selection, such as:

- **Gene swamping**, where there is a loss of variance at a specific locus under selection due

Figure 2.9: *Two blesbuck* (Damaliscus pygargus phillipsi) *displaying different facial markings (phenotypes) due to a genotypic variation.*

to high gene flow.

- **Local adaptation**, where individuals in a habitat perform better than average when compared to immigrants.
- **Migration load**, the decrease in mean fitness of a population due to immigration, as the phenotypic mean will be different from the local optimum.
- **Migration meltdown**, a self-reinforcing process where immigration introduces maladapted alleles, which can lead to more immigration by decreasing local density.
- **Migration-selection equilibrium**, a stable polymorphic equilibrium where the effects of migration are counteracted by the effects of selection.

Gene flow can occur naturally, or it could be introduced artificially by humans in cases where endangered animals lose genetic variation, potentially threatening their survival. This was the case with the Florida panther (*Puma concolor couguar*), where dwindling populations in South Florida, USA, in the early 1990s threatened its survival. A study was performed by Hedrick [49] to determine whether or not an introduction of a different subspecies of panther, specifically from Texas, would result in a better genetic variation among the population without risking *migration meltdown*. It was concluded that if approximately 20% of the first generation of panthers were descended from the Texas panthers, the *migration load* would be significant enough to remove most of the detrimental genes.

In a Bt scenario with a high dose/refuge strategy in place, many of these concepts are relevant, as the population that develops resistance displays some *local adaptation* to its environment, ie. being able to survive on the proteins expressed by the crop. Maintaining large populations of susceptible insects in refuge areas could promote migration of the susceptible insects into areas with resistant insects, leading to *migration load* and *gene swamping*, effectively keeping the *migration-selection equilibrium* stable and thus limiting development of resistance in the overall population [109].

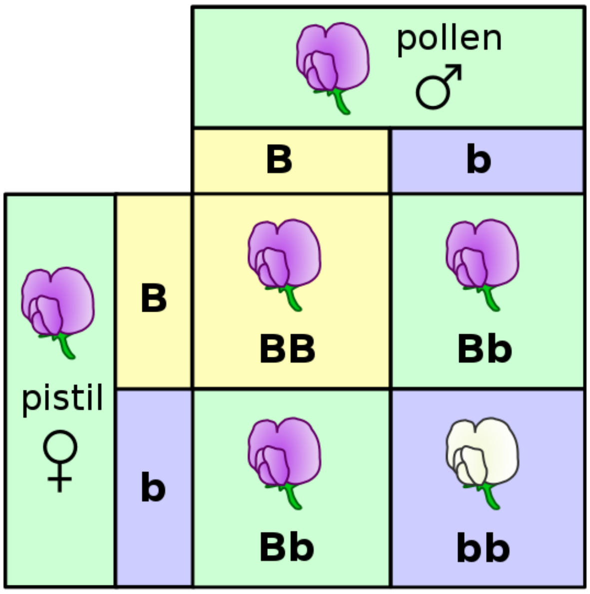


Figure 2.10: *The Punnett square shows the alleles for two pea plants with one dominant purple allele, B, and one recessive white allele, b.*

2.5 Chapter Summary

In this chapter, the most relevant literature pertaining to the biological background required for this study was discussed. A brief overview of the biology and cultivation of sugarcane was presented, highlighting the key farming methodologies, such as ratoon crops, that set it apart from other crops. This was followed by an overview of the life cycle of *E. saccharina*, its relationship with sugarcane and its threat as a pest for sugarcane growers as well as how the threat is currently being managed. An in-depth review of transgenic crops and the role of *Bacillus Thuringiensis* in developing insect resistant crops is presented, outlining its mechanisms, regulations surrounding its use as well as cases of resistance development observed so far and what steps are being taken to limit it. This also introduces the concept of a high-dose/refuge strategy. The chapter closes with a summary and glossary of genetic flow terminology and how it relates to the current study.

CHAPTER 3

Computer Simulation Modelling

Contents

3.1 Simulation modelling **17**
  3.1.1 The components of a simulation model 18
  3.1.2 Different classes of simulation modelling 19
  3.1.3 Simulation modelling paradigms 19
3.2 Steps in developing a simulation model **20**
3.3 Advantages and disadvantages of simulation modelling **22**
3.4 Simulation and mathematical modelling of ecological systems **24**
3.5 Chapter summary **26**

In this chapter, simulation modelling as a science and as a viable analytical tool is discussed. The different types of simulation models, the advantages and disadvantages of simulation modelling as well as the prescribed steps to develop a simulation model as an approach to solve problems are also discussed. Finally, the application of simulation modelling in the ecological context is discussed, focussing on agent-based and cellular automaton models.

3.1 Simulation modelling

A simulation can be defined as the implementation of a model that is an imitation of a real-world system or process, often over a period of time. Simulation models can be used to describe and analyse systems as well as generate artificial history of a system that can be studied in lieu of the actual history [9]. This makes it a potentially valuable tool when used to model systems that are conceptual, allowing for better decision making in a wide variety of real-world applications.

As an analytical tool, simulation modelling is useful in both prescriptive and predictive analytics as it may be used in conjunction with traditional optimisation tools to optimise an existing system or to predict how a new or modified system will behave. It can be used where traditional analytical methods, which are functionally reliant on the system input in the form of several parameters, would not be suitable due to the parameter values either not existing or being difficult to find or measure [14]. In essence, a simulation model is a set of rules, that could take the form of equations, flowcharts or any other construct, that governs how the system will progress over time, given its current state [120]. The state is then defined as the collection of all variables required to fully describe the system at any point in time [66].

As with any analytical solution to a given problem description, there are several questions that need to be asked to determine the suitability of a simulation-based solution. The most important question is whether or not applicable data to build such a model are available and, if not, how easy is it to obtain the required data [14]? Once it has been determined that the data does exist (or can be obtained) for a simulation model, it must be decided what the desired outputs are that one wants to obtain from the simulation model, which is often linked to the level of *abstraction* in the simulation model. There are three defined levels of abstraction in simulation modelling: *strategic*, *operational* and *physical* level, listed in increasing level of detail [132].

Simulations on the strategic level have the least amount of detail and the greatest amount of abstraction and are primarily used to analyse the strategic organisational aspects of a system. For example, strategic level simulations could be modelling the spread of a disease on a population level. No intricate information is required in this case in terms of individuals, but only considers the aggregate information for the population. The model will take into account the proportion of the population within each stage of the disease, for example, from susceptible to infected to recovered. The aggregate effect of different healthcare policies or intervention strategies on a population may be investigated using a strategic level approach.

If more detail is required to make tactical decisions, an operational level simulation can be employed. These typically have more detail and may include modelling of subsystems within a larger system to generate more detailed outputs. Systems that could benefit from operational level models include how flows of populations between residential areas and public spaces can influence the spread of a disease as well as systems where problems could be identified by analysing performance measurements.

Where lots of detail is required, simulations can be built on the physical level. At this level there is very little abstraction and aggregation and entities within the simulation can be modelled on the individual level if it is required to analyse the behaviour and characteristics of every single entity. Such detail in a model may be used to model how interpersonal contact can spread a disease between infected and susceptible individuals.

3.1.1 The components of a simulation model

What is contained within a simulation model will vary according to the required complexity, but Banks [10] lists the following elements in a typical simulation model: the system, the system state variables and the entities, including their individual attributes, activities and events that may result in changes to the state variables. The real-world process being modelled is defined as the overall system.

This section will be illustrated using a system where a kettle (a simple heating element inside of a containment vessel) full of water is connected to a device that randomly switches on and off by drawing from a known distribution. Every 10 seconds the device samples from a distribution and will switch states from on to off or vice versa if the sampled value is below a threshold value τ. The simulation is run until the water comes to a boil.

A *model* is defined as the representation of a system and consists of entities that interact over time. The components that make up the model will be limited to the elements that are relevant to the study and to the level of abstraction required. In the example, the system can be comprised of the physical space the kettle is in, the vessel, the element, the water in the kettle, the device that switches the power on and off as well as the power supply.

To know what is happening in the system at any given time, it is required that we know what the *state* of the system is. All the variables which make up the state of the system are collectively referred to as the *system state variables*. In the example, some of the key system state variables would be how long the simulation has run for, the current temperature of the water and the current state of the device.

An *entity* is any object of interest that is present in the model and can be dynamic, where it is able to move around in the simulation space, or static, where it usually serves other entities in the model. In this example, the element, the water and the device can be considered entities with their own *attributes*. These *attributes* are usually local variables that are specific to the entity they pertain to, such as the rate at which the element is able to heat water or the specific distribution that the device draws from.

An *activity* is some process within the simulation that has a defined beginning and end, with the duration, which can be constant or randomly sampled, being known before the commencement of the *activity*. In this example, the 10 second intervals between the device sampling from the distribution can be considered the *activity*. When there is a period of time that passes where the length of its duration cannot be determined beforehand, a *delay* occurs. In this example, the length of time before the kettle switches states can be considered a *delay*, as its duration is indefinite. The beginning and end of an *activity* can further be described as an *event*.

An *event* can be described as something that happens in the simulation that can alter its state. *Events* can be *endogenous*, where the event occurs within the simulation, such as the device switching its state after sampling successfully, or they can be *exogenous*, where the event occurs due to influences from outside the simulation. In the example, the power supply could be considered exogenous, as a power supply outage would technically happen outside of the simulation.

3.1.2 Different classes of simulation modelling

Simulations are divided into different classes based on three dimensions: *static* versus *dynamic*, *stochastic* versus *deterministic*, and *continuous* versus *discrete* as described by Banks [9].

A *static* or *analytical* model represents the real-world in a specific point in time, where a set of input variables lead to an output, such as a model of pipes and valves in a water reticulation system, where inputting the flow rates through the valves, the pressure within the system can be determined. A *dynamic* model evolves over time, and given the same initial setup could evolve and develop in a variety of different ways over time, given the variability of the activities and events within the system.

In *deterministic* models, all inputs are fixed and every set of inputs should hence correspond with a specific output. A *stochastic* model has inputs and variables that are stochastic, meaning that the final output of the model will also be stochastic. This also means that the model output cannot be predicted precisely, however, the average output from many simulation runs is considered an approximation of the expected output and system behaviour.

A *discrete* model is one where the model variables are updated in discrete time steps, whereas in a *continuous* model the variables are updated continuously in time. In many cases, models are a mix of discrete and continuous, with some system state variables being updated continuously and some local variables or entity attributes being updated in discrete time steps [66].

3.1.3 Simulation modelling paradigms

Depending on the level of abstraction or detail required in a simulation model, there exists four distinct simulation modelling paradigms, namely *system dynamics*, *discrete event*, *agent-based* and *dynamic systems* modelling. It should also be noted that these paradigms can be integrated within each other in a multi-paradigm architecture [14].

System dynamics modelling may be described as a model that assists in understanding the dynamics and interactions of complex systems by aggregating entities such as people, processes and products into their associated quantities, sacrificing detailed interactions for scale. These models are usually of a higher abstraction and are used to predict how decisions or policy changes affect the performance of a system over time [14, 132]. In these models, real-world processes are represented as

- stocks, eg. knowledge, money, and products,
- flows between these stocks, and
- and the information that determines the values of these stocks [14, 39, 132].

This modelling paradigm is similar to dynamic systems modelling, where a dynamic system is represented by a mathematical model (eg. differential equations) and is widely applied in engineering and physics applications.

Discrete event simulation is a paradigm where events occur at discrete points in time and it is assumed that nothing of interest happens between these points [121]. The model requires clear definitions of system states and events and activities that could lead the system to transition from one state to the other [39].

In agent-based models, the focus shifts to simulating the interactions between entities and then observing the effects of these interactions on the overall system [132]. These models generally have the lowest level of abstraction, requiring very clear definitions of entities and their potential interactions. In ecological models, agent-based models, where the agents are individual organisms, are often referred to as individual-based models, where interactions between the individual organisms are modelled [45].

3.2 Steps in developing a simulation model

Banks [9] and Sargent [101] define twelve steps that should be taken in the development of a successful simulation model. In this section the steps (and when they should be repeated) will be discussed as well as visually represented in Figure 3.1.

1. *Problem description.* In any model being developed, it is required to have a clear problem description that includes all the objectives and questions the model in development will aim to answer [9]. Having a clearly defined problem description can also assist in choosing the most applicable modelling paradigm [39].

2. *Project planning.* In this step the overall scope of the project is defined. The scenarios to be investigated will also be stated as well as the performance measurements used to evaluate the efficiency of the various system configurations must also be included. Project time-lines, including stages and desired outputs to be achieved at these stages, projected costs and all project hardware and software needs to be defined at this point.

3. *Model conceptualisation.* In this step, the real-world system under investigation (if applicable) is abstracted by a conceptual model that uses mathematical and logical relationships which govern the model components and structure of the system [9, 39, 120]. The best simulation models will only abstract the most important aspects of the system, setting and iteratively modifying basic assumptions and features until the model represents a useful approximation of the real-world systems. It is also recommended to begin modelling simply and then adding complexity as required or until the limitations of the hardware the model is being developed on is reached, which can be considered a hard constraint as the hardware specifications were set in step 2.

4. *Data collection.* This step occurs concurrently with model conceptualisation, as there is a constant interplay between defining the model to use and its potential data requirements,

where a more complex model may require more data. In general, the stated objectives will largely dictate the data requirements [9]. When collecting input data, it is also important to collect data pertaining to the performance of the current real-world system which will be used to validate the developed model [66].

5. *Model translation.* In this step, the model conceptualised in step 3 is translated into code using a suitable programming language or simulation package. The use of an existing simulation package can reduce development time, but allows for less control as most elements are immutable. Using an existing programming language to develop a simulation framework from the ground up, while more complex in terms of development, gives the developer much greater control over its functionality [9, 66].

6. *Model verification.* In this step the output of the model, or smaller modules within the model, is checked to confirm that the model or module behaves as intended. It is recommended that the model verification occurs throughout the development process, as it is generally easier to debug the system piece by piece rather than as a complex whole, where variable interdependencies can be masked to appear correct, concealing logical errors. The two basic approaches to model verification are static and dynamic testing. In static testing, verification can be done using structured walkthroughs and correctness proofs[1]. In dynamic testing, the model is run under different conditions and outputs such as *traces* can be obtained to check if the model performs as expected.

7. *Model validation.* This step determines whether or not a model is an adequate representation of the real-world system. Validation is often an iterative process where the model outputs are compared to real-world outcomes if possible, or scrutinised by subject-matter experts to determine if it has achieved an acceptable level of realism, also known as face validation. Validation techniques commonly used include animations that graphically display the model output, event validation where the 'events' that occur in the simulation are compared to the real system, and historical data validation (if historical data exists), where the model is built on some of the historical data (training set) and the validated against the rest of the data (test set).

8. *Experimental design.* With an acceptable model being established, it is necessary to decide on the different scenarios or system designs that are to be tested, as well as the parameters of the experiments which would include the duration of the simulation run, the number of simulation replications to run and the starting configurations of the system.

9. *Production run and analysis.* Using the designs of the previous step, production runs and their analysis should be used to estimate performance measures for the aforementioned system designs.

10. *More production runs.* This is not a fixed step, but may be a requirement based on the results of the initial production runs. If more production runs are required, more experimental design would have to follow to document the new scenarios.

11. *Documentation and reporting.* Documentation of the model serves many purposes, as design documents where the details of the coded model are logged would greatly assist future analysts that would want to use the model in understanding how the model works and how to potentially modify it. It is also critical to document the results obtained from the model, as well explained analyses of the model output is often, if not always, a requirement for the project stakeholders. It should be noted, however, that these do not

---

[1] Correctness is a computer science term where it is tested whether or not a given algorithm is correct with respect to a given specification. This is often achieved by testing the correctness of a set of outputs given some inputs.

need to be discrete documents, as in an academic study such as a thesis or dissertation would contain all of the above information.

12. *Implementation.* If the preceding steps have been implemented successfully and the stakeholders and key decision makers have found value in the outputs of the model and its accompanying documentation, it is likely that the processes and configurations obtained from the model will be implemented.

3.3 Advantages and disadvantages of simulation modelling

The rapid growth in computational power over the latter half of the twentieth century and early twenty-first century has made it possible to execute larger and more complex programs, leading to the development of powerful and robust simulation platforms that can execute instructions at a remarkable pace, leading to the development of models that can simulate larger and more complex systems. The advantages of using simulation models are described in detail by several experts in the field, including Banks [9] and Law [65].

One of the most frequently cited advantages of simulation modelling is the ability to provide practical feedback when designing real-world systems [34]. The user can determine the efficacy of a design before it exists or can predict the future effects of a strategy or process change in an existing system with no impact on the current system and without committing any expensive resources and time. Using simulation allows for the testing of a novel system at only a fraction of the cost of prototyping, which could lead to significant innovation as a larger proportion of the solution space can be explored without ever entering the construction phase. In industries where there is a large capital requirement for any production design changes, such as the petroleum refinery industry, the benefit of running simulations on the performance of proposed changes becomes clear, as in a study performed by Oh *et al.* [80].

Another key advantage in simulation modelling is the ability to manipulate time. There exists processes, especially in physics, where changes occur extremely rapidly, making them very difficult to observe in the real world, but can be stretched out and viewed 'frame-by-frame' in a computer simulation. Conversely, it is possible to execute the model in a very short time, hence processes that run over a very long time, such as gene flow in a population over many generations, can be observed in a matter of minutes.

A third advantage to simulation modelling is the ability to represent the model as an animated graphic, making it easier to understand for parties that are not necessarily as involved in the modelling process as the model developer themselves. It also allows for easier verification and validation of the model as some errors or unrealistic reactions are easier to verify when seen as an animation rather than as a string of output data.

Craig [34] also lists the ability to model over different levels of abstraction as an advantage, as it is possible to use a 'top-down' approach in modelling where high levels of abstraction model the interactions of the high level components of the system, which can then be used to verify and validate the results of lower level components that are modelled using lower levels of abstraction. This is useful where the model developer would otherwise have been overwhelmed by the sheer complexity of the lower level approaches.

There are, unfortunately, some drawbacks to using simulation. Craig [34] lists the main disadvantage of simulation modelling to be the time complexity of most non-trivial models, where processes that take a relatively short time to occur in the real world can take a very long time to execute in a simulation either due to high levels of complexity or the large number of entities contained in it. Banks [9] also argues that the rigorous scientific and practical knowledge

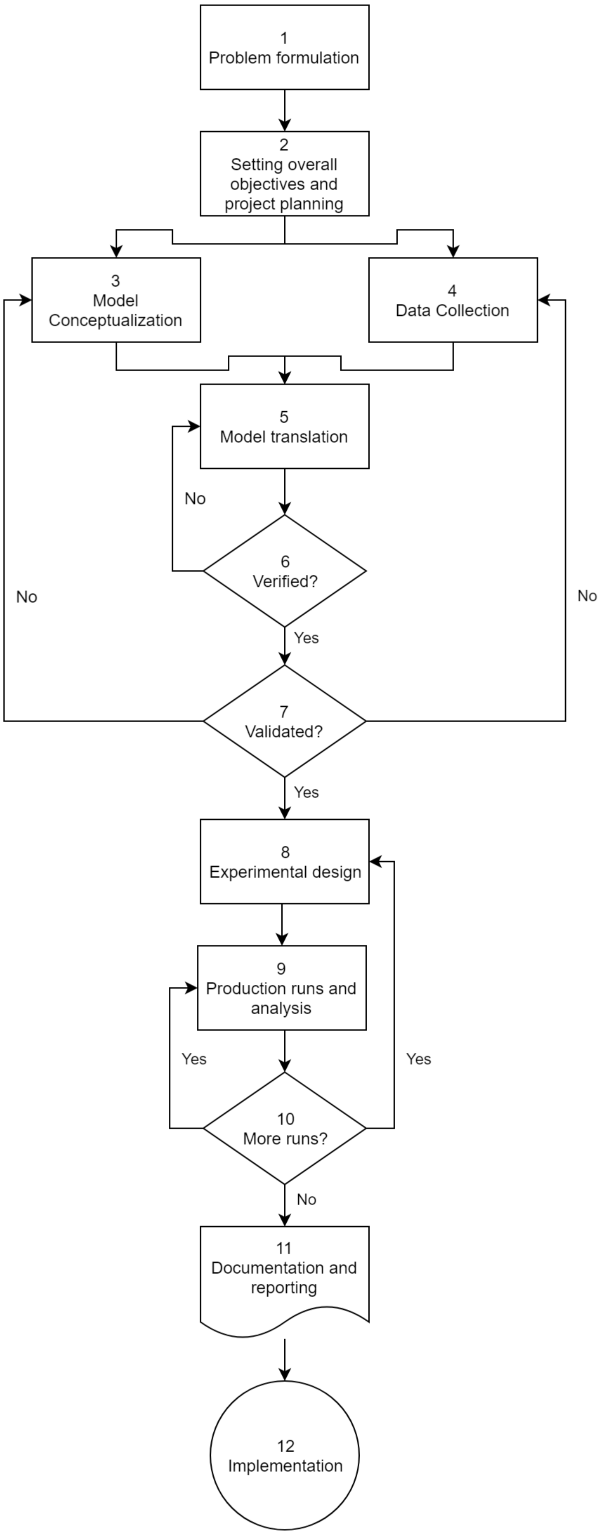


Figure 3.1: *The twelve steps in a simulation study as laid out by Banks [9]*

required to construct a feasible simulation model often requires significant skill and even training. This has been somewhat lessened by the development of complete simulation development packages such as AnyLogic, Simio and Simul8, that already contain many pre-built simulation solutions and analysis tools. Many of the proprietary software packages can be very expensive, but the cost is often outweighed by the analytical benefit the simulations can provide and many software packages are sold in tiers of complexity, so one does not necessarily have to pay for more professional features if they are not necessary [39]. In models with a large number of random processes interacting with each other, it also becomes increasingly difficult to analyse these models as they effectively become chaotic in nature.

3.4 Simulation and mathematical modelling of ecological systems

As ecological systems can be complex with many interacting elements, they are often targeted for the development of simulation models. An early form of ecological modelling include the dynamic systems models developed independently by Lotka [69] and Volterra [122] to describe the interactions between predator and prey organisms. Better technology has led to more ecological applications and models have been developed for topics such as forest management [84] and the movement and schooling of fish [56]. Several examples of individual-based modelling also exist in literature, some focusing on non-specific population behaviour such as dispersal of metapopulations within a species [113], while others focus on specific species, such as a study by Rose *et al.* [97], modelling population dynamics in walleyes and yellow perches in a predator-prey scenario.

With regards to modelling *E. saccharina*, two recent models utilising different paradigms are well described in literature. The first is a population-based reaction-diffusion model developed by Potgieter [86, 88] to study the feasibility of an SIT program as described in §2.2.2. This model can be considered as related to a spatial system dynamics model[2], and being based on partial differential equations would be deterministic in nature. The second is an individual-based, stochastic approach in the form of an agent-based simulation model developed by Van Vuuren *et al.* [120], which was an in-depth, discrete time, general model that aimed to capture as many behaviours of *E. saccharina* as possible to understand what the small-scale behaviours are that drives the large-scale infestation patterns in sugarcane fields. This study was very useful during the development of this thesis, as many complexities regarding how to implement certain parameters, such as aging, and behaviours were already solved for. Their validation using a panel of subject matter experts is also an approach utilised in this study. Due to the complexity of their model and the related computational effort required to run the model, it was deemed infeasible to use it as a starting point for this study, as it was only designed to work on a small scale over a relatively short time period. In this study, analyses over a larger spatial and temporal scale are required.

Ongoing research at Stellenbosch University with respect to the modelling of *E. saccharina* by the Surgor research group [87] include a cellular automaton model to study the effect of differently configured sugarcane fields on infestation levels as a follow up study on Potgieter *et al.* [89], as well as differential equation based models to study the refugia design problem addressed in this thesis, but using a population-based approach instead of individual-based approach. Output from the population-based and individual-based models may be compared, and possibly used for validation purposes. In one of the models developed, it was found that the level of initial infestation had a significant impact on the refuge requirements, with resistance developing much more readily in scenarios with high infestation rates. This would then lead to a much larger refuge requirement and indicates that an industry wide recommendation for refuge requirements may not be suitable [72]. The modelling has several limitations, which includes not being spatially explicit, which assumes that all individuals are able to interact with all other individuals. The model also assumes one mode of action, which is unlikely to be developed. This thesis aims to address both of these limitations and how they may affect the outcomes.

Several studies pertaining to the modelling of resistance development in pest species to Cry proteins in Bt crops have been performed since the mainstream introduction of Bt crops in the

---

[2] System dynamics models that have a spatial element added are labelled as *spatial system dynamics*, which is considered a sub-paradigm of system dynamics.

1990s. Some simple simulation models have been developed by Butterfield *et al.* [18], Roush [98] and Cristofoletti *et al.* [35]. Butterfield *et al.* developed a population-based model to determine what the effects of refuge size, dominance level, and initial resistance gene frequency would have in a scenario where there exists a Bt sugarcane with one mode of action. The results of the study indicated that having a low initial gene frequency with the resistance gene being recessive would require very little ($< 10\%$) refuge. If the gene was dominant and more common, upwards of 50% refuge would be required [18]. Roush developed a model that tested whether or not "pyramiding" different Cry proteins, where two independent modes of action were considered, would lead to lesser refuge requirements. It was found that pyramids could effectively reduce refuge requirements from 40–50% to as low as 10% [98]. Cristofoletti *et al.* performed a study that included a mathematical model that would assist in determining what combination of protein efficacy, number of modes of action and refuge size could curb resistance development in the stalk borer *Diatraea saccharalis* in a Bt sugarcane scenario. It was found that, given a 95% efficacy of the Bt protein, that approximately 10–20% refuge would be sufficient to limit resistance development for 100 generations (the expected lifetime of a variety of Bt sugarcane) where two modes of action was considered [35]. As with the model developed by Maselela [72], these models are all population-based and lack a spatial component.

Many spatially explicit refuge models have been developed, although they are generally focussed on a regional scale, with individual points in space being upwards of 4 hectares in size. An early implementation of such a model was described by Caprio & Tabashnik [22], which used a spatial system dynamic modelling approach to determine how stochastic factors influence the effects of migration on the development of resistance among several populations. Caprio [21] used a variation of this model to determine the effect that refuge strategies could have in IPM scenarios where a sprayed Bt pesticide is used. It was found that, when refuge areas were included, resistance would take 4.4 times longer to develop than cases where refuge was omitted. It was also found that low gene flow increased the risk of resistance development. Peck *et al.* [83] developed a spatially explicit model to explore the development of resistance in *Heliothis virescens* (Lepidoptera: Noctuidae) populations on Bt cotton on a regional level. It was found that refuge areas were highly effective at curbing resistance development and that higher migration and dispersal rates also delayed resistance development. Storer *et al.* [108] adapted this model for *Helicoverpa zea* (Lepidoptera: Noctuidae) in a mixed agroecosystem consisting of maize and cotton, where it was found that farm-level refuge requirements are important as resistance would readily develop in localised clusters in areas where Bt usage is high, even when the surrounding area has low Bt usage. Tyutyunov *et al.* [114] developed a spatially explicit reaction-diffusion model to study what refuge landscapes would prevent resistance development and it was found that larger, contiguous refuge areas were more effective than a large number of smaller refuge patches. Kang *et al.* [58] developed a population-based model to study the development of resistance in *Diatraea saccharalis* (Lepidoptera: Crambidae) in a mixed agrolandscapes consisting of maize (Bt and non-Bt), sorghum, sugarcane and rice. The heterogeneity of the agrolandscapes proved to make effective refuge planning more complicated as planting dates and maturation rates would differ, forcing migration of the insect populations. It was also found that an intermediate dispersal rate was often better at curbing resistance than extremely low or high dispersal rates.

A series of studies co-authored by Sisterson and Tabashnik focussed on the development of resistance in insect populations to Bt crops [103, 104, 112]. The general model can be described as a square grid of fields that are often very large, usually at least 10 hectares each. Each cell would be populated with a number of eggs, larvae and adults of the target species and would be assigned a daily mortality and maturation rate. Adults were allowed to move between fields only once in their lifetime and would happen either before mating or after mating, and the magnitude of their movement would be dependent on an appropriate distribution, such as a bivariate normal distribution as used by Sisterson *et al.* [104] to study the effects of field distribution and insect movement on resistance development. All insects were modelled as populations within a field, so in each time step, often one day, a proportion of eggs would hatch, die, mature to larvae and be laid by females, a proportion of larvae would mature or die and a proportion of moths would move, mate, die or lay eggs. The model used is technically a discretized reaction-diffusion model also related to spatial system dynamics, and reaction-diffusion models can be considered approximations of individual-based models in cases where movement of individual agents is modelled using a random walk. One of the key results from his studies include combining sterile insect releases with refugia to limit resistance development in pink bollworm populations in a Bt cotton scenario. The results obtained from the simulation model indicated that this combination would nearly render the refuge area useless, as continual, but sufficiently large, insect releases would limit resistance by itself. An eradication program instituted in Arizona from 2006 to 2009 found that the introduction of large scale sterile insect releases not only decreased the resistant allele frequency, but also in a 99.9% reduction in infestation levels [112]. The key results obtained from from Sisterson *et al.* [103, 104] included the importance of refuge distribution, population size and movement, as resistance was found to develop rapidly in scenarios where insect movement was low and Bt fields were isolated from refuge areas.

Whereas the spatial models described above were very large-scale models, smaller scale, individual-based models are rare due to the complexity of their implementation. In terms of modelling on a relatively microscopic scale, a model developed by Garcia *et al.* [41] focussed on the prediction of resistance development due to larval movement. The implementation modelled each stalk of a plant as an individual cell in a cellular automaton, with females able to oviposit on the stalk and move between stalks. It was found that larval dispersal had different effects on resistance development given different refuge layouts. Strip refuges fared better in scenarios with high larval movement, block refuges preferred intermediate dispersal and seed mixes ("refuge-in-a-bag") did best with low dispersal rates.

3.5 Chapter summary

A general overview of the history and theory surrounding computer simulation was presented in this chapter. Some core concepts such as the various simulation paradigms, including where each would possibly be most suitable, and components of simulations were discussed using a simple example. The steps one should follow in a successful simulation study were also presented. This was followed by a discussion of some of the most important advantages and disadvantages of simulation modelling and the chapter was concluded with a brief review of the applications of simulation modelling in ecological systems, including its applications to *E. saccharina* and refuge modelling in a Bt scenario.

CHAPTER 4

Simulation Specifications

Contents

4.1 Model paradigm **27**
4.2 Modules **28**
  4.2.1 Field object 28
  4.2.2 Egg and larval objects 30
  4.2.3 Moth object 35
4.3 Simulation framework **36**
  4.3.1 Life stage management 37
  4.3.2 Time step management 39
  4.3.3 Female mating and oviposition 42
  4.3.4 Moth movement between cells 43
  4.3.5 Moth movement between layers 46
4.4 Simulation outputs **46**
4.5 Model implementation **47**
4.6 Chapter summary **47**

The choice of paradigm, structure and specifications for the model is described in this chapter. The model can be split into two principle components: the simulation framework that handles the simulation space, manages the agents and maintains the timing elements, and the modules that represent the agents that populate the simulation. As explained in Chapter 3, there are many assumptions regarding processes, variables and parameters to include in a simulation model, especially when deciding on the level of abstraction. Due to the spatial scale of the proposed model, it was decided that a higher level of abstraction would be required than the model developed by Van Vuuren *et al.* [120]. Due to the computational complexity of the model, several elements of Van Vuuren's model had to be aggregated to expand the spatial scale to several hectares.

4.1 Model paradigm

The four main simulation modelling paradigms were discussed in §3.1.3, detailing the level of abstraction of each. To observe gene flow at a low level within a population, it was required that the entities, or moths, in the model be represented as individuals that can interact with each other and their environment. For this reason it was decided that the most applicable simulation paradigm for this model would be an agent-based model. Significant groundwork for modelling

E. saccharina as an agent-based model already exists in the work of Van Vuuren *et al.* [120], which further reinforces the choice of this modelling paradigm.

As discussed in §3.1.1, there are several components to a simulation model. The real-world system of *E. saccharina* on sugarcane can be represented as a model, with the moths, larvae, pupae, eggs and the sugarcane itself being represented as *entities*, each with their own *attributes* such as age and gender. There are *activities* that occur, such as the moths ageing or the sugarcane growing, as well as *events* such as a female moth laying eggs, which adds new *entities* to the model, or harvesting which removes some of the sugarcane. There are also *states* in the form of daily temperatures and time that passes. The technical specifications of how the system is translated to a *model* will be discussed in this chapter.

4.2 Modules

The model uses classes or objects to represent agents that can populate it and there are four main objects, known as modules, in the developed model:

- Field
- Eggs and Larvae (which includes pupae)
- Adult moths

Each module is an entity within the model and has a set of attributes and activities that can be queried or executed to drive the behaviour of the agent represented by the module. The attributes can either be set by the user or is set by the simulation during runtime.

