Integrating Genetic Control Tools for Insect Pest and Insecticide Resistance Management



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Format of Chapters

The thesis comprises three free-standing papers, each of which has been formatted to the requirements of the academic journal to which they have been, or are intended to be, submitted:

- Chapter 2: *Journal of Theoretical Biology* (Published)
- Chapter 3: *Parasites and Vectors* (In preparation)
- Chapter 4: *Parasites and Vectors* (In preparation)

Chapter 4 builds on the modelling work presented in Chapter 3 and there is, therefore, a degree of unavoidable overlap and repetition between the chapters. The supplementary materials for each chapter are presented in Appendices A-C.

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Statement of Authorship

The manuscripts presented in Chapters 2-4 are co-authored with my primary supervisor, Professor Michael. B Bonsall, with myself as first and corresponding author. In each case, I lead the development of the aims and methods, wrote the code for the models and analyses, and drafted the manuscript. My supervisor Professor Michael B. Bonsall provided guidance and input at each stage for each piece of work. I wrote the thesis introduction and discussion with comments from Prof. Michael B. Bonsall.

Publications

The following publication arose from this thesis and is presented in chapter 2:

- **Brewer, T.R.**, Bonsall, M. B., 2021. Combining refuges with transgenic insect releases for the management of an insect pest with non-recessive resistance to Bt crops in agricultural landscapes. Journal of Theoretical Biology 509, 110514. doi: 10.1016/j.jtbi.2020.110514.

Additional publications also arose from work completed during the funding period, but which fell outside of the scope of this thesis:

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Abstract

Pest insects that attack crops and vector disease continue to impose a tremendous burden on both global agricultural production and public health. Insecticidal interventions have greatly enhanced the control of pest insect populations, but their continued efficacy is threatened by the evolution of resistance. Developing novel tools and strategies to manage resistance to insecticides and strengthen population suppression will be essential for our continued ability to mitigate the impact of pest insects on human populations. Releases of insects genetically modified to possess a self-limiting, lethal transgene that conditionally terminates female offspring during development are a novel, species-specific tool that could be used alongside insecticidal interventions to manage resistance and enhance population suppression. However, while their potential as a pest suppression tool has been well characterised, more work is required to expand our understanding of their potential for resistance management. Using a spatially-explicit population dynamics and genetics model, I first evaluate the efficacy of landscape-level strategies combining the planting of refuge crops with self-limiting releases for the control of an agricultural pest and its resistance to insecticidal crops. I show that replacing area-wide releases with releases targeted at population hotspots can provide effective landscape-level population suppression and resistance management. I also demonstrate that reinforcing insecticidal crops with releases can compensate for violations of existing resistance management practices to maintain population and resistance control. I then expand considerations of these integrated strategies from agriculture to public health, investigating whether self-limiting releases can be deployed alongside insecticidal nets (LLINs) and indoor residual sprays (IRS). I present a population demographic and genetic model capable of simulating the response of a sex-, stage-, and genotype-structured anopheline population with overlapping generations to control strategies integrating pulsed releases of self-limiting insects with bed nets or indoor sprays. Using this model, I then investigate how resistance evolution and behavioural avoidance, recognised as current threats to the continued efficacy of nets and sprays, erode insecticide-only control. Finally, I demonstrate that plausible release strategies could reinforce population and resistance management to delay or reverse the evolution of resistance, and compensate for the reduced exposure to nets and sprays induced by behavioural avoidance.

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1 Introduction

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Globally, invertebrate pests and pathogens are estimated to decrease the yields of five major crop varieties by 20-30% (Oerke, 2006; Savary et al., 2019). In Brazil, annual losses of 25 million tonnes of agricultural production, worth approximately US$17 billion, are inflicted by pest insects (Oliveira et al., 2014), while in the USA approximately $11 billion is spent annually on pesticides (Fernandez-Cornejo et al., 2009). The combined cost of crop losses and management efforts attributable to a single pest species, the diamondback moth (*Plutella xylostella*), is estimated conservatively at US$4-5 billion annually (Zalucki et al., 2012). Global warming looks set to compound the problem, with losses to the world's three staple grains to invertebrates predicted to increase by 15-25% for every degree increase in mean global surface temperature (Deutsch et al., 2018). This erosive effect of agri-pests hinders humanity's capacity to sustainably and equitably feed a growing global population projected to exceed 10 billion by 2100 (United Nations, 2017) under a climatic regime

expected to destabilise agricultural production (Wheeler & Von Braun, 2013).

Pest invertebrates also impose a substantial toll on global public health, vectoring diseases that put at risk over 80% of the world's population and cause >700,000 deaths annually (World Health Organization, 2017). *Aedes* and *Anopheles* mosquitoes, widely recognised as the most important public health pests (Franklinos et al., 2019), serve as competent vectors of a number of high-burden infectious diseases, including malaria, dengue, chikungunya, yellow fever, and Zika virus (World Health Organization, 2017). In 2020, the World Health Organisation (WHO) estimated that there were 241 million cases of malaria, resulting in 627,000 deaths. Vectored by anopheline mosquitoes, the burden of morbidity and mortality imposed by malaria parasites fell disproportionately on the people of Africa, where 95% and 96% of these cases and deaths were reported respectively (World Health Organization, 2020). In response to the Zika virus epidemic, transmitted by *Aedes* mosquitoes, the WHO was forced to declare an international public health emergency after it spread to 60 countries in South and Central America (World Health Organization, 2016). Mosquito-borne diseases also impose significant economic costs. The combined cost of malaria control programmes totalled $3.3 billion in 2020 (World Health Organization, 2020), while the CDC estimates that, in Africa alone, the combined costs associated with illness, treatment and premature deaths total $12 billion (Centre for Disease Control and Prevention, 2022). Furthermore, countries with malaria experienced a reduction of up to 1.3% in economic growth per year between 1965-1990 (McCarthy et al., 2000). Estimates for the number of symptomatic dengue cases range from 58 million (Shepard et al., 2016) to 96 million (Bhatt et al., 2013), costing $8.9 billion in prevention and treatment (Shepard et al., 2016).

The management of invertebrate pests remains a significant challenge in the context of both food security and public health. The mass conversion of large areas of Earth's terrestrial surface from complex ecosystems into ecologically-simplified agricultural and residential systems has provided ideal conditions for a range of insects that attack crops and vector harmful pathogens to flourish (Matson et al., 1997; Schrama et al., 2020), while our global transport network continues to facilitate

their dispersal across the globe (Gippet et al., 2019). In 2015, Member States of the United Nations made ambitious commitments, enshrined in the Sustainable Development Goals, to achieve zero hunger, ensure good health and well-being for all, and protect and restore terrestrial ecosystems by 2030 (United Nations, 2015). Continuing to design and implement novel tools and strategies, while safeguarding the efficacy of existing technologies, that can manage agricultural pest insects and disease vectors in an evolutionarily, ecologically and economically sustainable way is likely to be essential if these ambitious goals are to be realised.

Pest insect control strategies

Efforts to minimise agricultural yield losses and mosquito-borne disease morbidity and mortality typically centre on the management of the agricultural pest and mosquito populations that inflict them. The most widely used management tool in both settings are insecticides, synthetic chemicals introduced, either prophylactically or responsively, into an area to suppress or eradicate the pest insect population. Genetics-based control is another technological approach in which genetically modified insects are released into the target population to introduce useful genetic traits, most commonly sterility. A range of additional approaches are also important for pest insect suppression, including larval site management, crop rotations, biological control, and spatial repellents. However, the focus of this thesis is on insecticide- and genetics-based interventions, which I review below.

Bt crops

In recognition of the need to reduce the dependence on insecticidal sprays and limit off-target effects on beneficial species, whose diversity and abundance are linked to the provision of agriculturally-beneficial ecological processes (e.g. biological control, pollination, nutrient cycling) (Dainese et al., 2019), crop varieties have been genetically engineered to express toxins, primarily crystal (Cry) proteins derived from the bacterium *Bacillus thuringiensis* (Bt), as a constitutive defence against herbivory. In contrast to broad spectrum insecticides such as Dichlorodiphenyltrichloroethane

(DDT), the Cry proteins expressed in Bt crops exhibit a higher degree of specificity against a narrow range of, typically lepidopteran or coleopteran, target insects. Furthermore, Bt crops eliminate only the larval stages of the insects which attack crop tissue, providing targeted suppression of crop pests and further limiting the potential for off-target effects on beneficial organisms (Lu et al., 2012).

Since coming to market in 1991, Bt crops have revolutionised agricultural pest control and by 2015 transgenic varieties of corn, cotton and soybean had been planted on more than 1 billion acres of cropland globally (Tabashnik et al., 2013). As well as providing effective, regional pest suppression (Carriere et al., 2003; Hutchison et al., 2010), Bt crops have been associated with reductions in insecticide spraying, enhanced biocontrol (Romeis et al., 2018) and increases in crop yields and profits (Carpenter, 2010; Edgerton et al., 2012). Area-wide adoption of Bt crops has seen these benefits extend beyond the fields in which they are planted, to the economic benefit of growers in the wider landscape (Dively et al., 2018). The continued efficacy of these transgenic crops has been argued to be essential to efforts to sustainably meet the food, fibre, and fuel demands of the increasingly consumptive human population (Tabashnik & Carrière, 2017).

Long-lasting insecticidal nets, indoor spraying, and larvicides

Insecticide-based interventions also form the core of strategies to manage populations of both *Aedes* and *Anopheles* mosquitoes, although differences in the typical ecologies of members of each genus are reflected in the primary means of insecticide delivery (Pates & Curtis, 2005). Efforts to control *Anopheles* mosquitoes centre on the widespread deployment of long-lasting insecticide nets (LLINs) and/or indoor residual spraying (IRS). LLINs and IRS suppress anopheline populations and reduce malaria transmission by protecting people from members of the *Anopheles gambiae* sensu lato (s.l.) species complex that preferentially feed indoors (endophagy), at night, on human hosts (anthrophagy), and rest on indoor walls (endophily) while their eggs mature. Treated bed nets and indoor spraying have been instrumental

to the success of malaria control in Africa, accounting for an estimated 68% (62-73% credible interval) and 10% (5-14% credible interval) of 663 million (542-753 credible interval) averted clinical cases respectively (Bhatt et al., 2015). *Aedes* vectors, on the other hand, are also crepuscular feeders, such that some bites occur before people are protected by bed nets, and are therefore less amenable to control by LLINs. Instead, *Aedes* management programmes combine larval source management and/or larviciding with adulticide sprays (peri-domestic and/or indoor). These combined approaches have previously been effective in suppressing *Aedes* populations and constricting dengue and yellow fever transmission across large parts of South and Central America and, more recently, reducing dengue transmission in Singapore (Morrison et al., 2008).

Sterile insect technique

Conceptualised in the 1950's, the Sterile Insect Technique (SIT) is a species-specific, environmentally non-polluting suppression strategy in which large releases of, preferably male, insects of the target species, mass-reared and sterilised via irradiation, are released into the population to seek and mate with wild insects (Knipling, 1955). Offspring from these matings fail to develop such that, if enough sterilised insects are released over a large enough area for long enough, the target population size declines through time. SIT has been used successfully to suppress both agricultural pest populations, most notably the New World screwworm, *Cochliomyia hominivorax*, in North and Central America, and mosquito populations, as in the case of *Anopheles albimanus* in El Salvador (Weidhaas et al., 1974).

However, traditional SIT suffers a number of drawbacks (Alphey, 2002). Irradiated insects display significantly reduced longevity and mating competence, necessitating the rearing and release of substantial numbers of insects to achieve suppression. For many species, it is also difficult and labour-intensive to separate males from females prior to release, introducing inefficiencies if released individuals mate with each other and, in the case of mosquitoes, contributing to the burden of bites experienced by the human population. Preventing the release of female

insects is also beneficial in agricultural settings if oviposition damage is a concern. Most problematically, if offspring die early during development, SIT programmes also risk releasing populations from density-dependent regulation, which can greatly undermine the benefits of release programmes (Alphey et al., 2010).

Self-limiting releases

Advances in molecular genetics facilitated the development of more modern implementations of SIT, in which effective sterility is induced through a dominant, repressible, lethal transgene (Thomas et al., 2000). Insects homozygous for the lethal transgene are released into the target population to mate with wildtype insects and produce heterozygous offspring that, in the absence of a dietary tetracycline repressor (or analogue), are terminated during development. The deleterious effect of the transgene on carriers renders it self-limiting - the transgene frequency in the wild population will naturally decline to extinction when releases cease (Harvey-Samuel et al., 2014). While bisex-lethal strains are expected to provide superior suppression (Gentile et al., 2015), female-specific strains have been developed in which the lethal phenotype is expressed only in female carriers, simplifying the sex-sorting process and facilitating reliable male-only releases (Fu et al., 2007). While female-specific strains provide weaker suppression relative to bisex-lethal strains, the survival of male carriers allows heterozygous male offspring to further propagate the lethal transgene, providing additional female suppression in the following generation(s).

The timing of the lethal phenotype can also be programmed, with early-acting and late-acting strains having been developed (Phuc et al., 2007). Early-acting strains are required in agricultural settings to limit crop damage caused by the larvae of pest insects and have been shown to provide effective suppression in laboratory (Harvey-Samuel et al., 2015) and field cage trials (Leftwich et al., 2014). For vector control (or agricultural pests in which the adults are responsible for crop injury) late-acting lethal strains are preferred to maximise competition for larval resources and enhance density-dependent larval mortality (Phuc et al., 2007), and self-limiting mosquito strains have also been used effectively in field trials (Carvalho

et al., 2015; Harris et al., 2012). While genetically modifying insects to introduce the transgene construct is expected to impose a fitness cost, evidence suggests that transgenic insects remain competitive relative to wildtypes in both the laboratory and field (Lacroix et al., 2012; Shelton et al., 2020), potentially enabling smaller releases to achieve equivalent results relative to releases of irradiated insects.

Self-sustaining releases

Self-sustaining genetic control tools designed for population replacement are also increasingly of interest for pest population and resistance management. Harnessing selfish genetic elements to drive 'cargo genes' into the target population at super-Mendelian rates, including those that impose a significant fitness cost to carriers, gene drive systems can permanently modify target populations to exhibit traits favourable to managers of crop and public health pests. While suppression is one option if, for instance, the cargo gene reduces female fertility (Deredec et al., 2011), more interesting applications include introducing genes for malaria refractoriness (Gantz et al., 2015) or insecticide susceptibility (Kaduskar et al., 2022). However, while the potential for this suite of tools is fascinating, the focus of the work presented in this thesis is on advancing our understanding of applications for self-limiting releases.

Existing strategies for managing insecticide resistance

The widespread adoption of insecticidal interventions, coupled with the strong, often continuous selection pressure they impose on pests, selects quickly for mechanisms of resistance, with approximately 8000 individual cases of resistance to insecticidal compounds now reported in over 500 species of invertebrate (REX Consortium, 2013). Defined as a heritable trait that reduces an individual's susceptibility to a control measure (Onstad, 2014), resistance can be either physiological, as in the case of the *kdr* mutation suspected to reduce the target-site binding efficiency of pyrethroids in mosquitoes, or behavioural, such as the prolonged diapause displayed by the Western Corn Rootworm (*Diabrotica virgifera*) to counter crop rotations (Levine

et al., 1992). Strategies to slow or prevent the evolution of resistance are therefore recommended (World Health Organisation, 2012), or even mandated (Gould, 1998), when insecticides are used to manage agricultural pests or disease vectors.