A key assumption is that the immature life stages (eggs, larvae and pupae) are essentially immobile and once the eggs are laid by a female moth it is assumed that the larval dispersion is so small that it can be ignored completely, meaning that an agent in its immature life stages will remain stationary until it emerges as an adult moth. This assumption significantly reduces the complexity of the immature life stages and effectively reduces it to maturation and mortality.

4.2.1 Field object

Due to the simplicity of the sugarcane as an organism as discussed in §2.1, a collection of sugarcane plants in a finite area is modelled as a single object. This collective module is referred to as a Field and the attributes and methods contained within the Field object are listed in Table 4.1.

The `Variety` (binary, can be either Bt or non-Bt) and `Stressed Ratings` are static attributes and are set at initialisation and can be changed manually by the user. The remaining attributes are dynamic and are updated each simulation step. The Field object receives two input parameters every time step that it uses to update these dynamic attributes: `days` and `temperature`. These values feed into the CANEGRO model's equations for sugarcane growth which is housed in `Set_Growth_Rate`. The output from `Set_Growth_Rate` is used to update the `Height` of the cane using the `Set_Height` method and to update the `Stalk Capacity` of the cane using the `Set_Capacity` method. `Days` is then used to update the `Age`, using the `Set_Age` method, of the cane which affects the `Moth Capacity`. A `Harvest` procedure can be called that sets all the parameters except for `Variety` and `Stressed Rating` to 0.

	Name	**Type**
Attributes	Age	Integer
	Variety	Binary
	Height	Float
	Stalk Capacity	Integer
	Moth Capacity	Integer
	Growth Rate	Float
	Stressed Rating	Float
Methods (Activities)	Set_Age	
	Set_Capacity	
	Set_Growth_Rate	
	Set_Height	
	Harvest	

Table 4.1: *The attributes and methods contained within the Field object.*

4.2.1.1 Sugarcane growth

Sugarcane, like other grasses, grow in terms of stalk length and leaf length, with stalk length being a function of the leaf length. The CANEGRO model is a comprehensive sugarcane growth simulation package that can predict sugarcane growth based on a variety of inputs. Part of the model is an equation for leaf growth, and it assumed that stalk growth is roughly 16% of that. The derived equation for stalk growth $l(t)$ is

$$l(t) = 0.16(r)(-1.32 + 0.176(\tau_t - 10)) \tag{4.1}$$

where r is 48 for a one year crop and 24 for a two year crop and τ_t is the temperature for day t and $l(t)$ is measured in millimetres [13]. Using the different r values will accelerate the growth for a one year crop, leading to a one year crop reaching similar heights than a two year crop in half the time. Using the stalk growth, $l(t)$, at time t, the new stalk `Height` can be set as $h(t) = h(t-1) + l(t)$. The model follows a similar approach to that of Potgieter [86] and Van Vuuren *et al.* [120] and assumes that this growth, variety (resistance rating) and the stressed rating are the only elements that are required to model the interaction between *E. saccharina* and sugarcane. The stalk growth is stored in and returned by `Set_Growth_Rate`, which is added to the height of the cane on day $t-1$ by `Set_Height` to give the new stalk `Height`.

4.2.1.2 Stalk capacity

The model sets the same assumption as that of Van Vuuren *et al.* [120] with regards to larval capacity of the sugarcane stalk, where a default value of 40mm uninfested cane is required for a larva to reach full maturity, as Atkinson [5] specifies between 2 and 8 centimetres is sufficient for a larva to reach maturity and be competitive in mating. This value can be scaled down to some degree by setting the `Stressed Rating` of the cane, which will result in the cane being slightly stunted in vertical growth, but ultimately being more nutritious leading to a decreased requirement for the feeding larvae. It is also assumed that a larva that cannot get sufficient nutrition to reach full maturity, i.e. there is less than 40mm of cane for it to feed on, will be uncompetitive as a moth when looking for a mate and is removed from the model. Another assumption in Van Vuuren *et al.* [120] regarding sugarcane capacity is that there are approximately 129600 sugarcane stalks in a cultivated hectare, which is the equivalent of 13 stalks per square metre. Based on the above assumptions, the larval capacity (lc) in the model is given by

$$lc(t) = \frac{13(\frac{ah(t)}{3})}{40s} \tag{4.2}$$

where a is the area in square metres, $h(t)$ the height of the stalks in millimetres at time t (`Height`) and $s \in \{0.5, 1\}$ is the `Stressed Rating` of the cane. The area is divided by 3 as the larvae show a distinct preference for the lower sucrose-rich third of the stalk. Once a batch of larvae are mature enough to bore into the stalks, the `Stalk Capacity` is reduced by the minimum of the remaining `Stalk Capacity` and the number of larvae attempting to bore into the stalk. Once the number of larvae is equal to $lc(t)$, any larvae attempting to bore into a stalk will be removed as it is assumed that the overcrowding will result in any new larvae failing to thrive. To account for new growth, the `Stalk Capacity` is also updated by an incremented capacity, ic, every time step t by

$$ic(t) = \frac{13(\frac{al(t)}{3})}{40s} \tag{4.3}$$

where $l(t)$ is the cane growth for time t as expressed in Equation 4.2. The new `Stalk Capacity` is then $lc(t) = lc(t-1) + ic(t)$.

4.2.2 Egg and larval objects

The objects that represent the immature life stages of the moth are very similar in their attributes and methods. The most significant difference is that the larval object is responsible for assigning the final genotype and is divided into two stages representing the larva outside and then inside the stalk.

4.2.2.1 Egg object

The eggs in the model are not modelled as individuals, but as clusters of eggs laid by the female. The attributes and methods contained within the Egg object is listed in Table 4.2.

	Name	**Type**
Attributes	Age	Float
	Female Gene	List of lists
	Male Gene	List of lists
	Number	Integer
	Survival Rate	Float
Methods (activities)	Set Age	

Table 4.2: *The attributes and methods contained within the Egg object.*

The current size of the cluster is stored in the `Number` attribute. The `Male Gene`, `Female Gene` and `Survival Rate` are static and are set at initialisation. `Male Gene` and `Female Gene` are simply lists that contain both the `Bt Resistance` arrays from the male and female parent, respectively. Details on these arrays are available n §4.2.3. The Egg object receives the `degree days` as a parameter each time step and uses it to calculate the maturation rate of the eggs as part of the `Set_Age` method, which is a function of time and temperature. The degree-day `Age` of the eggs is represented as a proportion of the accumulated degree-days necessary to mature, so eggs exposed to higher temperatures will mature and hatch faster than those exposed to lower temperatures. If the eggs do not reach full maturity in a given time step, the age is updated and a survival probability is applied to the eggs to simulate random natural mortality. When the eggs are matured, the Egg object returns the parameters which should be used as input for a new larvae object.

4.2.2.2 Larvae object

The Larvae object is similar to the Egg object, but as discussed in §2.2.1 the larval stage broadly consists of two phases. The first phase represents the larvae outside of the stalk and the second phase represents the larvae that has bored into the stalk as well as the pupae. As with the Egg object, the Larvae object represents a cluster of larvae from the same group of eggs and not individuals. This is done to limit the number of agents in the model at any given time and it can be assumed that larvae do not disperse far enough from where they hatch to justify modelling them as individuals. The distinction between larvae outside the stalk and larvae inside the stalk is kept due to their different survival rates. The attributes and methods contained in the Larvae object is listed in Table 4.3.

	Name	**Type**
Attributes	Age	Float
	Female Gene	List of lists
	Male Gene	List of lists
	Number	Integer
	Stage 2 Indicator	Binary
Methods	Set Age	

Table 4.3: *The attributes and methods contained within the Larvae object*

The `Male Gene` and `Female Gene` are static attributes and are set at initialisation. The Larvae object receives the `degree days` as a parameter for the `Set_Age` method each time step and uses it to calculate the maturation rate of the larvae, which is a function of time and temperature. The `Age` of the larvae is represented as a proportion of maturity, so larvae exposed to higher temperatures will mature faster than those exposed to lower temperatures. If the larvae do not reach full maturity in a given time step, the `Age` is updated and a survival probability is applied according to the phase it is in, its genetics and the sugarcane it is on.

In the first phase the larvae can only mature to the point where it has bored into a stalk. Once it reaches the second phase a survival probability is applied that represents random and natural mortality outside of the stalk, meaning that mortality is only applied once per cluster of larvae during this phase. Once the second phase is reached, the different genotypes relating to Bt resistance derived from the `Male Gene` and the `Female Gene` is created and stored as a list, resulting in four genetic sub-populations as seen in Table 4.4 if only a single MOA is considered, or if two MOAs are considered, 16 genetic sub-populations are generated as seen in Table 4.5. Survival rates are then calculated for the genotype clusters depending on whether or not they are resistant and whether or not the sugarcane they feed on is a Bt variety. All Larvae objects in the second phase theoretically reach full maturity and mortality is only applied once they are set to be promoted to moth objects. As levels of crop damage due to feeding activity are not within the scope of this thesis, only applying mortality at a single point in time is reasonable.

		Male	
		m	M
Female	f	fm	fM
	F	Fm	FM

Table 4.4: *A punnett square of the possible genetic combinations of parental alleles for a single allele.*

		Male			
		mm	mM	Mm	MM
Female	ff	ffmm	ffmM	ffMm	ffMM
	fF	fFmm	fFmM	fFMm	fFMM
	Ff	Ffmm	FfmM	FfMm	FfMM
	FF	FFmm	FFmM	FFMm	FFMM

Table 4.5: *A punnett square of the possible genetic combinations of two separate alleles.*

As the pupal stage does not add to the required summary statistics in the output of the model, its maturity rate has been combined with the larval stage. Hence the larva that has bored into the stalk will require more degree-days to reach maturity to compensate. Theoretically, the egg and larva objects could be combined into a single object, but to simplify on reporting and verification outputs it was decided to keep these two life stages separate.

4.2.2.3 Maturation rates of Eggs and Larvae

Extensive experiments on the maturation rates of *E. saccharina* were performed by Way [126] under controlled laboratory conditions. The maturation rates for all the immature life stages were found to be significantly correlated with the temperature experienced by the individual, with lower temperatures leading to significantly longer maturation times (as detailed in Table 4.6). To determine the maturation rates for the model, the experimental rates were plotted against the temperature and a polynomial was fit to the data. To keep computational time to a minimum, the polynomial of lowest degree was always used that would give a satisfactory fit. During each time step t, the daily temperature τ_t is read in by the immature life stage agents and the maturation rates are updated using the following equations:

$$r_e(t) = -0.0000226609268\tau_t^3 + 0.00149116532\tau_t^2 - 0.02255014329\tau_t + 0.15225212124 \tag{4.4}$$

$$r_l(t) = -0.000001147\tau_t^4 + 0.00008242\tau_t^3 - 0.0018976\tau_t^2 + 0.0180336\tau_t - 0.05135045 \tag{4.5}$$

$$r_p(t) = 0.0016\tau_t - 0.0166 \tag{4.6}$$

where $r_e(t)$ is the maturation rate for the eggs, $r_l(t)$ for the larvae outside the stalk and $r_p(t)$ the rate for larvae inside the stalk and pupae combined. The curves and how they fit to the experimental data in Table 4.6 is shown in Figure 4.1. It was decided to combine the life stages of the larvae inside the stalk with the pupae to simplify the model and because the pupal life stage is generally short compared to the larval stage.

Once an agent enters a new life stage, such as a newly laid egg or an egg hatching into a larva, its `Age` is set to 0. Every time step thereafter, the applicable maturation rate r is calculated daily for the appropriate daily temperature τ_t, measured as *degree days*. This value is added

	Eggs		**Larvae (outside stalk)**		**Larvae (inside stalk)**		**Pupae**	
°C	Days	Rate	Days	Rate	Days	Rate	Days	Rate
13	16.8	0.06						
15	13.2	0.076	85.1	0.01175	97	0.01031	38	0.026
20	8.6	0.116	36.32	0.02753	50.5	0.0198	19.6	0.051
25	6.1	0.164	19.4	0.05155	30.3	0.033	9.8	0.102
30	4.8	0.208	12.7	0.07874	22.9	0.04367	8	0.125
35	4.6	0.217	14.88	0.0672	19.9	0.05025	6.1	0.164

Table 4.6: *The average maturation rate (in days) of the different immature life stages of* E. saccharina *[126], with the maturation rate per day taken as its reciprocate value.*

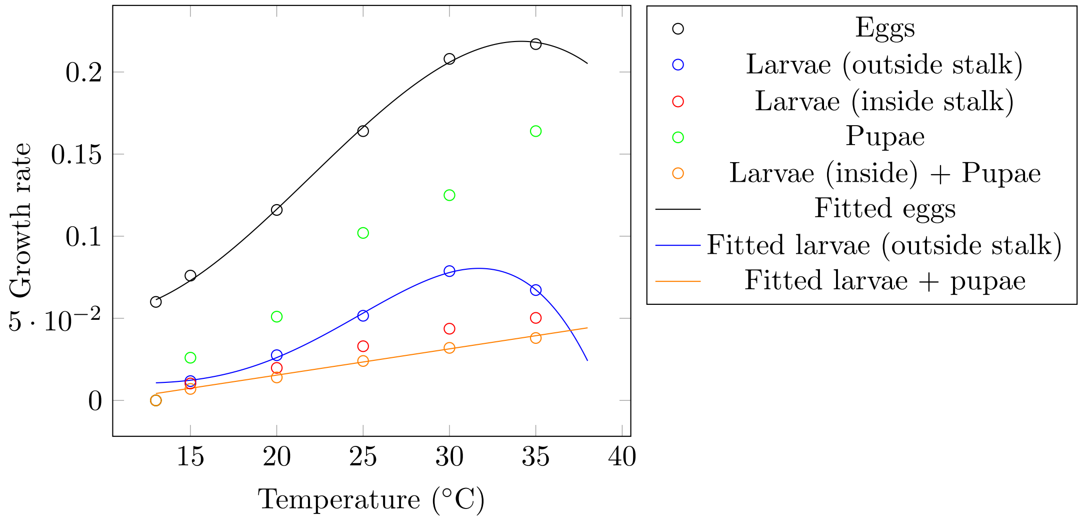


Figure 4.1: *The maturation rates of the immature life stages including the combined maturation rate of the inside stalk larvae and pupae as detailed in Table 4.6.*

to the current `Age` and checked by the model, if `Age` is equal to or greater than 1, the agent is considered matured and will move to the next life stage. The larva has two distinct life stages, when `Age` is equal to 1 it will progress from an outside-stalk larva to boring into the stalk. Once the larva is inside the stalk it will mature until `Age` is equal to or greater than 2, at which point it will mature to an adult moth.

4.2.2.4 Mortality rates of Eggs and Larvae

Through significant experimentation, the Entomology Department at SASRI could determine stage-specific mortality rates for *E. saccharina*. Natural and predator mortality is stage specific and calculated at the point where they would progress to the next life stage for larval objects and at each time step for egg and moth objects. Mortality is applied as a rate, where given a mortality rate $d \in (0, 1)$ and n insects in a batch, there will be $\lfloor \max(0, (1 - d)n) \rfloor$ insects in the next step for egg objects and in the next life stage for larva objects. Moth mortality is applied as a probability, with a moth having a $1 - d$ likelihood of surviving to the next day. The probabilities that determine the rates are derived from rates described by Van Coller [117] (see Table 4.7). The mortality rates are dependent on temperature, but to facilitate a single mortality event in the Larvae objects, the effect of temperature on mortality is ignored and could be explored in future work.

Due to the mortality function in the model being a once-off process for the Larvae objects, the values in Table 4.7 had to be converted into a value that would capture the mortality of the

	eggs	larvae (outside stalk)	larvae (inside stalk)	pupae	adult moths
Mortality rate (per day)	0.03	0.112	0.009	0.007	0.2

Table 4.7: *The daily stage specific mortality rates for* E. saccharina *at 26° C [117].*

objects through the entire life stage. In conversations with the entomologists at SASRI, it was speculated that the likelihood of a young larvae outside the stalk surviving to the point where it would bore into the stalk would only be approximately 10%. For larvae that have successfully bored into a suitable stalk, the survival rate would be much higher, with between 80 and 85% of larvae emerging as adult moths [32]. Based on the information obtained from Table 4.7 and from the entomologists, the mortality rates in the model are summarised as in Table 4.8.

	eggs	larvae (outside stalk)	larvae/pupae (inside stalk)	adult moths
Mortality rate	0.03	0.9	0.15	0.2
Frequency	Daily	Once	Once	Daily

Table 4.8: *The stage specific mortality rates for* E. saccharina *at 26° C [32, 117] as applied in the model.*

Mortality for larvae inside the stalk is also dependent on the type of sugarcane that it feeds on, as this is the point where the larva is expected to ingest the largest amount of Bt proteins if the sugarcane is of a Bt varietal. If it becomes known that the protein is also present in high doses within the leaf material, this assumption may be updated to include all larvae. At this point, the larvae are still represented as a batch of insects with genes inherited from both parents, hence the susceptibility of the larvae to Bt proteins will only be known once the genetic combinations have been assigned. The combinations are only assigned once the larva/pupa is about to emerge as an adult moth, where it is assigned a random combination of its parental genes as its own genome. This genome is then used to assign the applicable mortality rate given the variety of sugarcane it fed on. The effect of the Bt proteins on the feeding larva is not immediate and it takes time for the larva to starve to death, which serves as further justification for only applying mortality once at the end of the life stage. As with pesticides, the Bt sugarcane can also express high levels of multiple Cry proteins and it is common to have varieties that express two proteins, providing two modes of action (MOA) against the target pest. It is generally assumed that the multiple modes of action would have an additive effect, meaning that if one MOA is $x\%$ effective, two MOAs would be $x + x(100 - x)\%$ effective. In a Bt scenario, a 99% efficacy against susceptible insects is considered to be high dose for a single MOA [81], which would indicate that 2 MOAs should be 99.99% effective. To provide a conservative estimate, these values are reduced to 95% and 99.75% respectively. Using these values, the mortality rates for larvae with different genomes on normal versus Bt sugarcane is summarised in Table 4.9.

	Susceptible	Resistant 1 MOA	Resistant 2 MOA
Normal sugarcane	0.15	0.15	0.15
Bt sugarcane	0.9975	0.95	0.15

Table 4.9: *The stage specific mortality rates for* E. saccharina *larvae/pupae at 26° C [32, 117] as applied in the model assuming 1 or 2 MOAs.*

4.2.3 Moth object

The moth objects are the only agents that are modelled as individuals as they are the only stage in the *E. saccharina* life cycle that has significant mobility and interacts with other individuals. The adult moth no longer matures or eats and it is essentially a race against time for them to mate and lay eggs before perishing after a few days. The moth's purpose and actions can be reduced to a handful of key actions depending on the gender of the moth. Male moths would want to find other males to form *leks* with so they can display and mate, while female moths want to find a suitable *lek*, mate with a male in the chosen *lek* and then find suitable egg-laying sites. The parent genomes from the larvae objects that are promoted to moths are used to set some of the moth object's attributes that are listed in Table 4.10.

	Name	**Type**
Attributes	Age	Integer
	Gender	Binary
	Fertility	Array
	Bt Resistance 1	Array
	Bt Resistance 2	Array
	Times Mated	Integer
Methods	Set_Age	
	Set_Fertility	
	Lay_Eggs	

Table 4.10: *The attributes and methods contained within the Moth object*

`Gender`, `Bt Resistance 1` and `Bt Resistance 2` are static attributes and cannot be modified once the moth object has been initialised. `Fertility` is a parameter that only exists for female moths, and consists of an array with three elements: the number of eggs she can lay, her genome and her male mate's genome. The array is set after mating using the `Set_Fertility` method. A female that has a `Fertility` array can call on a method called `Lay_Eggs` that generates the necessary input for a new Egg object from her `Fertility` array. Both `Bt Resistance 1` and `Bt Resistance 2` represent the chromosome pairs that determine resistance to the two Bt proteins and are represented as an array with two elements. A value of 1 in the array indicates that the gene is present, with both elements needing to be 1 for a trait to express in the case where it is recessive. In the case where 1 MOA is considered, only `Bt Resistance 1` is used. Male moths have a `Times Mated` parameter that is incremented every time the male is selected by a female to mate with. This is necessary as males can only successfully mate a finite number of times. The `Set_Age` method is used to progress the `Age` attribute of the moth with each time step.

4.2.3.1 Moth mortality

The stage-specific mortality rates for all objects are described in Table 4.8. The daily mortality rate for adult moths is set at 20% per day and is mostly due to natural causes such as predation. Because a female moth in search of a mate that decides to move during a time step can do nothing else in that same time step, mortality is only applied to newly emerged females from the second day/time step. This arrangement serves as a proxy for the case where females may move and mate in the same day. To help curb the number of inactive moths in the model, i.e. female moths that are no longer searching for a mate or have laid all their eggs, a number of hard mortality rules are also included. These are:

- any moth over the age of 6 days is removed,

- any unmated female over the age of 4 days is removed, and
- any female that has laid all of her available eggs is removed.

The rationale for the rules lie in the general biology of *E. saccharina* as described by Walton & Conlong [125], where it has been observed that almost all mating and ovipositing activities occur within the first four days of the adult moth's life. Almost all females would have mated within the first two days post emergence and it is assumed that any female that remains unmated after the third day would be 'past her prime' and no longer be viable. It was also concluded that, in controlled laboratory conditions, females would mate more than once if given the opportunity, contradicting the findings of Atkinson [7]. Despite this, and considering that more than half of the females in the laboratory conditions only mate once, it is assumed that once a female has mated and started oviposition, she will not mate again and can be removed once she no longer has any eggs left to lay. Males, as discussed in §2.2.1.1, are assumed to be able to mate multiple times and remains in the model until mortality occurs naturally.

4.3 Simulation framework

The simulation space for the model is represented as two arrays of cells. The first level is an $m \times n$ array of cells that represents the ground which contains the field, egg and larva objects as well as the fertile females in the process of laying eggs. In the model, a ground cell is the smallest, discrete piece of the simulation space. The second array of cells is a $q \times r$ array of cells that represents the canopy of the sugarcane and the sky where adult moths interact, move and mate. The sky cells can only contain adult moth objects and is larger than the underlying ground cells. A sky cell contains a grid of 5×5 ground cells that lie below it, allowing for movement and communication between the two layers. A representation of how the sky cells and ground cells fit atop each other may be seen in Figure 4.2.

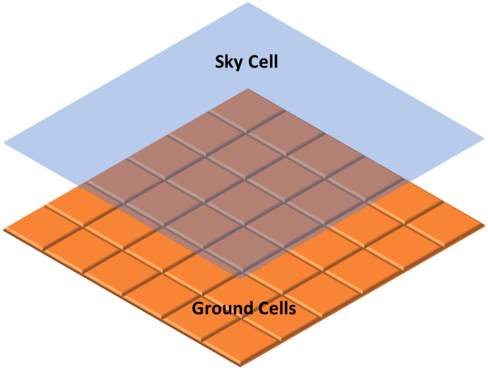


Figure 4.2: *An example representation of how the ground and sky cells are laid out.*

In the model, the cell is responsible for managing the agents within and facilitating the progress of agents from one type of module to the next, or life stage management, eg. a group of eggs hatching into larvae. The cell also assists with the movement capabilities of the adult moths.

To keep track of the agents contained within it, a cell maintains a lookup list of all agents in each object type. It also maintains separate lists for male and female moths, which simplifies the mating procedure. To facilitate movement in the neighbourhood of a given target cell, a reference list to the cells bordering the target cell, as well as all cells bordering the neighbouring cells, is stored during initialisation. The target cell is also given a reference list to every cell in the simulation space which can be used for modelling moths that fly far away from where it hatched. The cell can also call on procedures that can return certain statistics regarding its

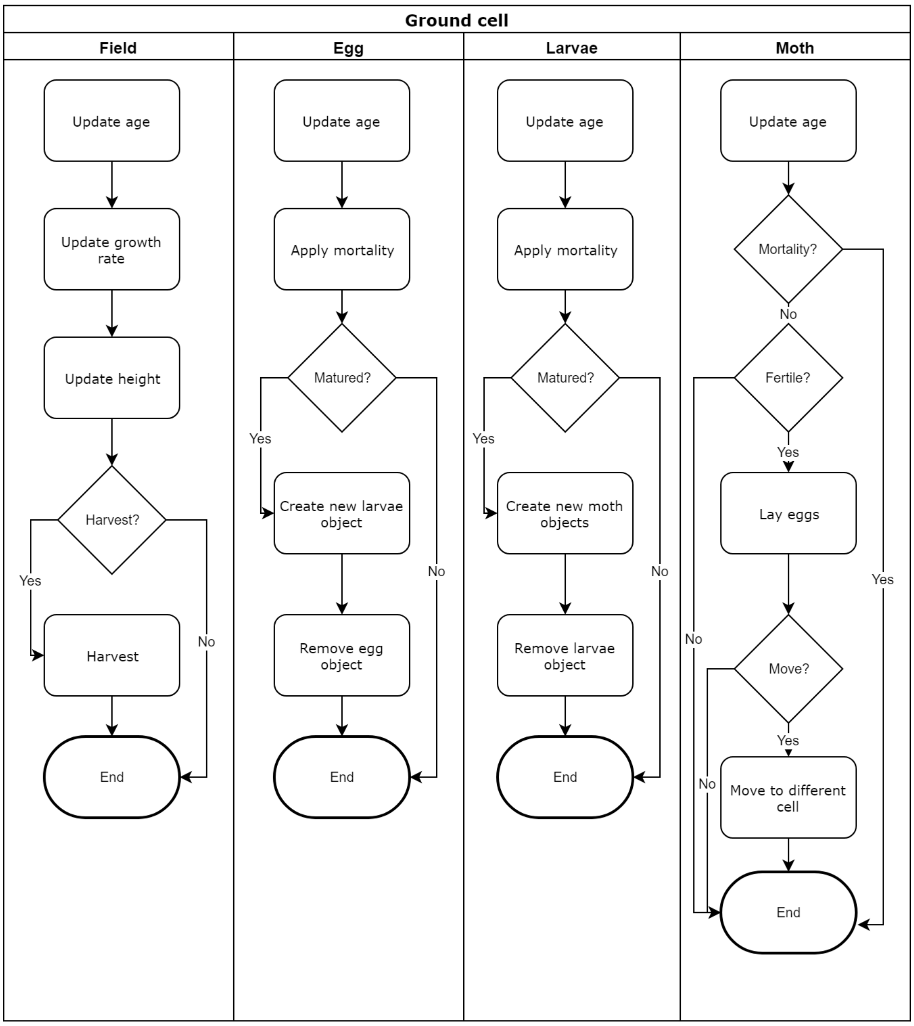


Figure 4.3: *Objects in a ground cell are all updated simultaneously and each object type is updated independently of the other.*

contents, including the total number of moths it contains and the number of resistant moths there are.

The boundary of the simulation space has been implemented as reflective, meaning that agents cannot leave or enter the simulation space across the border, but would be reflected back towards the centre of the simulation space. It was debated whether or not to include an absorbing boundary condition as done by Van Vuuren *et al.* [120], where moths were able to leave the simulation space, but without being able to allow agents to re-enter the simulation space such implementation would be problematic as the aim of the study is to study the dynamics of a local genetic population. Including migration would add significant complexity to the model and it was decided to keep a simple reflective boundary.

4.3.1 Life stage management

Moving agents from one life stage to the next is an event managed by the cell; the modules, or entities, described in §4.2 do not have their own function to progress through life stages. The model has three distinct life stages and subsequently has two processes for progressing life stages: egg to larvae and larvae (including pupae) to moth.

4.3.1.1 Egg to larvae

The egg and larvae life stages are similar in the information they contain, so progressing from the Egg module to the larvae module is merely collecting the data from the Egg object and using that as the initialisation variables for the Larvae object. The logic for the progression can be seen in Algorithm 4.1.

Algorithm 4.1: Progression from egg to larvae.

```
Data: An Egg object
Result: New cluster of larvae in a Larvae object
Call the Set_Age function for the Egg;
if eggs are mature then
    Remove Egg object from simulation;
    Write Egg attributes to new Larvae object and add to simulation;
else
    Continue;
end
```

4.3.1.2 Larvae to moth

In §4.2.2.2 it was stated that in the second stage of larval development, the larvae object generates its different genetic combinations and corresponding mortality rates according to the genes it inherits from its parents and the type of cane they fed upon. These genetic sub-populations are passed to a `Moth_Generator` method. The `Moth_Generator` takes the number of larvae and iteratively generates the number of moth objects, randomly assigning the final genetic code used to determine its resistance to the Bt protein(s), and adds them to the appropriate sky cell in the simulation space with a probability equal to one minus the given mortality rate. The logic of the `Moth_Generator` method is detailed in Algorithm 4.2.

Algorithm 4.2: Progression from larvae to moth.

```
Data: A Larvae object
Result: A set of individual Moths
for Each larva in the Larvae object do
    Randomly assign gender $G \in (0,1)$;
    Randomly assign genotype and split into chromosome pairs;
    Get mortality rate $s$;
    Generate uniform random number $r$;
    if $r \leq (1-s)$ then
        Set Gender as $G$;
        Set Bt_Resistance 1 from 1st chromosome pair;
        Set Bt_Resistance 2 from 2nd chromosome pair;
        Set Fertility to null;
        Add Moth to appropriate lookup list in sky cell;
    end
end
```

4.3.2 Time step management

In the model, time progresses in discrete daily time steps. Every step, each agent needs to be updated and this function is handled by the cell. When a new time step starts, a function called `Next_Step` is called that iteratively starts updating each agent in the cell by calling a subroutine that handles the agents of a specific module. Ground cells contain all Field, Egg and Larvae objects and can also be populated by fertilised female Moths. The process followed to update all objects in the ground cell are detailed in Figure 4.3. The sky cells can only be populated by adult moths and the process followed to update the moth objects are detailed in Figure 4.4.

4.3.2.1 Field module update

To update the field, the length of the time step in fractions of a day and the temperature in degrees Celsius is passed to the `Next_Field` function that sets the new age of the cane as well as the amount of growth that occurred in that time given the temperature according to equations from the *CANEGRO* model. There is a `harvest` flag that would result in the cane being harvested using the `Harvest` method, resetting all the values of the field object to `null`. The logic is detailed in Algorithm 4.3. `Set_Growth_Rate` and `Set_Height` contain the equations described in §4.2.1.1, while `Set_Capacity` updates the Stalk Capacity as described in §4.2.1.2.

Algorithm 4.3: Updating the field object (`Next_Field`)

```
Data: Length of time step, temperature
Call Set_Age method;
Call Set_Growth_Rate method, returns growth;
Call Set_Height with growth as input;
Call Set_Capacity with growth as input;
if Harvest flag is set then
    Call Harvest method;
end
```

4.3.2.2 Egg and larvae module update

The update process for both Egg and Larvae objects are similar as they both simply develop until they reach the next stage in their development, where the functions as specified in §4.3.1 are then called. The length of the time step in fractions of a day and the temperature in degrees Celsius is used to set the new age of the eggs or larvae, and if the `Set_Age` function within the Egg or Larvae object returns a flag stating that the development cycle is complete, the appropriate method from §4.3.1 is called. The logic for updating Egg and Larvae objects are detailed in Algorithms 4.1 and 4.2.

4.3.2.3 Moth module update

The moths are the most active agents in the model and have the most complex update process. This process differs depending on whether the moth is in the sky cells or in the ground cells. As moths are able to move from cell to cell as part of its update process, it is first necessary to check that a moth has not already been updated in a given time step. If the moth has not been updated, the update process continues, else it ends and the next moth is considered.