Resistance management strategies leverage an understanding of the selective pressures that drive the evolution of resistance and can be broadly separated into two classes. The first seeks to reduce or remove the selective pressure for resistance to promote susceptibility, typically by sheltering a portion of the population from the intervention. The second class of strategy attempts to eliminate resistance by exposing the pest to multiple interventions to increase sources of intra-generational mortality, thereby limiting the probability that the population will develop resistance to any one intervention (REX Consortium, 2013). This is typically achieved by deploying two or more insecticides in spatiotemporal patterns designed to increase the probability that individual insects are exposed to more than one insecticide during its lifetime (REX Consortium, 2013).

The high-dose/refuge strategy

The primary mechanism for managing resistance to Bt crops has been the high-dose/refuge (HDR) strategy, which seeks to promote susceptibility and dilute resistance before it can evolve to damaging frequencies. The HDR strategy involves planting the majority of a field to a Bt strain expressing a toxin dose sufficient to render resistance functionally recessive, such that only resistant homozygotes (RR) can survive exposure. A small portion of non-toxic crop is planted in close proximity to the Bt crop as a sacrificial refuge to promote the survival of susceptible insects (SS/RS). Providing resistance is initially rare and mating is non-assortative, the relatively small population of resistant insects will have a high probability of mating with susceptible insects and producing heterozygous offspring. If oviposition is non-preferential, the majority of offspring will emerge in Bt crop and be eliminated by the toxin, thereby diluting resistance and slowing its evolution (Tabashnik & Croft, 1982). The efficacy of this strategy is increased if, as is often assumed (Ffrench-Constant & Bass, 2017), resistance is associated with a fitness penalty (e.g.

reduced survival or fecundity) that allows susceptible homozygotes to out-compete heterozygotes and resistant homozygotes in the refuge.

The HDR strategy has been explored extensively using theoretical models and is generally expected to be effective in delaying resistance evolution when its assumptions of functionally recessive and initially rare resistance, and non-assortative mating and oviposition are met (Caprio, 2001; Huang et al., 2017; Peck et al., 1999; Sisterson et al., 2005; Tabashnik et al., 2004). Indeed, resistance management strategies relied almost exclusively on the projections from such models in the years proceeding the commercialisation of Bt crops (Tabashnik & Carrière, 2017). Although difficult to conclusively verify experimentally, the sustained suppression of economically important agricultural pests in the USA, China and Australia, coupled with the absence of widespread field-evolved resistance after decades of planting Bt crops, has been cited as evidence of its effectiveness (Tabashnik et al., 2008).

Bt crop pyramids

Another strategy for delaying resistance to Bt crops has been to modify strains to express two or more toxins to which the target species is vulnerable in 'pyramided' crops. First deployed in 2003, pyramided crops delay resistance evolution by requiring the pest species to independently acquire resistance to multiple toxins simultaneously (Tabashnik et al., 2009), and have largely replaced single-toxin crops. To efficiently delay resistance, resistance to one toxin must not reduce susceptibility to the other(s), pyramid crops must be planted in combination with a refuge crop, resistance to both toxins should be rare and recessive, and single-toxin crops expressing a compound used in the pyramid should not be planted in close proximity (Carrière et al., 2016; Zhao et al., 2005).

Insecticide rotations, mosaics, mixtures and combinations

The limited number of classes of insecticides with distinct modes of action (MoA) recommended for use in bed-nets and sprays, which account for an estimated 96% of the global insecticide usage in vector control (van den Berg et al., 2021),

renders the management of resistance to these tools an acute challenge. In recognition of the threat posed to insecticide-based vector control by the evolution of insecticide resistance, the WHO established a Global Plan for Insecticide Resistance Management in Malaria Vectors (World Health Organisation, 2012). The strategies recommended within this plan involve the deployment of two or more insecticides with non-overlapping MoAs in rotations, mosaics, mixtures or combinations to introduce spatiotemporal variation in the selective pressure for a given insecticide (Dusfour et al., 2019; REX Consortium, 2013).

Insecticide mixtures are the simplest strategy and involve mixing two or more insecticides into a single formulation for deployment in LLINs or IRS. Combination strategies are similar to mixtures, but present two or more different insecticides simultaneously in different interventions and have only recently been approved by the WHO (e.g. pyrethroid LLINs and non-pyrethroid sprays). Insecticide rotations introduce temporal variability in the selective pressure for an insecticide by rotating at fixed intervals to an alternative with a non-overlapping MoA. If the pest insect's generation time is greater than the rotation period, pest insects will be challenged by multiple insecticides over their lifetime, preventing the build up of resistance to each insecticide. Mosaics introduce spatial variation in the selective pressure for a given insecticide by deploying insecticides with exclusive MoAs over different, non-overlapping areas such that, if the average dispersal distance of a pest species sufficiently exceeds the scale of the mosaic, insects resistant to one insecticide will be eliminated by the other with high probability (REX Consortium, 2013).

The relative efficacy of these strategies have been explored in a range of theoretical studies, reviewed in REX Consortium (2013), with a general consensus that mixtures or combinations outperform mosaics and sequences, particularly if all insecticides are highly effective against susceptible insects, the probability of exposure is low, and resistance imposes a significant cost to bearers in the absence of the insecticide (South & Hastings, 2018). When the probability of exposure is high, modelling suggests that sequences could be favoured over mixtures, while, as with the HDR strategy, increasing the dominance of resistance erodes the efficacy of all

strategies (South & Hastings, 2018). While the integration of insecticide resistance management strategies into vector control programmes is widely recommended, evidence on the extent to which they are used in practice, or have been demonstrated to preserve susceptibility, is fragmented and inconclusive (van den Berg et al., 2021).

Resistance to Bt crops

While the HDR strategy has been effective in preventing the evolution of widespread resistance to Bt crop varieties, the number of cases of field-evolved resistance is rising (Tabashnik et al., 2013; Tabashnik & Carrière, 2017, 2019). In their most recent census, Tabashnik and Carrière (2019) identified nineteen cases of field-evolved resistance to Bt crops with consequences for population management. These cases included resistance to each of the most widely employed Cry toxins, identified in seven species across six countries. This rise in cases is particularly problematic as it appears to be driving a reduction in the time between the commercialisation of new varieties and the emergence of resistance, with the time-to-resistance now estimated at only 5.3 years, reducing the longevity of new Bt crops (Tabashnik & Carrière, 2017).

Cases of field-evolved resistance can usually be linked to violations of the principles of the high-dose/refuge strategy (Tabashnik & Carrière, 2017, 2019), which greatly reduces its potential to slow the evolution of resistance (Campagne et al., 2016). The assumption of high-dose was not met in any of the cases of resistance identified by Tabashnik and Carrière (2017), and cross-resistance - a violation of the assumption of rare initial resistance - has been heavily implicated in the reduced lifespan of new Bt crops (Tabashnik & Carrière, 2017). Failure to comply with recommended or mandated refuge planting, due to an unwillingness to sacrifice crop yield, has also resulted in the evolution of resistance in South Africa (Kruger et al., 2009, 2012), India (Naik et al., 2018), and the United States (Reisig, 2017). Establishing HDR strategy principles in these cases, either by switching to strains that express sufficiently high toxin doses, or by taking steps to enforce refuge compliance and/or increase the size of the refuges deployed, should be prioritised

and has the potential to reverse the evolution of resistance (Tabashnik & Carrière, 2019). However, the increasing frequency of cases of field-evolved resistance and decline in toxin lifespan suggest that it would be prudent to identify novel tools and strategies for enhancing the management of resistance to Bt crops.

Resistance to LLINs and IRS

Despite the awareness of the threat it poses, and the development of strategies to slow its evolution, (World Health Organisation, 2012), insecticide resistance has emerged in vector populations at an alarming rate (Hancock et al., 2020), and has now been reported for all major insecticide classes deployed in LLINs and IRS, in each of the main mosquito vectors, and in all but the European WHO region (Moyes et al., 2017; World Health Organization, 2020). Of the 82 countries for which resistance monitoring data was submitted, 28 reported resistance to all four of the main insecticide classes in at least one vector species, while resistance to at least three classes was reported in a further 17 countries (World Health Organization, 2020). While the evolution of resistance has so far not been explicitly linked to a reduction in malaria control, there is a concern that the observed plateauing of global malaria morbidity and mortality in high-transmission regions, which threatens efforts to achieve the ambitious targets outlined in the Global Technical Strategy for Malaria (GTS) of a 90% reduction from 2015 levels (World Health Organization, 2015, 2020), is linked to the widespread evolution of resistance. The speed and scale at which resistance has emerged has led to calls to accelerate the development of both new insecticides for use in LLINs and IRS and non-chemical vector control tools to be used alongside them (Hemingway et al., 2016).

Behavioural avoidance

Another threat to the continued efficacy of insecticide-based vector control is learned or natural avoidance of the LLINs and IRS that have formed the backbone of vector control efforts. Insecticide-avoidance behaviour can emerge as an evolutionary response to insecticide-induced selection. Following the distribution of LLINs in

Tanzania, Kreppel et al. (2020) reported evidence of behavioural shifts towards cattle feeding (zoophily) and outdoor resting (exophily) in *Anopheles funestus* and *Anopheles arabiensis* populations. Behavioural avoidance can also result from ecological shifts in vector communities as species which naturally avoid insecticidal interventions due to differences in host choice, feeding times, and resting behaviour replace species eliminated by LLINs/IRS (Pates & Curtis, 2005). In a recent study across 12 sites in Burkina Faso, Sanou et al. (2021) found no evidence of within-species behavioural adaptations over the two year study period, but did find evidence of shifts in vector community composition towards those increasingly represented by exophagic species which naturally avoid LLINs. The potential for behavioural avoidance to further erode the suppressive capacity of the leading vector control tools highlights the need for additional interventions that target mosquitoes in different spatial and/or temporal settings if negative impacts on disease prevention are to be avoided.

Resistance management potential of self-limiting releases

Management strategies reinforcing the HDR strategy with female-specific, self-limiting releases have been suggested as a novel strategy that could both enhance suppression and delay or reverse the evolution of insecticide resistance. If the released males are homozygous for an allele conferring susceptibility to the insecticide, releases introgress genetic susceptibility into the target population through the surviving male offspring impervious to the lethal transgene. Spatially-implicit deterministic models have demonstrated the capacity for strategies supplementing refuge planting with continuous releases to provide greater population suppression (Watkinson-Powell & Alphey, 2017), delay resistance evolution, and reduce the proportion of refuge crop required to sustain susceptibility (Alphey et al., 2009; Alphey et al., 2007). Harvey-Samuel et al. (2015) demonstrated experimentally that combining small releases with an insecticide treatment can provide similar levels of suppression to larger releases, while also slowing the rate of insecticide resistance

evolution relative to insecticide-only treatments. Similarly, Zhou et al. (2018) used laboratory cage experiments to demonstrate that self-limiting releases both suppressed populations of diamondback moth and slowed the evolution of resistance when integrated with the HDR strategy. SIT has also been used in combination with BT crops in the field, achieving a 99.92% reduction in pink bollworm infestation in Arizona over 3-4 years and facilitating a reduction in the proportion of crops planted to refuge (Tabashnik & Carrière, 2019; Tabashnik et al., 2021).

SIT, and by extension transgenic releases, has traditionally been considered as a tool for long-term, landscape-level eradication, requiring significant and sustained economic and logistical input (Dyck et al., 2006). However, the ability of genetic control tools to achieve rapid suppression and resistance management when used with insecticidal crops has turned the discussion towards the potential for smaller-scale, targeted releases if population and resistance suppression rather than elimination is an acceptable outcome (Zhou et al., 2019). For instance, where violations of HDR strategy principles yields growing populations of resistant insects, short-term releases of self-limiting insects could be used to suppress the population and dilute resistance back below economic or resistance frequency thresholds that can be maintained by the HDR strategy and/or while efforts are made to restore HDR-based management (e.g. increasing compliance or toxin dose). Zhou et al. (2019) investigated this possibility using an experimental mesocosm system but found that, while this approach may provide short-term benefits to suppression, ultimately area-wide releases may be necessary to manage resistance. However, these experiments were naturally limited to assess a limited number of management strategies and resistance scenarios over a small number of generations. A theoretical approach would enable a wider range of scenarios to be investigated over more meaningful time horizons to more comprehensively explore the feasibility of these strategies.

Finally, the potential of these combined strategies has been theoretically and empirically demonstrated in agricultural pests, but their potential as an integrated vector population and resistance management tool remain under-explored. Given the threats posed to established vector control tools by insecticide resistance

and behavioural avoidance, and the emphasis increasingly placed on developing integrated vector management (IVM) strategies that combine multiple, preferably chemical and non-chemical, interventions to optimise pest control (European Union, 2020; World Health Organization, 2017), the potential for self-limiting releases to be used in combination with LLINs and IRS warrants exploration.

Thesis outline

The central aim of the work presented in this thesis is to advance our understanding of the potential for self-limiting releases to be used alongside insecticide-based interventions, which underpin pest insect control in both agricultural and public health settings, to reinforce population suppression and manage resistance to the deployed insecticide. In particular, I focus on scenarios in which the continued efficacy of insecticide-based control may be compromised by non-compliance with mandated management policies, the evolution of resistance, or the behavioural avoidance of insecticide-based interventions.

In Chapter 2, I develop a spatially-explicit implementation of an existing population dynamics and genetics model to investigate how self-limiting releases can be integrated with refuge planting to manage a theoretical diamondback moth (*Plutella xylostella* L.) population across an agricultural landscape. Using this framework, I investigate the possibility of deploying localised releases in combination with the high-dose/refuge (HDR) strategy, in place of area-wide releases, to suppress a pest population and manage resistance to Bt crops. Cases of field-evolved resistance to Bt crops are generally attributable to violations of the assumptions of the HDR, so I assess the robustness of these integrated management strategies when resistance is non-recessive and refuge compliance across the landscape is incomplete.

Chapter 3 is motivated by the threats posed to ongoing vector management programmes centred on the deployment of LLINs and IRS by the evolution of insecticide resistance, low coverage, and behavioural avoidance. I develop a mathematical framework for investigating mosquito population and resistance management strategies that reinforce insecticide-based vector control with releases of

late-acting, female-specific, self-limiting releases. The model is used to demonstrate the effects of insecticide resistance and exposure probability (a proxy for the effects of coverage and behavioural avoidance) on population and resistance management. This enables me to identify conditions under which LLINs and IRS may require reinforcement, and to develop a baseline against which to compare the efficacy of strategies combining LLINs and IRS with releases.

In Chapter 4, I use the modelling framework developed in Chapter 3 to investigate the extent to which reinforcing LLIN and IRS deployment with self-limiting releases can enhance population and resistance management under the scenarios identified in Chapter 3, in which the efficacy of insecticide-only control is compromised. Specifically, I assess whether releases can slow or reverse the evolution of resistance and maintain population control when resistance is widespread and recessive, and when resistance is dominant with recessive fitness costs. I also investigate whether releases can compensate for reduced costs of resistance and low insecticide exposure probability.

2

Combining transgenic insect releases with the high-dose/refuge strategy for the management of insect pests and resistance to Bt-crops in agricultural landscapes

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Abstract

Reinforcing the high-dose/refuge strategy with releases of transgenic insects has been posited as a method for simultaneously managing agricultural pest populations and resistance to transgenic crops. Theoretical and empirical studies have shown that these approaches can work when deployed against closed populations and the assumptions of the HDR strategy are met. However, field-evolved resistance is often linked to non-recessive resistance or refuge non-compliance, and pest management regimes are likely to take place at the landscape-level. It is therefore important to understand how effective such strategies are when resistance is non-recessive, and how they could be employed in agricultural landscapes. We developed a spatially-explicit model to investigate the efficacy of strategies combining refuges with transgenic insect releases to manage a pest with non-recessive resistance in agricultural landscapes. We compared two release strategies, area-wide releases and localised releases targeted at population hotspots, and analysed the effects of refuge and release parameters on population and resistance dynamics. Area-wide releases reliably achieved landscape-level pest eradication. Localised releases also eradicated the pest when low release thresholds were combined with high release ratios, and maintained the pest at low densities when insufficient to achieve extinction. Reinforcing refuges with localised releases also greatly enhanced the probability of resistance extinction. However, when resistance remained in the population, localised releases prevented resistance from reaching fixation rather than greatly delaying or reversing resistance evolution. Our work indicates that combining refuges with simple release policies is effective for landscape-level pest suppression when the HDR assumptions are violated, but more nuanced release strategies may be required to enhance the benefits to resistance management.

Introduction

Transgenic crops, genetically engineered to express insecticidal crystal (Cry) proteins derived from the bacterium *Bacillus thuringiensis* (Bt), have revolutionised agricultural pest insect control and are now planted on more than a billion acres worldwide (Tabashnik et al., 2013). This expanding suite of biotechnologies (Parisi et al., 2016), which includes varieties for many of the world's staple crops, provides targeted suppression of lepidopteran and coleopteran pests, enhances yields and profits (Carpenter, 2010; Edgerton et al., 2012), and decreases applications of insecticide sprays (Tabashnik et al., 2010). Furthermore, Bt-crops eliminate only insects which feed on crop tissue, reducing off-target effects on beneficial organisms, notably natural enemies (Romeis et al., 2018) and pollinators, the diversity and abundance of which are essential to the provision of agriculturally-important ecosystem services (Dainese et al., 2019).

The strong, continuous selection pressure imposed by the toxin(s) promotes the evolution of resistance, defined as a genetically-based reduction in the susceptibility of insects to Bt-toxins (Tabashnik & Carrière, 2019; Tabashnik et al., 2009), threatening the long-term efficacy of these technologies (McGaughey & Whalon, 1992; Tabashnik et al., 2013). The primary mechanism for managing resistance has been the high-dose/refuge (HDR) strategy. Assuming the toxin dose is sufficient to eliminate resistant heterozygotes (RS), rendering resistance functionally recessive, only homozygous-resistant (RR) individuals survive on Bt crop. A sacrificial, non-toxic refuge crop is planted in close proximity to promote the survival of susceptible (SS / RS) insects. If the resistance allele is rare the population of resistant homozygotes in the Bt crop will be small, enhancing the probability of matings with susceptible insects from the larger refuge population. Any resulting heterozygous offspring that feed on the Bt crop are eliminated before they can reproduce, thereby diluting resistance. The absence of widespread resistance to Bt crops is indicative of the success of the HDR strategy in delaying the evolution of Bt resistance (Huang et al., 2011; Tabashnik et al., 2013; Tabashnik & Carrière, 2019).

Despite this success, the number of cases of Bt resistance is rising while the time between the commercialisation of new Bt crop strains and the emergence of resistance is decreasing, largely attributable to cases in which the assumptions of the HDR strategy are not met. A recent census identified nineteen cases of field-evolved resistance linked to either cross-resistance between Bt strains or Bt crops that failed to meet the high-dose requirement (Tabashnik & Carrière, 2017, 2019). The evolution of field-evolved resistance has also been linked to cases of non-recessive resistance (Campagne et al., 2013; Zhang et al., 2012), which greatly reduces the efficacy of refugia. Another issue is refuge non-compliance due to an unwillingness to sacrifice crop yields, resulting in the early development of resistance in South Africa (Kruger et al., 2009, 2012), India (Naik et al., 2018), and the United States (Reisig, 2017). To maintain Bt crops as a viable technology it would be prudent to develop additional resistance management tools to reinforce refuges, particularly for scenarios in which assumptions of the HDR strategy are violated.

Genetically-modified male insects bearing a self-limiting, dominant, female-lethal transgene are a species-specific pest suppression tool (Ant et al., 2012; de Valdez et al., 2011; Leftwich et al., 2014) that could supplement refuges to manage Bt resistance (Alphey et al., 2007; Harvey-Samuel et al., 2015; Zhou et al., 2018). Males, homozygous for both the lethal transgene construct (LL) and the toxin susceptibility allele (SS), are released into a target population, mate with wildtype females (++), and produce heterozygous offspring (+L). The transgene terminates female offspring early during larval development, suppressing the population's size and reproductive capacity, while the introgression of susceptibility alleles dilutes genetic resistance to the toxin. The self-limiting nature of the construct ensures that, when releases cease, the fitness penalty conferred by the transgene drives it to extinction (Alphey, 2014).

Early evidence from spatially-implicit models indicated that combining the HDR strategy with releases of transgenic insects can suppress target populations, delay or reverse resistance evolution, and reduce the proportion of refuge crop required (Alphey et al., 2009; Alphey et al., 2007; Harvey-Samuel et al., 2015; Zhou et al., 2018). Promisingly, these findings have been validated experimentally in

single-population cage experiments (Harvey-Samuel et al., 2015; Zhou et al., 2018). However, cases of field-evolved resistance are often linked to violations of the HDR assumptions (Tabashnik & Carrière, 2017, 2019), and ascertaining to what extent these population and resistance management benefits are robust to low toxin doses, non-recessive resistance, and refuge non-compliance remains an important task.

Furthermore, while the effects of landscape structure and pest ecology on the efficacy of the HDR strategy have been explored extensively (Huang et al., 2017; Peck et al., 1999; Storer et al., 2003; Vacher et al., 2003), how transgenic releases could be integrated into landscape-level management strategies is less well characterised. Sterile Insect Technique, the forerunner of modern transgenic release technologies, was typically deployed in area-wide eradication schemes. However, these programmes are typically expensive and require coordination across stakeholder groups over large areas (Dyck et al., 2006). If population suppression and resistance management rather than eradication is an acceptable outcome, localised releases targeted at population hotspots could provide an effective alternative to area-wide release programmes (Zhou et al., 2019). The smaller-scale and enhanced flexibility afforded by localised releases recommend them as a tool in resistance management scenarios in which violated HDR assumptions render refuge-only strategies vulnerable, but more work is required to characterise the short and long-term population and evolutionary dynamic consequences of these strategies.

Here, we developed an existing spatially-implicit population genetics and dynamics model (Alphey et al., 2009) into a metapopulation model for the investigation of pest management strategies combining transgenic insect releases with refuges in agricultural landscapes. We used this model to simulate a generic agricultural pest with non-recessive resistance under a range of management strategies. Our objectives were to: 1) assess whether supplementing refuges with transgenic insect releases represents a viable strategy when the high-dose assumption is not met; 2) compare the dynamics observed when refuges are paired with area-wide and localised release regimes; and 3) determine the effect of refuge non-compliance on the efficacy of refuge and release strategies. In addressing these objectives, we

aimed to expand on work investigating the potential of transgenic insect releases as a resistance management tool by considering their use in scenarios linked with cases of field-evolved resistance.

Methods

To investigate the effect of management strategies combining refuges with transgenic insect releases in agricultural landscapes on the dynamics of a pest population and its genetic resistance to a single-toxin Bt crop, we developed a spatially explicit, individual-based (see Appendix A.1) metapopulation model. Our framework extends the spatially implicit population genetics and dynamics model developed by Alphey et al. (2009) across an agricultural landscape lattice and simulates area-wide and localised transgenic insect release strategies.

Landscape model

The agricultural landscape is simulated using a coupled map lattice composed of 11 x 11 square fields of equal dimension and area. Each field can support a discrete population of the pest insect and is assumed to be managed by an independent farmer. Individual fields are planted with proportions of Bt crop (F_{xy}^{Bt}) and non-Bt refuge crop (F_{xy}^{Ref}) such that:

$$F_{xy}^{Bt} + F_{xy}^{Ref} = 1 \tag{2.1}$$

where F_{xy}^{Bt} and F_{xy}^{Ref} represent the proportions of Bt crop and refuge crop in the xy^{th} field respectively. The proportion of Bt crop and refuge crop planted in each field is determined by the farmer's compliance with the mandated refuge (C_{xy}). We assume farmers adopt one of two actions: i) comply with the mandated refuge proportion ($C_{xy} = 1$), or ii) fail to comply with the mandated refuge and plant their entire field with Bt-crop ($C_{xy} = 0$), such that the proportions of Bt crop and refuge crop in the xy^{th} field are calculated as follows:

$$F_{xy}^{Ref} = \begin{cases} \phi, & C_{xy} = 1 \\ 0, & C_{xy} = 0 \end{cases} \tag{2.2}$$

$$F_{xy}^{Bt} = \begin{cases} 1-\phi, & C_{xy} = 1 \\ 1, & C_{xy} = 0 \end{cases} \tag{2.3}$$

where ϕ represents the mandated refuge crop proportion. The landowner's planting choice is assumed to be independent and invariant through time such that a proportion of fields in the landscape, $\bar{C}$, are compliant with the refuge. Toroidal boundaries are modelled to avoid edge-effects and approximate the dynamics observable on larger landscape lattices on a smaller, less computationally-expensive system (Ives et al., 2011).

Population dynamics model

We modelled a pest insect metapopulation in which each field in the landscape can support a discrete population. Within individual fields we assume no internal spatial population structuring due to preference for a given crop type, and all populations are linked by dispersal. We simulate an insect with discrete, non-overlapping generations and a simple life cycle, in which larvae hatch from eggs, develop, are subject to density-dependent regulation and fitness costs imposed by their genotype and natal crop-type. The larval stage, in which individuals are exposed to the effects of the Bt toxin, is assumed to be non-motile, with individuals developing on their natal crop type. Surviving larvae mature into adults, mate, disperse, and then die.

The population dynamics are described by a discrete-generation, density-dependent model:

$$N'_{xy,t} = \sigma_{xy,t} N_{xy,t} \tag{2.4}$$

where $N_{xy,t}$ represents the number of eggs in the xy^{th} field, $N'_{xy,t}$ and $\sigma_{xy,t}$ represents the number and proportion of larvae in the xy^{th} field that survive the combined effects of their genotype and natal crop type respectively. After the genotypic fitness costs have been applied, the surviving larvae experience density-dependent, intraspecific

competition within their natal fields. We adopt a flexible, two-parameter form of density-dependence (Bellows, 1981; Maynard Smith & Slatkin, 1973):

$$\frac{1}{1+(\alpha N'_{xy,t})^{\beta}} \tag{2.5}$$

where β is a shape parameter that determines the form of density-dependence, and α is a scaling constant proportional to a theoretical within-field carrying capacity:

$$K = \frac{\sqrt[\beta]{R_0 - 1}}{\alpha}. \tag{2.6}$$

The number of larvae that survive to maturity, $N''_{xy,t}$, is therefore given by:

$$N''_{xy,t} = \frac{\sigma_{xy,t} N_{xy,t}}{1+(\alpha \sigma_{xy,t} N_{xy,t})^{\beta}} = \frac{N'_{xy,t}}{1+(\alpha N'_{xy,t})^{\beta}}. \tag{2.7}$$

Survivors mature and proceed to mate. Each female mates with a single male and produces $2R_0$ eggs, where R_0 represents the number of female offspring produced per adult female. Mating is panmitic and produces male and female offspring with equal probability. The number of offspring produced during the mating step is given by:

$$N_{xy,t+1} = 2R_0 F''_{xy,t} \tag{2.8}$$

where $F''_{xy,t}$ represents the number of females in the xy^{th} field that survive to mate in the t^{th} generation. We model the worst-case scenario in which every adult female reproduces.

Population genetics model

Resistance to single-toxin Bt crops

Resistance to the Bt crop is conferred by a resistance allele, denoted R, at a single, autosomal, diallelic locus (herein referred to as the S/R locus). The second allelic variant, denoted S, renders carriers susceptible to Bt crops. The frequencies of the resistance and susceptibility alleles in the xy^{th} field in the t^{th} generation are given by $p_{xy,t}$ and $q_{xy,t}$ respectively, where $p_{xy,t} + q_{xy,t} = 1$. The genotype at the S/R locus, i, has three possible allelic combinations, SS, RS, and RR, with each individual's

probability of surviving from larvae to adulthood in Bt or refuge crop influenced by its genotype at the S/R locus. This genotype- and host crop-dependent survival occurs before density-dependent mortality and is captured by the relative fitness of each genotype i at the S/R locus, $\Omega_{xy,t,i}$, calculated as:

$$\Omega_{xy,t,i} = F^{Bt}_{xy,t}\omega_i + F^{Ref}_{xy,t}\upsilon_i \tag{2.9}$$

where ω_i and υ_i represent the relative fitness of larvae with genotype i at the S/R locus on Bt crop and refuge crop respectively.

Transgenic Construct

The transgene construct is modelled as an un-linked, diallelic, autosomal locus, with lethal, L, and wildtype, W, allelic variants with frequencies l and w respectively. The possible genotypes at the W/L locus, j, are WW, WL, and LL. We simulate a transgene with an early-acting, dominant, female-lethal action. Male carriers of the L allele are unaffected, while female carriers are terminated early in the larval stage. As female carriers of the L allele do not persist to adulthood, when combined with the S/R locus, six viable genotypes can arise from mating events: $SSWW$, $RSWW$, $RRWW$, $SSWL$, $RSWL$, $RRWL$, of which three produce viable females ($SSWW$, $RSWW$, and $RRWW$). Note that, while homozygous lethal males are viable, they fail to arise post release due to the lethal action in females. The relative fitnesses at the W/L locus, j, are calculated as follows:

$$\Omega_{xy,t,j} = (1 - \epsilon_j). \tag{2.10}$$

where ϵ_j is the fitness penalty of the lethal construct to carriers. The lethal phenotype is assumed to be dominant and fully penetrant, such that ϵ_{sswL}, ϵ_{rswL} and $\epsilon_{rrwL} = 1$ for females and 0 for males, and ϵ_{ssww}, ϵ_{rsww} and $\epsilon_{rrww} = 0$ for both sexes. When combined with the equation for calculating relative fitnesses at the S/R locus, this gives:

$$\Omega_{xy,t,ij} = (1 - \epsilon_j)[F^{Bt}_{xy,t}\omega_i + F^{Ref}_{xy,t}\upsilon_i]. \tag{2.11}$$

Thus, when $\epsilon_j = 1$, $\Omega_{xy,t,ij} = 0$. Though in reality there may be additional energetic and fitness costs associated with the lethal allele that affect important ecological pest parameters (e.g. fecundity, dispersal distance, longevity), we opt to model the simpler scenario in which the lethal allele incurs no additional fitness penalties to carriers.