For moths in the sky cells, the first phase of the update process is checking for random mortality and removing the moth from the simulation if true. If the moth survives, a `Movement` function

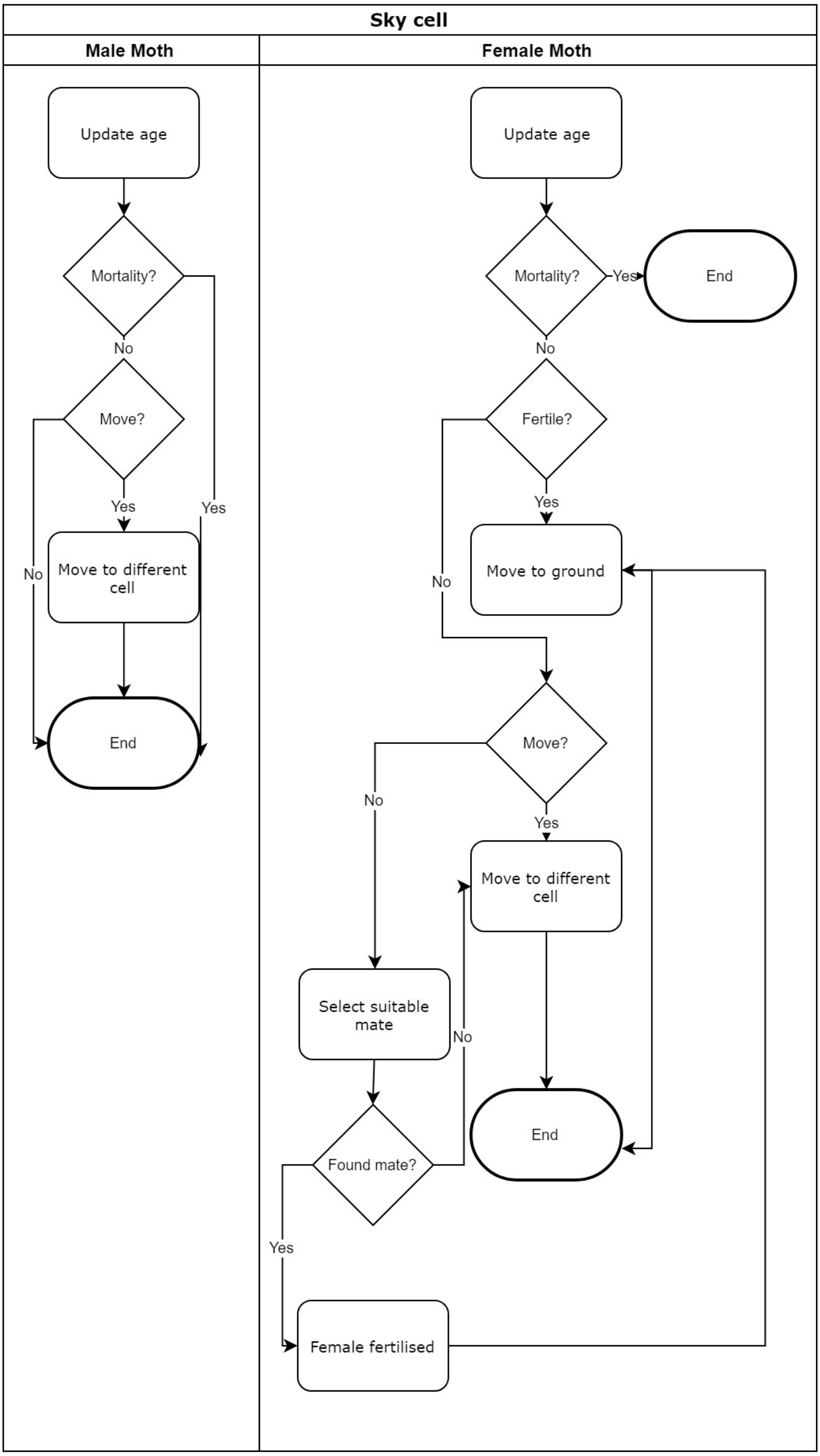


Figure 4.4: *Objects in a sky cell are all updated simultaneously and each object type is updated independently of the other.*

is called that determines whether or not the moth will move from its current cell and if so, to which cell. If the moth moves the update process ends. If the moth does not move, its gender is checked and if it is a male moth, its `Times Mated` attribute is checked. If it has reached the maximum threshold, the moth is removed, else the process ends. All females in the sky cells are yet to be fertilised and search the cell for a male moth to mate with, where a male could possibly mate more than once in an evening. If she finds a mate, she is fertilised and is able to lay eggs. Once fertilised, the female descends from the sky cell into a randomly selected ground cell connected to the specific sky cell, otherwise the update process ends. The update process for the moths in the sky cells is given in Algorithm 4.4.

For moths in the ground cells, the first phase of the update process is checking for random mortality and removing the moth from the simulation if true. If the moth survives a `Movement` function is called that determines whether or not the moth will move from its current cell and if so, to which cell. If the moth moves the update process ends. All moths in the ground cells are fertilised females that search for sites to lay eggs. If a female still has eggs left to lay, she will lay a batch in her current cell, else she is removed from the simulation. A female only has a finite number of days where she can lay eggs and this may be shorter than her lifespan, but the moth is still removed to reduce the model complexity as it will no longer have a purpose. The update process for the moths in the ground cells is given in Algorithm 4.5.

Algorithm 4.4: Updating a moth in a sky cell during a time step

```
Data: A list of moths in the cell
for Each moth do
    if moth already updated this time step then
        Skip this Moth;
    else
        Continue with update;
    end
    Call Set_Age method;
    Apply random mortality;
    Call Movement routine;
    if Moth chooses to move then
        Move moth to desired cell;
        Remove moth from cell;
        End update;
    end
    if Moth is female AND there are males in cell then
        Select random male;
        Increment Times Mated for selected male;
        Call Set_Fertility;
        Move female from sky cell to randomly selected ground cell;
        End update;
    end
    if Moth is male then
        End update;
    end
end
```

4.3.3 Female mating and oviposition

An unmated female in the sky cells can choose any male in the same sky cell to mate with. As described in §2.3, males usually form *leks* of 3–6 individuals to display. Due to the cells being so large, implementing the *lek* system would add unnecessary complexity to the model and is for the most part disregarded completely, except that if a cell does not have enough males to form a *lek*, she will move from her current cell. As a result, a female moth's choice in male is mostly a random one, except that she will not mate with a male that has already mated more than 5 times, as it was shown by Walton & Conlong [125] that this was the upper limit of times a male would mate in laboratory conditions. The male moths that have already mated 5 times are not removed from the model once they reach this limit, as they should still be able to influence the female moth's biased flight.

Once the female has mated with a male in the sky cells, she will descend into the ground cells and start ovipositing eggs. In §2.2.1 it was stated that the average fecundity of a female was approximately 500 eggs, but the mean fertility ratio of the eggs was 63.2% [125], meaning that the average mated female would be able to produce just over 300 fertile eggs in her lifetime. In the model, a female that mates with a male would be set as fertilised with between 300 and 350 eggs, drawn from a uniform distribution, and would be able to start ovipositing the day after fertilisation. While the female oviposits her eggs in batches of up to 20 eggs, she will usually oviposit between 30 and 150 in a day, with most eggs (50%) oviposited on the second day after emergence as may be seen in Figure 4.5. The variance of the percentage of eggs oviposited per day was also found to be small, with a standard error of 3.9%.

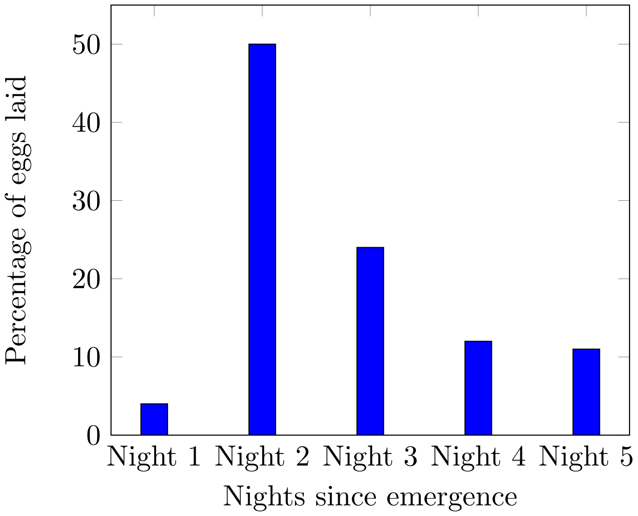


Figure 4.5: *The average percentage of eggs laid by a fertile female per night post-emergence [125].*

Due to the model implementation not allowing female moths to oviposit on the first day, the proportion of eggs oviposited on the first night is distributed equally to the remaining four nights. Hence a female fertilised with a number of eggs n_e, will oviposit a specific number of eggs for a given number of days after fertilisation as described in Table 4.11.

Once the fertilised female enters a ground cell, she starts ovipositing. She has the option to move to neighbouring cells in the form of a random walk, but due to the female's movement during this time being very localised, there is only a very small likelihood of her doing so. If she does decide to move, she will oviposit first as to avoid moths simply moving from cell to cell of viable egg-laying sites without ever ovipositing.

Days after emergence	Days after fertilisation	Batch size
2	1	$0.5n_e$
3	2	$0.24n_e$
4	3	$0.14n_e$
5	4	$0.12n_e$

Table 4.11: *Given a total number of fertile eggs* n_e*, the number of eggs the female will oviposit given a number of days since fertilisation is set according to a fixed ratio.*

4.3.4 Moth movement between cells

There are different `Movement` routines available to the moths depending on their gender and whether they are in the sky cells or in the ground cells. The routines available are:

- Male moths
  - **Random walk** – A male moth is able to move to a random sky cell in its immediate neighbourhood with likelihood $m_m \in (0, 1)$.
  - **Biased random walk** – The random walk is applied in the sky cells, but is weighted according to the average age of the underlying sugarcane.
- Female moths
  - **Random walk** –A female moth is able to move to a random sky cell in its immediate neighbourhood with likelihood $m_f \in (0, 1)$.
  - **Biased random walk** – A random walk is applied in the ground cells, but is weighted according to the available stalk capacity of the underlying sugarcane.
  - **Biased flight** – A female moth in a sky cell during a specific time step can fly from the specific cell to a neighbouring cell up to two cells away with likelihoods (m_s^1, m_s^2), where $m_s^1 \in (0, 1)$ is the likelihood of the moth moving to a neighbouring cell in the immediate neighbourhood, marked 'B' in Figure 4.6(b), and $m_s^2 \in (0, 1)$ is the likelihood of the moth moving to a neighbouring cell two cells away, marked 'C' in Figure 4.6(b).

4.3.4.1 Biased flight

The rationale, as discussed in §2.2.1.2, behind the functionality of the biased flight of the female moth lies in *E. saccharina* generally being considered to be a weak flier, but is capable of flying long distances in search of a mate. Generally females should emerge in areas where there are other male moths, as the sky cell should cover between 2500 and 10,000 square meters. By this logic, the need for the female to migrate to a different cell should be small compared to staying in the cell it emerged into. Based on this fact, the assumption is made that $1 - m_s^1 - m_s^2 > m_s^1 > m_s^2$, if $1 - m_s^1 - m_s^2$ is replaced with m_s^0, the likelihood of movement has a ratio similar to that as seen in Figure 4.6(a).

If there are no neighbouring cells that offer better mating selections, the female will not fly. If the female moth does decide to fly to a neighbouring sky cell of radius one or two, the likelihood of choosing a neighbouring cell in that radius is determined by a biased random selection, with the probability of selecting a sky cell being proportional to the number of males within it when compared to the number of males in her current cell. There are eight cells in the red (radius

Algorithm 4.5: Updating a moth in a ground cell during a time step

```
Data: A list of moths in the cell
for Each moth do
    if moth already updated this time step then
        Skip this moth;
    else
        Continue with update;
    end
    Call set_age method;
    Apply random mortality;
    Call movement routine;
    if Moth chooses to move then
        Move moth to desired cell;
        Remove moth from cell;
        End update;
    end
    if Moth is fertilised then
        Check how many eggs it has left to lay;
        if More than 0 eggs left then
            Call lay_eggs method;
            Add eggs to cell;
        else
            Remove female from simulation;
        end
    end
end
```

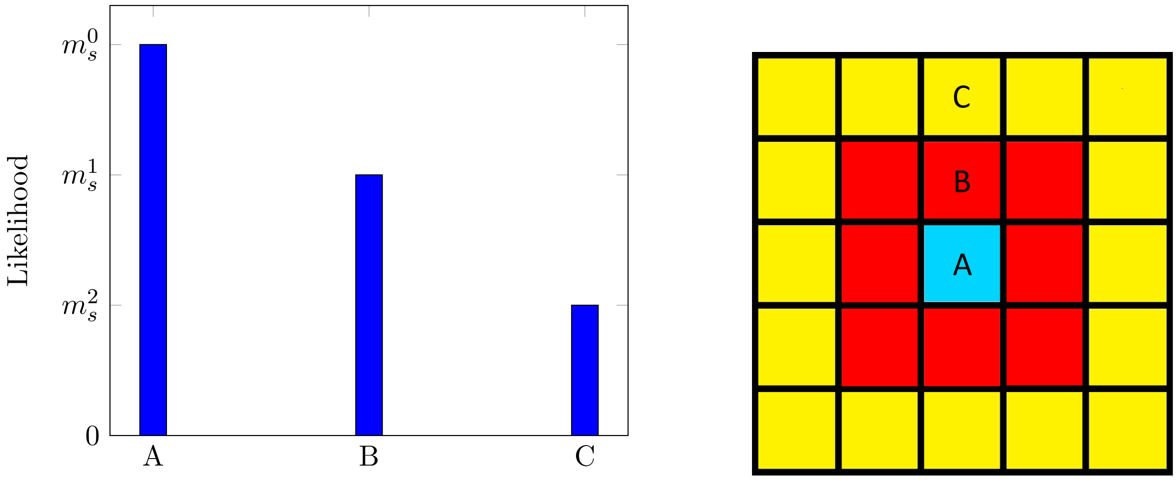
one) neighbourhood, or set A in Figure 4.6(b) and sixteen cells in the yellow (radius two) neighbourhood, or set B. if the number of males in cell i of a neighbourhood is equal to X_i and the number of males in her current cell is X_0, then the probability π_i of the female selecting cell i is

$$\pi_i = \begin{cases} 0 & \text{if } X_i < X_0 \\ p\left(\frac{X_i}{\sum_1^n X_i}\right) & \text{if } X_i \geq X_0 \end{cases} \tag{4.7}$$

where n is equal to 24 (the maximum number of neighbours within a 2 cell radius) and $p = 1$ if X_i is in set B and $p = m_s^2/m_s^1$ if X_i is in set C. This biased random selection aims to partially mimic the actual behaviour of the female as she would be attracted to areas where there are more males displaying as there would be a higher concentration of pheromones in the air. As females prefer to fly on nights with little to no wind [7], it can be assumed that the number of males displaying will be the only determining factor of the female's choice.

4.3.4.2 Biased random walk – Male

The male's biased random walk in the sky cells works in a manner similar to the female's biased flight, but is limited to cells in set B as they are considered weaker fliers than the females, as discussed in §2.2.1.2. The bias is applied as a variable likelihood for a male to move to a given cell, where the likelihood is modelled as the average age of the sugarcane in the ground cells beneath the neighbouring sky cell relative to the sky cell it is currently in. The average age for the sugarcane for the k ground cells beneath a given sky cell, Y_i, is calculated as



(a) *A symbolic representation of the movement likelihood of the female moth searching for a mate.*

(b) *The focus cell, labelled 'A', surrounded by cells in its Moore neighbourhood of radius 1, labelled 'B' in red and those cells surrounded by the Moore neighbourhood of radius 2, labelled 'C' in yellow.*

Figure 4.6: *Graphical representation of the female moth's biased flight in the sky cells.*

$$Y_i = \frac{\sum_{m=1}^{k} G_m}{k} \tag{4.8}$$

where G_m is the `Age` in days of the sugarcane in ground cell m. Similarly to Equation 4.7, if the current sky cell that a male occupies is labelled Y_0, then the probability λ_i of a male selecting a cell is

$$\lambda_i = \begin{cases} 0 & \text{if } Y_i < Y_0 \\ \frac{Y_i}{\sum_1^l Y_i} & \text{if } Y_i \geq Y_0 \end{cases} \tag{4.9}$$

where l is equal to 8 (the maximum number of immediate neighbours). The rationale for the inclusion of the biased random walk for males is further detailed in §4.3.5 and the impact will be discussed as part of the sensitivity analysis of Chapter 5.

4.3.4.3 Biased random walk – Female

The female moth in a ground cell can also embark on a biased random walk/flight if she does decide to move. The female appears to prefer cryptic oviposition sites generally found in older sugarcane [33] and may prefer to lay eggs where the cane is not already heavily infested. To account for this, an ovipositing female will choose a neighbouring cell m in set B with a likelihood proportional to the remaining stalk capacity of the sugarcane in cell m. This implicitly gives her a bias towards older cane, as older cane generally has a higher stalk capacity, and a bias towards lower density areas, as less infested sugarcane will have higher stalk capacity available. If the stalk capacity of a given cell m at time t is $lc_m(t)$ and the stalk capacity of the cell the moth is currently in is $lc_0(t)$, then the probability, β_m of the moth moving to a given cell in their neighbourhood is

$$\beta_m = \begin{cases} 0 & \text{if } lc_m(t) < lc_0(t) \\ \frac{lc_m(t)}{\sum_1^j lc_m(t)} & \text{if } lc_m(t) \geq lc_0(t) \end{cases} \tag{4.10}$$

where j is equal to 8 (the maximum number of immediate neighbours). As with the biased flight and the male biased random walk, the probability is in relation to the stalk capacity of the current cell, hence the probability of a female moving if no other cell is better than the current cell is 0.

4.3.5 Moth movement between layers

To facilitate the movement of the female moth between layers after she has mated, it is necessary to make an assumption of which ground cell beneath her current sky cell she would descend to. It is known that the female prefers to lay eggs in older sugarcane, where there is an abundance of dead leaf material that could serve as concealed egg-laying sites [33]. It was highlighted in the work of Van Vuuren *et al.* [120] that there is still some debate on whether the clustering of *E. saccharina* around older, higher quality sugarcane was due to male moths forming leks there or if the female had a preference for higher quality cane and actively sought it out. To simulate the behaviour of the female seeking the higher quality sugarcane, the female's choice of a ground cell is a biased selection based on the age of the sugarcane in days. As in Equation 4.7, the likelihood, ρ_{im}, of the female in sky cell i selecting one of the k possible ground cells m is

$$\rho_{im} = \frac{G_m}{\sum_1^k G_m} \tag{4.11}$$

where G_m is the `Age` of the sugarcane in cell m in days. Using this method, we can expect females to descend into a ground cell with more mature cane more often, or choose a completely random cell if all the surrounding cane is the same age.

If the choice of ground cells was limited to the cells directly underneath the sky cell, as seen in Figure 4.2, the moth populations would form straight and jagged edges where the borders of the sky cells would be. To soften the edges of the descending females' dispersal pattern, the available ground cells under a sky cell is determined by the cells within a circle of radius r that is the smallest enclosing circle of the square that represents the sky cell. A representation of the 57 cells inscribed in such a circle may be seen in Figure 4.7. This also allows for a marginally greater rate of dispersal for the moths.

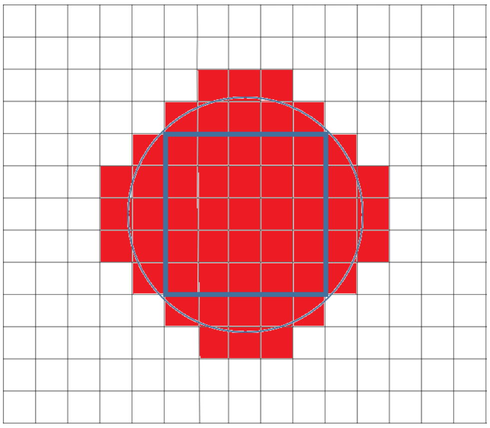


Figure 4.7: *A sky cell atop the underlying ground cells with the smallest circle inscribing the cell. The red cells are the cells the female can choose from.*

4.4 Simulation outputs

To track and monitor the outputs of the model, there are several variables available that can be called at any time during a simulation run. Every attribute in the modules detailed in §4.2

can be read and saved during a simulation run, but there are also specific functions available that assist in keeping track of the various populations within the model. The ground and sky cells have a function called `count_moths` that loop through all the moths in the given cell and return the total number of moths, the number of resistant moths and the number of heterozygote moths. The outputs of this function for all cells is then aggregated per time step into arrays `moth_run_array`, `resistant_run_array` and `carrier_run_array` respectively. A sky cell also has a `count_genotype` function that returns the frequency of each genotype within that cell. This output can then be used to determine, in conjunction with the `count_moths` function, how a gene spreads throughout the population.

The ground cells have two functions, namely `count_eggs` and `count_larvae`, that will loop through the Egg and Larvae objects within the cell and sum the `number` attributes, outputting the actual population size. These values are then aggregated per time step over all ground cells into the arrays `eggs_run_array` and `larvae_run_array` respectively.

4.5 Model implementation

The model was developed using Python 3.6 with statically typed variables, which was compiled using the Cython 0.24 compiler to aid in performance. Early verifications and validations were performed on a 64-bit personal desktop computer with an Intel Core i7-6700 CPU running at 3.40GHz with 8GB of DDR3 memory. Full simulation runs were performed on the Lengau cluster, a super computer hosted by the Centre for High Performance Computing of the CSIR (*http://www.chpc.ac.za*).

4.6 Chapter summary

In this chapter, the basic structure of the simulation model was discussed without emphasis on the underlying assumptions, starting with the rationale behind the choice of simulation type and paradigm. In §4.2 class structures of the various modules were discussed, with emphasis on the attributes and methods contained within the class to help drive agent behaviours. This section also contains details about the underlying assumptions made for each module. In §4.3 the algorithms and logic that the model employs to run the simulation was discussed, with particular focus on how the life cycles of the sugarcane and the moths are handled and how information is passed from one module to the next. The different ways in which an adult moth can move through the simulation space is also discussed. The chapter concludes with the technical model implementation details.

CHAPTER 5

Verification, validation and sensitivity analysis

Contents

5.1 Verification 49
    5.1.1 Verification of cane growth 50
    5.1.2 Verification of larval capacity in the stalk 50
    5.1.3 Verification of immature life stage maturation and mortality 52
    5.1.4 Verification of adult moth life cycle 53
5.2 Validation of immature life stage maturation and mortality 58
5.3 Face validation 60
5.4 Sensitivity analysis 62
    5.4.1 General experimental set-up 63
    5.4.2 Results 66
    5.4.3 Discussion 70
5.5 Chapter summary 74

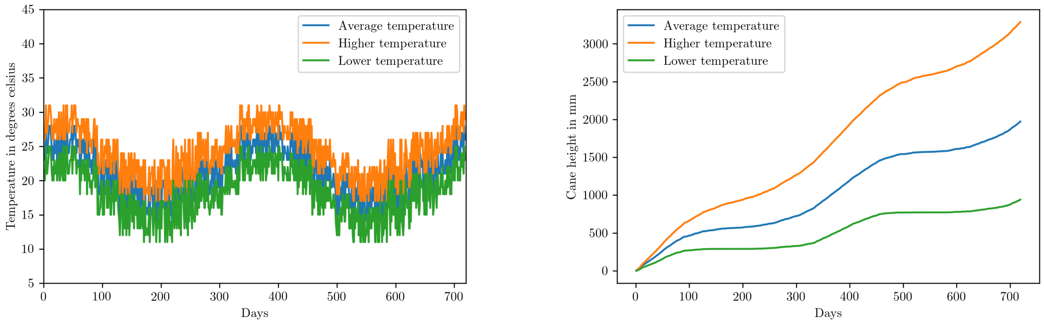
With the model developed as in Chapter 4, it is necessary to perform verifications on the behaviour of these objects as part of the steps to developing a simulation model as discussed in §3.2. The next step is then to validate its performance. Validation is done by means of comparison with data available, expert feedback in the form of a face validation, and sensitivity analysis. The sensitivity analysis includes a detailed discussion on the experimental set-up that will be used to test the sensitivity of the model to parameter variations in two sets. The first set of parameters will focus on variables pertaining to the moth agents, while a second set will focus on parameters related to the sugarcane field. The results from these experiments will help guide the study in developing insightful case studies for the following chapter by highlighting what changes appear to have the largest impact on the model.

5.1 Verification

Sargent [101] describes computerized model verification, or simply verification, as the process of ensuring that the computer programming and implementation of the conceptual model is correct. They also note that models developed in high level programming languages, such as C, Java or Python, are generally more difficult to verify than purpose built simulation languages, but do offer more flexibility. To verify the components of the developed model, output is taken from the program after a certain input given. The output is scrutinised to confirm whether or not the behaviours observed are expected as implemented.

5.1.1 Verification of cane growth

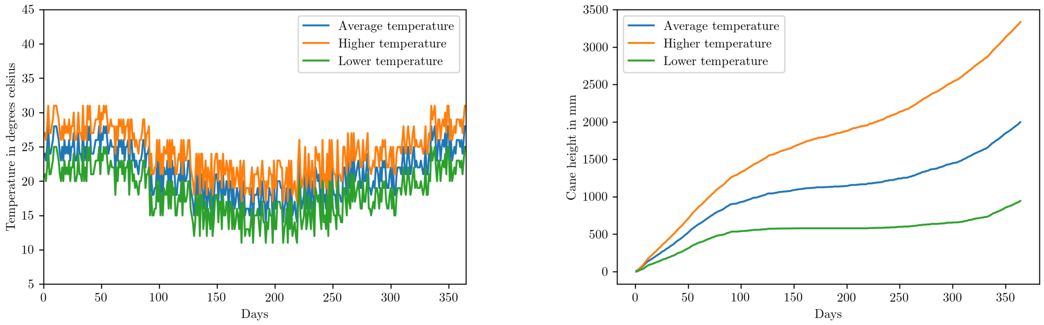
The stalk growth equation from the CANEGRO model describes a linear relationship between daily temperatures and growth rate. Higher temperatures should lead to higher growth rates and vice versa.



(a) *Temperature experienced by the model.* (b) *Corresponding sugarcane height over time.*

Figure 5.1: *Sugarcane growth as an output from the CANEGRO model equations over two years for average inland KwaZulu-Natal temperatures.*

In Figure 5.1(b) the simulated sugarcane stalk height (`Height`) over time may be seen over three different temperature ranges, as seen in Figure 5.1(a). The higher and lower temperatures are a 3 degree inflation and deflation of the average temperature, respectively. Growth rates accelerate during the warmer months and flatten out during the colder winter months, with growth essentially flattening out where the temperatures were deflated, meaning that minimum temperatures were closer to the 10 degrees celsius threshold at which sugarcane does not grow new leaf material. In Figure 5.2(b) the same eventual growth may be seen over a single year as the CANEGRO equation for a single year crop increases growth rate for the same temperature by exactly a factor of 2.



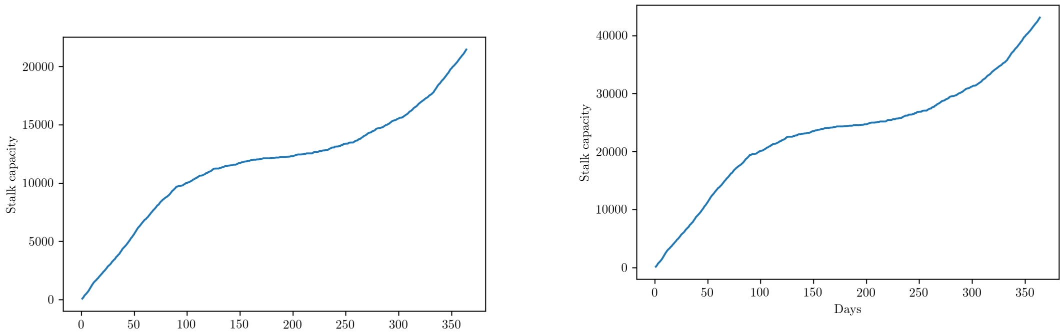
(a) *Temperature experienced by the model.* (b) *Corresponding sugarcane height over time.*

Figure 5.2: *Sugarcane growth as an output from the CANEGRO model equations over one for average inland KwaZulu-Natal temperatures.*

5.1.2 Verification of larval capacity in the stalk

For `Stalk_Capacity` there are more complex interplays involved between different aspects of the model, but most importantly it needs to be verified that the stalk capacity (1) increases as the cane grows, (2) decreases as the larvae infest it, and (3) scale appropriately should the cane become stressed.

It is expected that the `Stalk_Capacity` should increase proportionately with stalk growth, so for cane that is not stressed and is not infested with larvae the increase in `Stalk_Capacity` should follow the same curve as seen in Figures 5.1(b) and 5.2(b). In this test, the cane height was not reduced proportionally to the `Stressed_Rating` to simplify the verification process.



(a) *The total stalk capacity of all stalks in a ground cell with unstressed cane ($s = 1$)*

(b) *The total stalk capacity for all stalks in a ground cell with maximally stressed cane ($s = 0.5$).*

Figure 5.3: *Sugarcane stalk capacity for minimally and maximally stressed cane for the same temperatures as experienced in Figure 5.2(a).*

As may be seen in Figures 5.3(a) and 5.3(b), the shape of the curves for stalk capacity over one year follows the same shape as the sugarcane height in Figure 5.2(b) for average temperatures as expected. The curves are identical apart from the scale of the y-axis in Figure 5.3(b) being exactly double that of Figure 5.3(a). These results satisfy both points 1 and 3 above. To demonstrate the effect of larvae boring into the stalks, the above experiment is repeated, but 50000 larvae are added after 50 simulated days. Once they mature enough to bore into the stalk a noticeable drop of about 5000 in capacity should be observed approximately 14 simulated days later as 90% of larvae outside the stalk perish before being able to drill into the stalk.

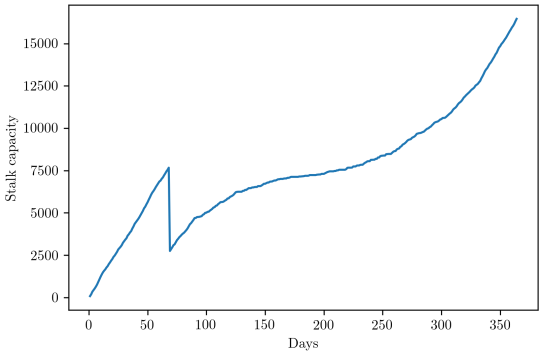


Figure 5.4: *The total stalk capacity for a ground cell with minimally stressed cane ($s = 1$) with the introduction of larvae at 50 days.*

In Figure 5.4, the effect of the larvae may clearly be seen between the 50 and 100 day marks, where the entire batch of 5000 remaining larvae simultaneously bored into the sugarcane stalks, verifying point 3 above.

5.1.3 Verification of immature life stage maturation and mortality

To verify the maturation and mortality processes of the immature life stages in the developed model, a series of experiments were conducted at different constant temperatures to observe whether or not the actual maturation and mortality rates are in line with the predicted rates. The initial setup of the experiment will include four batches of 500 fertilised eggs on a single cell of sugarcane. The first set of experiments will run on normal sugarcane and the second batch on Bt sugarcane. The parental genes for the four batches are detailed in Table 5.1.

Batch number	male gene 1	male gene 2	female gene 1	female gene 2
1	0,0	0,0	0,0	0,0
2	1,0	0,0	0,1	0,0
3	1,1	0,0	1,1	0,0
4	1,1	1,1	1,1	1,1

Table 5.1: *The genetic makeup of the four egg batches in the experiment, where 1 indicates a resistant allele and 0 a susceptible allele.*

The experiments will also run for two temperatures, 20°C and 30°C, which should highlight the difference in maturation rates. The duration of each experiment is set to a maximum of 120 days, which is the theoretical time it should take for a newly laid egg to mature to adulthood at 20°C. Each experiment will be repeated ten times and the average value over those ten runs will be analysed to verify the model performance. The experimental design is summarized in Table 5.2.

Experiment number	Temperature °C	Sugarcane variety
1	20	Non-Bt
2	20	Bt
3	30	Non-Bt
4	30	Bt

Table 5.2: *The experimental design to test the performance of the model with regards to egg and larva maturation and mortality.*

When considering the time to maturation, the only influencing factor should be the temperature. In Table 5.3 it may be seen that, for experiments 3 and 4, the time to maturation is significantly shorter than in experiments 1 and 2. This indicates that an increase in temperature is influencing the maturation rates as expected.

	Days to maturation		
Experiment number	Eggs	Larvae 1	Larvae 2
1	9	37	66
2	9	37	66
3	5	13	32
4	5	13	32

Table 5.3: *The experimental results showing the number of days required to mature with regards to Egg and Larvae objects, where experimental pair 1 and 2 and pair 3 and 4 are identical due to only temperature influencing maturation rates.*

The overall mortality for eggs is directly proportional to the temperature, as the temperature influences the maturation rate. A higher maturation rate means that more eggs will mature each day. As mortality is applied to eggs daily, this leads to fewer eggs to apply mortality to. This will lead to fewer eggs succumbing to natural mortality, hence in hotter temperatures it is expected that more eggs will survive to hatching. In this way, the model implicitly models the higher mortality rates experienced by eggs in colder temperatures. This is evident in Table 5.4, where approximately 10% more eggs mature to larvae where temperatures were increased by 10°C.