Table 2.1: Summary of the complete set of model parameters, their descriptions, and the values or value ranges used in this paper.

Parameter	**Description**	**Value(s)**
F_{xy}^{Bt}	Proportion of xy^{th} field planted with Bt-crop	$0 \leq F_{xy}^{Bt} \leq 1$
F_{xy}^{Ref}	Proportion of xy^{th} field planted with refuge	$0 \leq F_{xy}^{Ref} \leq 1$
ϕ	Mandatory refuge crop proportion (per field)	$0 \leq \phi \leq 1$
C_{xy}	Farmer's decision to comply with planting the mandated refuge in the xy^{th} field	$C_{xy} = 0$ or 1
$\overline{C}$	Proportion of farmers compliant with the mandatory refuge	$0 \leq \overline{C} \leq 1$
i	Genotype at the S/R locus	*WW, WL, or LL*
j	Genotype at the W/L locus	*SS, RS, or RR*
$p_{xy,t}$	Frequency of the R allele in the xy^{th} field in the t^{th} generation	$0 \leq p_{xy,t} \leq 1$
p_0	Frequency of the R allele in the initial population ($N_{66,0}$)	0.005
$q_{xy,t}$	Frequency of the S allele in the xy^{th} field in the t^{th} generation	$0 \leq q_{xy,t} \leq 1$
ω_i	Relative fitness of genotype i on Bt-crop	$0 \leq \omega_i \leq 1$
υ_i	Relative fitness of genotype i on refuge crop	$0 \leq \upsilon_i \leq 1$
$\Omega_{xy,ij}$	Average relative fitness of genotype ij in the xy^{th} field	$0 \leq \Omega_{xy,ij} \leq 1$
$w_{xy,t}$	Frequency of the W allele in the xy^{th} field in the t^{th} generation	$0 \leq w_{xy,t} \leq 1$
$L_{xy,t}$	Frequency of the L allele in the xy^{th} field in the t^{th} generation	$0 \leq L_{xy,t} \leq 1$
ϵ_j	Fitness penalty of lethal construct to carriers	0, 1
$\sigma_{xy,t}$	Proportion of the population in the xy^{th} field that survive the fitness cost imposed by their genotypes and crop type	$0 \leq \sigma_{xy,t} \leq 1$
R_0	Lifetime average number of female offspring produced per female	10
ρ	Release ratio of transgenic males to wildtype males	0, 0.5, 1, 2, 5, 10
α	Density-dependence scaling constant	0.0001
β	Density-dependence shape factor	1
κ	Dispersal proportion	0.1
$N_{xy,t}$	Population size in the xy^{th} field in the t^{th} generation	
N_{thresh}	Release threshold	0, 500, 1000, 2000
$D_{xy,t}$	Number of dispersing females from the xy^{th} field in the t^{th} generation	

Releases of transgenic males

Transgenic males, homozygous for the transgene construct ($ij = SSLL$) are released into wild adult populations at a ratio of ρ transgenic males to males in the target population. Localised release policies are simulated by targeting transgenic releases at populations which exceed a fixed threshold ($N_{thresh} < N_{xy,t}$). Insects are released into the populations prior to the mating phase and transgenic males mate with females with equal probability as wildtype males. The number of transgenic males released in the xy^{th} field in the t^{th} generation ($T_{xy,t}$) when these conditions are met is calculated by:

$$T_{xy,t} = \rho M''_{xy,t} \tag{2.12}$$

where $M''_{xy,t}$ is the number of adult males surviving to adulthood in the current time step. Releases do not occur when the population density prior to mating is lower than the release threshold ($N_{xy,t} < N_{thresh}$). Area-wide release regimes are simulated by setting $N_{thresh} = 0$, which triggers releases in all fields with extant pest populations.

Dispersal

After mating, but prior to oviposition, a fixed proportion, κ, of the females in each population disperses from their natal field. Dispersal probability and ability are assumed to be independent of genotype, sex, natal-patch density and habitat type, and dispersal inflicts no penalty on either the survival or fecundity of dispersing individuals. Adult males at this stage of the life-cycle have no further impact on the following generation, so only female dispersal is modelled. The number of dispersing females departing from the xy^{th} field in t^{th} generation ($D_{xy,t}$) is calculated by:

$$D_{xy,t} = \kappa F''_{xy,t}. \tag{2.13}$$

We implemented a dispersal mechanism that approximates continuous dispersal in discrete-space landscapes (Slone, 2011). In the absence of a good approximation for an agricultural pest insect dispersal distance kernel, for each dispersing female a

dispersal distance and angle (θ) is generated from an inverse Gaussian distribution ($\mu = 0.4$, $\lambda^2 = 0.2$) and uniform distribution ($0 \leq \theta \leq 2\pi$) respectively. This parameterisation reflects our assumptions that dispersal direction is unbiased, the vast majority of individuals disperse short distances in agricultural landscapes with plentiful resources, while a minority of individuals undertake longer flights. Following dispersal, all females oviposit $2R_0$ eggs. All adult individuals then die, signalling the end of the generation and beginning of the next.

Simulations

This system was used to simulate the control of an agricultural pest using strategies combining refuges with transgenic insect releases under a challenging scenario in which Bt crops fail to eliminate 99.9% of heterozygote resistant individuals, thus violating the high-dose assumption of the HDR strategy. The central field was initiated with a population of 1000 individuals with an even sex-ratio and an initial resistance allele frequency of 0.5% ($p_0 = 0.005$), with all resistant individuals homozygous for the resistance allele (RRWW). The remaining fields begin uncolonised and the landscape is naive to transgenic insects ($L_0 = 0$).

We model a pest in which resistance is non-recessive ($\omega_{rs} > 0$) using, where available, published estimates for ecological and relative genotypic fitness parameters on single-toxin Bt-crop and refuge crop for the Diamondback moth, *Plutella xylostella L.*, a major pest of Brassica crops worldwide (Table 2.2). In the absence of published estimates for dispersal proportions, we assumed a constant dispersal proportion (κ) of 0.1. Estimates for carrying capacities and density-dependence parameters in agricultural landscapes are notoriously difficult to obtain, so we imposed an arbitrary field-level carrying capacity of 10,000 individuals to limit the computational cost of individual simulations. Simulations were run for 30 generations and 20 realisations were generated per parameterisation.

We first determined the extent to which refuge-only strategies could delay pest population growth and resistance evolution in landscapes with a range of refuge proportions (ϕ) and refuge compliance proportions ($\bar{C}$). Next we investigated the

Table 2.2: Summary of ecological and relative fitness parameters for which estimates for the Diamondback moth (*Plutella xylostella*) were derived from the literature.

Parameter	Estimate	Reference
ω_{rr}	0.82	Tabashnik et al. (1997)
ω_{rs}	0.205	Tabashnik et al. (1997)
ω_{ss}	0	-
υ_{rr}	0.29	Liao et al. (2019)
υ_{rs}	0.77	Liao et al. (2019)
υ_{ss}	1	-
R_0	10	Liao et al. (2019)

extent to which reinforcing refuges with transgenic insect releases could enhance both pest and resistance management, looking at the effects of release regime (area-wide ($N_{thresh} = 0$) vs. localised releases) and release ratio. As localised releases are likely to be more effective when deployed early in outbreaks, we also simulated a range of release thresholds (N_{thresh} = 500, 1000, and 2000) to determine the effect of target population size on the efficacy of localised release strategies.

For combined strategies, we determined the conditions under which the resistance allele and/or the pest population are eradicated. Where combined approaches were insufficient to eradicate the pest population, we investigated how the refuge and release parameters affected the dynamics of pest population growth and resistance evolution. Lastly, we assessed the longer-term consequences of different refuge and release strategies by comparing the distribution of terminal mean population size and resistance allele frequencies under different control strategies.

Results

Figure 2.1 presents the number of landscape-level pest population (Fig. 2.1a) and resistance allele (Fig. 2.1b) extinction events observed for a subset of the refuge and release parameters simulated. The across-field mean resistance allele frequency, across-field mean population size, and landscape colonisation dynamics are presented in Figures 2.2-5. Figures 2.5-7 show the distribution of across-field mean resistance allele frequency, across-field mean population size, and landscape colonisation proportions after 30 generations. To ease legibility, the figures presented in the main

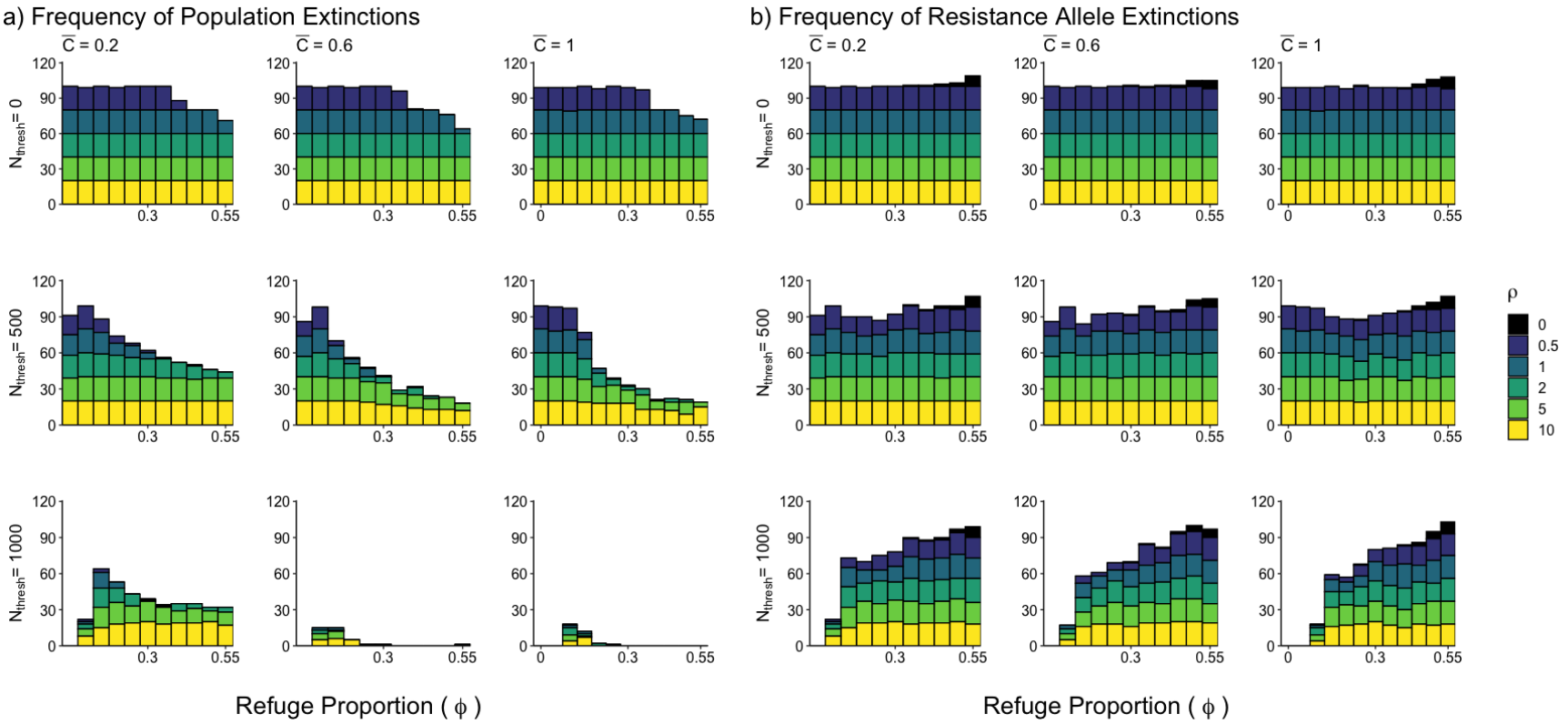


Figure 2.1: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level resistance allele and pest population extinctions. Individual plots within each panel present, for a subset of the combinations of release threshold (N_{thresh}), refuge compliance proportion ($\bar{C}$), refuge proportion (ϕ), and release ratio (ρ) simulated, the number of realisations in which the a) pest population and b) resistance allele were driven to landscape-wide extinction. Each parameterisation was run for 20 realisations on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K =$ 10,000, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

text show the results for a subset of the parameter combinations. Figures presenting the results for the full set of parameter combinations modelled are available in the supplementary materials in Appendix A.2.

The effect of refuge implementation and release regime on the frequency of population and resistance allele extinctions

Area-wide releases ($\rho > 0$, $N_{thresh} = 0$) successfully eliminated the pest population across all release and refuge parameter combinations, except when the largest refuges ($\phi > 0.45$) were deployed with the lowest release ratio ($\rho = 0.5$). Localised releases also eliminated the pest population for all release ratios when $N_{thresh} = 500$ and $\phi < 0.1$. However, as the refuge proportion increased, a $\rho \geq 5$ was increasingly required to reliably achieve population extinction. The frequency of landscape-wide population extinctions declined dramatically when $N_{thresh} \geq 1000$ and $\bar{C} \geq 0.6$, but increased as $\bar{C}$ decreased for $\rho \geq 5$. Refuge-only strategies ($\rho = 0$) were insufficient to eliminate the pest population across all simulations.

Resistance allele extinction events were rare under refuge-only strategies, but increased in frequency as ϕ increased from 0.2 to 0.55 (Fig. 2.1b). Combining refuges with transgenic insect releases drove the resistance allele to extinction across the full combinatorial suite of refuge compliance proportions, refuge proportions, and release ratios for both area-wide ($N_{thresh} = 0$) and localised releases with an N_{thresh} of 500 (Fig. 2.2a, Fig. A.2). When $N_{thresh} \geq 1000$, transgenic releases achieved landscape-wide resistance extinction when $\phi \geq 0.35$. However, when $\phi \leq 0.3$, the frequency of resistance extinctions declined with decreasing ρ and increasing N_{thresh}. Decreasing $\bar{C}$ had little effect on the frequency of resistance allele extinctions.

Combined release strategies provided enhanced resistance management in Bt crop dominated landscapes

Under refuge-only strategies, the resistance allele sweeps rapidly to fixation when $\phi \leq 0.2$. Increasing ϕ slows the evolution of resistance and eventually maintains the resistance allele at low frequencies. Decreasing $\bar{C}$ when $\phi \leq 0.3$ greatly increased

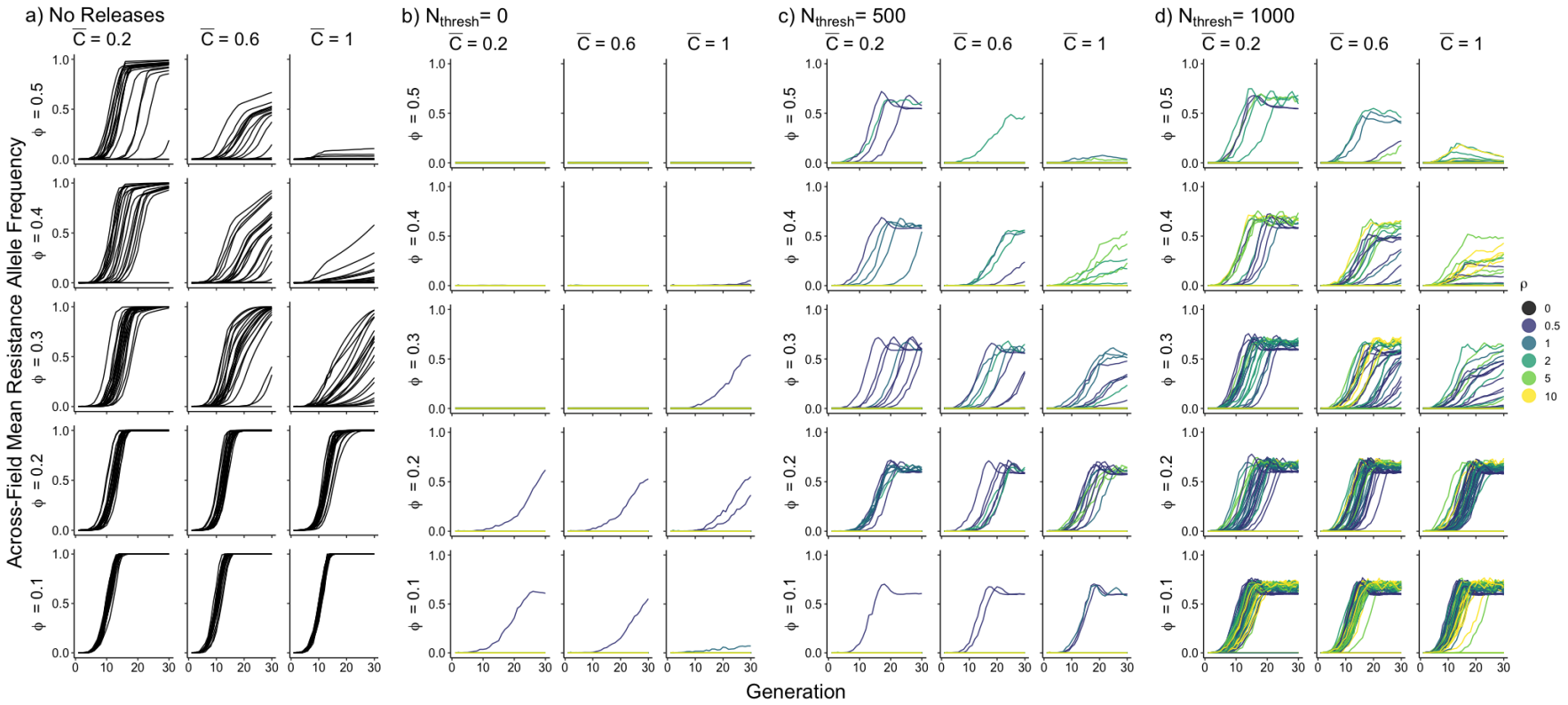


Figure 2.2: Across-field mean resistance allele frequency dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the across-field mean resistance allele frequency dynamics observed under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the across-field mean resistance allele frequency through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

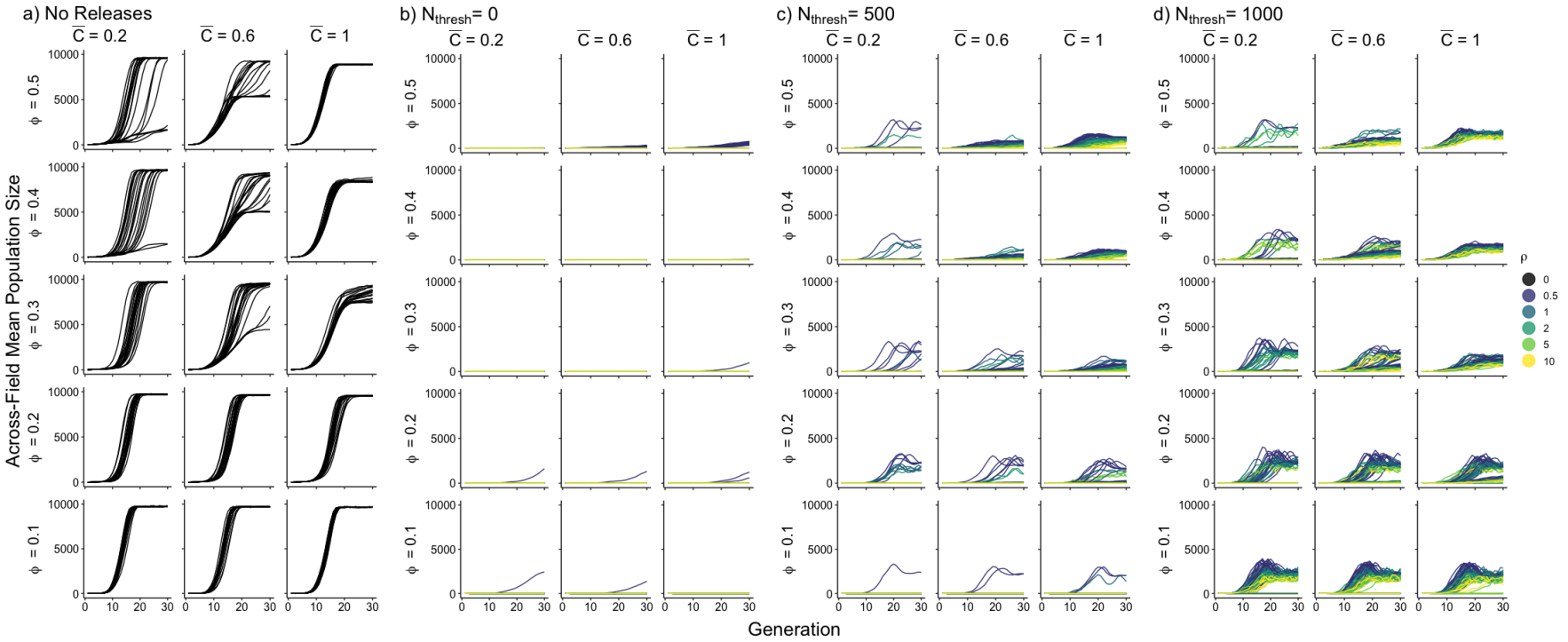


Figure 2.3: Across-field mean population size dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the across-field mean population size dynamics observed under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the across-field mean population size through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

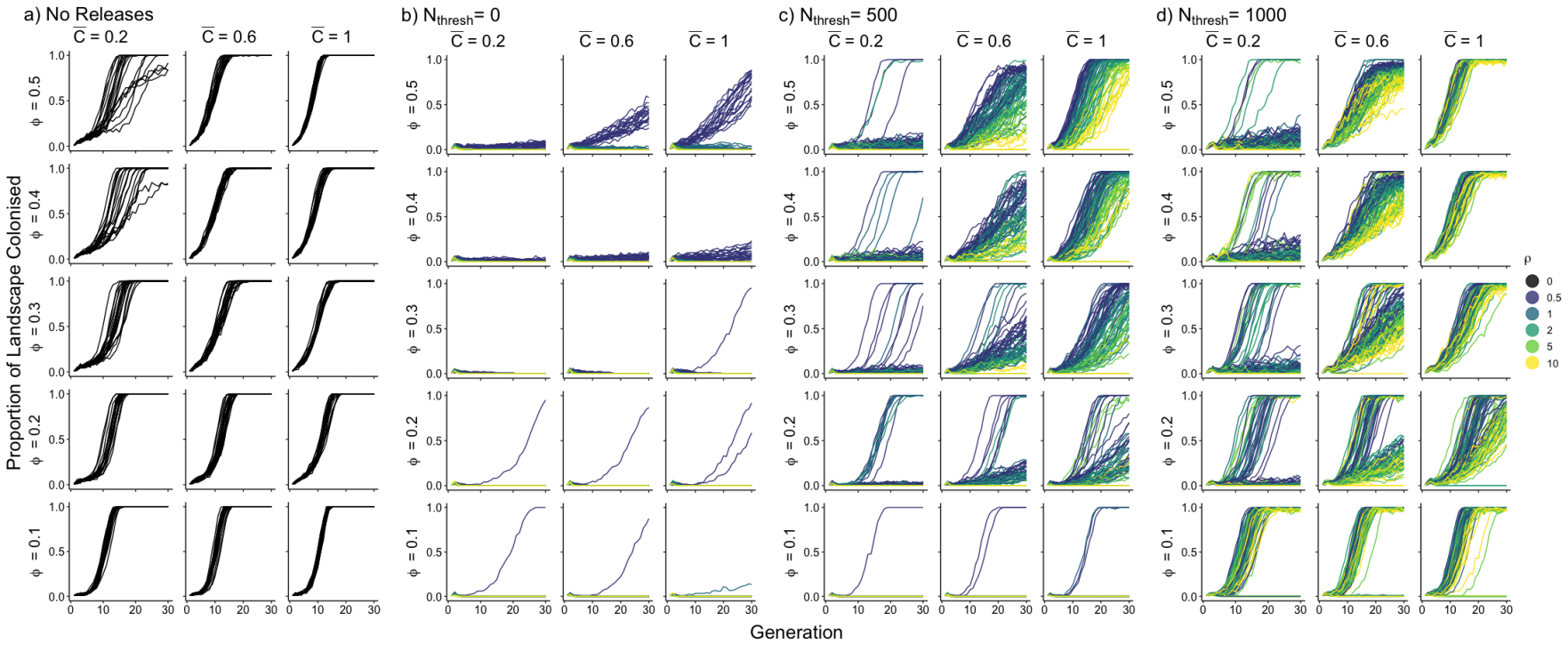


Figure 2.4: Landscape colonisation dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the proportion of the fields in the landscape populated through time under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the colonisation dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the proportion of the landscape colonised through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

the rate of resistance evolution, enabling the resistance allele to reach fixation for values of ϕ which prevented its spread in fully compliant landscapes ($\bar{C} = 1$), but had little effect when $\phi \leq 0.2$.

When insufficient to drive resistance to extinction, the degree to which releases enhanced resistance management depended on the refuge proportion and refuge compliance proportion. In landscapes dominated by Bt crop ($\bar{C} \leq 0.6$ and/or $\phi \leq 0.3$), supplementing refuges with transgenic insect releases slowed the rate of evolution (Fig. 2.2) and maintained the mean resistance allele at substantially lower frequencies (Fig. 2.5). However, as refuge crop becomes more prevalent in the landscape ($\bar{C} > 0.6$ and/or $\phi > 0.3$), the benefits to resistance management benefits offered by transgenic insects diminish. Increasing N_{thresh} increased both the rate of resistance evolution and the terminal mean resistance allele frequency, while increasing ρ slowed the rate of resistance evolution (Fig. 2.2), but increased the terminal mean resistance allele frequency.

Population suppressive effect of localised releases enhanced with low release thresholds and higher release ratios

Refuge-only control strategies provided little protection against the pest population which, for both the largest ($\phi \geq 0.4$) and smallest ($\phi \leq 0.15$) refuge proportions proliferated rapidly to a stable mean population equilibria that approached the fixed carrying capacity ($K = 10{,}000$)(Fig. 2.3a, Fig. 2.6a), and rapidly colonised the entire landscape (Fig. 2.4a, Fig. 2.7a). Planting intermediate refuge proportions ($0.2 \leq \phi \leq 0.3$) increased the frequency of heterozygotes, which had the lowest mean fitness across the two crop types, slowing the rate of both population growth and landscape colonisation (Fig. 2.3), but was insufficient to prevent the populations achieving high densities and colonising the entire landscape (Fig. 2.7).

Decreasing $\bar{C}$ had little effect on the population dynamics in refuge-only strategies when $\phi \leq 0.2$. However, for $\phi \geq 0.3$, when the resistance allele was extant, decreasing $\bar{C}$ increased the rate of population growth and terminal across-field mean population

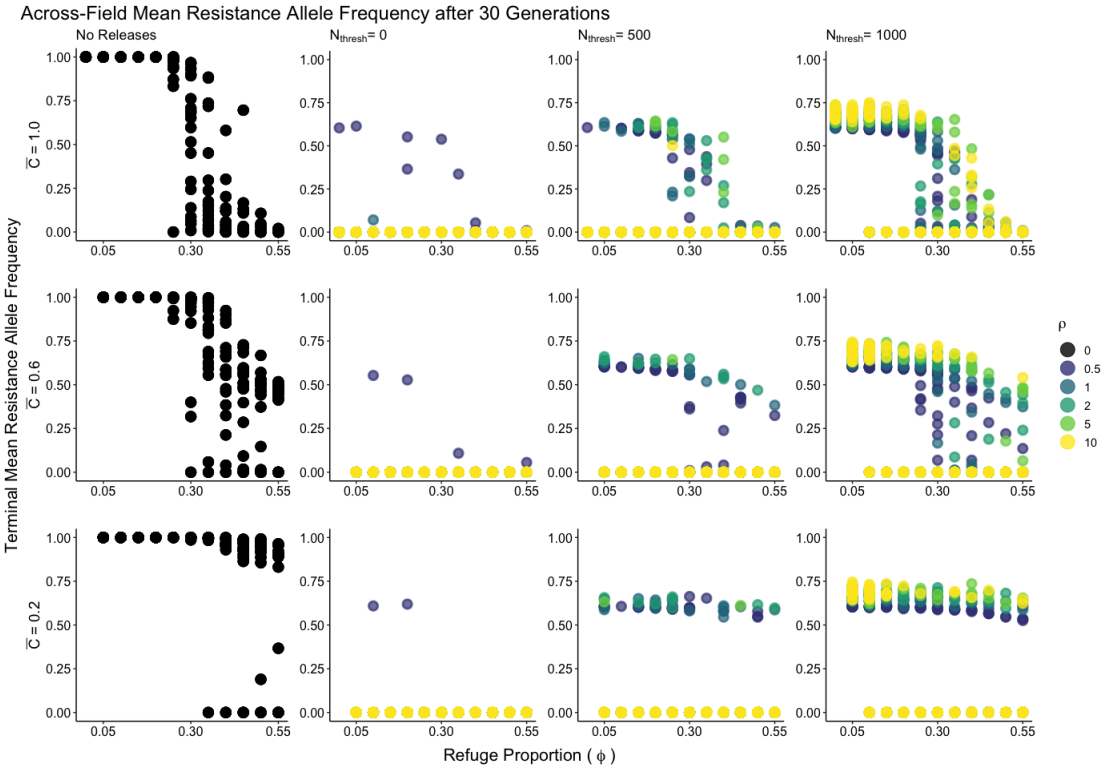


Figure 2.5: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean resistance allele frequency. Individual panels present the across-field mean resistance allele frequencies after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

size. Conversely, when resistance was absent from the population, decreasing $\bar{C}$ slowed population growth (Fig. 2.3a) and maintained the populations of susceptible insects at increasingly lower densities (Fig. 2.6a).