Experiment number	Eggs Actual survival rate
1	0.786
2	0.786
3	0.884
4	0.884

Table 5.4: *The experimental results with regards to egg mortality, showing the proportion of eggs that survived to maturity.*

The larval stage has mortality that is dependent on the genome, hence it was necessary to calculate how many moths of each phenotype given a set of parental genes were expected to emerge and apply the appropriate mortality rate. In Table 5.5, where there was no Bt sugarcane in experiments 1 and 3, it may be seen that the proportion of surviving larvae remains fairly constant. This aligns with the proportions surviving in experiments 2 and 4 for batch 4, where homologous resistant for both alleles larvae should have the same mortality rates in the Bt sugarcane as susceptible larvae do in non-Bt sugarcane. The proportion of surviving larvae in batches 1, 2 and 3 should increase as observed due to the likelihood of the larvae being heterozygotes are 0% for batch 1, 25% for batch 2 and 100% for batch 3.

	Larval survival rates			
Experiment number	Batch 1	Batch 2	Batch 3	Batch 4
1	0.0837	0.0862	0.0852	0.0842
2	0.00051	0.001	0.00402	0.079
3	0.0882	0.0846	0.0837	0.0833
4	0.000	0.00181	0.00543	0.0841

Table 5.5: *The experimental results with regards to larval mortality (survival rate) for the different genetic combinations in Table 5.1.*

5.1.4 Verification of adult moth life cycle

To verify that the implementation of the adult moths was done correctly, it is necessary to test several scenarios to measure various outputs. Some of the core outputs that need to be verified are

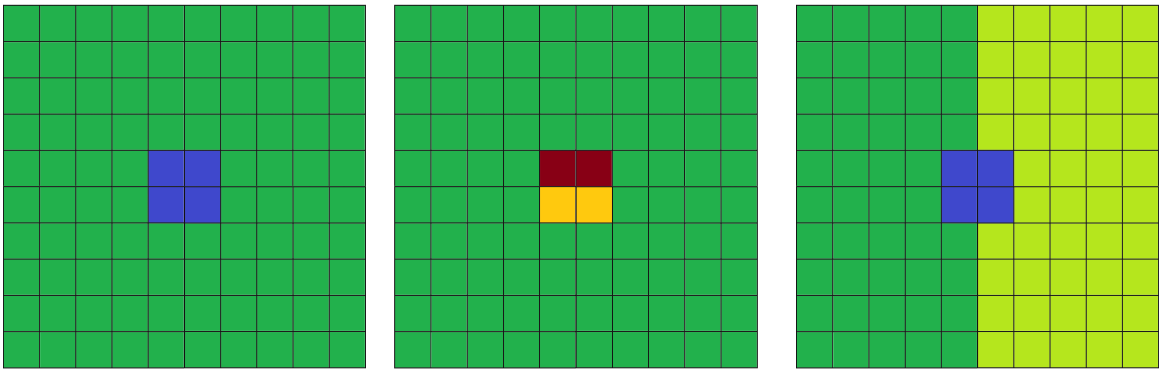
1. that in an environment with no control measures, the population should, within the available stalk capacity, grow nearly exponentially due to their high fecundity,
2. moths are removed when mortality conditions are met,
3. males are only allowed to mate up to 5 times,
4. females should move towards areas with more males,

5. females should move towards older sugarcane when starting to oviposit, and
6. sugarcane age/capacity biases should have an influence on how the moths move around, if implemented.

To test these outputs, three distinct scenarios were implemented and the relevant outputs were stored and analysed or visualised where applicable. These scenarios were:

1. **The one year population**: A population of 100 newly emerged adult moths are inserted into the middle of the simulation space. The temperature is kept constant at 30 degrees Celsius and the sugarcane throughout is of a uniform age, starting at six months old and the simulation is run for 365 simulated days. The scenario is displayed in Figure 5.5(a).
2. **The single generation**: A population such as the one in the previous scenario is created, but with males being focused on a single area. The simulation only runs for 10 simulated days. The scenario is displayed in Figure 5.5(b).
3. **The edge of two fields**: The first scenario is repeated, except that one half of the sugarcane is newly planted and one half is 3 months old in the first iteration. In the second iteration, one half is 3 months old, and the other half is 4 months old. The scenario is displayed in Figure 5.5(c).

A combination of scenarios 1 and 2 was used to verify outputs 1, 2 and 3, scenario 2 was used to test for output 4 and scenario 3 was used to test for outputs 5 and 6. To run the verification tests, a set of fixed parameters was used as specified in Table 5.6 and a set of 10 simulation runs were executed. The average of these runs was analysed.



(a) *The sugarcane age and type is homogeneous throughout and the male and female moths are equally distributed in the centre four sky cells.*

(b) *The sugarcane age and type is homogeneous throughout, but the male and female moths are split up with males in the red cells and females in the gold cells.*

(c) *The sugarcane type is homogeneous throughout, but the darker green half is three months old, compared to the light green that is newly planted. The male and female moths are equally distributed in the centre four sky cells.*

Figure 5.5: *A graphical representation of the scenario layouts for the verification of the adult moths, showing only the sky cells in detail.*

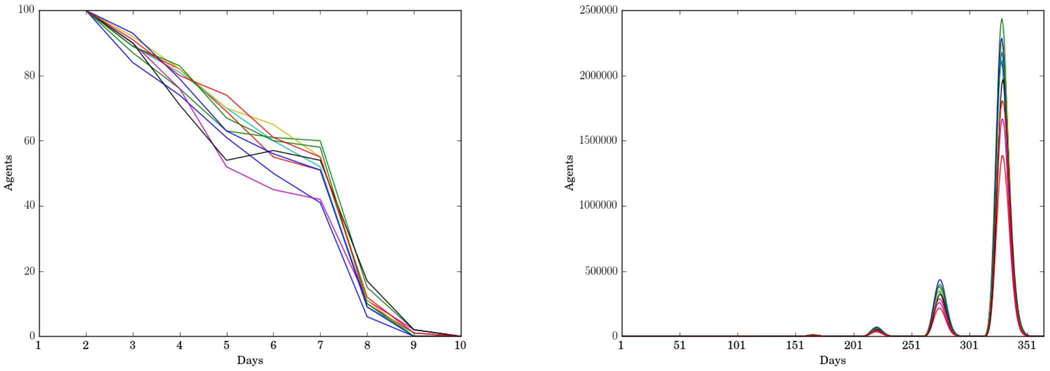
Parameter	Value	Unit
Temperature	30	°C
Ground cell		
Size	25	m^2
Grid	50×50	N/A
Sky cell		
Size	$25\ (5 \times 5)$	ground cells
Grid	10×10	N/A
Field		
Harvest cycle	24	months
Starting age	180	days
Bt proportion	0	percent
Eggs		
Initial population	0	agents
Mortality (daily)	3	percent
Larvae/pupae		
Initial population	0	agents
Mortality (outside, once-off)	90	percent
Mortality (inside, once-off)	15	percent
Female moth		
Initial population	50	agents
Proportion susceptible	100	percent
Proportion heterozygotes	0	percent
Proportion resistant	0	percent
m_s^1	10	percent
m_s^2	1	percent
m_f	10	percent
Mortality (daily)	20	percent
Ground cell movement	Random walk	N/A
Male moth		
Initial population	50	agents
Proportion susceptible	100	percent
Proportion heterozygotes	0	percent
Proportion resistant	0	percent
m_m	10	percent
Mortality (daily)	20	percent
Sky cell movement	Random walk	N/A

Table 5.6: *The parameter values that are fixed for the output verification.*

5.1.4.1 Scenario 1: The one year population

When considering a cohort of moths that emerge on the same day, it is expected that none of the moths should remain after the eighth day, as all males should be removed when they are six days old and all females should be removed by day seven if they are laying eggs, else, if unmated, day three. In Figure 5.6(a), where the populations are measured at the start of the day and given a one day warm-up before running, it may be seen that on day 8, where males would turn six days old, there is a significant decrease in population as they are removed. By day 10, no moths are left as the last of the egg-laying females have been removed from the simulation after spending four days in the ground cells and having no more eggs to lay on day five.

As implemented, a female can lay up to 350 eggs in her lifetime, meaning that an uncontrolled



(a) *The first days of a newly emerged cohort shows where some of the hard removal rules are triggered.*

(b) *The population growth over one year for ten simulation runs shows a population explosion, reaching a maximum of nearly* 2,500,000 *adult moths.*

Figure 5.6: *The output of the one year population scenario, showing total adult moth population.*

population would explode even if less than 10% of the eggs would reach maturity. If all 50 females in the starting cohort were fertilised and was able to lay the maximum of 350 eggs, the following should hold:

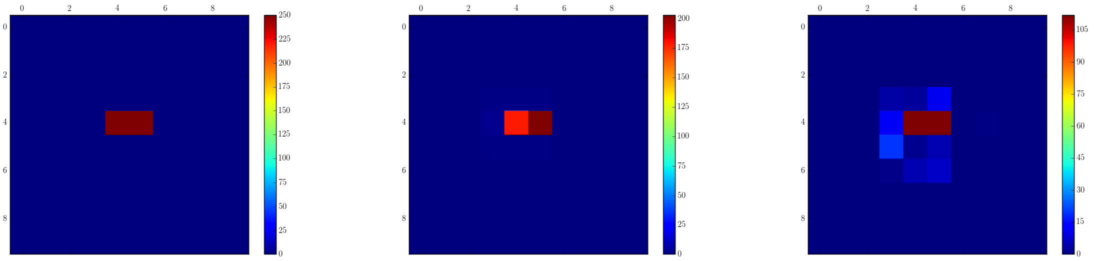
- The eggs should mature in 5 days, with 85% surviving,
- only one in ten larvae will bore into a stalk,
- but 85% will pupate and emerge as adults.

Given that 50% of moths are female, for each adult moth in a generation it can be expected that there will be up to $0.5 \times 350 \times 0.85 \times 0.1 \times 0.85 = 12.64$ moths in the next generation, or an order of magnitude increase. This would mean the maximum population after seven generations, as in this scenario, would be over 400 million adult moths. The first three generations are small compared to the last four, making them difficult to see in Figure 5.6(b). As seen in Figure 5.6(b), the maximum population was still below 2500000 adults after seven generations, a growth rate of approximately 4.4 moths per generation. This is expected, as not all females will mate and be able to lay eggs, not all moths will have 350 fertile eggs to lay, and not all females will lay all their fertilised eggs before dying.

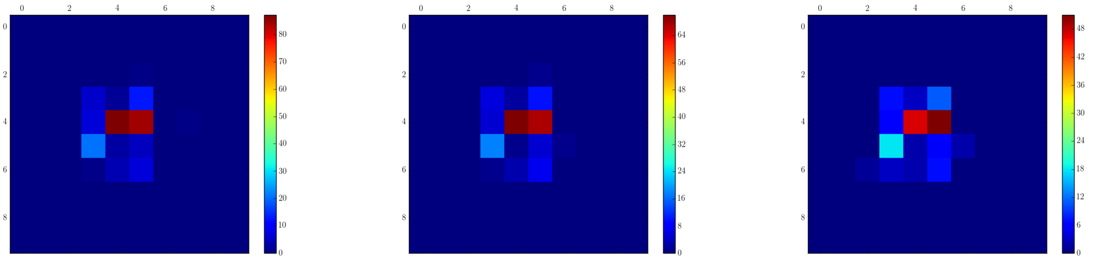
5.1.4.2 Scenario 2: The single generation

The male moth should disperse randomly and in low numbers throughout the sky cells. In Figure 5.7, the male's random dispersal may be seen over the six days that the cohort survives, showing a random dispersal around the two sky cells they emerged from. The volumes per sky cell are taken as the sum of the moths per cell over all 10 runs, leading to the colourbars displaying higher numbers than expected. This was done simply to create a larger value range for plotting purposes. In Figure 5.8, it may be seen that by day 2 nearly every female has migrated from their original cells in row 5, to the much more densely male populated cells in row 4, indicating that the females do show significant bias towards cells with more males as well as the pressure to migrate when there are no males works as intended.

In laboratory conditions when presented with newly emerged virgin females every night, males were observed to mate an average of 3.3 times in their lives [125], which serves as an upper



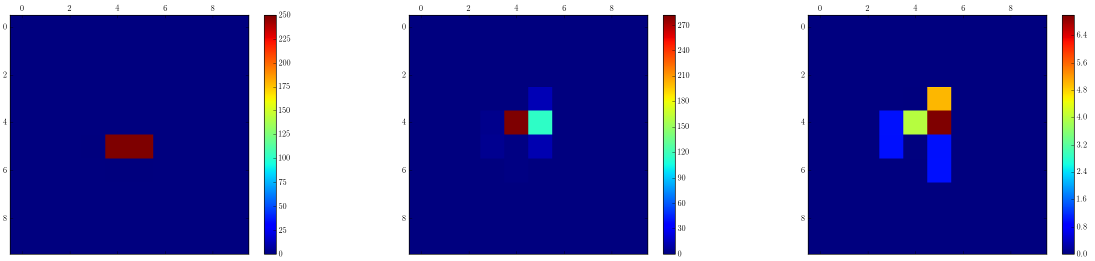
(a) *Day 1* (b) *Day 2* (c) *Day 3*



(d) *Day 4* (e) *Day 5* (f) *Day 6*

Figure 5.7: *The random dispersal of the male moths, shown as the sum of the moths in each sky cell over 10 runs.*

bound for male mating frequency. It was found that, in all runs there would always be at least one male that would mate the maximum of 5 times, but in the 10 runs it was found that a male would mate an average of 1.36 times, which in a competitive environment such as that in the scenario is reasonable and well within the upper bound.



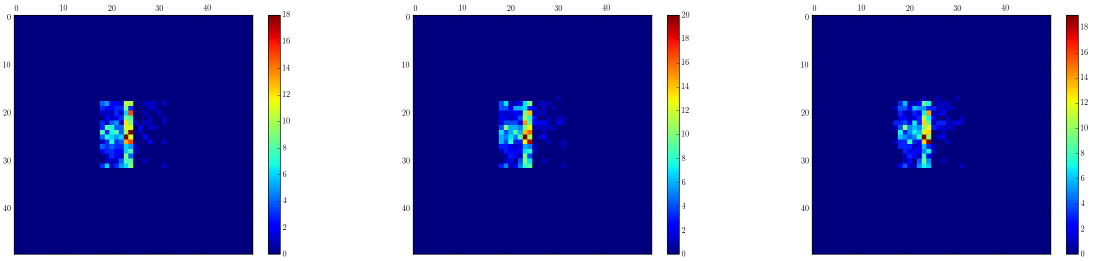
(a) *Day 1* (b) *Day 2* (c) *Day 3*

Figure 5.8: *The biased dispersal of the female moths towards where the male moths were, shown as the sum of the moths in each sky cell over 10 runs.*

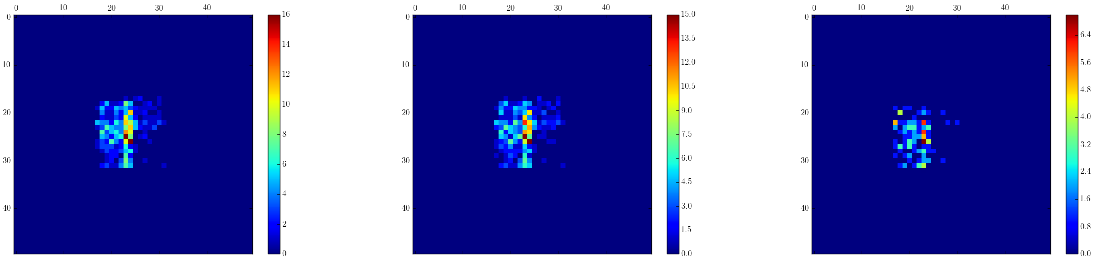
5.1.4.3 Scenario 3: The edge of two fields

In this scenario, the functionality of the female moth descending into more mature sugarcane and the male congregating around more mature sugarcane is tested. As in Figure 5.5(c), the left half of the field is older than the right half, with the first iteration having cane 90 days and 1 day old, and the second iteration having cane 120 and 90 days old.

For iteration 1, even though females and males were evenly distributed in the middle of the simulation space, the female moth's preference for the more mature sugarcane on the left is shown clearly in Figure 5.9. When the experiment is repeated in iteration 2, there is still a bias towards the left as seen in Figure 5.10, but it is not nearly as dramatic as in the first iteration. This is preferable, as it is not realistic to assume that the moths can accurately differentiate between cane that is relatively close in age.



(a) *Day 2* (b) *Day 3* (c) *Day 4*



(d) *Day 5* (e) *Day 6* (f) *Day 7*

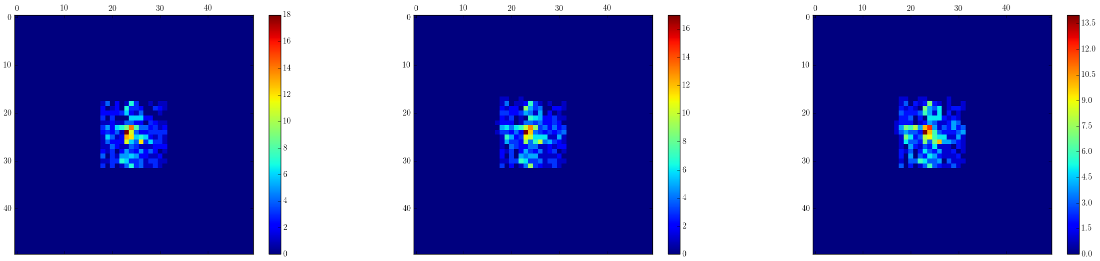
Figure 5.9: *The female moths prefer the older sugarcane much more than the newly planted cane, staying mostly on the left of centre.*

This scenario can also be used to test the male moth's biased random walk. Iteration 1 is repeated with male moth movement set to a biased random walk and their dispersal can be seen in Figure 5.11. Although males have a very low likelihood of moving, it may be seen that most dispersal outside of the centre is focused on the left of the simulation space, indicating that the males do tend to prefer the more mature sugarcane.

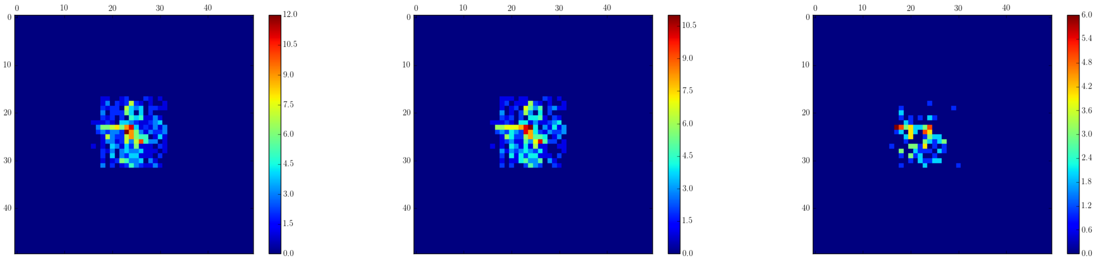
5.2 Validation of immature life stage maturation and mortality

As there is some data available regarding the maturation and mortality rates of the immature life stages, it is possible to perform a validation of the model outputs to the existing data. The experiments of §5.1.3 are repeated and the outputs are compared to their expected values.

When considering the time to maturation, a slight variation in maturation rate between the values in Table 4.6 and the output from Equations 4.4, 4.5 and 4.6 could result in some small deviation between the expected values from Table 4.6 and actual values from the simulation output. This is evident in Table 5.7, where there is a difference of several days between the actual and expected values for the larva/pupa stage (Larvae 2). Upon further investigation, the maturation rate for the larva/pupa stage from the table at 20°C would equate to 0.0142 given the values in Table 4.6, but equates to 0.0154 when taken as output from Equation 4.6, leading to a marginally shorter maturation time. The actual proportion of surviving eggs closely matches the expected values, with deviations likely due to how integer rounding is handled in the model.



(a) *Day 2* (b) *Day 3* (c) *Day 4*



(d) *Day 5* (e) *Day 6* (f) *Day 7*

Figure 5.10: *The female moths prefer the 4 month old sugarcane slightly more than the 3 month old cane, staying slightly on the left of centre.*

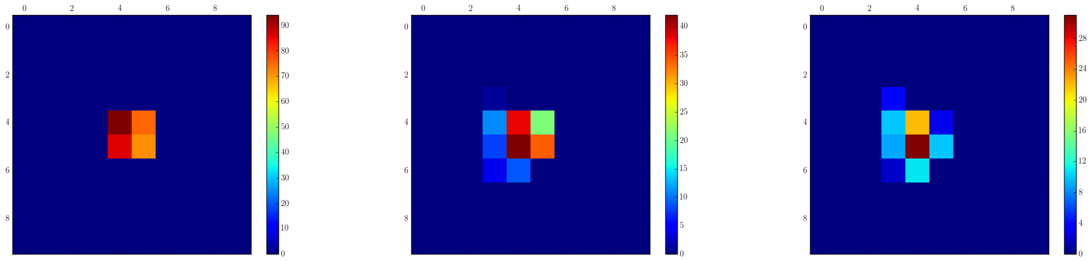
	Days to maturation					
Experiment number	Eggs Predicted	Eggs Actual	Larvae 1 Predicted	Larvae 1 Actual	Larvae 2 Predicted	Larvae 2 Actual
1	8.6	9	36.32	37	70.4	66
2	8.6	9	36.32	37	70.4	66
3	4.8	5	12.7	13	30.9	32
4	4.8	5	12.7	13	30.9	32

Table 5.7: *The experimental results showing the actual number of days required from the simulation results to mature with regards to Egg and Larvae objects compared to the expected results from Table 4.6.*

	Survival rates	
Experiment number	Eggs Predicted	Eggs Actual
1	0.7696	0.786
2	0.7696	0.786
3	0.8639	0.884
4	0.8639	0.884

Table 5.8: *The experimental results with regards to egg mortality, showing the expected proportion from Table 4.6 versus the actual proportion of eggs reaching maturity from the simulation output.*

In Table 5.9, where there was no Bt sugarcane in experiments 1 and 3, the actual moth emergence was in line with the expected values. Where the expected value was very small (less than 1% survival), the actual values tended to deviate more as it was not uncommon for zero moths to emerge from some gene combinations. Overall the actual model performance matches closely



(a) *Day 1* (b) *Day 4* (c) *Day 6*

Figure 5.11: *The biased dispersal of the male moths towards where the mature cane lies, shown as the sum of the moths in each sky cell over 10 runs.*

with the theoretical expected values, indicating that the model is handling maturation and mortality as designed.

	Survival rates							
Experiment number	Batch 1 Predicted	Batch 1 Actual	Batch 2 Predicted	Batch 2 Actual	Batch 3 Predicted	Batch 3 Actual	Batch 4 Predicted	Batch 4 Actual
1	0.085	0.0837	0.085	0.0862	0.085	0.0852	0.085	0.0842
2	0.00025	0.00051	0.00144	0.001	0.005	0.00402	0.085	0.079
3	0.085	0.0882	0.085	0.0846	0.085	0.0837	0.085	0.0833
4	0.00025	0.000	0.00144	0.00181	0.005	0.00543	0.085	0.0841

Table 5.9: *The experimental results with regards to larval mortality for the different genetic combinations in Table 5.1, showing the expected proportions from Table 4.6 versus actual proportions from the simulation outputs of larvae that survived.*

5.3 Face validation

While *E. saccharina* may be a topic of significant research due to its pest status, there are limited data available that would allow for a full validation, as laid out in §3.2, to be performed without the input of subject matter experts. While existing literature could guide the development of the model in some areas, such as potential lifespan, female fecundity and behaviour during searching for egg-laying sites, many aspects of how *E. saccharina* moves as an adult moth is poorly understood and documented. For this reason, the model was also validated at face value by subject matter experts to ensure that the behaviour of the system matches as closely as possible to the actual observed behaviours.

Model development occurred in several phases with consistent feedback and validation being provided throughout, with most of the feedback being provided by two senior researchers, henceforth collectively referred to as the validators, from SASRI. Dr. Des Conlong [32], a senior entomologist specialising in the biology of *E. saccharina*, assisted in guiding the development of the behaviour of the agents within the model and provided many key parameters necessary to implement the life cycle of the insect. Dr. Sandy Snyman [106], the principle biotechnologist, assisted in developing the scenarios that are of interest and defined the outputs that are expected from the study and also acted as the liaison between the University of Stellenbosch researchers and the SASRI researchers. Once the model verification process was completed, the draft model was presented to the validators in a workshop held at the SASRI offices in Mount Edgecomb, KwaZulu-Natal, in December 2018, the attendees are shown in Figure 5.12.

The workshop was held in three parts: the presentation of the model and accompanying as-

Figure 5.12: *The author (middle) with Dr. Des Conlong (left) and Dr. Sandy Snyman (right) at the validation workshop.*

sumptions, the presentation of a series of animated model outputs, and a final feedback session. The following points were discussed during the model presentation:

- **Life stages** – The life stages and their implementation in the model was discussed, with specific focus on the validity on combining the pupal stage with the larval stage after it had bored into the stalk. It was noted that, due to their similar mortality rates, it is a sound assumption and should not affect the outcome of the model.
- **Mortality rates** – The implementation of the mortality rates and processes were discussed, with the general consensus being that the chosen parameter values were sufficient, but that the mortality rate of the combined life stage mentioned above was too high and should rather be reduced from the default 15% to a more conservative 7.5% once off.
- **Maturation rates** – The implementation of the maturation rates as a function of temperature was well received, with commendations for slowing maturation rates at very high temperatures.
- **Female fecundity** – Feedback on the implementation of the female reproductive cycle was generally well received, with most default parameters remaining unchanged. It was pointed out by Dr. Conlong that some research and observations suggest that the female would lay the vast majority of her eggs in the first two nights after mating, and suggested modifying the ratios to those seen in Table 5.10.

Days after emergence	Days after fertilisation	Batch size
2	1	$0.5n_e$
3	2	$0.4n_e$
4	3	$0.05n_e$
5	4	$0.05n_e$

Table 5.10: *Using feedback from entomologists, during validation, the values in Table 4.11 is modified to the above values.*

- **Male movement** – Dr. Conlong agreed that not having explicit *lek* formations would be a suitable assumption given the large areas the male moths reside in. It was noted while discussing the biased walk that the males tend to seek taller cane, but that age was not always the best indicator for this as very mature cane tends to ”lodge” in high winds, meaning they collapse and then curve to grow upwards again. But it was mentioned that this was not something that happened consistently and that age as a proxy for cane height was still a suitable assumption. When queried whether the random walk or the biased walk was more suitable, it was concluded that the biased walk would be the default method.

- **Female movement - sky cell** The nature of the female's flight to find a mate was discussed, and the feedback was positive. The validators agreed that females would tend to fly towards higher concentrations of males and would prefer males that are closer than those that are further away. It was agreed that the parameters for this flight would be one of the key variables in the sensitivity analysis, as this is not an aspect that is well studied.

- **Female movement - sky to ground** The mechanics of the female moving from the sky cell to a ground cell was discussed and favourably received. It was noted that the assumptions of why the female would behave this way is sound and that the slight outward dispersal pattern appeared to be a logical approach to the problem.

- **Female movement - ground cells** The discussion of females moving along the ground cells, specifically the implementation of the biased walk, led to a lengthy discussion on the magnitude of the differences in preference towards older and younger cane. It was highlighted that, for two cohorts of older cane, for example 12 and 15 month old cane, the difference in the likelihood of a female choosing either would be much smaller than between two cohorts that are 1 and 4 months old. The validators were in favour of this assumption and again preferred the biased walk over the random walk.

- **Sugarcane field** The sugarcane field is a relatively simple object in the model, and it was pointed out that if the model could accommodate for a one or two year harvest cycle then they would be happy with all the assumptions made.

With the workshop concluded and the recommended changes implemented, the model was prepared for the final step in the validation process, a sensitivity analysis on the key input variables.

5.4 Sensitivity analysis

In a simulation model, a sensitivity analysis is the study of how variances in the input to the model parameters can influence the output of the model, and to what magnitude the output will be affected [99]. Results from a well-executed sensitivity analysis study can be used by the modeller for many purposes, Pannell [82] cites several:

- parameter variation can be used as a form of model validation [101],
- identifying important and sensitive variables,
- developing flexible recommendations that depend on certain circumstances,
- improve communication between the modellers and decision makers, and
- potentially simplifying the model by identifying insensitive variables that can be removed.

For this study, the sensitivity analysis had a lesser focus on parameters related to the insect life cycle, for example mortality rates and maturation rates, as most of the parameter values have been extensively researched and are generally well understood. Four input types were identified as being important if the model was to be used for decision making purposes:

- **Initial population** – This includes the initial population size, gender make-up and allele frequency.
- **Dispersal rates** – This includes the female's likelihood of travelling in search of a mate, how likely males are to move to different patches of sugarcane and how likely a female is to move between ground cells when laying eggs.
- **Underlying sugarcane structure** – This includes having fields of different levels of maturity, varieties of refuge layouts and sizes as well as one and two year harvest cycles.
- **Bt protein** – This includes the numbers of modes of action, whether the resistance trait is dominant or recessive, and how effective the protein is.

5.4.1 General experimental set-up

For the sensitivity analysis, some parameters were kept constant throughout to ensure that the model outputs consistent results. In this section, the parameter values that were kept constant through all experiments are listed, as well as the default values for the four input types.

5.4.1.1 Constant parameters

There are two types of constant parameters in the model, namely the parameters that govern the simulation, and parameters that impact the life cycle of *E. saccharina*. The values for the constant parameters that govern the simulation may be seen in Table 5.11. Parameters that remained constant in all experiments are labelled as non-variable. All experiments were run for a simulated time of 10 years, with the first four years serving as a warm-up period.

5.4.1.2 Input type default values

The default values for the identified input types are listed in Table 5.11 and are marked as parameters that are variable. These are the values that the varied parameter values will be compared to when assessing the sensitivity of the model.

Underlying sugarcane structure

The 25 hectare square space is divided into four equal quadrants as in Figure 5.13. Quadrant 1, 3 and 4 is of a Bt variety and quadrant 2 is a refuge area. Quadrants 1 and 3 are newly planted sugarcane, being only 10 days old and quadrants 2 and 4 being 120 days old. The cane is on a two year harvesting cycle and will be harvested once the cane age reaches 730 days.

Bt protein

The resistance trait was set as recessive and the Bt crop was set to two independent modes of action. The Bt protein was set to have a 95% efficacy per MOA, making it effectively 99.75% effective against agents with no resistance to either MOA.

Parameter	Value	Unit	Variable
Temperature	See Appendix	°C	No
Ground cell			
Size	100	m^2	No
Grid	50×50	N/A	No
Sky cell			
Size	25 (5×5)	ground cells	No
Grid	10×10	N/A	No
Field			
Harvest cycle	24	months	Yes
Bt MOA	2	N/A	Yes
Bt efficacy	95%	N/A	Yes
Gene inheritance	Recessive	N/A	Yes
Eggs			
Initial population	0	agents	No
Mortality (daily)	3	percent	No
Larvae/pupae			
Initial population	0	agents	No
Mortality (outside)	90	percent	No
Mortality (inside)	10	percent	
Female moth			
Initial population	500	agents	No
m_s^1	18	percent	Yes
m_s^2	2	percent	Yes
m_f	20	percent	Yes
Mortality	20	percent	No
Ground cell movement	Biased random walk	N/A	Yes
Proportion susceptible	100	percent	Yes
Proportion heterozygotes	0	percent	Yes
Proportion resistant	0	percent	Yes
Male moth			
Initial population	500	agents	No
m_m	20	percent	Yes
Mortality	20	percent	No
Sky cell movement	Biased random walk	N/A	Yes
Proportion susceptible	100	percent	Yes
Proportion heterozygotes	0	percent	Yes
Proportion resistant	0	percent	Yes

Table 5.11: *The parameter values are either constant for non-variable or default values for variable parameters in the sensitivity analysis.*

5.4.1.3 Warm-up

In a simulation study, one way to reduce initialisation bias in a model where starting conditions are unrealistic is to use a warm-up period. This can be defined as a time that the model is

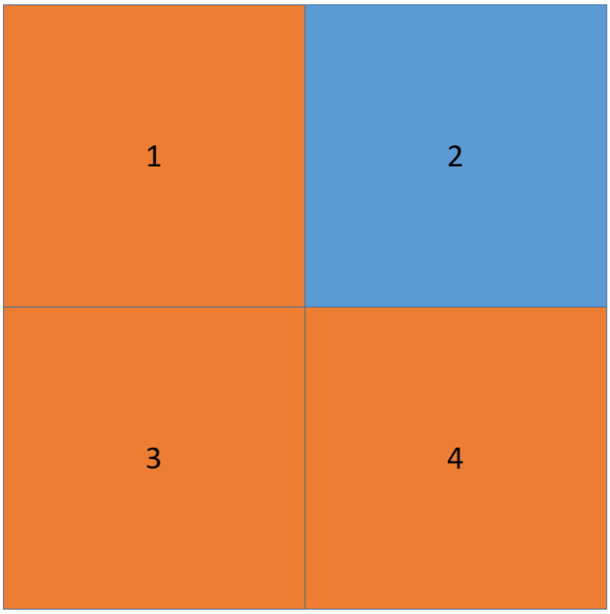


Figure 5.13: *The default 25ha field layout with the Bt quadrants shown in orange and the refuge area shown in blue.*

required to run to reach a steady state in non-terminating simulations and can be deleted when analysing the output of the model [61]. There are several ways to determine how long the warm-up period should be, ranging from graphical methods such as time series inspection, to heuristic approaches such as Kimbler's double exponential smoothing method [50]. For this study, a simple time series inspection of the adult moth population was used to determine where to terminate the warm-up time. The baseline model was run for 10 years and the average population size per day is plot for 15 independent runs in Figure 5.14. The population appears to be very unstable before the 1500th day, with a cyclical pattern emerging afterwards with the population size steadily growing due to the development of resistance. To eliminate the worst instability, it was decided that the first four years, or 1460 days, would be considered a warm-up period and results would only be derived from the remaining 6 years.