Supplementing refuges with releases of transgenic insects dramatically decreased the population growth rate and maintained the across-field mean population at substantially lower sizes relative to refuge-only strategies across all combinations of ϕ, $\bar{C}$, N_{thresh}, and ρ simulated (Fig. 2.3, Fig. 2.6). The population suppressive effect of combined management strategies were maximised by reducing N_{thresh} and increasing ρ (Fig 2.3b-d, Fig 2.4b-d, Fig. 2.5, Fig. 2.6). As in the refuge-only strategy, the magnitude of the suppressive effect of combined strategies was dependent on the

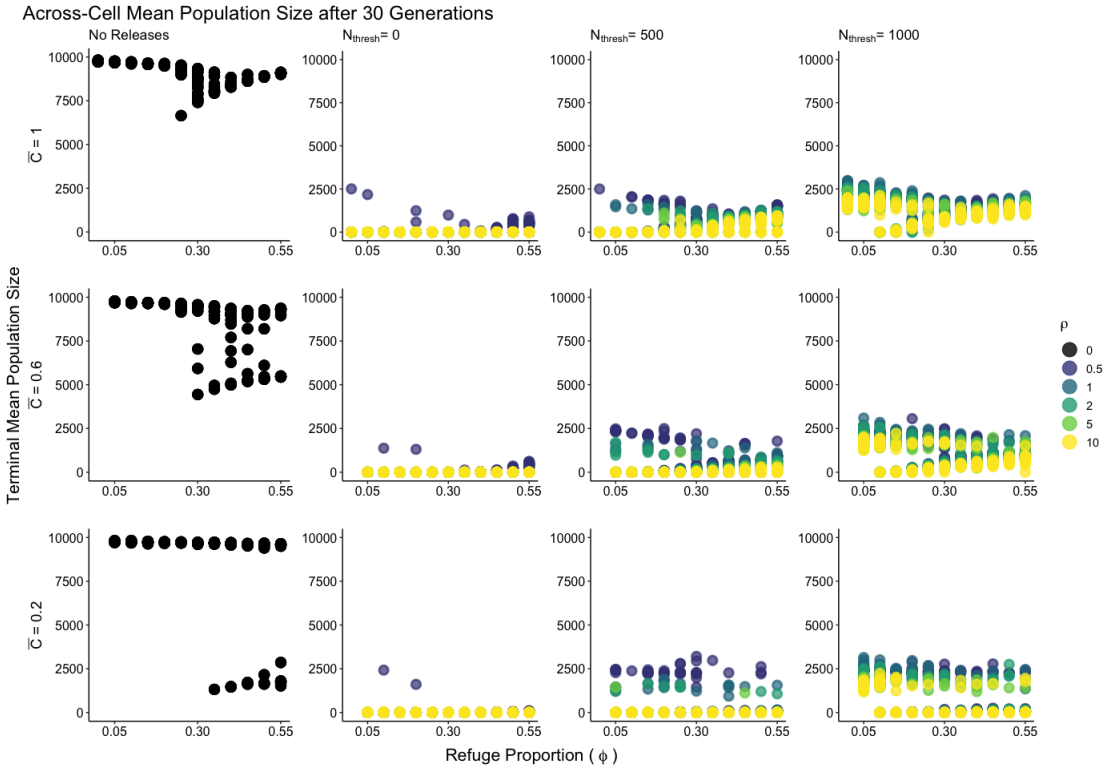


Figure 2.6: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean population size. Individual panels present the across-field mean population sizes after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

extinction status of the resistance allele. Reducing $\overline{C}$ increased the suppressive effect of localised releases of transgenic releases when the resistance allele had been driven to extinction. When releases failed to eliminate resistance, the pest density increased as $\overline{C}$ declined (Fig. 2.3b-d, Fig. 2.6b-d). When $\overline{C} \geq 0.6$, releases consistently provided superior suppression relative to the HDR-only strategy. In landscapes with $\overline{C} \leq 0.4$, when resistance remained within the population the mean population density differed little between the refuge and combined strategies (Fig. 2.6).

The effect of $\overline{C}$ on landscape colonisation rate also depended on the resistance allele status (Fig. 2.4b-d, Fig. 2.7). When releases drove the resistance allele to extinction, decreasing $\overline{C}$ and increasing ρ slowed the rate of landscape colonisation

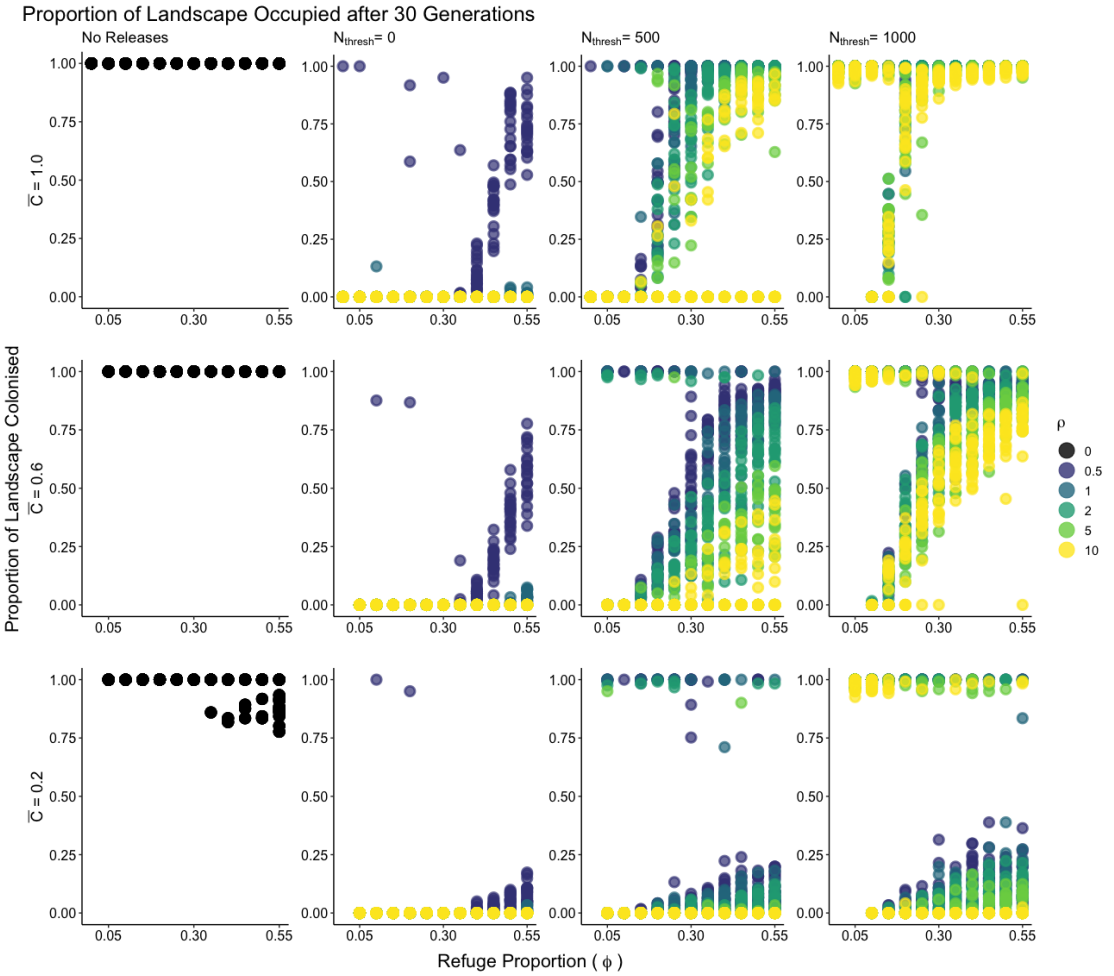


Figure 2.7: The effect of refuge implementation and transgenic insect release regime on the terminal proportion of the landscape colonised. Individual panels present the proportion of fields in the landscape colonised after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

(Fig. 2.5), preventing the pest from colonising the entire landscape when $\overline{C} \leq 0.4$ (Fig. 2.7). When releases failed to eliminate resistance, the rate of landscape colonisation differed little between the refuge-only and refuge and releases strategies as $\overline{C}$ decreased and the pest colonised the entire landscape within the simulated number of generations (Fig. 2.7).

Discussion

We developed a spatially-explicit population dynamics and genetics framework to facilitate investigations of landscape-level agricultural pest management strategies using transgenic insect releases. Here, the model was used to investigate the effects of refuge composition and compliance, as well as release strategy, on the efficacy of management regimes combining non-toxic refuges with transgenic insect releases for the landscape-level control of a pest with non-recessive resistance to Bt crops. While the effects of landscape composition, pest ecology, and refuge design on the evolution of Bt resistance have been studied extensively at the landscape scale (Huang et al., 2017; Peck et al., 1999; Sisterson et al., 2004; Sisterson et al., 2005; Storer et al., 2003; Vacher et al., 2003), our work represents, to the best of our knowledge, the first theoretical investigation of the utility of strategies combining refuges with transgenic releases at the landscape-level. The modular nature of this model facilitates its adaptation to consider different landscape structures (e.g. the effects of non-crop habitat), pest ecologies (e.g. over-lapping generations, alternative dispersal timings, additional density-dependence forms), and transgenic insect and crop technologies (e.g. pyramided crops, gene drive systems).

As anticipated, combining refuges with area-wide releases ($N_{thresh} = 0$) eliminated the pest for the majority of refuge proportion and release ratio combinations tested (Fig. 2.1a, Fig. A.1). That continuous releases of self-limiting, female-lethal transgenic males provides effective pest suppression, particularly when combined with an insecticide treatment or Bt crops, is theoretically (Alphey et al., 2009, 2011) and empirically (Ant et al., 2012; Harvey-Samuel et al., 2015; Leftwich et al., 2014; Zhou et al., 2018) well established. We also made some simplifying assumptions that favoured the efficacy of transgenic releases, including that the transgene works with perfect efficiency ($\epsilon_j = 1$), incurs no fitness cost to the carrier, and that released males mate with equal probability as wildtype males. However, recent evidence from laboratory and field trials suggests that self-limiting strains appear to exhibit dispersal ability, survival, and mating competency equal to that of a wildtype strain (Shelton et al., 2020). Though, in our simplified system, area-wide releases with

low release ratios relied to some extent on the additional suppressive power of the transgenic crop, as evidenced by the failure of the lowest release ratios with the largest refuges to achieve eradication, eradication was always achieved when deployed with sufficiently large release ratios. Given that area-wide applications of SIT have been used successfully to eradicate pest insects, including screwworm (*Cochliomyia hominivorax*) from the southern United States (Scott et al., 2017), melon fly (*Bactrocera cucurbitae*) from the Okinawa island chain (Kakinohana et al., 1997), and medfly (*Ceratitis capitata*) from Mexico and Guatemala (Hendrichs et al., 1983), despite radiation-sterilised insects typically suffering greatly reduced competence, our results are consistent with the expectation that area-wide releases of transgenic insects are likely to be an effective technology for landscape-wide eradication programmes, particularly if integrated with additional management tools.

When insufficient to eliminate the pest population, reinforcing refuges with localised releases of transgenic insects enhanced population suppression and slowed landscape colonisation relative to refuge-only strategies across the full combinatorial range of refuge proportions, refuge compliance proportions, release ratios, and release thresholds simulated (Figs 2.3-4, Figs A.3-6). Recently, Zhou et al. (2019) showed, using a system of three cage populations of diamondback moth linked by manual dispersal, that targeting the cage with the largest resistant population significantly reduced the total pest population size across the three-cage metapopulation relative to no-release strategies within three generations. Our theoretical work builds on their results to show that the suppressive benefits they observed in the short-term are likely to be maintained over longer-time frames, even as resistance becomes prevalent in the population (Figs. 2.2-3, Fig. A.3-6). Furthermore, localised releases targeted at population hotpots (Pulliam & Danielson, 1991) greatly enhances suppression even when non-recessive resistance reduces the contribution of toxic crops to pest mortality. We also found that the most effective method for enhancing the efficacy of localised release strategies is to reduce the release threshold, while increasing the release ratio also enhances their suppressive effect. Taken together, our results support the suggestion of Zhou et al. (2019) that localised releases

could represent a viable alternative to area-wide release regimes when population suppression, rather than eradication, is an acceptable outcome.

In Bt-dominated landscapes with small refuges and/or low refuge compliance, the resistance allele evolved rapidly to fixation in the absence of releases. The efficacy of refuges for resistance management is greatly diminished when the HDR assumptions of recessive-resistance, rare resistance, and random mating are violated (Campagne et al., 2013; Carriere et al., 2010) and, as expected, large refuges ($\phi \geq 0.3$) were required to delay or prevent the rapid evolution of non-recessive resistance, with even the largest refuges insufficient to drive resistance to extinction (Fig. 2.1b, Fig. 2.2, Fig. A.2, Fig. A.7). While increasing the abundance of refuge crop represents one potential solution to enhance resistance management (Tabashnik & Carrière, 2019), particularly when refuges are compromised by non-recessive resistance, this may result in economically unacceptable yield sacrifices. To facilitate the planting of smaller refuges, strategies that incorporate additional insect control measures, such as releases of transgenic insects, are likely to be necessary to protect both short-term yields and the longer-term efficacy of transgenic crops (Alphey et al., 2009).

Management strategies combining refuges with release policies based on local population densities provided mixed results for the management of resistance to a single-toxin Bt crop (Fig. 2.1b. Fig. 2.2, Fig. A.2, Fig. A.4). When refuges were reinforced with transgenic insect releases, the resistance allele was driven to extinction when $N_{thresh} \leq 500$, or when $N_{thresh} \geq 1000$ was combined with large refuges ($\phi \geq 0.3$). However, combined strategies provided only a moderate delay to resistance evolution relative to refuge-only strategies when resistance was not driven to extinction and, although they did prevent resistance reaching from fixation, were unable to maintain resistance below 50%. Furthermore, increasing the release ratio had the undesirable consequence of increasing the mean resistance allele frequency.

This phenomenon was an artefact of the population-dependent release strategy; greater release ratios suppressed the population further below the threshold, increasing the amplitude and wavelength of population oscillations and therefore the number of generations between releases.

The decreased frequency of releases reduced the introgression of susceptibility alleles and subjected the population to longer periods of selection for resistance between releases, enabling the resistance allele to reach greater frequencies. While our model shows that population-centred release strategies can enhance resistance management efforts, even when assumptions of the HDR strategy are violated, developing more nuanced management programmes that find optimal solutions which minimise both the short-term crop losses and longer term resistance costs (Hackett & Bonsall, 2019) would greatly enhance the benefits to both population and resistance management of transgenic insect releases.