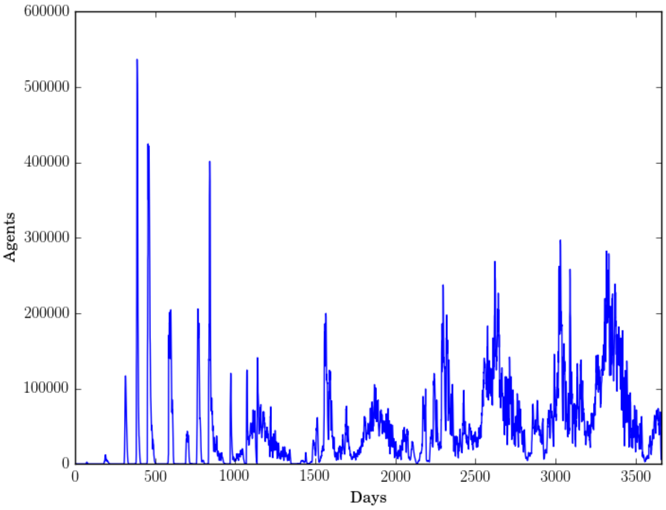


Figure 5.14: *A time series plot of the number of adult moths in the sky cells for each day of the 10 year simulation.*

There was some debate around whether the resistance genes should be introduced at the start of the warm-up period, or at the end as a random mutation in a proportion of the eggs in the model at that point in time. It was decided that the resistance genes would be introduced in the starting population, allowing the genes to naturally flow through the growing population so that there is an even distribution of resistance alleles by the time the warm-up period ends. It was also found that, in the baseline test, the development of resistance was not severely impacted in either scenario, with resistance development occurring only slightly later in the case where the gene was introduced later. The one key difference was the manner in which the moth population was perturbed, with the time series in Figure 5.15 showing significant population spikes when compared to Figure 5.14, but then stabilizing after day 3000 to the point where the two populations are nearly identical. This is likely due to the long time scale, more than four years, which allows the introduced gene to percolate through the population, eventually reaching an equilibrium point.

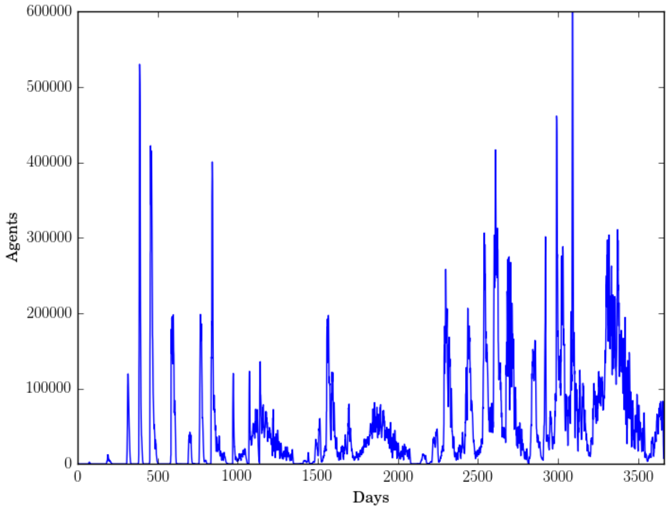


Figure 5.15: *A time series plot of the number of adult moths in the sky cells for each day of the 10 year simulation where the resistance gene was added after the warm-up.*

5.4.2 Results

The model generates a very large amount of data, so to analyse the outputs it is necessary to determine what statistics should be saved for every run, as each parameter combination is run 15 times to account for the inherent stochasticity of a simulation model. The coefficient of variation for control runs with no resistance is less than 0.05, so for a confidence interval width of 0.05, only 7 simulation runs are technically required [19]. The mean and standard deviation used was based on the average number of adult moths in a run. More runs were added to account for the added variability when resistance development is included. A number of key outputs have been identified in conjunction with the validators, namely the average population sizes for all types of agents, the maximum population sizes for all types of agents, the number of runs that developed resistance, the severity of resistance, or the average size of the resistant population, and where resistant populations were focused.

For the purpose of the sensitivity analysis, the first four outputs were considered important. The chosen parameters were varied in small increments and the changes in the summary statistics were compared as an average of all 15 runs. For the summary statistics related to the insect population, the symbols used to denote a specific statistic is listed in Table 5.12. Due to how mortality rates are applied to the immature life stages, the key metric used was the adult moth population, as the population sizes for both the eggs and the larvae/pupae are grossly inflated.

Statistic	Agent	Symbol
Average population	Adult moths	μ_m
	Resistant moths	μ_r
Maximum population	Adult moths	ω_m
	Resistant moths	ω_r
Number of runs with resistance development	–	ν

Table 5.12: *The symbols that are used to identify summary statistics for agents from the model output.*

The number of runs that developed resistance, denoted as ν, is an important metric that gives an indication of how successful a scenario was in curbing the development of resistance. For the purposes of the study, a run that develops resistance is defined as a run that, in the last 6 months, has at least 1000 fully resistant individual adult moths. This was chosen as the threshold as it indicates an order of magnitude growth for the initial resistant population and was found to be a good indicator of future resistance development as all runs with 1000 resistant moths developed large resistant populations. This point is discussed in greater detail in §6.1.1.3. The combination of ν and μ_r should provide a good metric of how likely it is for resistance to develop in a given scenario, as two scenarios that develop resistance the same number of times can be differentiated by the severity of the developed resistance, as a high μ_r indicates that there was a consistently large resistant population or that resistance developed earlier in the run. It is also important to consider these resistance measurements when observing variations in the statistics in Table 5.12, as a scenario with more resistance should drastically increase the number of agents in the model. The baseline test has all parameter values set as in Table 5.11. As there is a significant amount of stochasticity involved, the results where there was a large change (approximately 50%, denoted by ⇑ for an increase or ⇓ for a decrease) from the baseline were considered much more significant as it would be less likely that such a result would be a random occurrence.

Parameter	Value	μ_m	ω_m	Change from baseline
Gene inheritance	Recessive (Baseline)	61875	659192	–
	Dominant	111694	658024	⇑
Modes of action	Single MOA	112749	674364	⇑
Male movement (m_m)	10%	59645	555178	↓
	30%	41872	319327	↓
Male movement type	Random	31763	318979	⇓
Female movement type	Random	64282	579633	↑↓
Starting resistance	0.1%	27957	223228	⇓
	10%	112869	654485	⇑
Female ground movement (m_f)	10%	42212	388276	↓
	30%	46973	438246	↓
Female sky movement (m_s^1, m_s^2)	10%, 1%	51950	541242	↓
	40%, 10%	28466	199785	⇓

Table 5.13: *The sensitivity of the adult moth population to variations in the input parameters, with double arrows denoting a very large change (approximately 50%) and two arrows indicating changes in opposite directions.*

When considering the average daily adult moth population and the maximum moth population, parameter changes that have the greatest influence on μ_m and ω_m are often very intuitive, as it is expected that any scenario that benefits the moth in terms of resistance development (dominant gene, one mode of action, a higher resistant starting population) should result in the moth population being able to expand into the Bt crops. This expansion increases total available stalk capacity, leading for more larvae to be able to mature into adult moths, increasing the number of moths in the model. The parameter that had the smallest impact on the model is the female ground movement type, with values that are very similar to the baseline, with only one fewer case of resistance developing. Some parameter variations resulted in no or little development of resistance, including having male movement set to random, having a sufficiently low starting resistance, and having female sky movement likelihoods high. It should be noted that increasing both the male and the female's likelihood to move resulted in less resistance development, indicating that a higher dispersal rate could have a positive influence on refuge efficacy. It should be noted that, in all runs where resistance did not develop, the model was very stable and the coefficient of variation of the average population size across all the runs was $< 1\%$. This proves that the changes in population sizes is solely due to the development of resistance.

Parameter	Value	ν	μ_r	Change from baseline
Gene inheritance	Recessive (Baseline)	11	116619	–
	Dominant	15	242602	⇑
Modes of action	Single MOA	15	242984	⇑
Male movement (m_m)	10%	9	112068	↓
	30%	4	131354	⇓↑
Male movement type	Random	3	27945	⇓
Female movement type	Random	10	154358	↓↑
Starting resistance	0.1%	0	0	⇓
	10%	15	244975	⇑
Female ground movement (m_f)	10%	6	85896	⇓
	30%	6	51192	⇓
Female sky movement (m_s^1, m_s^2)	10%, 1%	8	118073	↓
	40%, 10%	0	0	⇓

Table 5.14: *The sensitivity of the adult moth population to variations in the input parameters when considering the development of resistance. Double arrows denote a very large change (approximately 50%) and two arrows indicate changes in opposite directions.*

When combining the outputs seen in Table 5.13 with the ν and μ_r values in Table 5.14, it can be seen that the potential likelihood for developing resistance in the model is affected by the parameters in the following way:

- **Increases likelihood**
  - Dominant gene,
  - only one mode of action, and
  - high starting resistant population.
- **Decreases likelihood**
  - Higher male movement likelihood,
  - random male movement,
  - low starting resistance,

  - lower or higher female ground movement likelihood, and
  - higher female sky movement likelihood.

- **No real change**
  - Random female ground movement likelihood,
  - lower female sky movement likelihood, and
  - lower male movement likelihood.

These results support the assumptions of Ostlie *et al.* [81], as it was observed that the cases where the resistance gene frequency was more than one in 1000, or the genes were not recessive, resistance development occurred frequently. It was also observed that moths with higher dispersal rates were less likely to develop resistance, serving as evidence for their third point that the refuge areas must be within reach of the moth's typical dispersal area.

The field object also has some parameters that can be varied that could potentially influence the development of resistance. It was mentioned when speaking to the validators that crops are often harvested earlier to try and avoid large infestations, as this limits female egg laying sites and wipes out a large part of the larval infestation. There is also the case for crops grown in a one year cycle, which generates stalk capacity much quicker and could potentially house larger infestations. Apart from growth and harvesting, it is also important to consider how effective the protein is in terms of larval mortality, the hypothesis being that a lower efficacy protein might result in pockets of susceptible insects surviving alongside the resistant insects, limiting the spread of the resistance gene, but allowing more insects to live. To see the effect of having refuge that is not all harvested at the same time, the refuge quadrant is further divided into its own quadrants. Using Figure 5.13 as a reference, quadrants 1 and 3 are started at 120 days old and 2 and 4 are set to 60 days old. This provides a "staggered" refuge scenario.

Parameter	Value	μ_m	ω_m	Change from baseline
Crop cycle	Two year (Baseline)	61875	659192	–
	One year	67026	564098	↑↓
Harvesting	22 months	41540	420746	↓
	10 months	46815	696059	↓
Protein efficacy	85%	44560	508920	↓
	75%	57585	543760	↓
Staggered refuge	–	41665	515407	↓

Table 5.15: *The sensitivity of the adult moth population to variations in the input parameters directly related to the field. Double arrows denote a very large change (approximately 50%) and two arrows indicate changes in opposite directions.*

When combining the outputs seen in Table 5.15 with the ν and μ_r values in Table 5.16, it can be seen that the potential likelihood for developing resistance in the model is affected by the parameters in the following way:

- **Increases likelihood**
  - Lowered protein efficacy.
- **Decreases likelihood**
  - Shorter time to harvest,
  - one year harvest times, and
  - staggered refuge ages

Parameter	Value	ν	μ_r	Change from baseline
Crop cycle	Two year (Baseline)	11	116619	–
	One year	5	114204	⇓
Harvesting	22 months	4	151777	⇓↑
	10 months	0	0	⇓
Protein efficacy	85%	7	87025	↓
	75%	12	91108	↑↓
Staggered refuge	–	7	71493	↓

Table 5.16: *The sensitivity of the adult moth population to variations in the input parameters directly related to the field when considering the resistant population. Double arrows denote a very large change (approximately 50%) and two arrows indicate changes in opposite directions.*

5.4.3 Discussion

As briefly discussed in Section 5.4.2, there are some parameter values that appear to place the model at greater or lesser risk of developing resistance. Setting the resistance gene as dominant, removing a mode of action and drastically increasing the initial resistant population all led to significant resistance development and these results are intuitive when paired with the number of resistant runs produced by the baseline test, as is the lack of resistance development in the case where the initial resistant population was very small. Some of the results are less intuitive and require some more analysis to determine why a given result would occur and what it could mean when applied to a real-world scenario.

5.4.3.1 Random male movement

When setting male movement to a random walk, it was observed that the development of resistance became much less likely, which was considered a significantly unexpected result and more runs were scheduled to confirm the initial result. The results remained the same during the second set of runs and the model output was scrutinised. It was found that, if the male would have a bias towards older and taller sugarcane as in the baseline test, when the refuge area is harvested it drives the male moth population towards the Bt sugarcane in quadrants 1 and 3 in Figure 5.13, which will contain relatively mature sugarcane. The female moth, being attracted to areas of high male moth density, will start following the males into the Bt sugarcane and also start laying eggs in the Bt sugarcane. This is problematic, as this massively inflates the fitness of individuals carrying the resistance gene and drastically increasing the likelihood of a large concentration of resistant insects developing. Given the refuge area in the test was a single block, if a resistant population starts developing far enough from the refuge area, it is nearly impossible to contain the spreading of the resistant gene.

If the male movement is simply a random walk, this migration towards the Bt sugarcane is much less likely to happen, mitigating these risks. This result provides some insights into how refuge should, or rather should not be planted to avoid this problem, as it was the very sudden loss of refuge during harvesting which drove the male moths (in the more likely case of a biased random walk) towards the Bt sugarcane. Hence for a successful refuge strategy it is recommended that refuge areas be harvested in a staggered fashion, or even after all the surrounding Bt sugarcane, as to limit the migration towards any neighbouring unharvested Bt sugarcane.

5.4.3.2 Greater female and male sky movement

These results are in line with the third assumption for successful refuge strategies as set out by Ostlie *et al.* [81], where refuge should be planted in such a way that it is accessible within the known dispersal area of the target pest. Increasing the values for m_m, m_s^1 and m_s^2 effectively increases the dispersal rate of the moth, making the refuge area much more accessible for any potential carrier or resistant moths that start emerging in nearby Bt sugarcane. The effect of the higher dispersal rate is significant enough that it mostly counteracts the effects discussed in Section 5.4.3.1. This result aligns with the results obtained by Potgieter [86], where the efficacy of sterile insect releases in populations of *E. saccharina* were positively influenced by a higher dispersal rate. Unfortunately this does indicate that both control strategies suffer from the same weakness and the combination of the two strategies as an integrated pest management strategy may not be as complementary as hypothesised by the authors. It is still possible for the two strategies to be complimentary in scenarios where sterile insects releases are focussed on areas furthest away from refuge areas.

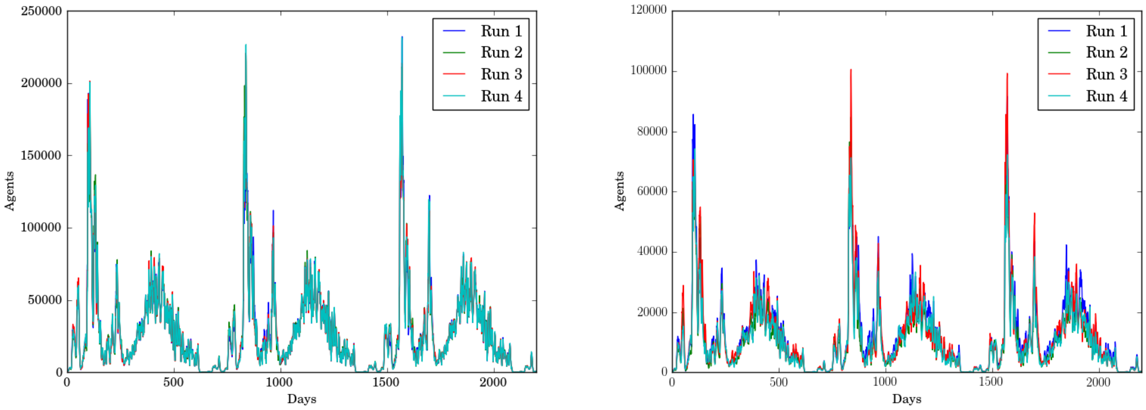
5.4.3.3 Female ground movement

For sensitivity related to female ground movement, it was found that both increasing and decreasing the value for m_f resulted in a decreased likelihood of resistance development. It was not clear from the output of the model exactly why this would be the case, but the hypothesis is that, where m_f is lower, females moving around the edges of refuge areas are less likely to stumble into Bt sugarcane, helping to keep the population within the refuge bounds. On the contrary where the likelihood is higher, any female that is in the Bt area is more likely to move back into the refuge area. This behaviour requires more data to fully understand and it may require future work on variations of the size of the ground cells, as the magnitudes of m_f tested would still lead to very low relative movement, making the result less significant.

5.4.3.4 Stable runs versus resistant runs

A run that does not result in resistance development has been observed to be incredibly stable, and the differences between two stable runs with the same parameter values are almost negligible. In Figure 5.16(a), the number of adult moths in the sky cells per simulated day for the four runs from the baseline test that did not develop resistance are shown. Here it can be seen that the population falls into a very predictable cycle that is mostly centred around harvesting as the entire refuge area is harvested at once. The insect population has reached an equilibrium and is relatively uniformly spread throughout the roughly 6.25 hectares of refuge and, given resistance does not develop in the future, will continue to cycle indefinitely. This is supported by Figure 5.16(b), where the number of adult moths carrying at least one allele of the resistance genes is plot for the same four runs, showing the gene flow reaching an equilibrium and cycling along with the size of the population in Figure 5.16(a). Where resistance develops, the populations become very unstable as may be seen in Figures 5.17(a) and 5.17(b). It does appear that, for runs where resistance develops earlier, the population once again reaches a stable cycle as may be seen in Figure 5.17(b), but it does take longer to reach stability when compared to the instability seen in Figure 5.15.

A problem that arises with having very stable runs when resistance does not develop, is that runs that do develop resistance very likely do so at random and at any point during the simulation run. The 11 runs that developed resistance are shown in Figure 5.17, with Figure 5.17(c) showing the resistant population of runs that developed later and Figure 5.17(d) showing runs that developed earlier. In all runs it can be seen that, once any real sizeable resistant population is allowed to establish itself, the resistant population grows exponentially. Given enough time, as seen in



(a) *Sky moth population*

(b) *Resistance gene carrier population*

Figure 5.16: *Time series plots of the number of adult moths for each day of the 6 year simulation where resistance did not develop, showcasing how similar the runs end up being after the initial warm-up.*

runs 10 and 11, the resistant population reaches an equilibrium point and also starts falling into a cyclical pattern. There is a significant range between earliest resistance development, as seen in run 11 at less than one year and in run 1 at over 4 years, showing that the development is seemingly sporadic and random. It is interesting to note that runs 1 through 5 only develop resistance well after the first stable cycle completed at approximately day 500, hence it is worth investigating the dynamics of the carrier population to see how it differs from the population in Figure 5.16(b).

In Figure 5.18, the carrier population of the runs seen in Figure 5.17(c) is shown. Up until the 4 year mark, the population size looks very similar to the population in Figure 5.16(b) apart from having slightly higher peaks. Unfortunately this may indicate that there will be little to no warning to imminent development of resistance, as only after all 5 runs have developed significant resistance does this reflect on the carrier population.

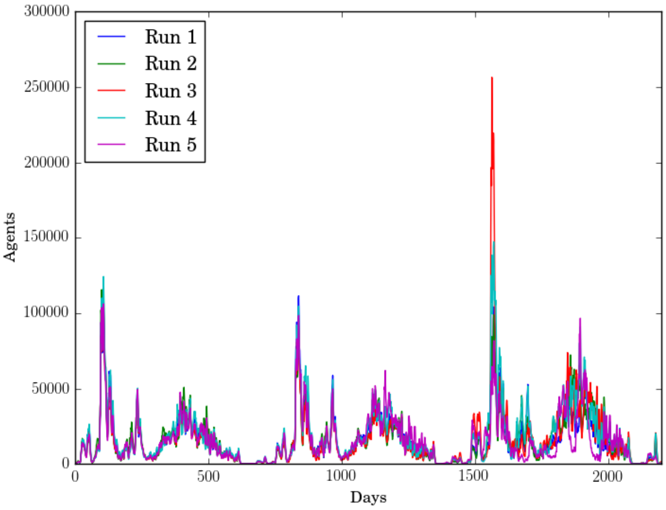
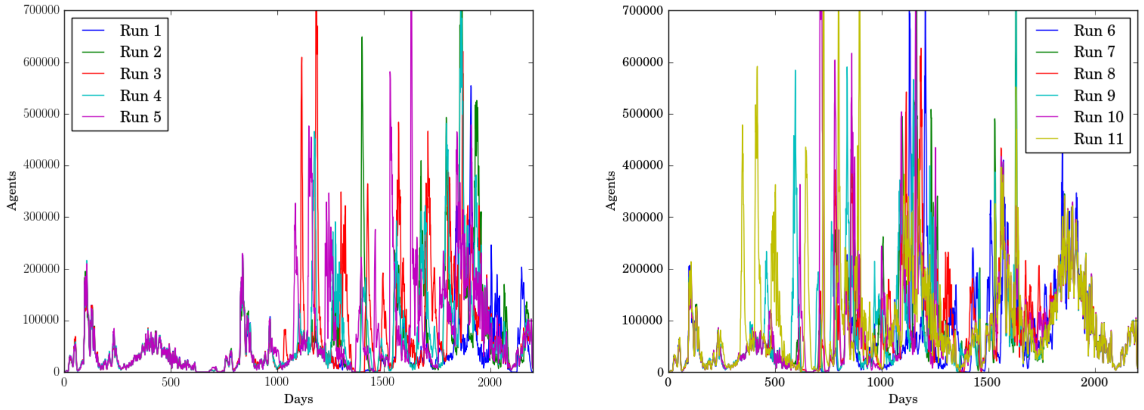
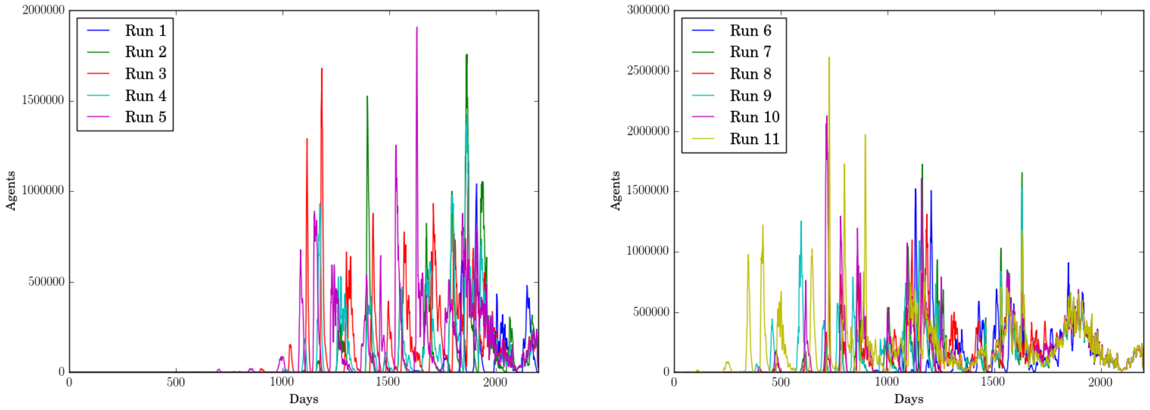


Figure 5.18: *Time series plots of the number of carrier adult moths (heterozygotes) for each day of the 6 year simulation where resistance did develop.*



(a) *Later resistance development – total population*

(b) *Earlier resistance development – total population*



(c) *Later resistance development – resistant population*

(d) *Earlier resistance development – resistant population*

Figure 5.17: *Time series plots of the number of adult moths for each day of the 6 year simulation where resistance did develop, showcasing how resistance starts developing at different times and how it introduces instability in the cycles.*

5.4.3.5 Harvesting methods and Bt protein efficacy

The results from differing the harvesting parameters were generally expected and intuitive. Unfortunately the hypothesis stated in §5.4.2 regarding lower efficacy proteins did not hold as well as expected, largely due to the two modes of action resulting in a 93.75% mortality rate for susceptible insects even at 75% efficacy, leading to a resistant to susceptible insect ratio that is too high to curb the spread of the resistance genes. When testing the 75% efficient protein in a scenario that only has one mode of action, it was found to be even less effective, performing as poorly as the original run with only one mode of action. Based on this, it is highly recommended that very high doses of Bt protein be used in any scenario.

As observed in §5.4.3.1, when all the refuge gets harvested at the same time, the male moth's drive to seek out older sugarcane can push the moth population into the Bt sugarcane. Staggering the refuge ages could prevent this, but the effect of staggered refuges on the model is not as significant as the random male movement parameter, but it does make the model less likely to develop resistance, providing evidence to support the recommendation of harvesting in a staggered fashion.

It does seem that harvesting earlier results in smaller average population sizes and crops grown on a one year cycle are able to house larger infestations due to their increased overall stalk capacity. A scenario that proved very effective in limiting the development of resistance, was a one year harvest scenario where the sugarcane was harvested at 10 months old. This scenario was effective due to the moth population not being able to reach a proper equilibrium point as in the two year case and having the population effectively being reduced to 0 several times during the run. In Figure 5.19, the average population for all the runs shows that the population never reaches a stable equilibrium and is prone to severe peaks and troughs, likely helping to curb the development of resistance.

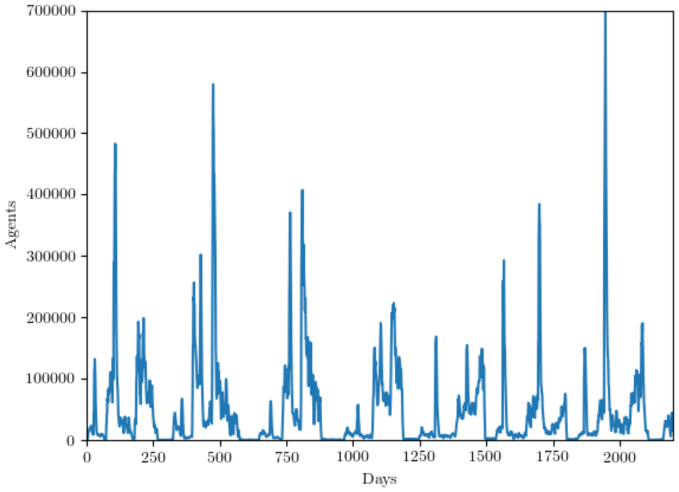


Figure 5.19: *Time series plots of the number of adult moths for each day of the 6 year simulation where sugarcane is harvested at 10 months old.*

5.5 Chapter summary

In this chapter, the process of verifying the model outputs as well as validating some aspects against historical data was discussed. The face validation with the key stakeholders was also discussed, including any changes they recommended that were implemented into the final model. This was followed by the general experimental set-up that would be used in the sensitivity analysis, including the identification of key parameters of interest in both the insect population and the underlying field. The chapter concludes with a summary of the sensitivity analysis results, a discussion of some of the results and their impact on the model and the course of this study, as well as some initial recommendations that can be presented to stakeholders. These include staggering the harvest times of refuge areas, keeping harvests early and using a high dose Bt sugarcane.

CHAPTER 6

Case studies and a decision support tool

Contents

6.1 Case studies 75
6.1.1 Case study 1 – Refuge distribution 76
6.1.2 Case study 2 – Refuge shapes 81
6.1.3 Case study 3 – Gradually introducing Bt sugarcane 85
6.2 Decision support tool 90
6.2.1 Necessary assumptions 90
6.2.2 Risk factors and recommendations 91
6.3 Chapter summary 94

There is an infinite number of possible scenarios that can exist in reality, making exhaustive testing using the developed model impossible. To compensate for this fact, several fictional case studies based on realistic scenarios were tested that can be used to develop a decision support tool. These case studies focus specifically on refuge layout, size and planting strategies to try and find the best combination of results to provide an informed recommendation on what the potential refuge requirements may be in a realistic scenario.

6.1 Case studies

In this chapter, three distinct case studies are presented, each focussing on different aspects of real-world refugia planning. The case studies are:

- finding the right size and distribution of refugia,
- finding a suitable shape for refugia, and
- introducing Bt sugarcane in a phased approach, slowly reducing refugia.

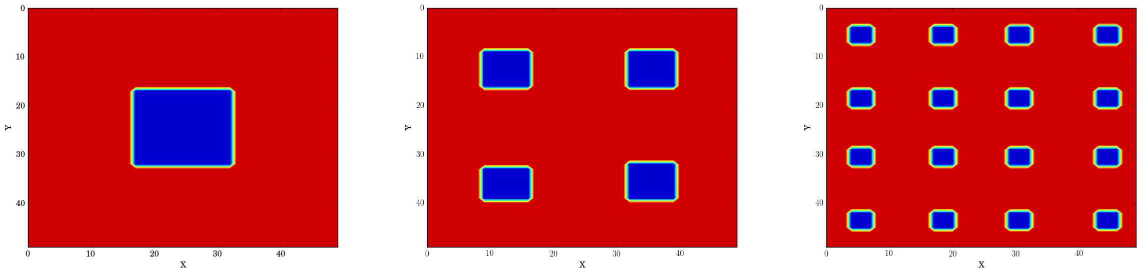
In each case study the same parameter values as in the base case defined in Chapter 6 are, except for the proportion of initial resistant insects being reduced to 0.5% of the total population as the original 1% was found to result in resistance developing too often. The number of runs per scenario is held at 15, but where stated may be reduced to 10 to account for memory limitations. The parameters and initial values for the case studies may not be reflective of a realistic scenario, in §5.3 it was highlighted that there is very little data to work with. For this reason all scenarios developed were designed to be pessimistic. This does lead to results that are more conservative, but it also makes them more interesting, as overly optimistic scenarios were found to be uninteresting as seen in §5.4.2, specifically where initial resistance was very low. Some of the more interesting results from Chapter 5 regarding the spatial movement were incorporated into the case studies, specifically parameters that were observed to have a positive effect on curbing resistance development. For some of the experiments in the case studies, the movement parameters were set to a low movement and a high movement scenario, the values of which may be seen in Table 6.1.

Parameter	Low	Average	High
m_s^1	9%	18%	40%
m_s^2	1%	2%	10%
m_f	10%	20%	30%
m_m	10%	20%	30%

Table 6.1: *The values of the different movement parameters for the low, average and high movement scenarios. Here, m_s^1 and m_s^2 is the likelihoods of female movement one or two sky cells away, respectively, and m_f and m_m is the likelihood of female and male movement to neighbouring ground and sky cells, respectively.*

6.1.1 Case study 1 – Refuge distribution

In the first case study, the focus is on the distribution of refugia in a given area. In this scenario, given a proportion of refugia, $h \in \{10\%, 20\%, 30\%\}$, three layouts are tested. The h values were chosen to be less than or equal to 30% as a higher proportion of refuge is expected to result in significant push-back from growers [106]. The first layout is a single large block in the centre of the simulation space, the second divides the simulation space into equal sized quadrants and places a quarter of the original layout's refuge in the centre of each quadrant, resulting in four equal sized refuge areas. The third layout applies the same transformation to the quadrants of the second layout, resulting in 16 refuge areas. Visual representations of the refugia layouts and their fractal expansion may be seen in Figure 6.1.



(a) *Layout 1* (b) *Layout 2* (c) *Layout 3*

Figure 6.1: *The layouts expand in a fractal manner, starting with a single block of refuge and expanding into 4 and then 16 blocks, increasing the distribution, but not the overall size, of the refugia (shown in blue).*

To ensure that moths are able to reach any part of the simulation space, the starting conditions for the warm-up period is also changed from having a single point starting population, to having an evenly distributed population, with 20 batches of 1000 adult moths inserted into the simulation. There will thus be a total of 20000 moths in the simulation at the start of the warm-up period, with 100 being fully resistant. The distribution of the 20 batches may be seen in Figure 6.2.

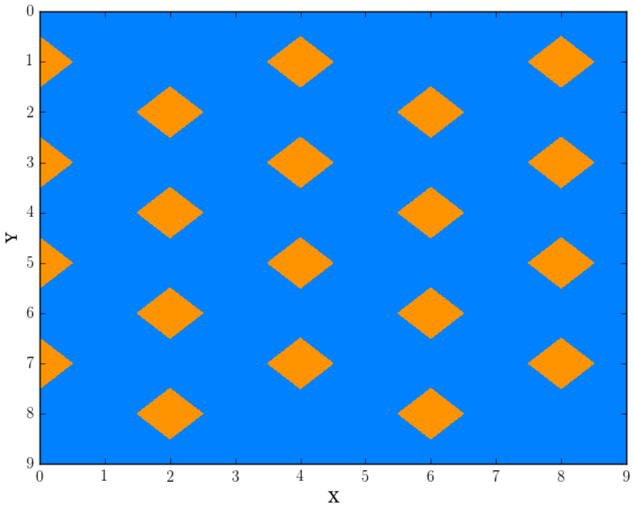


Figure 6.2: *For this case study, 20 batches of 1000 moths are equally distributed throughout the simulation space as indicated by the orange diamonds.*

Before analysing the results, it is important to identify which questions this case study are trying to answer. The key outcome of this case study is to evaluate whether or not having more distributed refuge areas would help curb development of resistance. The second key outcome, based on the assumptions of Ostlie *et al.* [81] as discussed in §2.3.2.1 and §5.4.2, is to determine whether or not, for any distribution, enough refugia has been allocated to help curb resistance development. In these scenarios, having $h = 10\%$ split over 16 blocks of refuge may not perform well due to the individual refuge areas being too small to support a significant susceptible insect population. For larger total refuge areas, it is expected that more refuge areas should provide better protection against resistance development, as the average distance between any point in the Bt sugarcane to a refuge area will be smaller as the number of refuge areas is increased.

6.1.1.1 Results

For these results, the same set of symbols used in §5.4.2 as detailed in Table 5.12 are used. For the 9 combinations of h and the three layouts, the key result is the number of runs (out of 10) that developed resistance, ν, as well as the average number of resistant moths, μ_r. A lower value for both ν and μ_r is considered a more successful combination.

The most noticeable and arguably most intuitive trend, as may be seen in Table 6.2, is that a higher h value is correlated with fewer cases of resistance development. However, there does not seem to be such an obvious trend in the different layouts, with some results being particularly counter-intuitive. For $h = 10\%$, the best result is observed where there are 4 blocks of refuge, but the exact opposite is observed in the cases where $h = 20\%$ and $h = 30\%$. When excluding $h = 10\%$, considering the results in Tables 6.2 and 6.3 as a whole, it would appear that either having a single, large refuge area or many smaller refuge areas would be most effective. Although $\nu = 3$ for $h = 20\%$ distributed over 16 blocks, upon further investigation it was found that two of the three resistant runs were barely within the threshold for resistance, as both had a maximum resistant population of about 2000 moths. For the case where $h = 10\%$, individual refuge areas are too small in the case of 16 blocks ($< 1\%$ of total area) to maintain a significant susceptible population. For $h \geq 20\%$, the large, single blocks of refuge reduce the need and the likelihood of the moths moving into Bt sugarcane, resulting in it being an effective strategy.

To determine what led to the results obtained for the 4 blocks of refuge, it is best to consider

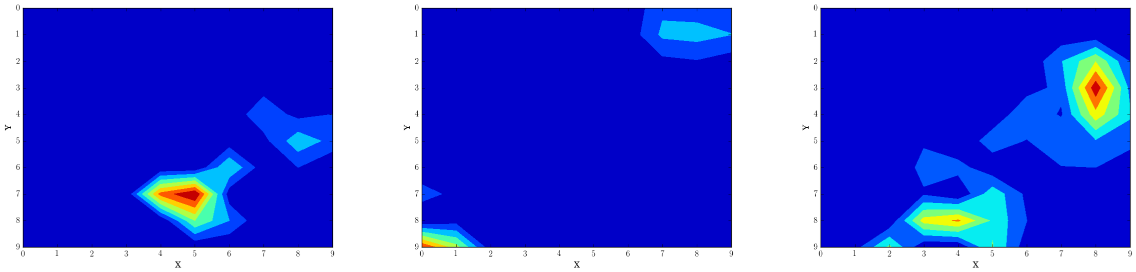
Layout	$h = 10\%$	$h = 20\%$	$h = 30\%$
1 block	6	2	2
4 blocks	3	5	3
16 blocks	7	3	1

Table 6.2: *The number of runs that develop resistance, ν, out of 10 simulation runs per combination of refuge size, h, and layout.*

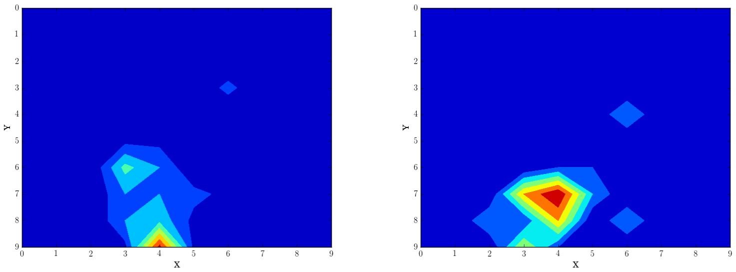
Layout	$h = 10\%$	$h = 20\%$	$h = 30\%$
1 block	60285	21114	5054
4 blocks	25498	35790	21832
16 blocks	49816	11050	743

Table 6.3: *The average number of resistant moths, μ_r, for 15 simulation runs per combination of refuge size, h, and layout.*

where resistance first developed. For this investigation, the grid of sky cells is considered at the earliest point in time that there were more than 1000 resistant moths and plotting where they are concentrated. In Figure 6.3, the locations of initial resistance development may be seen. It does appear that there is a preference for resistance to start developing on the edge of the simulated space, between the two blocks of refuge as seen in Figures 6.3(a), 6.3(c), 6.3(d) and 6.3(e). Only in the run shown in Figure 6.3(b) did resistance develop in a corner of the space.



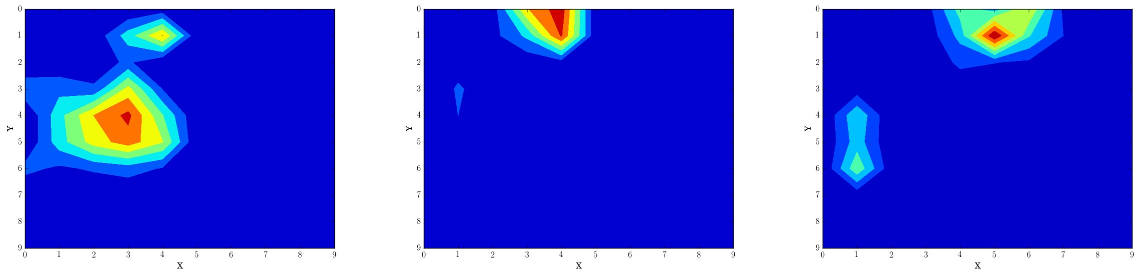
(a) *Resistant run 1* (b) *Resistant run 2* (c) *Resistant run 3*



(d) *Resistant run 4* (e) *Resistant run 5*

Figure 6.3: *The concentrations of resistant insects in the sky cells at the first point in time of resistance development for the 5 runs that developed resistance in the case where $h = 20\%$ and 4 blocks of refuge.*

For $h = 30\%$, only 3 runs developed resistance as may be seen in Figure 6.4. As with the case where $h = 20\%$, the development of resistance appears to be focused on the edge of the simulated space, as may be seen in Figures 6.4(b) and 6.4(c). The only case where resistance development seems to have happened more towards the centre of the space is the run seen in Figure 6.4(a). From these results, it would appear that, for the case where there are 4 blocks of refuge, the increased number of moths that can migrate towards the edge of the simulated space allows for greater survival of the resistance gene. Given enough time, the clusters of moths carrying the resistance gene that do make it to the edges would eventually be in high enough concentrations to create large populations of resistant insects. The same is not observed for the single block cases, as there is too great a distance between the edge of the refuge and the edge of the simulation space, making it far less likely for moths to get "stuck" on the edges. Where there are 16 blocks of refuge, the refuge areas are close enough to the edge for the susceptible and resistant insects to interact regularly, leading to less resistance development. It may be necessary to further investigate how the boundary conditions affect these results in future work.



(a) *Resistant run 1* (b) *Resistant run 2* (c) *Resistant run 3*

Figure 6.4: *The concentrations of resistant insects in the sky cells at the first point of resistance development for the 3 runs that developed resistance in the case where* $h = 30\%$ *and 4 blocks of refuge.*

6.1.1.2 Low/high movement

The best results obtained from the average movement cases was for $h = 30\%$ and 16 blocks of refuge. To stress test these results, the experiments are repeated for the high and low movement scenarios for all values of h. From the results in §5.4.2, it was found that having the movement parameters individually set either high or low resulted in fewer cases of resistance development, hence it would be interesting to note what effect having all movement parameters biased towards a high or low likelihood of movement. Considering that the refuge is both a different shape and size as in Chapter 6, it is to be expected that the results obtained in §5.4.2 may not be repeatable.

For these tests two sets of experiments were run. The first set compares the different movement parameters to the 16 blocks of refuge cases for $h \in \{10\%, 20\%, 30\%\}$, while the second compares the movement parameters to the 1, 4 and 16 blocks cases where $h = 20\%$, which was the median case of the previous results. The combination of these experiments should indicate how resistance development correlates with both moth movement and refuge size and distribution.

From Tables 6.4 and 6.5, it may be seen that for all three h values, the low movement cases were much more prone to resistance development. This indicates that, where the moths are not likely to move very far, there is a point where even highly distributed refugia cannot guarantee that susceptible and resistant insects will ever meet, and resistance can develop quickly. In general, where movement parameters were very high, resistance did not develop as easily, hence there is a correlation between likelihood of movement and likelihood of resistance development. Where $h = 20\%$ in the high movement case, one run spontaneously developed resistance near the end of the 10 year run (including warm-up), which resulted in it not being better than the average case. This single run of resistance development begged the question of how rapidly resistance would spread through a population once it starts gaining momentum. This is further investigated in §6.1.1.3.

Movement	$h = 10\%$	$h = 20\%$	$h = 30\%$
Low movement	11	6	2
Average	7	3	1
High movement	3	4	0

Table 6.4: *The number of runs that develop resistance, ν, out of 15 simulation runs per combination of refuge size, h, distributed over 16 blocks, and high/average/low movement.*

Movement	$h = 10\%$	$h = 20\%$	$h = 30\%$
Low movement	79808	23920	11487
Average	49816	11050	743
High movement	10926	30142	0

Table 6.5: *The average number of resistant moths, μ_r, for 15 simulation runs per combination of refuge size, h, distributed over 16 blocks, and high/average/low movement.*

In Tables 6.6 and 6.7, it may be seen that lower movement led to resistance development more often, which is in contrast to the results obtained in §5.4.2, whereas the low ratio of resistance development in the higher movement case is similar to results obtained in §5.4.2. However, there is not a very clear distinction on which layout performed better, except that the results for a single large block did appear to be slightly better at curbing resistance. This result does coincide with those obtained in Tables 6.2 and 6.3 where it was stated that a very large or many smaller refuge areas are the most effective. Due to constraints on the CHPC, only 10 simulation runs were performed for this test and not 15 as in earlier scenarios.

Movement	1 block	4 blocks	16 blocks
Low movement	4	7	6
Average	2	5	3
High movement	2	3	4

Table 6.6: *The number of runs that develop resistance, ν, out of 10 simulation runs per combination of refuge layout and high/average/low movement where $h = 20\%$.*

Movement	1 block	4 blocks	16 blocks
Low movement	55882	59456	23920
Average	21114	35790	11050
High movement	30893	32882	30142

Table 6.7: *The average number of resistant moths, μ_r, for 10 simulation runs per combination of refuge layout and high/average/low movement where $h = 20\%$.*

6.1.1.3 Rapid development of resistance

All experiments in this case study started with exactly 100 resistant moths, and in runs where resistance did not develop, the initial 100 moths remains the maximum number of resistant moths throughout the run. For all the runs in this case study that developed resistance, it was found that from the point where the resistant population goes over 100, to the point where it reaches 100000 took an average of 709 days, or approximately one harvest cycle. In Figure 6.5 it may be seen that, for a run in the high movement case with $h = 20\%$ and four blocks of refuge, the time from having less than 1% resistance in the entire population to having over 75% resistance was less than 800 days.

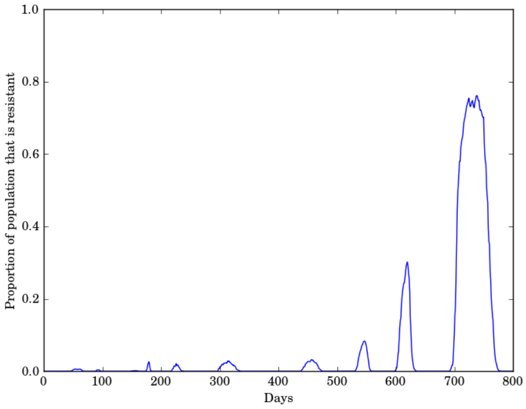


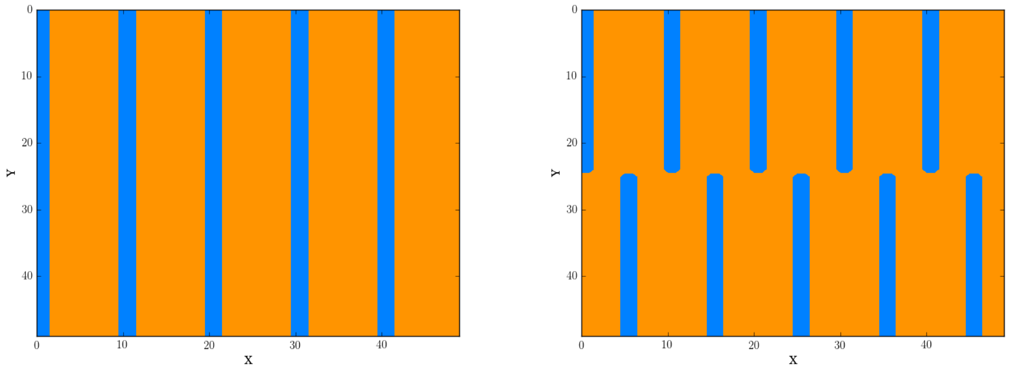
Figure 6.5: *The proportion of resistant moths over time for* $h = 20\%$ *and high movement with four blocks of refuge.*

As the initial 100 resistant moths are evenly distributed among the 20 starting populations, it is likely that there exists a critical point. This point is where either the number or concentration of resistant moths in the same area becomes large enough that resistance becomes inevitable. It is difficult to determine what this point may be, but it can be approximated by taking the maximum number of resistant moths that appear in a sky cell in all models that do not develop resistance, as well as the highest ratio of resistant to susceptible insects in a sky cell. Taking the results from the previous experiment where no resistance was observed, with $h = 30\%$ and high movement, the maximum number of resistant moths in a sky cell was only 13. The maximum ratio of resistant to susceptible moths, where there are at least 50 total moths in a sky cell, was approximately 1 to 9. When considering the high movement case where $h = 20\%$, if the first point in time after initiation is considered where the total number of resistant insects reach 100 for runs that did develop resistance, the maximum ratio of resistant to susceptible insects was 1 to 5. For the resistant runs from $h = 10\%$ and high movement, this ratio was 1 to 4 resistant to susceptible insects. From this, it can be assumed that the critical point/ratio would then lie between 1:4 and 1:9 resistant to susceptible insects in a single sky cell.

6.1.2 Case study 2 – Refuge shapes

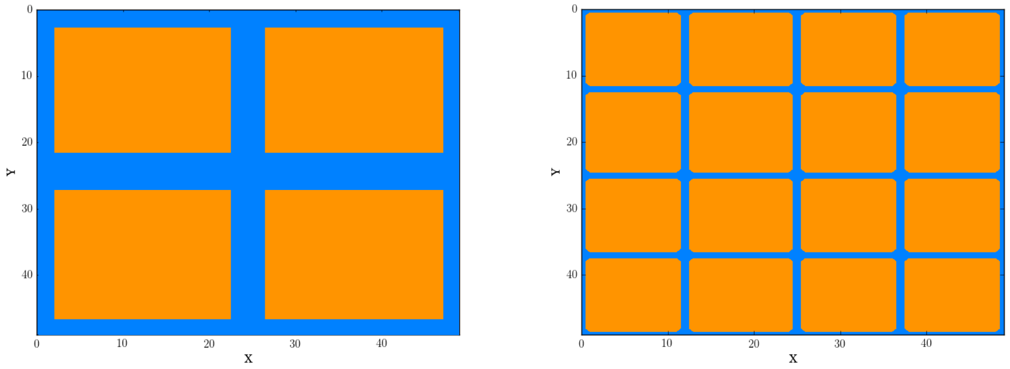
As discussed in §2.3.2.1, there are various approaches to planting refuge. All previous experiments used a form of block structured refuge, but as may be seen in Figure 2.7 there are several possible planting strategies. As the block strategy has been tested in the first case study, this case study focusses on the linear block/bracket and border strategies. Strip planting was considered infeasible [32, 106] and does not form part of the scope of this project.

For the linear blocks, two different layouts, as seen in Figure 6.6, were tested for the median h value of 20%. The rationale behind the layout in Figure 6.6(b) is that the more distributed refuge areas may allow for more overlapping of susceptible populations, especially on the edges of the simulated space, an area identified as particularly vulnerable in the first case study. For the border, the simulation space is first divided into 4 equal squares as may be seen in Figure 6.7(a), and then into 16 squares as in Figure 6.7(b). The same initial population distribution and parameter values as in §6.1.1 apply.



(a) *Linear block 1* (b) *Linear block 2*

Figure 6.6: *The two linear block refuge layouts that are considered for this case study, both with* $h = 20\%$. *The orange represents Bt sugarcane, whereas the blue represents refugia.*



(a) *Border 1* (b) *Border 2*

Figure 6.7: *The two border refuge layouts that are considered for this case study, both with* $h = 20\%$. *The orange represents Bt sugarcane, whereas the blue represents refugia.*

6.1.2.1 Results

As observed in the first case study, the general trend seems to be that more mobile insects led to fewer cases of resistance developing across all layouts. As may be seen in Tables 6.8 and 6.9, Linear 1 and Border 2 or arguably the worst performing layouts. Staggering the linear blocks as in Linear 2 does appear to have the desired effect, with the cases of low and average movement outperforming those of Linear 1 and the case for high movement only being slightly worse. The layout that weakly dominates all others is Border 1, with the lowest ν values for all movements and lowest μ_r for two of the three movements. When compared to the case where $h = 20\%$ for 1 and 16 blocks as in §6.1.1.2, Border 1 compared favourably, having ν and μ_r values that fall in between the block refuge results.

The fact that Border 2 performs worse than Border 1 again speaks to the third point made by Ostlie *et al.* [81], where it is stated that refuge areas should (1) be able to sustain susceptible insects and (2) should allow for susceptible insects to come into contact with resistant ones. For Border 2, the refuge areas are much more distributed, which does agree with the second point, but the refuge areas are only a single ground cell wide, which may not be large enough for the first point to hold. It is also interesting to note that for the high movement cases, the shape of the refuge made very little difference, supporting the result that, where insect dispersal is high, only the size of the refuge has a significant impact. This result supports the model implementations of Sisterson *et al.* [104] and Peck *et al.* [83] where only refuge size was considered, as the target pests were considered to disperse over large areas.

Layout	Linear 1	Linear 2	Border 1	Border 2	1 block	16 blocks
Low movement	12	8	5	8	4	6
Average	6	3	3	8	2	3
High movement	2	3	2	2	2	2

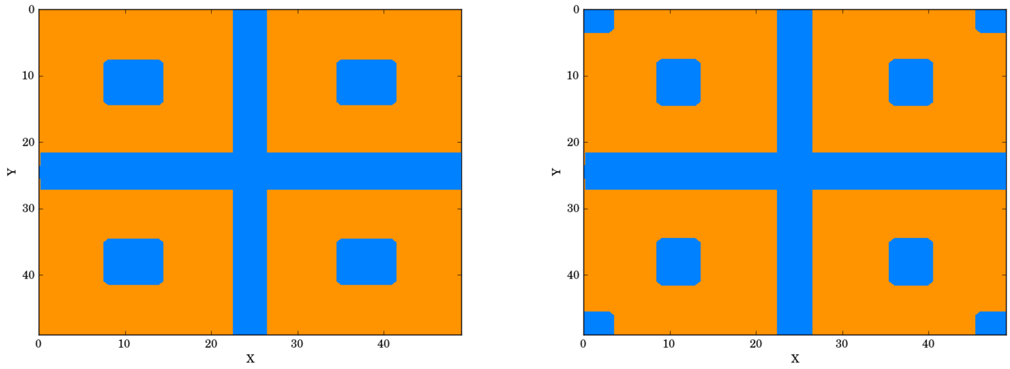
Table 6.8: *The number of runs that develop resistance, ν, out of 15 simulation runs per combination of refuge layout and high/average/low movement for $h = 20\%$.*

Layout	Linear 1	Linear 2	Border 1	Border 2	1 block	16 blocks
Low movement	59793	54350	49135	55082	55882	23820
Average	37068	13302	18341	46262	21114	11050
High movement	6994	20483	953	12986	30893	30142

Table 6.9: *The average number of resistant moths, μ_r, for 15 simulation runs per combination of refuge layout and high/average/low movement for $h = 20\%$.*

6.1.2.2 Combining block and border refuge

As, for $h = 20\%$, block and border refuge performed the best so far, it was decided to experiment with combining the two layouts. In Border 1, the borders were only one ground cell wide and this was found to be insufficient for maintaining a healthy susceptible population. For this experiment, the cells making up the border of Border 1 are aggregated into blocks of refugia as may be seen in Figure 6.8. In §6.1.1 it was found that resistance was most likely to develop towards the middle of the edges as in Figure 6.3. With the combination refuge, there are now refuge areas extending into those edges that can help curb the development of resistance.



(a) *Border and Block* (b) *Border and Block revised*

Figure 6.8: *The border and block refuge layouts that are considered for this case study with $h = 20\%$.*

This layout does appear to be effective in preventing the development of resistance across all movements. The ν and μ_r values in Tables 6.10 and 6.11 are mostly improved when compared to those achieved by the Border 1 test or the block refuge test where $h = 20\%$. This indicates that two different refuge structures with distinct weak spots can be combined to form a more effective hybrid. The 4 block refuge had weaknesses at the centre of the edges and Border 1 had points in the middle of the Bt crops that were very far from refuge. In the hybrid, the border portion provided refuge near the edges, addressing the 4 block weakness, and the 4 blocks covered the vulnerable centres of the Border 1 layout.

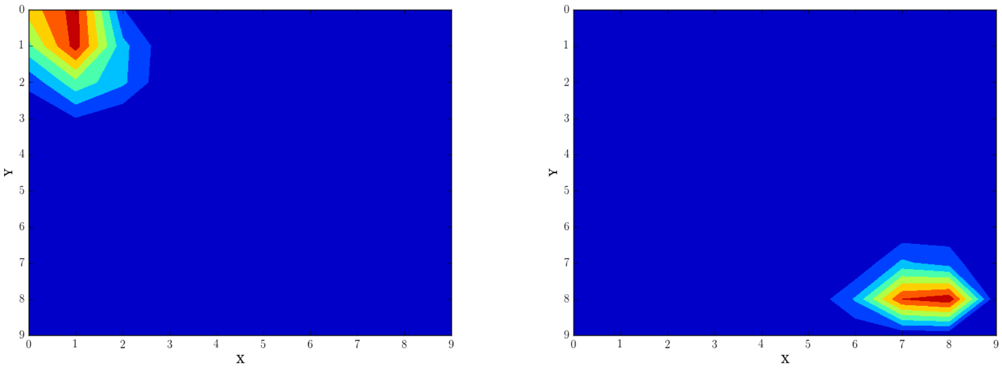
Layout	Border and Block	Border and Block Revised	Border 1
Low movement	3	4	5
Average	4	5	3
High movement	2	0	2

Table 6.10: *The number of runs that develop resistance, ν, out of 15 simulation runs per combination of refuge layout and high/average/low movement.*

Layout	Border and Block	Border and Block Revised	Border 1
Low movement	14350	29037	49135
Average	3474	11881	18341
High movement	6994	0	953

Table 6.11: *The average number of resistant moths, μ_r, for 15 simulation runs per combination of refuge layout and high/average/low movement.*

With the edges and the vulnerable centres covered, it was hypothesised that resistance would develop in the corners of the simulated space. To investigate, the two runs from the high movement case where resistance developed were considered, as it was assumed that these runs would highlight the biggest flaws in the refuge strategy. In Figure 6.9(a) it may be seen that this is indeed the case, with both initial resistant populations tending towards the corners of the simulated space.

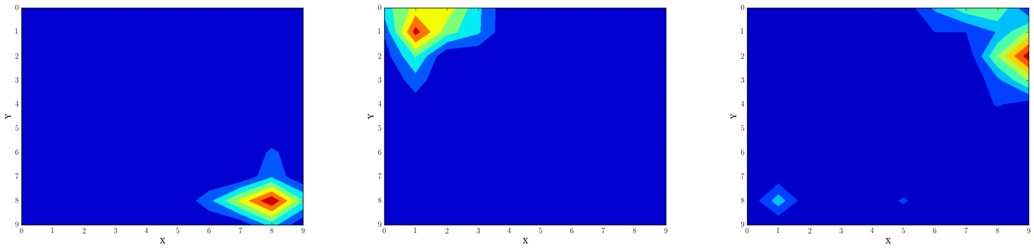


(a) *First resistant run*

(b) *Second resistant run*

Figure 6.9: *Initial resistance development in the two border and block refuge runs with high movement.*

To see if placing refuge areas in the corners would counteract this result, the original experiment was revised to include refuge areas in the corners as may be seen in Figure 6.8(b). For the high movement scenario, as may be seen in Tables 6.10 and 6.11, there was no resistance development at all. Unfortunately in both the low and average movement scenarios the revised layout performed worse than the initial layout. Taking a sample of 3 runs that developed resistance, the points of initial resistance development may be seen in Figure 6.10. The resistance seems to develop just off of the edges of the corner refuge areas, indicating that these refuges may simply be too small to have a real impact in low or average movement scenarios.



(a) *First resistant run* (b) *Second resistant run* (c) *Third resistant run*

Figure 6.10: *Initial resistance development in three of the revised border and block refuge runs.*

6.1.2.3 Random male movement

One parameter found to have a surprisingly significant impact as discussed in §5.4.3.1 was setting the male moth's movement pattern to a random walk. To confirm whether or not this effect is universal, the experiments for the average movement cases of Linear 1 and Border 2 are repeated, but with male movement set to random. For both scenarios, there is a significant reduction in both ν and μ_r values, which indicates that there is a definite correlation between the implementation of male movement and likelihood of resistance developing. In §5.4.3.1, the effect of males moving into Bt sugarcane when the refuge is harvested was discussed, but in this scenario not all refuge was harvested at the same time, which indicates that there are more factors at work than simply driving males into the Bt areas. The full explanation of this behaviour is left for future research.

Layout	Linear 1	Border 2
ν	4	3
μ_r	25735	16306

Table 6.12: *The number of resistant runs, ν, and the average number of resistant insects, μ_r, values for 15 simulation runs for Linear 1 and Border 2 for the average movement case where male movement is random and $h = 20\%$.*

6.1.3 Case study 3 – Gradually introducing Bt sugarcane

In §2.1, the process of*ratooning* sugarcane after a harvest is discussed. As these regrowings usually occur three or four times before planting a new stock of sugarcane, it is unlikely that a commercial grower would harvest and replant their entire crop with a Bt variety in a single harvest. It is more likely that a variety would only be replaced when it has reached the end of its viable ratooning cycle. Most commercial farms have sugarcane that have been planted at different times [106], hence any farmer that plans to introduce a Bt sugarcane variety would likely introduce it in stages as their current ratoons reach their replacement age. Such a phased introduction of Bt sugarcane could potentially negate the need for any structured refuge, as the remaining non-Bt crops would act as natural refuge areas.

In this case study, two hypothetical scenarios are presented. The first is a scenario where the farmer replants all their crops with a Bt variety as they are scheduled to be replaced. The second scenario also has the farmer plant Bt sugarcane, but they include a 20% refuge block in the middle of it. An auxiliary scenario (Scenario 2b) in the two year harvest is included where the 20% refuge is included as in the 1 block scenarios from the first case study. The initial farms will be identical, with four quadrants of ratoons due to be replaced at different times. Each quadrant is further divided into quadrants of slightly different ages. The quadrants are shown in Figure 6.11, with the replacement schedule of the quadrants listed in Table 6.13.

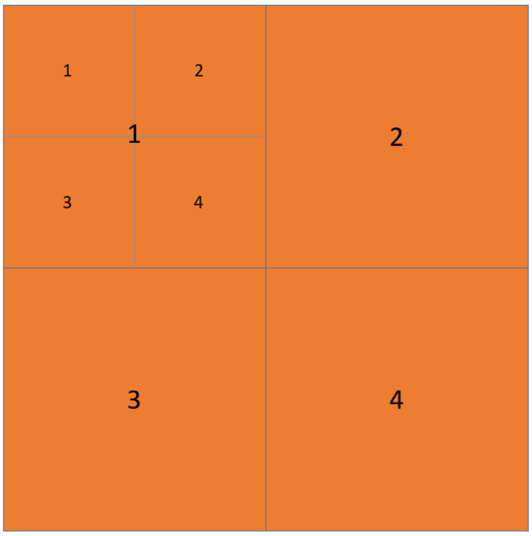


Figure 6.11: *For this case study, there are four quadrants of sugarcane that are due to be replaced at different times, each with a further four quadrants of slightly different ages.*

		Sub-quadrant			
		1	2	3	4
Quadrants	1	1470	1500	1530	1560
	2	2200	2230	2260	2290
	3	2930	2960	2990	3020
	4	3660	3690	3720	3750

Table 6.13: *The replacement schedule (in days) of each sub-quadrant.*

Due to the entire farm being non-Bt sugarcane during the four year warm-up period, the initial population is limited to 1000 adult moths centred around the midpoint of the farm and only 10 simulation runs are considered, as tests showed that 15 runs would overrun the CHPC's 128 gigabytes of memory. The simulations were run for a total of 14 simulated years, as the final quadrant would only be replaced at the 10 year point. Both scenarios will be tested with low and high movement as well as on a one and two year crop harvest cycle. The starting population will have 0.5% insects fully resistant and 1% that are heterozygotes. All other parameter values not mentioned are set as in Table 5.11.

6.1.3.1 Results

For this case study, the results of the one and two year harvest cycles are discussed separately. For the two year harvest cycle, it may be seen in Tables 6.14 and 6.15 that there were no cases of resistance development for the high movement cases in any scenario. This outcome is particularly interesting in the first scenario, as it means that the entire *E. saccharina* pest population was eradicated. As may be seen in Figure 6.12, the adult moth population for both scenarios look very similar up until the introduction of Bt sugarcane in the 3rd quadrant, at which point the population for the first scenario goes extinct (in all cases where resistance did not develop) whereas the refuge areas in Scenarios 2 and 2b keep a healthy, but significantly smaller, susceptible population alive. When considering the low movement cases, the first scenario performed consistently better than the second set of scenarios, which indicates that Bt introduction with the intent to cause extinction is likely a feasible approach.

Layout	Scenario 1	Scenario 2	Scenario 2b
Low movement	1	3	2
High movement	0	0	0

Table 6.14: *The number of runs that develop resistance, ν, out of 10 simulation runs per scenario for high and low movement cases for a two year harvest cycle.*

Layout	Scenario 1	Scenario 2	Scenario 2b
Low movement	2858	3858	4667
High movement	0	0	0

Table 6.15: *The average number of resistant insects, μ_r, for 10 simulation runs per scenario for high and low movement cases for a two year harvest cycle.*

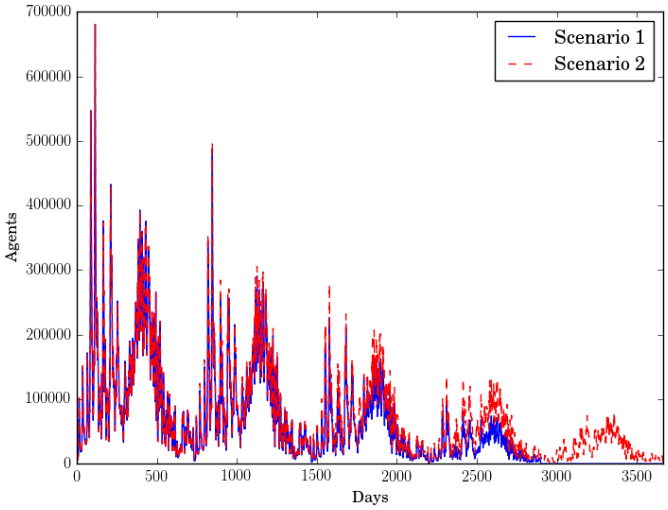


Figure 6.12: *The moth population can be seen steadily declining as Bt sugarcane is introduced, leading to total eradication in Scenario 1 when high movement is considered.*

When considering the scenarios applied to a one year harvest cycle, the results, as may be seen in Tables 6.16 and 6.17, are very similar to that of the two year scenarios. The greatest difference is that the sole resistant run from Scenario 1 developed resistance much earlier than the sole resistant run of Scenario 2. These results support the results from §5.4.2 where it was found that single year harvest cycles do not develop resistance as easily and could possibly have less stringent refuge requirements.