As the proportion of global land area planted with Bt crops, and therefore the selection pressure for resistance, continues to increase, efforts to update and develop new resistance management strategies will be increasingly important. Our findings provide further evidence that releases of self-limiting transgenic insects have real potential as a tool for landscape-level population suppression and resistance management that can be integrated effectively with existing management strategies, even in the challenging scenarios which appear to be underpinning the increase in frequency of cases of field-evolved resistance (Tabashnik & Carrière, 2017). Our results are consistent with existing theoretical (Alphey et al., 2009; Alphey et al., 2007) and empirical (Harvey-Samuel et al., 2015; Zhou et al., 2018; Zhou et al., 2019) investigations and, in light of the increasing frequency of incidences of field-evolved Bt resistance (Tabashnik & Carrière, 2019), decreasing longevity of new insecticides (Tabashnik & Carrière, 2017), and urgent need to reduce the environmental impacts associated with broad-spectrum insecticides (Ali et al., 2019), provide further evidence that self-limiting insect releases could be a valuable addition to the Integrated Pest Management (IPM) toolkit for both area-wide and localised population and resistance management.

3

A mathematical model for the investigation of resistance management strategies using transgenic insect releases in anopheline mosquitoes

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Prepared for submission to *Parasites and Vectors*

Abstract

Background: The control of anopheline vectors centres on the mass deployment of insecticidal bed nets (LLINs) and indoor sprays (IRS), but the continued efficacy of these interventions is threatened by insecticide resistance and behavioural avoidance. Reinforcing insecticidal interventions with releases of self-limiting insects could enhance suppression and delay the evolution of resistance. However, while the resistance management potential of these integrated approaches has been investigated in agricultural pest management, they have yet to be explored for vector control.

Methods: We extended a population dynamics and genetics model to simulate an anopheline population and its control using strategies combining LLINs or IRS deployment with self-limiting releases. By targeting the effects of interventions on the appropriate life-stage(s), sex(es), and genotype(s), this framework facilitates investigations of the effect of a range of management strategies on vector population and resistance dynamics, the effect of resistance on population control, and the effect of releases on the evolution of resistance. Parameterising the model using estimates for *Anopheles stephensi*, we assessed the sensitivity of single-intervention strategies to ecological and operational parameters and identified scenarios in which integrated strategies could be required to maintain population control.

Results: Under insecticide-only strategies, resistance evolved quickly to erode population control when recessive and initially widespread, dominant with recessive costs, and when the costs of resistance declined. Reducing the exposure probability slowed the evolution of resistance, but at the cost of suppression. Release-only strategies provided effective suppression, eliminating the population when sufficiently intense, but were sensitive to reductions in mating competence and transgene penetrance. Releases restored insecticide susceptibility in the absence of insecticides, rapidly driving resistance from fixation to extinction.

Conclusions: Our single intervention simulations identified conditions under which population control is undermined by the evolution of resistance or avoidance, and provide a baseline against which to assess integrated approaches. The model

developed will be used to investigate strategies combining releases with LLINs/IRS, but its flexible design also supports its use by others to investigate a host of integrated vector control strategies.

keywords: Mosquito, Insecticide, Resistance, Management, Genetics, Modelling.

Introduction

Global efforts to reduce the burden of malaria have made significant progress since the turn of the century, with the number of cases falling from approximately 262 million in 2000 to 214 million in 2015 (World Health Organisation, 2015). A core component of strategies to combat malaria has been the mass deployment of insecticide-treated nets (ITNs) / long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). By 2012, over 50% of households in the malaria endemic region of Africa possessed treated-nets, while IRS applications provided protection to more than twice the number of people they did in 2003 (World Health Organisation, 2012). These insecticidal interventions leverage an understanding of the ecology of members of the *Anopheles gambiae* senso lato (s.l.) species complex, exploiting the indoor feeding (endophagic) and resting (endophilic) preferences of the adult females whose bites are responsible for disease transmission (Takken & Knols, 1999). Between 2000-2015, strategies incorporating these interventions are estimated to have averted 663 (542 - 753 credible interval) million clinical cases of malaria, with 68% (62%-73%) and 10% (5%-14%) of averted cases attributed to LLINs and IRS respectively (Bhatt et al., 2015). However, progress appears to have stalled since 2015, as malaria-induced morbidity and mortality reductions fell short of the World Health Organisation's (WHO) Global Technical Strategy (GTS) targets for 2020, and the number of cases in 2019 increased to 229 million (World Health Organization, 2020).

While a number of causes have been cited for the observed stagnation, including static funding (fixed at approximately $3 billion per year since 2013) and a reduction in bed net coverage, bioefficacy and longevity (Lindsay et al., 2021), there is growing concern that resistance to the insecticide-based interventions that have formed the backbone of vector-control strategies may be responsible (Hemingway et al., 2016; Ranson & Lissenden, 2016; World Health Organisation, 2012; World Health Organization, 2020). There is currently limited evidence explicitly linking the evolution of insecticide resistance to epidemiological control failures (Kleinschmidt et al., 2018; Lindsay et al., 2021). However, an over-reliance on the limited number of

insecticide classes recommended for use in LLINs and IRS, and the still more limited number of unique modes of action (MoA), has resulted in the evolution of resistance, defined as a genetically-based reduction in insecticide-induced mortality, in most/all major anopheline and *Aedes* vectors (Hemingway et al., 2016; Moyes et al., 2020) to all of the primary insecticide classes (World Health Organization, 2020).

Another potential threat to the continued efficacy of LLIN- and IRS-based vector control strategies are anopheline species whose exposure to nets and sprays is reduced through natural or learned behavioural avoidance (Gatton et al., 2013; Kreppel et al., 2020). A notable example is *Anopheles stephensi* (Liston), which has recently expanded from its native range in Southeast Asia and the Arabian Peninsula into the Horn of Africa (Takken & Lindsay, 2019; World Health Organisation, 2019b). First documented in Djibouti in 2012 (Faulde et al., 2014), it has since been detected in Ethiopia (Carter et al., 2018), Sudan, and Somalia (Balkew et al., 2021). *An. stephensi* exhibits a number of ecological traits which could frustrate efforts to monitor and control its spread into Africa using existing tools and practices. Firstly, in contrast to other anopheline vectors which typically prefer rural areas, *An. stephensi* is able to establish in urban environments (Balkew et al., 2021; Sinka et al., 2011; Subbarao et al., 1987) which, given the trend towards urbanisation in sub-Saharan African populations and the traditional focus of malaria control efforts towards rural settings, could put at risk an estimated additional 126 million people in African cities (Sinka et al., 2020). Secondly, *An. stephensi* is an exophillic, zoophilic, crepuscular feeder and may evade traps and insecticide-based interventions designed to protect people from indoor, night-biting species (Sinka et al., 2020). Thirdly, *An. stephensi* is a competent vector of both *Plasmodium falciparum* and *Plasmodium vivax*, and has been implicated in the outbreak of malaria cases in Djibouti City in 2012 where transmission of both species was detected (de Santi et al., 2021; Seyfarth et al., 2019). Lastly, resistance to the insecticide classes commonly used in LLINs and IRS has been reported in this species across its native range (Enayati et al., 2020; Hemingway, 1982; Safi et al., 2017) and in Ethiopia (Balkew et al., 2021; Yared et al., 2020). Managing the invasion of *An. stephensi*

into Africa, and other species whose ecology could similarly undermine existing controls, therefore represents a significant challenge that could benefit from the integration of novel control tools (Pates & Curtis, 2005).

Existing strategies for managing resistance to the insecticides used in LLINs and IRS attempt to modulate the selection pressure they exert on the target population by altering their usage across space and/or time (Levick et al., 2017; South & Hastings, 2018). These strategies include using complimentary insecticides with different modes of action (MoA) (to minimise the risk of cross resistance) in temporal sequences, spatial mosaics, and/or mixtures to increase sources of intra-generational mortality and prevent the accumulation of resistance to any one insecticide (REX Consortium, 2013). However, these strategies can be difficult to coordinate, exceed the financial capacity of the authorities tasked with implementing them, and rely on the existence of a selection of insecticide chemistries with exclusive MoAs (Mnzava et al., 2015). Increasing the suite of approved insecticides, preferably with a greater number of unique MoAs, to be used in LLINs and IRS, as well as identifying synergists such as Piperonyl butoxide (PBO) which enhance the efficacy of existing formulations, is undoubtedly important to preserve the efficacy of these insecticide-based tools (IVCC, 2021). It would also be prudent to develop novel alternatives that can be used in place of, or alongside, chemical-control measures in support of both integrated vector (IVM) and resistance management (IRM).

One promising option is the release of genetically-modified insects bearing a dominant, self-limiting, lethal transgene, a modernisation of the Sterile Insect Technique, which is emerging as a population suppression tool for agricultural pests and disease vectors (Wang et al., 2021). Non-biting male mosquitoes, homozygous for a transgenic construct with a lethal phenotype, are released into a target population and compete with wildtype males for mating opportunities. Offspring from these matings are eliminated during early development such that, if releases are large or frequent enough to mate with a threshold proportion of wild females, the population will be suppressed or eliminated (Thomas et al., 2000). Releases exploit the highly-evolved mate-seeking behaviour of the target species to introgress

this lethal payload and could therefore compensate for insecticides when the target species exhibits behavioural avoidance. The timing and specificity of the lethal phenotype is programmable and insect strains have been developed that terminate bearers during the egg phase (early-acting) or during late larval development (late-acting) (Phuc et al., 2007), and target either both sexes (bisex) or only females (female-specific) (Fu et al., 2007). To facilitate mass rearing, the transgene construct includes a repressor that, in the absence of dietary tetracycline (or analogue), eliminates female carriers to enable efficient sex-sorting of males prior to release. This prevents the release of biting females and increases the efficiency of release strategies by preventing matings between released insects. The efficacy of such releases for controlling insect populations has been demonstrated using theoretical modelling (Alphey, 2014; Gentile et al., 2015; White et al., 2011; White et al., 2010), laboratory assays (Harvey-Samuel et al., 2015; Leftwich et al., 2014) and field trials (Carvalho et al., 2015; Harris et al., 2012).

Female-specific self-limiting releases could also have utility as a tool for genetic insecticide resistance management (Alphey et al., 2007). If released males are homozygous for the allele that confers susceptibility to the insecticide, transgenic releases can introgress genetic susceptibility into the target population through the viable male offspring resulting from crosses between released males and wild females. In the context of agricultural pest management, both theoretical and empirical work has demonstrated the capacity for self-limiting releases to slow or reverse the evolution of insecticide resistance, particularly when used in combination with non-toxic refugia (Alphey et al., 2009; Alphey et al., 2007; Zhou et al., 2018; Zhou et al., 2019). The use of releases for managing resistance in mosquitoes remains underexplored, but differences in the requirements for vector management mean that the findings from agriculturally-focused studies are not necessarily generalisable. Firstly, in agricultural systems insecticides are designed to control the larval population which feeds on crop tissue and reduces yield, whereas LLINs and IRS in vector control programmes target the adult females responsible for transmission. The survival of susceptible males could, in theory, increase the

efficiency of releases as a resistance management tool, as neither released males or their viable male offspring are likely to be eliminated by the insecticide. Furthermore, as mosquito larvae are not in themselves problematic, transgenes with a late-acting phenotype can be deployed which eliminate female carriers late during larval development to maximise the mortality imposed by density-dependent regulation. As a first step, a theoretical approach would be useful to assess the viability of releases for resistance management in mosquito populations.

In light of these concerns regarding the continued efficacy of LLIN- and IRS-based vector control due to limited coverage, behavioural avoidance, and the evolution of resistance, we developed a population dynamic and genetic modelling framework that can simulate integrated mosquito population and resistance management strategies that combine LLIN/IRS with releases of transgenic insects with a dominant, late-acting, female-specific lethal phenotype. In the present work, we investigated the impact of reducing the exposure to insecticides (either due to low coverage or behavioural avoidance) and the evolution of insecticide resistance on population and resistance management under a range of resistance scenarios. We also determined the effects of release intensity, transgenic male mating competence, and transgene penetrance, on the capacity for release-only strategies to suppress an anopheline population and manage resistance. Finally, we identified scenarios in which combined strategies are likely to be necessary to overcome the weaknesses of single-intervention population and resistance management strategies, and generated a series of baselines against which to compare single- and dual-intervention approaches.

Methods

To capture the effects of both insecticide-based interventions and transgenic insect release control strategies on population dynamics and resistance evolution, we developed the demographic and population genetics model presented by Barbosa et al. (2018). This framework is a system of discrete-time difference equations that describe a stage-, age-, sex-, and genotype-structured population of an anopheline mosquito with overlapping generations. To explicitly model both genetic resistance

to LLIN/IRS and the self-limiting transgene construct, the population genetic component of the framework was extended to track two independently segregating diallelic loci. This highly structured model allows the differential mortality and selective pressure associated with the different control interventions to be applied to the relevant subsets of the population, and captures the lagged effects of self-limiting releases on the population. All population model indices and parameters are summarised in Table 3.1.

Juvenile stages

The life cycle of anopheline mosquitoes begins with an aquatic juvenile phase when adult females oviposit eggs into a water source. The juvenile classes are described using the notation $J_a^{sj}(t)$, which represents the number of juvenile individuals of sex s, genotype j, and age a (days), alive at the end of day t. The age of juvenile individuals can take values between 1 and ζ, where ζ is the combined duration, in days, of the egg, larval and pupal stages. Age increases by 1 with each iteration of the model and is indicative of the individual's current life-history stage. The number of new eggs of sex s and genotype j that survive to the end of their first day ($a = 1$) on day t is given by:

$$J_1^{sj}(t) = \gamma \Lambda^j(t-1)\rho_e^j \tag{3.1}$$

where $\Lambda^j(t-1)$ is total number of eggs of genotype j oviposited the previous day, ρ_e^j is the daily survival proportion of eggs of genotype j, and γ is the sex ratio. The egg phase has a duration of θ_e days and the remaining days spent as eggs are tracked using:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\rho_e^j \tag{3.2}$$

for $2 \leq a \leq \theta_e$. For simplicity, the four larval instars that anopheline mosquitoes progress through after hatching are combined into a single larval stage of duration θ_l days. Larvae are assumed to be subject to density-dependent regulation, which we modelled using the flexible two-parameter form described by Maynard Smith and

Table 3.1: Summary of the insect model parameters.