Layout	Scenario 1	Scenario 2
Low movement	1	1
High movement	0	0

Table 6.16: *The number of runs that develop resistance, ν, out of 10 simulation runs per scenario for high and low movement cases for a one year harvest cycle.*

Layout	Scenario 1	Scenario 2
Low movement	13133	755
High movement	0	0

Table 6.17: *The average number of resistant insects, μ_r, for 10 simulation runs per scenario for high and low movement cases for a one year harvest cycle.*

In Figure 6.13, it may be seen that, as with the two year harvest cycle, Scenario 1 experiences total extinction. The more regular harvesting schedule leads to blocks of sugarcane being replaced much more often, leading to a more pronounced difference between the insect populations of the two scenarios, with Scenario 1 showing a noticeable decrease at the four year mark. This also leads to extinction occurring at least a year earlier in the one year scenario when compared to the two year scenario.

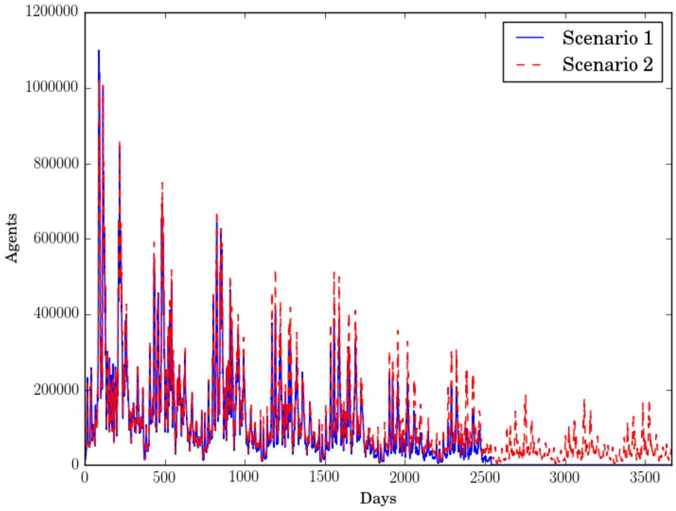


Figure 6.13: *The moth population can be seen steadily declining as Bt sugarcane is introduced, leading to total eradication in Scenario 1 when high movement is considered, as well as a stable population for Scenario 2.*

When compared to the results in §6.1.1 where $h = 20\%$ and four blocks of refuge, the phased approach did perform significantly better. The only caveat being that there was time in this case study for the gene to flow through the population before being exposed to Bt sugarcane, so a direct comparison is not possible. A direct comparison may serve as evidence that there may be less stringent refuge requirements should the Bt sugarcane be phased in rather than planted all at once. To test this hypothesis, the two year harvest case of Scenario 2 was rerun with $h = 10\%$ and low movement, and the case for $h \in \{10\%, 20\%\}$ and four blocks of refuge was rerun for low movement, except that refuge was introduced at the very first harvest after the warm-up period (Scenario 3). The simulated time, initial farm layout, and starting population was kept the same as for the previous experiments in case study 3. Low movement was chosen as it is historically the parameter value set that is most likely to develop resistance.

As may be seen in Table 6.18, for both cases the lesser refuge at 10% performed significantly better than the 20% refuge cases. This indicates that, where Bt sugarcane is to be planted in a field with an existing infestation, smaller refuge may be more effective, but further testing is required to confirm. With either value for h, the phased approach still performed the best at curbing resistance development.

Scenario	Scenario 3		Scenario 2	
Parameter	ν	μ_r	ν	μ_r
10%	4	14081	1	5180
20%	6	17530	3	3858

Table 6.18: *The number of runs that develop resistance, ν, out of 10 simulation runs and μ_r, the average number of resistant moths for the 10 runs per scenario for the low movement case for a two year harvest cycle.*

To determine whether or not the phased approach scales with how gradually the Bt sugarcane is introduced, two more scenarios are run for $h \in \{10\%, 20\%\}$ and low movement, but in the first scenario (Scenario 4) the replacement schedule now combines quadrants 1 and 2 and quadrants 3 and 4, giving a new replacement schedule as may be seen in Table 6.19. This effectively splits the field into two halves that are replaced four years apart. The second scenario (Scenario 5) has 8 blocks that are sequentially replaced a year apart. The modified replacement table for this scenario may be seen in Table 6.20, where, in essence, sub-quadrants 1 and 3 and sub-quadrants 2 and 4 are combined in each quadrant in Figure 6.11.

		Sub-quadrant			
		1	2	3	4
Quadrants	1	1470	1500	1530	1560
	2	1470	1500	1530	1560
	3	2930	2960	2990	3020
	4	2930	2960	2990	3020

Table 6.19: *The replacement schedule (in days) of each sub-quadrant for the first scenario.*

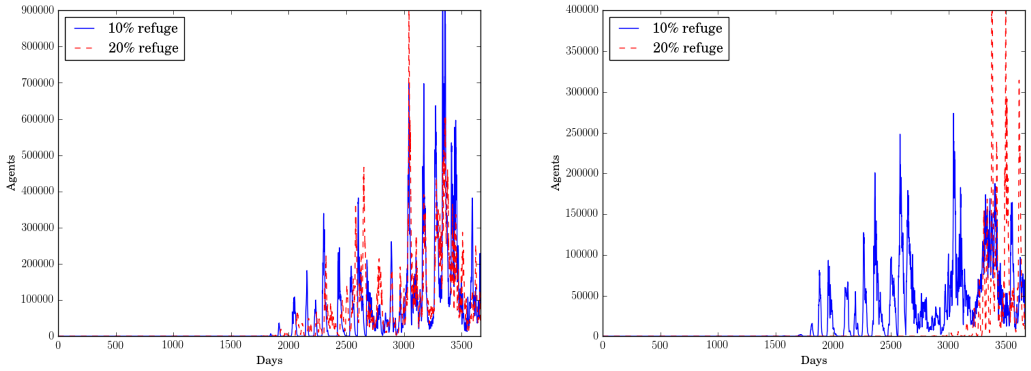
		Sub-quadrant			
		1	2	3	4
Quadrants	1	1470	1865	1530	1925
	2	2200	2595	2260	2655
	3	2930	3325	2990	3385
	4	3660	4055	3720	4115

Table 6.20: *The replacement schedule (in days) of each sub-quadrant for the second scenario.*

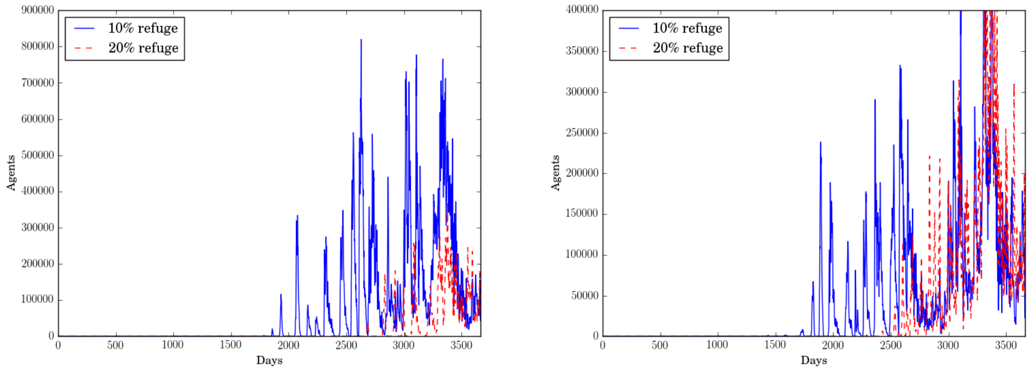
From Table 6.21 it may be seen that the results for the replacement in halves and eighths that 10% refuge delivers very unstable results, whereas there does seem to be a weak correlation between the replacement schedule and ability to curb resistance development for $h = 20\%$. As may be seen in Figure 6.14, except for Scenario 3, where $h = 10\%$ resistance tended to develop significantly earlier than for the runs where $h = 20\%$. In contrast to the discussion of the results for Table 6.18, this provides strong evidence against the notion of requiring less refuge, at least by the end of the phasing, if a phased approach is implemented.

Scenario	Scenario 4		Scenario 5	
Parameter	ν	μ_r	ν	μ_r
10%	4	6768	5	15580
20%	3	2094	2	4625

Table 6.21: *The number of runs that develop resistance, ν, out of 10 simulation runs and μ_r, the average number of resistant moths for the 10 runs per scenario for the low movement case for a two year harvest cycle.*



(a) *100% replaced (Scenario 3)* (b) *50% replaced at a time (Scenario 4)*



(c) *25% replaced at a time (Scenario 2)* (d) *12.5% replaced at a time (Scenario 5)*

Figure 6.14: *The average resistant population for runs that developed resistance for the four replacement scenarios tested for* $h \in \{10\%, 20\%\}$.

6.2 Decision support tool

The decision support tool in this study is presented as a summary of all the results obtained in the sensitivity analysis of Chapter 5 and those obtained from the case studies presented in this chapter. Unfortunately, as with any simulation study, it was impossible to test the combinations of all variables, but the results presented in this thesis should still allow for making high-level recommendations of how to approach refugia planning in a Bt sugarcane scenario. In this section, the key results are summarised and, where applicable, presented in a simplified infographic form.

6.2.1 Necessary assumptions

For a successful refuge strategy, there are some key basic assumptions that need to hold about the Bt sugarcane and the target pest, *E. saccharina*, before a refuge planting strategy can even be considered. These assumptions are summarised in the infographic as may be seen in Figure 6.15. Most of these assumptions were already discussed in detail in Chapter 2, but the results obtained in this study provide further evidence of their validity. The four key assumptions identified are:

- **High dose, high efficacy** – The development of resistance was best curbed where the efficacy of the Bt protein was at least 85%. Many of the most successful scenarios tested were very effective at quickly killing as many moths as possible, hence a very high dose Bt sugarcane would more likely result in a delay in resistance development.

- **Multiple modes of action** – One mode of action was found to be highly susceptible to resistance development. The default in this study was two modes of action, but as discussed in §2.3.2.2, more is necessarily better when considering the number of modes of action.

- **Recessive resistance genes** – In the scenario where the genes for resistance was dominant, even with two modes of action resistance developed readily. For the successful introduction of Bt sugarcane, the genes conferring resistance must be nearly or completely recessive.

- **Low initial gene frequency** – Most scenarios in this study had a relatively high initial resistance gene frequency in an effort to stress test the scenario set-up. In reality, the gene should be sufficiently rare ($<= 0.1\%$) to successfully curb resistance. In tests where the starting resistant population was 0.1%, resistance never developed. This is arguably the most important assumption as having too many insects initially resistant would most likely make any realistic refuge strategy fail.

6.2.2 Risk factors and recommendations

If the four basic assumptions hold, refugia planning may be considered. Based on the results obtained from the sensitivity analysis in Chapter 5 and the case studies presented in this chapter, the **maximum** proportion of a planted field to be kept as refuge is set at 30%, which is slightly higher than recommendations found in literature. This can be used as the conservative estimate for the refuge required in nearly any scenario. The recommended minimum is set at 10%, although this should only be considered if there is high confidence that the resistance gene is very rare ($< 0.1\%$). This corresponds with results obtained by Maselela [72] and Cristofoletti *et al.* [35], both recommending a refuge size of 10–20%. The key risk factors that could increase the risk of resistance development and recommendations of how to minimise the risk of resistance development is discussed in this section and a simplified infographic is presented in Figure 6.16.

6.2.2.1 Initial gene frequency

Although initial gene frequency is included as one of the four basic assumptions, a lower initial gene frequency is less likely to develop into a large resistant population and is supported by the results obtained by Butterfield *et al.* [18]. To confirm beyond the results in §5.4.2 whether or not a low initial resistant gene frequency would curb resistance development, two tests from the case studies are rerun for 10 runs each with an initial resistant population of 0.1%. The first scenario is from the first case study, where $h = 10\%$ in 16 blocks with average movement, and scenario 2 is Linear 1 with low movement from the second case study. The two chosen scenarios performed poorly before, having a ν value of 7 and 11 respectively. The first scenario had $\nu = 0$ and $\mu_r = 0$, indicating that 10% refuge would be sufficient in such a scenario even when the individual refuge areas are relatively small. Linear 1, arguably the worst performing scenario in all of the case studies, also had $\nu = 0$ and $\mu_r = 0$, hence it may be assumed that a low initial resistance gene frequency is one of the key drivers in curbing resistance development.

6.2.2.2 Moth movement

A key indicator of how successful a refuge strategy was, was what movement assumption was made about the moth. Moth populations that tend to be very localised would benefit from a

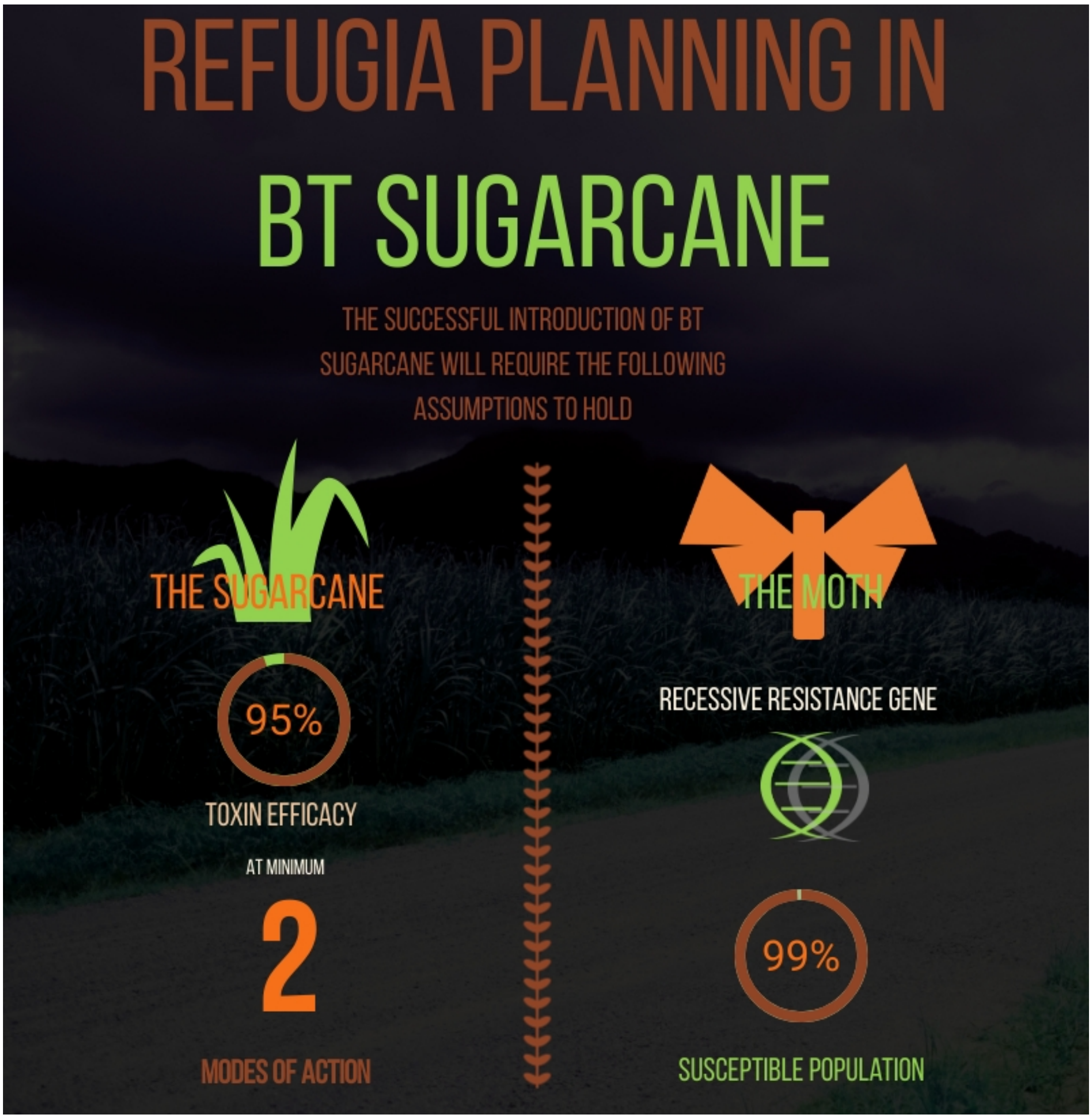


Figure 6.15: *An infographic summarising the four basic assumptions that must hold for the successful introduction of Bt sugarcane*

more conservative refuge strategy, whereas moths that were more likely to disperse over greater distances allows for a more lenient refuge requirement. The layout of the refuge is also less important where moths tend to disperse over greater distances, and most strategies performed nearly equally well when high moth movement was considered. If the moth is assumed to be a low movement species, the best strategies have larger individual refuge areas and it is recommended that a layout such as a central, single block or Border 1 be considered.

6.2.2.3 Planting and harvesting

One of the more minor factors identified was the increased risk of resistance development for sugarcane on a two year harvest cycle. Growers in these regions are recommended to be more conservative with their refuge strategy and should consider harvesting earlier if there are signs of infestation, as this was shown to help reduce the number of resistant runs. Growers on a one year harvest cycle could also benefit greatly from early harvesting even when infestation levels are high, as the sensitivity analysis tests had a very high initial resistant population. As observed in part by Kang *et al.* [58], it is also recommended that individual refuge areas not be harvested all at once and as far as possible after the Bt sugarcane was harvested, as a complete harvest

Figure 6.16: *An infographic summarising the risk factors that may increase the risk of resistance development, as well as some recommendations on how to minimise the risk.*

can drive moths into the Bt sugarcane, which increases the risk of resistance development.

6.2.2.4 Refuge shapes and distribution

Experiments in the first and second case studies provided evidence that not all refuge shapes are equally effective. Block refuges were found to be very effective in most cases and may potentially be improved upon by adding an element of the border refuge approach, but the difference was not significant enough to justify such a complex structure. The linear structures did not perform very well overall and may not warrant any further consideration. When considering the distribution of refuge areas, having large refuge areas may be more effective at curbing resistance development than having many small ones, especially if the infestation tends to be more localised (low movement). This correlates strongly with the results obtained by Tyutyunov *et al.* [114], where for small to medium farms it was also found that fewer, contiguous refuge areas were more effective. If a population of moths exist within a large refuge area, the surrounding Bt would act as a wall, reflecting the population towards the centre of the refuge area. If the refuge area is sufficiently large enough, the moths will not have any reason to leave the refuge area, reducing the risk of resistance development.

6.2.2.5 Gradual introduction of Bt sugarcane

In a more realistic scenario, Bt sugarcane would be introduced gradually as new sugarcane stocks are brought in to replace old ratoons. In this study, the gradual introduction was a very effective way of curbing resistance development. The reduction of non-Bt sugarcane, a natural refuge, from 100% to an eventual 0% was found to be very effective at completely eradicating the moth population. For this case, it is possible that structured refuges are not required, especially if newer Bt sugarcane varieties can be developed within 10 years of the first commercial release and planting of Bt sugarcane. If a structured refuge had to be recommended in a gradual introduction of Bt sugarcane, the same size and distribution recommendations would still be applicable.

6.3 Chapter summary

In this chapter, three case studies were presented that were designed to test the efficacy of refuge areas given several parameters such as size, distribution, shape, and how it is introduced. The results from these case studies and the results from §5.4.2 are then summarised as a set of risk factors (gene frequency, harvesting schedules) and recommendations (early harvesting, phased introduction of Bt sugarcane) presented as a decision support tool for refugia planning in a Bt sugarcane scenario. Simplifying infographics that visually summarise the results are also presented.

CHAPTER 7

Conclusion

Contents

7.1 Thesis summary **95**
7.2 Contributions of this thesis **97**
7.3 Proposals for future work **98**
  7.3.1 Recommendations for future entomological research 98
  7.3.2 Proposals for future simulation work 99

This concluding chapter opens with a summary of the work presented in this thesis, followed by a discussion of its main contributions with respect to computer simulation modelling and refuge planning in a sugarcane scenario. The chapter concludes with a list of propositions for future work to further this study.

7.1 Thesis summary

The introductory chapter opens with a background of historical pest management. This is followed by a review of the South African sugar industry, including its size and economic impact, and the status of *E. saccharina* as a pest in sugarcane and measures that have been employed to combat it. The notion of Bt crops is introduced, as well as the concept of refuge planting is described as a method to curb resistance development in target pests to the Bt crops. Chapter 1 also includes the formal problem description, scope and objectives of this thesis.

In Chapter 2, the biological literature is summarised in order to provide a background on: sugarcane as an organism, the general biology and life cycle of *E. saccharina*, the history and mechanisms involved in transgenic crops, and a short introduction on genetic flow. This is done in accordance with the Objective 1 as stated in §1.2. The background on *E. saccharina* includes discussions of its general biology, mating behaviours and dispersal patterns, as well as a summary of current and proposed control strategies. This is followed by a history of genetically modified organisms and specifically how insecticidal properties in *Bacillus thuringiensis* is used to create pest resistant crops. The potential for resistance development in target pests is also discussed, followed by how planting strategies such as structured or unstructured refuge areas could curb such resistance development. The chapter concludes with a brief introduction to genetics and how it can influence an organism and be passed on to future generations as well as introducing some key terms in understanding how genes flow in a population of organisms.

In Chapter 3, various topics on computer simulation modelling were discussed, including the components of a typical computer simulation model as well as the different classes and paradigms of simulation modelling found in literature. This is done in accordance with Objective 1 as stated in §1.2. The typical steps in developing a computer simulation model is discussed, followed by a review of some of the advantages and disadvantages of simulation modelling. The chapter concludes with a review of simulation modelling in ecological systems, with a specific focus on current pest management simulation models. Models that aimed to simulate resistance development in a Bt crop scenario were identified and it was found that none were focussed on the spatial layout of refuge, a key shortcoming that this thesis aimed to address.

Chapter 4 detailed the technical implementation of the agent-based model of *E. saccharina* on sugarcane. This includes a description of how the various organisms were implemented as objects and how they were allowed to interact with each other, as required by Objective 2. Information from Chapter 2 is then used to assist with setting parameter values for each agent (such as the mortality rates for eggs or the growth rate of sugarcane) in accordance with Objective 3. A detailed discussion on the implementation of the cellular structure of the simulation space, how the cells manage the agents within them, and performance benefits of using cells is presented, followed by descriptions of how movement of agents is facilitated given this cellular structure. The assumptions made in implementing moth movement was also discussed in detail, as this was an area of research that was found to be lacking. The chapter concludes with a review of the outputs that can be collected after running the model and a description the infrastructure used to run the models.

The model verification and validation is discussed in Chapter 5, two key steps in the development of computer simulation modelling as detailed in §3.2 and forms part of Objective 4. The chapter opens with the verification of the model to confirm that it does perform as designed and acts in a predictable way when presented with certain inputs. Once the model has been verified, a short validation of the maturation and mortality rates of the immature life stages is performed using historical data. To supplement this validation step, the process followed to perform a face validation using expert opinion is discussed and the outcomes of the discussion listed. The general outcome was that the experts did approve of how the model was implemented after being shown animations of the model outputs. This is followed by a sensitivity analysis, where a given experimental set-up was tested with several variations of parameters related to both the moth and the sugarcane to see how the model would behave. The chapter concludes with a discussion on some of the more interesting results obtained from the sensitivity analysis, such as the effects of random male movement on curbing resistance development and how unpredictable resistance development was when it did develop.

Chapter 6 aimed to fulfil Objective 5 by presenting three case studies that experiment with different refuge sizes, layouts and planting schedules. One of the most noteworthy results from the case studies was that an effective refuge strategy should have refuge areas that are individually large enough to house a significant susceptible population. It was also found that most strategies do not provide sufficient protection if the pest has a low dispersal rate. Another key result from this chapter was that a phased introduction could be a default strategy for nearly any scenario, as this was found to lead to the complete extinction of the pest even if no further refuge was planted. In terms of structured refuge, it was found that a combination of block and border refuge areas would likely be the most effective in the *E. saccharina* on sugarcane scenario where a phased approach is not implemented. The outputs and results from these case studies were compiled into a single summarised decision support tool in the form of an infographic that can aid in making recommendations to industry on what refuge strategy may work best in their specific scenarios by highlighting some of the biggest risk factors identified during the course of this study.

7.2 Contributions of this thesis

The main contributions of this thesis on refugia planning in a Bt sugarcane scenario, specifically with *E. saccharina* as a target pest and agent-based simulation as the modelling paradigm, are outlined in this section.

Contribution 1 *The development of an agent-based simulation framework that allows for the simulation of millions of agents over a very large area.*

The problem statement of this thesis required a very large, spatially explicit simulation model of *E. saccharina* on sugarcane. Existing models were found to be either too detailed to scale to the area and time scale required or were not granular enough to account for different refuge layouts. In addition, most models developed for refuge requirement analysis were population-based and did not consider the impact of refuge layout at all. The cellular structure of the developed simulation model allowed for scaling up to a farm scale, while still being granular enough to allow for more intricate refuge layouts as was demonstrated in Chapter 6. The aggregation of egg batches into single agents that could progress through all the immature life stages also assisted in reducing the memory and processing requirements of the model, further enabling very large scale scenarios.

Contribution 2 *A modelling framework for agroecological food web modelling which has significant potential use regarding the evaluation of integrated pest management strategies in agroecosystem management.*

The developed framework allows for the easy inclusion and testing of other pest control strategies, which could assist in better understanding how to develop successful integrated pest management systems on a spatial and temporal scale. Most farming decisions are spatially explicit problems. Few models in literature facilitate both multi-species modelling as well as spatial dynamics due to the computational difficulty, and also the difficulty in understanding and including the interaction between different species and pest control strategies. The model developed considered both temporal and spatial dynamics, interaction between sugarcane and insects, and climate, which has significant application in agroecosystem management.

Contribution 3 *Detailed investigations related to the shape, size and distribution of refuge areas and how they affect resistance development.*

In literature, it was found that most refuge recommendations were only focussed on the overall size of the refuge area. The case studies presented in Chapter 6 highlighted that not all refuge areas are equally effective. Having highly distributed refuge areas may mean that individual refuge areas may not be large enough to sustain a sufficiently large susceptible population, as was highlighted in the first case study of Chapter 6. It was also found that there is a significant difference between different layouts, even if they were overall the same size. Another key outcome that was discussed in this study is the effect of dispersal rates on how effective refuge strategies were, as it was found that there was an inverse relationship between the insects likelihood of movement (dispersal rate) and resistance development. In the scenario specific to how sugarcane is planted and harvested, it was also found that there is a possibility of foregoing the requirement for a refuge area if the Bt sugarcane is planted in phases, a result that the author could not find in currently available literature.

Contribution 4 *Using agent-based simulation to provide decision support in refugia planning and not as a "what-if" analysis tool.*

Many simulation models are designed as a tool to be used by the end-user to investigate what might happen to a system given a certain set of input parameters. The model of *E. saccharina* on sugarcane developed by Van Vuuren *et al.* [120] did not aim to provide any direct decision support, but was intended to be used as a "what if" analysis tool to test various pest management scenarios on a relatively small scale. In this study, the aim was to provide explicit decision support on refugia planning using the combined output of the model for several parameter variations and scenarios, supporting the case for using simulation models, specifically agent-based simulation models, to aid in decision making, especially in an ecological context.

Contribution 5 *A detailed description of how the movement of adult moths is facilitated in a cellular structure.*

The cellular structure of the model, especially the concept of layered cells, is not one seen often in literature and one of the challenges faced by the author was on how to model the moth's movement between cells in the same layer and how they may move between layers. The inclusion of a grid of larger cells representing the sugarcane canopy allowed for the desired dispersal rates of adult moths looking to mate, while the finer grid representing the ground level allowed for the localised movement of females searching for sites to lay eggs. While there is a cost in memory to store the extra grid of cells, the reduced processing burden of performing a very large neighbourhood search for pre-mated female moths was considered to be worth the cost.

7.3 Proposals for future work

As with all research projects, especially theses and dissertations, time does not allow for the exploration and investigation of all research questions and ideas that may arise during the completion of the study. The presented model cannot be considered as complete as methodologies, approaches and assumptions could be modified or replaced completely. In fulfilment of Objective 6, this section contains suggestions for possible future improvements and possible directions for future research from a simulation modelling and entomological point of view.

7.3.1 Recommendations for future entomological research

Most of the assumptions made when setting parameter values were supported by past research of *E. saccharina*'s general biology and how it infests sugarcane. Some behaviours of the moth were implemented as a "best guess" estimate and presented to subject matter experts to evaluate. Future research into these behaviours in particular would greatly benefit the development of improved models.

Proposal 1 *Measure the frequency of the resistance genes* in vitro.

One of the main assumptions highlighted in §6.2.1 was the requirement for a low initial resistance gene frequency and that the gene must be recessive. *In vitro* studies on the gene frequency and inheritance could be done before the development of the actual Bt sugarcane and would allow for greater accuracy within the model.

Proposal 2 *Measure accurate dispersal rates and dispersal triggers of E. saccharina moths.*

A point of research that would have greatly benefited this study and a point mentioned by Potgieter *et al.* [88] and Van Vuuren *et al.* [120] was the lack of experimental data related to how the *E. saccharina* moth disperses once it emerges. It was assumed that the female was able to disperse further and more readily than the male and that males tend to move more towards more mature sugarcane to form *leks* based on observation by experts [32]. The lack of experimental data on the validity of these behaviours leads to the implementation of moth dispersal in this study being only a rough estimate of their true behaviour.

Proposal 3 *Measure reproductive fitness of moths of different genotypes and how the Bt protein affects them.*

Due to the proposed Bt sugarcane still being in the research phase at the point in time when this study was carried out, there was very little known about how the Bt protein would affect a surviving adult moth's ability to reproduce. For this reason, the assumption was made to exclude reproductive fitness as a part of the *E. saccharina* life cycle. The availability of this data would make the model more accurate, as it would be able to more realistically simulate the interactions between resistant and susceptible populations.

Proposal 4 *Measure how the inclusion of Bt sugarcane may affect the female moth's egg laying sites.*

While it could be realistically assumed that there is no outward difference between a normal and a Bt sugarcane varietal, it is not currently known whether or not *E. saccharina* will display some notable bias when choosing an egg laying site. It is currently assumed that the female is agnostic and would equally likely choose an egg laying site, but future research into any potential bias would allow for a more realistic future model.

7.3.2 Proposals for future simulation work

The model developed in Chapter 4 contains several simplifying assumptions and, as such, cannot be considered a complete and accurate representation of the real-world interactions between *E. saccharina* and sugarcane, but rather as an approximation. The model may benefit from some refinement and the inclusion of better techniques, assumptions, validations or even modelling approaches. Possible future modelling work may include the following proposals.

Proposal 5 *Investigate the economical and practical impact of different refuge strategies.*

This study did not consider an economical analysis of different refuge strategies. Future research could focus on the risk-benefit analysis of planting less refuge and having smaller infestations, but possibly having to switch to new Bt variants sooner, or planting more refuge and possibly being able to use the same Bt variant for several years. Replanting with Bt would require significant capital investment, so it would be beneficial to investigate how a grower should approach planting Bt in a way that would effectively manage the pest and be financially feasible. As an example, a farm with a severe infestation could possibly find that the net present value over five years for replacing all their sugarcane with Bt regardless of ratoon age now could be significantly higher than using a phased approach.

Proposal 6 *Incorporate GIS shapefiles and topological information into the simulated space.*

The developed model uses a simple square space to represent the sugarcane field. In reality, there are many different layouts and structures within a sugarcane field such as roads and other unplanted areas. Being able to read in distinct geographic information system (GIS) shapefiles[1] would allow for customised refuge recommendations according to the layout of a specific sugarcane field. Due to the topic of the study focussing on general recommendations, the inclusion of shapefiles did not fall within the scope of this study, but is recommended for future research.

During the face validation of the developed model as discussed in Chapter 5, the notion of adding topological information to the simulated space was discussed. It was mentioned that infestations are observed to focus around the tops of hills more often than in the bottom of a valley, mostly due to the male moths wanting to reach the highest point for optimal pheromone

---

[1] Shapefiles are vector files that contain nontopological geometric information of geographic features such as rivers, lakes and natural boundaries.

dispersal [32, 106]. As with the inclusion of shapefiles, modelling the effect of land topology was not included as part of the original scope, but is considered an important topic for future improvements of the model.

Proposal 7 *Investigate how different boundary implementations may affect the model performance.*

The model was developed with a reflective boundary, with no agents being allowed to leave or enter the simulated space. This was done due to the lack of information regarding the frequency of the resistance genes, which complicates the inclusion of external migrants. Once there is more research available on the gene frequency in populations of *E. saccharina*, the model can be expanded with different boundary conditions, which would allow for the further investigation on the effects of gene swamping, migration load and migration meltdown as discussed in §2.4.

Proposal 8 *Introduce a fitness function for male and female moths.*

The inclusion of a fitness function that directly impacts reproductive success in adult moths could improve the accuracy of the developed model, given the research from Proposal 2 becomes available. It would also open up avenues of research related to scenarios where mating fitness is affected, such as the introduction of sterilised insects in an SIT scenario, where it was found that males and females that have been irradiated to induce sterility could be less competitive than their wild counterparts.

Proposal 9 *Implement more control strategies in the pursuit of a true integrated pest management system.*

The scenarios tested in this study assumed that there were no other control strategies in place other than the use of Bt sugarcane. In reality, it is much more likely that the grower would incorporate a variety of control strategies and the inclusion of these strategies in a future model could assist in finding good combinations for pest management. Tabashnik *et al.* [112] discussed the efficacy of combining sterile insect releases with the usage of Bt cotton where there were infestations of pink bollworm (*Pectinophora gossypiella*) and found that refugia became redundant. The combination of the model developed in this study with the SIT models developed by Potgieter *et al.* [88] is thus highly recommended for future work.

It would also be interesting to develop a model that combines a refuge strategy with push-and-pull plants, specifically pull plants that are more attractive to *E. saccharina* such as reeds from the family *Cyperaceae*. Using pull plants as natural refuges may result in lesser refuge requirements, as the moths are less likely to move out of the refuge areas and into the neighbouring Bt sugarcane. Future research could investigate how effective a combination of SIT, Bt and pull plants as a natural refuge would be as a truly integrated pest management strategy.

Proposal 10 *Compare the model outputs to existing deterministic and population-based models.*

To determine the degree to which the output of the model developed in this study corresponds with the outputs of existing mathematical models such as those described in §3.4, it is proposed that a comparison be performed. As model outputs for different research projects differ according to the specific scenarios they were testing, it may be necessary to create a single real-world scenario with parameter values that can be implemented across all the models included in the comparison. Such a real-world case study would ideally have measured data that would allow for a true comparison of the model outputs.

Proposal 11 *Implement better sugarcane parameters and behaviours.*

The current model reduces the complexity of the sugarcane to an organism that grows according to temperature and is able to house an infestation of *E. saccharina*. In §4.2.1.1, a

`Stressed Rating` was included and described, but time constraints did not allow for the proper implementation and testing of its function. It was also mentioned during the face validation that older sugarcane may “lodge”, or essentially fall over under its own weight as it matures, before regrowing upwards again [32]. It is proposed that future work add these complexities to the model to more accurately portray the true layout of a sugarcane farm, as both these parameters would influence the moth behaviour.

Proposal 12 *Expand the model from a farm scale to an area-wide scale.*

When demonstrating the model to James Rhodes of Biosafety SA [96], the notion of modelling an entire area rather than a single farm was discussed. Sugarcane farms exist in large expanses and commercial growers can be located adjacent to small-scale growers. As a risk management tool, it is proposed that the model be expanded to an area covering several square kilometres that would include several large commercial farms and smaller subsistence farms, each with their own planting strategies and Bt sugarcane not being available to all. This would also align the model with the current regional models developed by Sisterson *et al.* [103, 104], Peck *et al.* [83] and Caprio & Tabashnik [21, 22].

Bibliography

[1] *Diamond v. Chakrabarty*, 1980, volume 447, Supreme Court.

[2] AgInnovators, *Harvard scientists use PACE to fast-track discovery of bt toxins to target super-bugs*, Available from `http://www.aginnovators.org.au/news/harvard-scientists-use-pace-fast-track-discovery-bt-toxins-target-super-bugs`, [Online: accessed 7 May 2018].

[3] Aroian R, *History of Bt*, Available from `http://www.bt.ucsd.edu/index.html`, [Online: accessed 8 May 2018].

[4] Aronson AI, Beckman W & Dunn P, 1986, *Bacillus thuringiensis and related insect pathogens*, Microbiological reviews, **50(1)**, p. 1.

[5] Atkinson PR, 1979, *Distribution and natural hosts of Eldana saccharina Walker in Natal, its oviposition sites and feeding patterns*, Proceedings of the 53rd South African Sugar Technologists Association, pp. 111–115.

[6] Atkinson PR, 1980, *On the biology, distribution and natural host-plants of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Journal of the Entomological Society of Southern Africa, **43(2)**, pp. 171–194.

[7] Atkinson PR, 1981, *Mating behaviour and activity patterns of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Journal of the Entomological Society of Southern Africa, **44(2)**, pp. 265–280.

[8] Atkinson PR & Carnegie AJM, 1989, *Population dynamics of the sugarcane borer, Eldana saccharina Walker (Lepidoptera: Pyralidae), in Natal, South Africa*, Bulletin of Entomological Research, **79(1)**, pp. 61–80.

[9] Banks J, 1998, *Principles of simulation*, Handbook of simulation: Principles, methodology, advances, applications, and practice, pp. 3–30.

[10] Banks J, 1999, *Introduction to simulation*, Proceedings of the 31st Winter Simulation Conference, volume 1, pp. 7–13.

[11] Berg P, Baltimore D, Brenner S, Roblin RO & Singer MF, 1975, *Summary statement of the Asilomar conference on recombinant DNA molecules*, Proceedings of the National Academy of Sciences, **72(6)**, pp. 1981–1984.

[12] Berry SD, Leslie GW, Spaull VW & Cadet P, 2010, *Within-field damage and distribution patterns of the stalk borer, Eldana saccharina (Lepidoptera: Pyralidae), in sugarcane and a comparison with nematode damage*, Bulletin of Entomological Research, **100(4)**, pp. 373–385.

[13] Bezuidenhout CN, 2000, *A model review and proposed mechanistic tiller model for the CANEGRO sugarcane crop model*, Doctoral Dissertation, Durban University of Technology.

[14] Borshchev A & Filippov A, 2004, *From system dynamics and discrete event to practical agent based modeling: reasons, techniques, tools*, Proceedings of the 22nd International Conference of the System Dynamics Society, electronically published without page numbers.

[15] Bourguet D, Chaufaux J, Séguin M, Buisson C, Hinton JL, Stodola TJ, Porter P, Cronholm G, Buschman LL & Andow DA, 2003, *Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, Ostrinia nubilalis*, Theoretical and Applied Genetics, **106(7)**, pp. 1225–1233.

[16] Bruening G & Lyons J, 2000, *The case of the FLAVR SAVR tomato*, California Agriculture, **54(4)**, pp. 6–7.

[17] Bushak L, *A Brief History Of Genetically Modified Organisms: From Prehistoric Breeding To Modern Biotechnology*, Available from `https://www.medicaldaily.com/brief-history-genetically-modified-organisms-prehistoric-breeding-modern-344076`, [Online: accessed 11 May 2018].

[18] Butterfield MK, Brokensha MA & Wood RA, 2002, *Genetic models to assess the development of counter-resistance in insect pests exposed to Bt-sugarcane*, Proceedings of the 76th South African Sugar Technologists Association, pp. 329–335.

[19] Byrne MD, 2013, *How many times should a stochastic model be run? An approach based on confidence intervals*, Proceedings of the 12th International Conference on Cognitive Modeling, pp. 445–450.

[20] CANEGROWERS, *CANEGROWERS - How sugarcane is grown - paddock to plate*, Available from `http://www.canegrowers.com.au/page/archived\-pages/about\_australian\_sugarcane`, [Online: accessed 21 June 2016].

[21] Caprio MA, 1998, *Evaluating resistance management strategies for multiple toxins in the presence of external refuges*, Journal of Economic Entomology, **91(5)**, pp. 1021–1031.

[22] Caprio MA & Tabashnik BE, 1992, *Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance*, Journal of Economic Entomology, **85(3)**, pp. 611–620.

[23] Carnegie AJM, 1974, *A recrudescence of the borer Eldana saccharina Walker (Lepidoptera: Pyralididae)*, Proceedings of the 48th South African Sugar Technologists Association, pp. 107–110.

[24] Carnegie AJM, 1981, *Combating Eldana saccharina Walker: A progress report*, Proceedings of the 55th South African Sugar Technologists Association, pp. 116–119.

[25] Carnegie AJM & Smaill RJ, 1982, *Pre-trashing of sugarcane as a means of combating the borer Eldana saccharina Walker*, Proceedings of the 56th South African Sugar Technologists Association, pp. 78–81.

[26] Clayton WD, Vorontsova MS, Harman KT & Williamson H, *GrassBase - The Online World Grass Flora*, Available from `http://www.kew.org/data/grasses-db.html`, [Online: accessed 21 June 2016].

[27] Cohen SN, Chang ACY, Boyer HW & Helling RB, 1973, *Construction of biologically functional bacterial plasmids in vitro*, Proceedings of the National Academy of Sciences, **70(11)**, pp. 3240–3244.

[28] Conlong DE, 1990, *A study of pest-parasitoid relationships in natural habitats: an aid towards the biological control of Eldana saccharina (Lepidoptera: Pyralidae) in sugarcane*, Proceedings of the 64th South African Sugar Technologists Association, pp. 111–115.

[29] Conlong DE, 1994, *A review and perspectives for the biological control of the African sugarcane stalkborer Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Agriculture, ecosystems & environment, **48(1)**, pp. 9–17.

[30] Conlong DE, 1997, *Biological control of Eldana saccharina Walker in South African sugarcane: Constraints identified from 15 years of research*, International Journal of Tropical Insect Science, **17(1)**, pp. 69–78.

[31] Conlong DE, 2001, *Biological control of indigenous African stemborers: What do we know?*, International Journal of Tropical Insect Science, **21(4)**, pp. 267–274.

[32] Conlong DE, 2018, [Private Communication], Senior Entomologist at the South African Sugarcane Research Institute (`Des.Conlong@sugar.org.za`).

[33] Conlong DE, Kasl B & Byrne M, 2007, *Independent kids, or motherly moms? Implications for integrated pest management of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Proceedings of the 26th The International Society of Sugar Cane Technologists, pp. 787–796.

[34] Craig DC, 1996, *Extensible hierarchical object-oriented logic simulation with an adaptable graphical user interface*, Doctoral Dissertation, Memorial University of Newfoundland.

[35] Cristofoletti PT, Kemper EL, Capella AN, Carmago SR, Cazoto JL, Ferrari F, Galvan TL, Gauer L, Monge GA, Nishikawa MA *et al.*, 2018, *Development of transgenic sugarcane resistant to sugarcane borer*, Tropical Plant Biology, **11(1-2)**, pp. 17–30.

[36] Dannie M, *What Are the Health Benefits of Sugarcane?*, Available from `https://www.livestrong.com/article/495010-what-are-the-health-benefits-of-sugarcane`, [Online: accessed 7 May 2018].

[37] de Maagd RA, Bravo A & Crickmore N, 2001, *How Bacillus thuringiensis has evolved specific toxins to colonize the insect world*, TRENDS in Genetics, **17(4)**, pp. 193–199.

[38] Department of Agriculture, Forestry & Fisheries, *Sugar Fact Sheet*, Available from `http://www.nda.agric.za/docs/FactSheet/Sugar06.pdf`, [Online: accessed 21 June 2016].

[39] Einhorn MD, 2015, *Self-Organising Traffic Control Algorithms at Signalised Intersections*, Doctoral Dissertation, Stellenbosch University.

[40] Farias JR, Costa EC, Guedes JVC, Arbage AP, Neto AP, Bigolin M & Pinto FF, 2013, *Managing the sugarcane borer, Diatraea saccharalis, and corn earworm, Helicoverpa zea, using Bt corn and insecticide treatments*, Journal of Insect Science, **13(1)**, p. 109.

[41] Garcia AG, Ferreira CP, Cônsoli FL & Godoy WA, 2016, *Predicting evolution of insect resistance to transgenic crops in within-field refuge configurations, based on larval movement*, Ecological Complexity, **28**, pp. 94–103.

[42] Gassmann AJ, Petzold-Maxwell JL, Keweshan RS & Dunbar MW, 2011, *Field-evolved resistance to bt maize by western corn rootworm*, PloS one, **6(7)**, p. e22629.

[43] Gould F, Martinez-Ramirez A, Anderson A, Ferre J, Silva FJ & Moar WJ, 1992, *Broad-spectrum resistance to Bacillus thuringiensis toxins in Heliothis virescens*, Proceedings of the National Academy of Sciences, **89(17)**, pp. 7986–7990.

[44] Gouse M, Pray CE, Kirsten J & Schimmelpfennig D, 2005, *A GM subsistence crop in Africa: the case of Bt white maize in South Africa*, International Journal of Biotechnology, **7(1-3)**, pp. 84–94.

[45] Grimm V & Railsback SF, 2013, *Individual-based modeling and ecology*, Princeton University Press.

[46] Hall DG, 1988, *Insects and mites associated with sugarcane in Florida*, The Florida Entomologist, **71(2)**, pp. 138–150.

[47] Hannay CL & Fitz-James P, 1955, *The protein crystals of Bacillus thuringiensis Berliner*, Canadian Journal of Microbiology, **1(8)**, pp. 694–710.

[48] Heathcote RJ, 1984, *Insecticide testing against* Eldana saccharina *Walker*, Proceedings of the 57th South African Sugar Technologists Association, p. 155.

[49] Hedrick PW, 1995, *Gene flow and genetic restoration: the Florida panther as a case study*, Conservation Biology, **9(5)**, pp. 996–1007.

[50] Hoad K, Robinson S & Davies R, 2010, *Automating warm-up length estimation*, Journal of the Operational Research Society, **61(9)**, pp. 1389–1403.

[51] Höfte H & Whiteley HR, 1989, *Insecticidal crystal proteins of bacillus thuringiensis*, Microbiological Reviews, **53(2)**, pp. 242–255.

[52] Hokkanen HMT, 1991, *Trap cropping in pest management*, Annual Review of Entomology, **36(1)**, pp. 119–138.

[53] Huang F & McGaughey WH, 1999, *Inheritance of resistance to Bacillus thuringiensis toxin (Dipel ES) in the European corn borer*, Science, **284(5416)**, pp. 965–967.

[54] Huang J, Hu R, Pray C, Qiao F & Rozelle S, 2003, *Biotechnology as an alternative to chemical pesticides: a case study of Bt cotton in China*, Agricultural Economics, **29(1)**, pp. 55–67.

[55] Hutchison WD, Burkness E, Mitchell P, Moon R, Leslie T, Fleischer SJ, Abrahamson M, Hamilton K, Steffey KL, Gray M *et al.*, 2010, *Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers*, Science, **330(6001)**, pp. 222–225.

[56] Huth A & Wissel C, 1992, *The simulation of the movement of fish schools*, Journal of theoretical biology, **156(3)**, pp. 365–385.

[57] Ibrahim MA, Griko N, Junker M & Bulla LA, 2010, *Bacillus thuringiensis: a genomics and proteomics perspective*, Bioengineered Bugs, **1(1)**, pp. 31–50.

[58] Kang J, Huang F & Onstad D, 2014, *Modeling evolution of resistance of sugarcane borer (Lepidoptera: Crambidae) to transgenic Bt corn*, Environmental entomology, **43(4)**, pp. 1084–1104.

[59] Keen H, Pickup JC, Bilous RW, Glynne A, Viberti GC, Jarrett RJ & Marsden R, 1980, *Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men*, The Lancet, **316(8191)**, pp. 398–401.

[60] Keeping MG & Rutherford RS, 2004, *Resistance mechanisms of South African sugarcane to the stalk borer Eldana saccharina (Lepidoptera: Pyralidae): A review*, Proceedings of the 78th South African Sugar Technologists Association, pp. 307–311.

[61] Kolahi S, 2011, *Simulation Model, Warm-up Period, and Simulation Length of Cellular Systems*, Proceedings of the 2nd International Conference on Intelligent Systems, Modelling and Simulation, pp. 375 – 379.

[62] Kota M, Daniell H, Varma S, Garczynski SF, Gould F & Moar WJ, 1999, *Overexpression of the Bacillus thuringiensis (Bt) Cry2Aa2 protein in chloroplasts confers resistance to plants against susceptible and Bt-resistant insects*, Proceedings of the National Academy of Sciences, **96(5)**, pp. 1840–1845.

[63] Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M & Kadwell S, 1993, *Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis*, Nature Biotechnology, **11(2)**, p. 194.

[64] Kramer MG & Redenbaugh K, 1994, *Commercialization of a tomato with an antisense polygalacturonase gene: The FLAVR SAVR^{TM} tomato story*, Euphytica, **79(3)**, pp. 293–297.

[65] Law AM, 1990, *How to build valid, credible and appropriately detailed simulation models*, Industrial Engineering, **22(6)**, pp. 16–17.

[66] Law AM & Kelton WD, 2007, *Simulation modeling and analysis*, volume 3, McGraw-Hill New York.

[67] Lenormand T, 2002, *Gene flow and the limits to natural selection*, Trends in Ecology & Evolution, **17(4)**, pp. 183–189.

[68] Leslie GW, 1990, *The influence of dead leaf material on the oviposition behaviour of Eldana saccharina (Lepidoptera: Pyralidae) in sugarcane*, Proceedings of the 64th South African Sugar Technologists Association, pp. 100–102.

[69] Lotka AJ, 1920, *Undamped oscillations derived from the law of mass action.*, Journal of the American Chemical Society, **42(8)**, pp. 1595–1599.

[70] Luttrell RG, Ali I, Allen KC, Young III SY, Szalanski A, Williams K, Lorenz G, Parker Jr CD & Blanco C, 2004, *Resistance to Bt in Arkansas populations of cotton bollworm*, Proceedings of the Beltwide Cotton Conferences.

[71] Mahakrushi, *Growth Morphology of Sugarcane*, Available from `https://www.mahakrushi.com/2011/07/propagation-of-sugarcane`, [Online: accessed 7 May 2018].

[72] Maselela M, 2018, *Refugia planning in a Bt sugarcane scenario*, Honours project at Stellenbosch University.

[73] McGaughey WH, 1985, *Insect resistance to the biological insecticide Bacillus thuringiensis*, Science, **229(4709)**, pp. 193–195.

[74] Mendel G, 1965, *Experiments in plant hybridisation*, Harvard University Press.

[75] Milner RJ, 1994, *History of Bacillus thuringiensis*, Agriculture, ecosystems & environment, **49(1)**, pp. 9–13.

[76] Monsanto, *IRM Grower's Guide*, Brochure, Available from `https://monsanto.com/app/uploads/2017/05/irm-grower-guide.pdf`.

[77] Mudavanhu P, Conlong DE, Addison P *et al.*, 2013, *Releases of irradiated moths to suppress wild populations of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Proceedings of the 86th South African Sugarcane Technologists Association Congress, pp. 311–320.

[78] Ni Y, 1995, *Genetic variation of European corn borer in Delaware and genetic comparison with Asian corn borer*, Doctoral Dissertation, University of Delaware.

[79] Oberemok VV, Laikova KV, Gninenko YI, Zaitsev AS, Nyadar PM & Adeyemi TA, 2015, *A short history of insecticides*, Journal of Plant Protection Research, **55(3)**, pp. 221–226.

[80] Oh PP, Rangaiah GP & Ray AK, 2002, *Simulation and multiobjective optimization of an industrial hydrogen plant based on refinery off-gas*, Industrial & Engineering Chemistry Research, **41(9)**, pp. 2248–2261.

[81] Ostlie KR, Hutchison WD & Hellmich RL, 1997, *Bt corn and European corn borer*, Faculty Publications: Department of Entomology, **597**.

[82] Pannell DJ, 1997, *Sensitivity analysis of normative economic models: theoretical framework and practical strategies*, Agricultural Economics, **16(2)**, pp. 139–152.

[83] Peck SL, Gould F & Ellner SP, 1999, *Spread of resistance in spatially extended regions of transgenic cotton: implications for management of heliothis virescens (lepidoptera: Noctuidae)*, Journal of Economic Entomology, **92(1)**, pp. 1–16.

[84] Peng C, 2000, *Understanding the role of forest simulation models in sustainable forest management*, Environmental Impact Assessment Review, **20(4)**, pp. 481–501.

[85] Phillips L, *Sugar cane: prevent soil compaction and improve yield*, Available from `https://www.farmersweekly.co.za/crops/field-crops/sugar-cane-prevent-soil-compaction-improve-yield/`, [Online: accessed 8 May 2018].

[86] Potgieter L, 2013, *A mathematical model for the control of Eldana saccharina Walker using the sterile insect technique*, Doctoral Dissertation, Stellenbosch University.

[87] Potgieter L, *Surgor Research Areas*, Available from `https://www.surgor.org.za/research`, [Online: accessed 8 November 2018].

[88] Potgieter L, Van Vuuren JH & Conlong DE, 2013, *A reaction–diffusion model for the control of Eldana saccharina Walker in sugarcane using the sterile insect technique*, Ecological modelling, **250**, pp. 319–328.

[89] Potgieter L, Van Vuuren JH & Conlong DE, 2015, *The role of heterogeneous agricultural landscapes in the suppression of pest species following random walk dispersal patterns*, Ecological Modelling, **306**, pp. 240–246.

[90] Potgieter L, Van Vuuren JH & Conlong DE, 2016, *Simulation modelling as a decision support in developing a sterile insect-inherited sterility release strategy for eldana saccharina (lepidoptera: Pyralidae)*, Florida Entomologist, **99(1)**, pp. 13–23.

[91] Pray C, Ma D, Huang J & Qiao F, 2001, *Impact of Bt cotton in China*, World Development, **29(5)**, pp. 813–825.

[92] Qiao F, Huang J, Rozelle S & Wilen J, 2010, *Natural refuge crops, buildup of resistance, and zero-refuge strategy for Bt cotton in China*, Science China Life Sciences, **53(10)**, pp. 1227–1238.

[93] Ramgareeb S, McFarlane SA, Conlong DE & Rutherford RS, 2010, *Identification of herbivore induced plant volatiles from push-pull plants and Fusarium species: aids for the management of Eldana saccharina Walker (Lepidoptera: Pyralidae) in sugarcane?*, Proceedings of the 83rd South African Sugar Technologists Association, pp. 262–266.

[94] Rangel G, *From Corgis to Corn: A Brief Look at the Long History of GMO Technology*, Available from `http://sitn.hms.harvard.edu/flash/2015/from-corgis-to-corn-a-brief-look-at-the-long-history-of-gmo-technology/`, [Online: accessed 11 May 2018].

[95] Reynolds A & Martinez J, *Insect Resistance Management for Bt Plant-Incorporated Protectants*, Available from `https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/insect-resistance-management-bt-plant-incorporated`, [Online: accessed 8 May 2018].

[96] Rhodes J, 2017, [Private Communication], Analyst at Biosafety SA (`james@biosafety.org.za`).

[97] Rose KA, Rutherford ES, McDermot DS, Forney JL & Mills EL, 1999, *Individual-based model of yellow perch and walleye populations in oneida lake*, Ecological Monographs, **69(2)**, pp. 127–154.

[98] Roush RT, 1998, *Two–toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not?*, Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, **353(1376)**, pp. 1777–1786.

[99] Saltelli A, 2002, *Sensitivity analysis for importance assessment.*, Risk Analysis: An International Journal, **22(3)**, pp. 579 – 590.

[100] Sampson MA & Kumar R, 1985, *Life history, development and behaviour of Eldana saccharina Walker on sugar-cane in southern Ghana*, International Journal of Tropical Insect Science, **6(2)**, pp. 135–143.

[101] Sargent RG, 2009, *Verification and validation of simulation models*, Proceedings of the Winter Simulation Conference, volume 1, pp. 162–176.

[102] SASRI, *Industry overview*, Available from `http://www.sasa.org.za/sugar_industry/industryoverview.aspx`, [Online: accessed 21 June 2016].

[103] Sisterson MS, Antilla L, Carrière Y, Ellers-Kirk C & Tabashnik BE, 2004, *Effects of insect population size on evolution of resistance to transgenic crops*, Journal of Economic Entomology, **97(4)**, pp. 1413–1424.

[104] Sisterson MS, Carrière Y, Dennehy TJ & Tabashnik BE, 2005, *Evolution of resistance to transgenic crops: interactions between insect movement and field distribution*, Journal of Economic Entomology, **98(6)**, pp. 1751–1762.

[105] Slatkin M, 1978, *Spatial patterns in the distributions of polygenic characters*, Journal of Theoretical Biology, **70(2)**, pp. 213–228.

[106] Snyman S, 2018, [Private Communication], Principal Biotechnologist at the South African Sugarcane Research Institute (`Sandy.Snyman@sugar.org.za`).

[107] Snyman SJ, Baker C, Huckett BI, McFarlane SA, Van Antwerpen T, Berry S, Omarjee J, Rutherford RS & Watt DA, 2008, *South African Sugarcane Research Institute: embracing biotechnology for crop improvement research*, Sugar Tech, **10(1)**, p. 1.

[108] Storer NP, Peck SL, Gould F, Van Duyn JW & Kennedy GG, 2003, *Spatial processes in the evolution of resistance in Helicoverpa zea (Lepidoptera: Noctuidae) to Bt transgenic corn and cotton in a mixed agroecosystem: a biology-rich stochastic simulation model*, Journal of Economic Entomology, **96(1)**, pp. 156–172.

[109] Tabashnik BE, 2008, *Delaying insect resistance to transgenic crops*, Proceedings of the National Academy of Sciences, **105(49)**, pp. 19029–19030.

[110] Tabashnik BE, Carrière Y, Dennehy TJ, Morin S, Sisterson MS, Roush RT, Shelton AM & Zhao JZ, 2003, *Insect resistance to transgenic Bt crops: lessons from the laboratory and field*, Journal of Economic Entomology, **96(4)**, pp. 1031–1038.

[111] Tabashnik BE, Cushing NL, Finson N & Johnson MW, 1990, *Field development of resistance to Bacillus thuringiensis in diamondback moth (Lepidoptera: Plutellidae)*, Journal of Economic Entomology, **83(5)**, pp. 1671–1676.

[112] Tabashnik BE, Sisterson MS, Ellsworth PC, Dennehy TJ, Antilla L, Liesner L, Whitlow M, Staten RT, Fabrick JA, Unnithan GC *et al.*, 2010, *Suppressing resistance to Bt cotton with sterile insect releases*, Nature Biotechnology, **28(12)**, p. 1304.

[113] Travis JMJ & Dytham C, 1998, *The evolution of dispersal in a metapopulation: a spatially explicit, individual-based model*, Royal Society of London B: Biological Sciences, **265(1390)**, pp. 17–23.

[114] Tyutyunov Y, Zhadanovskaya E, Bourguet D & Arditi R, 2008, *Landscape refuges delay resistance of the European corn borer to Bt-maize: A demo-genetic dynamic model*, Theoretical Population Biology, **74(1)**, pp. 138 – 146.

[115] United States Environmental Protection Agency, 1998, *Bacillus thuringiensis*, United States Environmental Protection Agency.

[116] US National Library of Medicine, *What is a Gene?*, Available from `https://ghr.nlm.nih.gov/primer/basics/gene`, [Online: accessed 8 November 2018].

[117] Van Coller LM, 1992, *Optimum biological control strategies for a problem in the sugar industry*, MSc Thesis, University of Natal.

[118] Van den Berg J, 2017, *Insect resistance management in bt maize: wild host plants of stem borers do not serve as refuges in Africa*, Journal of Economic Entomology, **110(1)**, pp. 221–229.

[119] Van Rensburg J, 2007, *First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt-transgenic maize*, South African Journal of Plant and Soil, **24(3)**, pp. 147–151.

[120] Van Vuuren BJ, Potgieter L & Van Vuuren JH, 2017, *An agent-based simulation model of Eldana saccharina Walker*, Natural Resource Modeling, **31(2)**, p. e12153.

[121] Varga A, 2001, *Discrete event simulation system*, Proceedings of the 15th European Simulation Multiconference.

[122] Volterra V, 1928, *Variations and fluctuations of the number of individuals in animal species living together*, ICES Journal of Marine Science, **3(1)**, pp. 3–51.

[123] Walker F & Gray JE, 1855, *List of the Specimens of Lepidopterous Insects in the Collection of the British Museum*, volume 5, Order of the Trustees.

[124] Walton AJ, 2011, *Radiation biology of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, MSc Thesis, Stellenbosch University.

[125] Walton AJ & Conlong DE, 2016, *General biology of Eldana saccharina (Lepidoptera: Pyralidae): A target for the sterile insect technique*, Florida Entomologist, **99(sp1)**, pp. 30–35.

[126] Way M, 1995, *Developmental biology of the immature stages of Eldana saccharina Walker (Lepidoptera: Pyralidae)*, Proceedings of the 69th South African Sugar Technologists Association, pp. 83–86.

[127] Way MJ, 2001, *Characteristics of sugarcane bored by Eldana saccharina (Lepidoptera: Pyralidae)*, Proceedings of the 75th South African Sugar Technologists Association, p. 257.

[128] Webster TE & Conlong DE, 2005, *An integrated pest management system for Eldana saccharina in the Midlands North region of KwaZulu-Natal*, Proceedings of the 79th South African Sugar Technologists Association, p. 347.

[129] Whiteley HR & Schnepf HE, 1986, *The molecular biology of parasporal crystal body formation in Bacillus thuringiensis*, Annual Reviews in Microbiology, **40(1)**, pp. 549–576.

[130] Wikipedia, the free encyclopedia, *Bacillus thuringiensis*, Available from `https://en.wikipedia.org/wiki/Bacillus\_thuringiensis`, [Online: accessed 7 May 2018].

[131] Wright S, 1965, *The interpretation of population structure by F-statistics with special regard to systems of mating*, Evolution, **19(3)**, pp. 395–420.

[132] XJ Technologies Company, *AnyLogic 7 user's guide*, Available from `http://www:xjtek:com/`, [Online: accessed 28 June 2018].

[133] Zadoks J & Waibel H, 2000, *From pesticides to genetically modified plants: history, economics and politics*, NJAS-Wageningen Journal of Life Sciences, **48(2)**, pp. 125–149.

APPENDIX A

Contents of the disc accompanying the thesis

This thesis is accompanied by a compact disc that contains the Python implementation of the simulation model developed in Chapter 4. Python is free to download and it is recommended that the Anaconda Python distribution be used as it already contains the required libraries to run the model. Please note that Python 3 is required to run the included code. The contents of the disk consists of three folders or directories, namely *Thesis*, *Simulation model* and *Results*. The contents of each directory is described below.

Thesis – This folder contains this thesis as a *PDF* file as well as the infographic from Chapter 6 as an image file.

Simulation model – This folder contains several files required to run the simulation model. The main file that executes the model is `Simulation_main.py` and the model framework is found in `framework_cython_biased_5.pyx`. This file is currently compiled for usage on a Unix-based operating system, but can be recompiled using `setup.py`. The *CSV* files are the parameter files the model reads in to run and contain the following:

- `field.csv` – the field age and type per cell in the ground cells. A number in the file is the age in days of the initialised field. If the field is set to be Bt, the age is added to 10000.
- `moths.csv` – The initial number of moths in each sky cell.
- `parameters.csv` – The input parameters that were required to be easily variable for the sensitivity analysis. A full description of each parameter is available in `Simulation_main.py`.
- `temperature.csv` – The 730 days of base temperatures used in this study.

`Grid_funcs.py` contains a set of helper functions used by `Simulation_main.py` to determine neighbourhood values for cells in the model.

Results – The raw output obtained from the model used to compile the results for Chapters 5 and 6. The actual ground cell grids are excluded as each experiment generated grid files of approximately 500 megabytes each. As the eggs and larvae were also not analysed, these outputs are also excluded. The results are separated into four sets, one for the sensitivity analysis and one for each of the case studies. The output folders or subdirectories are named as follows:

- `results_grids_sky` – The sky grids containing the count of female and male moths, in that order, per sky cell for every 7th day.
- `results_grids_res` – The number of resistant and carrier moths, in that order, per sky cell for every 7th day.

- `results_summary_sky` – The total number of moths in the sky cells per simulated day.
- `results_summary_res` – The total number of fully resistant moths in the sky and ground cells.
- `results_summary_car` – The total number of heterozygotes in the sky and ground cells.

Each subdirectory has a plaintext file named `reference.txt` that details which experiments each set of results corresponds to. The folders contain the raw CSV/XLSX output files generated by the model.