Symbol	Description	Value(s)
Indices		
a	Age of the individual	1:ζ
i	Insecticide Treatment	LLIN\IRS
j	Genotype	
k	Genotype of adult females' male mate	
p	Phase of the gonotrophic cycle	1:τ
s	Sex	f, m
t	time step (days)	
Juvenile Stages		
θ_e	Duration of egg stage (days)	2
θ_l	Duration of larval stage (days)	10
θ_p	Duration of pupal stage (days)	2
ζ	Total duration of juvenile stages (days)	14
ρ_e^j	Proportion of eggs of genotype j that survive per day	0.895
ρ_l^j	Proportion of larva of genotype j that survive per day	0.972
ρ_p^j	Proportion of pupa of genotype j that survive per day	0.975
α	Strength of density dependence	1×10^{-6}
β	Density-dependence shaping parameter	1
Adult Stages		
τ	Duration of the gonotrophic cycle (days)	3
ρ_m^j	Proportion of adult males of genotype j that survive per day	0.9
ρ_s^j	Proportion of host-seeking adult females of genotype j that survive per day	-
ρ_r^j	Proportion of resting adult females of genotype j that survive per day	0.9
σ^j	Mating competency of adult males of genotype j	1
H^j	Proportion of host-seeking females that successfully attain a blood meal per day	0.67
ϵ^j	Number of eggs of of genotype j laid per female per gonotrophic cycle	100
γ	Sex ratio of eggs	0.5

Slatkin (1973), in which the scaling constant α determines the strength of density-dependent regulation, while the shaping parameter β can be tuned to simulate

competition along a spectrum from scramble to contest. While density-dependence can exhibit additional effects on other important components of larval ecology, such as development rate (Walker et al., 2021), for simplicity we model only its effects on larval mortality. Progression through the larval stages is described by:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^{\beta}}\right)\rho_l^j \tag{3.3}$$

for $(\theta_e+1) \leq a \leq (\theta_e+\theta_l)$, where ρ_l^j is the daily, density-independent larval survival probability and $L(t-1)$ is the total larval density at time $t-1$. Total larval density is calculated by summing across all larval age, sex, and genotype cohorts:

$$L(t) = \sum_{j\in\{SSWW-RRLL\}} \sum_{a=\theta_e+1}^{\theta_e+\theta_l} \sum_{s\in\{m,f\}} J_a^{sj}(t). \tag{3.4}$$

After θ_l days larva pupate and remain in the pupal stage for θ_p days. Pupa are assumed not to experience density-dependent mortality and transition through the pupal stage is captured by:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\rho_p^j \tag{3.5}$$

for $(\theta_e+\theta_p+1) \leq a \leq \zeta$, where $\zeta = \theta_e+\theta_l+\theta_p$ and represents the total duration of the juvenile phase, and ρ_p^j is the daily pupal survival probability. Individuals that survive to the end of the pupal phase eclose into adult mosquitoes.

Adult life stages

Whereas juvenile males and females are assumed to exhibit the same basic dynamics and are therefore governed by the same equations, the behaviour of adult males and females differ in ways that affect their exposure to LLINs and IRSs and, therefore, the insecticide-induced mortality and selection pressure they experience. The dynamics of adult males and females are thus driven by different sets of equations.

Adult male dynamics

Anopheline adult males do not seek blood meals and are therefore assumed to avoid exposure to treated nets and indoor residual sprays. Instead, it is assumed that they spend the majority of their lives seeking mating opportunities. Adult male dynamics are described by:

$$A^{mj}(t) = \left(J_{\zeta}^{mj}(t-1) + A^{mj}(t-1)\right)\rho_m^j \tag{3.6}$$

which represents the number of newly emerged males, $J_{\zeta}^{mj}(t-1)$, and males surviving from the previous day, $A^{mj}(t-1)$, that survive the current day with probability ρ_m^j. For simplicity, we assume no effect of age on survival or mating probability and, therefore, only segregate the adult male classes by genotype.

Adult female dynamics

To enable the effects of the insecticide-based interventions to be segregated, the gonotrophic cycle is incorporated into the adult female dynamics model. The gonotrophic cycle is divided into three phases (p): host-seeking and feeding ($p = 1$); resting and egg maturation ($2 \leq p \leq (\tau - 1)$); and oviposition ($p = \tau$). The average duration (days) of a single gonotrophic cycle is given by τ and, after ovipositing and completing a cycle, females are assumed to begin another. The female population compartments are denoted $A_p^{fjk}(t)$, which represents the number of adult females of genotype j in the p-th phase of the gonotrophic cycle that mated with a male of genotype k alive at the end of day t.

Gonotrophic cycle phase 1: host-seeking and feeding

After eclosing, females are assumed to mate once and store sufficient sperm to produce offspring for the duration of their lives. Females mate with males of each genotype with a probability M^j, which is a function of the relative frequency of the genotype in the adult male population and the mating competency of adult males of that genotype σ^j:

$$M^j(t) = \frac{\sigma^j(J_\zeta^{mj}(t-1) + A^{mj}(t-1))\rho_m^j}{\sum_j \sigma^j(J_\zeta^{mj}(t-1) + A^{mj}(t-1))\rho_m^j}. \tag{3.7}$$

In the first phase of the gonotrophic cycle ($p = 1$), unfed adult females seek a host and attempt to obtain a blood meal. The number of adult females in the host-seeking phase, $A_1^{fjk}(t)$, is given by:

$$A_1^{fjk}(t) = \left(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{A_1^{fjk}(t-1)(1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \right) \rho_s^j \tag{3.8}$$

which represents the number of newly emerged and mated females, the number of females that failed to obtain a blood meal the previous day, and the number of females that completed a gonotrophic cycle the day before, scaled by the daily survival probability of host-seeking females, ρ_s^j. Host-seeking females successfully obtain a blood meal with probability H^j, and fail to feed with probability $1 - H^j$.

Gonotrophic cycle phase 2: resting and egg maturation

After successfully obtaining a blood meal, females rest on surfaces while their eggs undergo maturation. The number of females that enter the resting phase ($p = 2$) in each time step is the number of host-seeking adult females that successfully obtained a blood meal and survived the previous day:

$$A_2^{fjk}(t) = A_1^{fjk}(t-1)H^j\rho_r^j \tag{3.9}$$

where ρ_r^j is the probability that a female of genotype j survives the day while resting. Resting females progress through the remainder of the resting phase as follows:

$$A_p^{fjk}(t) = A_{p-1}^{fjk}(t-1)\rho_r^j. \tag{3.10}$$

for $3 \leq p \leq \tau$.

Gonotrophic cycle phase 3: oviposition

On the final day of the gonotrophic cycle ($p = \tau$), females seek a water source into which they oviposit ϵ^j eggs. The genotypic composition of the egg batches laid are governed by parental genotypes and follow standard Mendelian patterns of inheritance. The equations governing offspring genotype proportions and the number of eggs of each genotype laid per day ($\Lambda^j(t)$) are provided in Appendix B. 1 and B.2. The day after oviposition has been completed, females re-enter the host-seeking phase and a begin a new gonotrophic cycle.

Control interventions

We adapted the base population model described above to simulate the effect of the deployment of three population control interventions, long-lasting insecticide nets (LLINs), indoor residual spraying (IRS), and transgenic insect releases, on mosquito population and resistance allele dynamics. Table 3.2 summarises the parameters governing the control interventions.

Long-lasting insecticide nets (LLIN)

Bed nets are designed to protect people from host-seeking females and so we restrict the effects of LLIN deployment to the host-seeking phase. The host-seeking population is subdivided into unexposed (A_1^{fjk}) and exposed ($\hat{A}_1^{fjk}$) classes. Newly-emerged adult females and those beginning new gonotrophic cycles enter the exposed class with probability ϕ_{LLIN} and experience additional, genotype-dependent survival probability of surviving exposure LLIN, μ_{LLIN}^j. Individuals that fail to find a host restart their search the following day and are able to switch between unexposed and exposed hosts. To capture this, equation 8 is updated to:

$$A_1^{fjk}(t) = \Bigg(\underbrace{(1-\phi_{LLIN})J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)(1-\phi_{LLIN})}_{Repeat\ Feeders} + \underbrace{(1-\phi_{LLIN})A_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg)\rho_s^j \tag{3.11}$$

$$\hat{A}_1^{fjk}(t) = \Bigg(\underbrace{\phi_{LLIN}J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)\phi_{LLIN}}_{Repeat\ Feeders} + \underbrace{\phi_{LLIN}A_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg)\rho_s^j\mu_{LLIN}^j \tag{3.12}$$

Resting-females are assumed to avoid exposure to LLINs and, as we do not here investigate the effect of strategies combining LLINs with IRS, individuals from the unexposed and exposed classes are recombined when entering the resting phase:

$$A_2^{fjk}(t) = \Big(A_1^{fjk}(t-1) + \hat{A}_1^{fjk}(t-1)\Big)H^j\rho_r^{fj}. \tag{3.13}$$

with the remainder of the resting phase described by equation 10.

Indoor residual sprays (IRS)

Indoor residual sprays target adult female mosquitoes resting on surfaces and thus IRS-induced mortality is applied to adult females in the resting phases of the gonotrophic cycle ($2 \leq p \leq \tau$). Assuming females rest on IRS treated surfaces with probability ϕ_{IRS}, progress from the host-seeking phase into the unexposed and exposed resting phases is governed by:

$$A_2^{fjk}(t) = A_1^{fjk}(t-1)H^j\rho_r^j(1-\phi_{IRS}) \tag{3.14}$$

$$\hat{A}_2^{fjk}(t) = \hat{A}_1^{fjk}(t-1)H^j\rho_r^j\phi_{IRS}\mu_{IRS}^j \tag{3.15}$$

where $\hat{A}_2^{jfk}(t)$ and $A_2^{jfk}(t)$ represent the number of resting females in unexposed and exposed populations, and μ_{IRS}^j is the genotype-dependent probability of surviving exposure to IRS. We assume that resting mosquitoes remain exposed for the duration of the resting phase with no transition between the unexposed and exposed states:

$$A_p^{fjk}(t) = A_{p-1}^{fjk}(t-1)\rho_r^j. \tag{3.16}$$

$$\hat{A}_p^{fjk}(t) = \hat{A}_{p-1}^{fjk}(t-1)\rho_r^j\mu_{IRS}^j \tag{3.17}$$

It is assumed that ovipositing females from unexposed and exposed areas use oviposition sites with uniform probability and, therefore, the eggs laid by females in treated and untreated populations are combined into a single first-day egg class:

$$J_1^{sj}(t) = (\Lambda^j(t-1) + \hat{\Lambda}^j(t-1))\rho_e^j \tag{3.18}$$

where $\Lambda^j(t-1)$ and $\hat{\Lambda}^j(t-1)$ represent the number of eggs of each genotype laid by ovipositing females in the unexposed and exposed classes respectively. Finally, as host-seeking females are assumed to avoid exposure to sprays, females beginning new gonotrophic cycles are pooled into a single, untreated class:

$$A_1^{fjk}(t) = \left(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{A_1^{fjk}(t-1)(1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1) + \hat{A}_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \right) \rho_s^j \tag{3.19}$$

Releases of self-limiting transgenic male insects

Release campaigns typically employ pulsed releases of a fixed number of transgenic males deemed sufficient to achieve suppression based on estimates of the pre-intervention adult male population (Carvalho et al., 2015; White et al., 2010). In contrast to the adaptive release sizes modelled in chapter 2, we here model pulsed releases of $\kappa_r A^{m*}$ males that occur with a per week frequency κ_f, where A^{m*} is the pre-intervention equilibrial adult male population size and κ_r is the ratio of released males to wildtype males. For example, a release campaign with $\kappa_f = 2$ and $\kappa_r = 3$

would involve biweekly releases of three times the number of transgenic males as there were wildtype males in the pre-intervention population. On release days, transgenic males ($j = SSLL$) are released directly into the adult male population class:

$$A^{mSSLL}(t) = (\underbrace{J_{\zeta}^{mj}(t-1)}_{New-emergers} + \underbrace{A^{mSSLL}(t-1)}_{SurvivingMales} + \underbrace{\kappa_r A^{m*}}_{Releases})\rho_m^{SSLL} \tag{3.20}$$

The transgene construct has a late-acting, female-lethal action that terminates both wL and LL female larvae on the final day of larval development ($a = \theta_l$), but has no effect on male carriers:

$$J_{\theta_l}^{f--wL}(t) = J_{\theta_l-1}^{f--wL}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^{\beta}}\right)\rho_l^{f--wL}\mu_{LL} \tag{3.21}$$

$$J_{\theta_l}^{f--LL}(t) = J_{\theta_l-1}^{f--LL}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^{\beta}}\right)\rho_l^{f--LL}\mu_{LL}. \tag{3.22}$$

where μ_{LL} is probability of surviving the transgene-induced mortality and is itself equal to $1 - \upsilon$, where υ is the transgene penetrance.

Population genetics

Dominance of genetic resistance

The probability that resistance allele bearers survive exposure to insecticides is increased such that $\mu_i^{RR--} \geq \mu_i^{RS--} \geq \mu_i^{SS--}$. The probability that resistant heterozygotes survive exposure is determined by the dominance of resistance, h_{RR}:

$$\mu_i^{RS--} = (1 - h_{RR--})\mu_i^{SS--} + h_{RR}\mu_i^{RR--} \tag{3.23}$$

where $h_{RR} = 1$ represents dominant resistance ($\mu_i^{RS--} = \mu_i^{RR--}$), and $h_{RR} = 0$ gives recessive resistance ($\mu_i^{RS--} = \mu_i^{SS--}$).

Table 3.2: Summary of the intervention parameters and their default values.

Symbol	Description	Base Value(s)
Insecticides		
ϕ_i	Probability of exposure to LLIN/IRS	0.8
μ_i^{SS--}	Proportion of homozygous susceptible individuals that survive exposure to insecticide intervention i	0.01
μ_i^{RR--}	Proportion of homozygous resistant individuals that survive exposure to insecticide intervention i	0.9
c_{RR}	Fitness cost of carrying two copies of resistance allele	0.05
g_{RR}	Dominance of costs of resistance	0.5
h_{RR}	Dominance of resistance	1
p_0	Initial resistance frequency	0.01, 0.5
Releases		
υ	Penetrance of the lethal transgene	1
μ_L	Probability of surviving the lethal transgene phenotype	0
κ_r	Release ratio of transgenic males	1
κ_f	Frequency of releases (per week) of transgenic males	2
c_{LL}	Fitness cost of carrying two copies of the transgene construct	0.5
g_{LL}	Dominance of costs of the transgene construct	0.5

Costs of genetic resistance

The resistance allele is assumed to impose an energetic fitness cost, c_{RR}, to carriers relative to homozygous wildtype individuals ($j = SSww$). Though this fitness penalty has been linked to changes in a number of key biological traits, including developmental rate, size, and fecundity, for simplicity we impose it on the daily survival probability of all life-history stages (ρ):

$$\rho^{RR--} = (1 - c_{RR})\rho^{SS--} \tag{3.24}$$

The degree to which heterozygotes experience the fitness costs associated with the resistance allele is determined by the dominance of costs of resistance, g_{RR}:

$$\rho^{RS--} = (1 - g_{RR})\rho^{SS--} + g_{RR}\rho^{RR--} \tag{3.25}$$

where $g_{RR} = 1$ represents dominant costs ($\rho^{RS--} = \rho^{RR--}$) and $g_{RR} = 0$ represents recessive costs ($\rho^{RS--} = \rho^{SS--}$). We assume that the lethal allele does not affect daily survival beyond the effect intended by the transgene construct, such that the values for wL and LL genotypes are given by their ww equivalents (e.g. $\rho^{SSww} = \rho^{SSwL} = \rho^{SSLL}$).

Fitness costs of the lethal transgene

Transgenic males reportedly display a reduced ability to obtain a mate. We therefore impost a cost, c_{LL}, with a dominance g_{LL}, on the mating competency (σ^j) of adult male carriers of the transgene, where the mating competency of transgenic homozygotes is calculated as:

$$\sigma^{--LL} = (1 - c_{LL})\sigma^{--ww} \tag{3.26}$$

and the mating competency of insects bearing a single copy of the transgene is:

$$\sigma^{--wL} = (1 - g_{LL})\sigma^{--ww} + g_{LL}\sigma^{--LL}. \tag{3.27}$$

It is assumed that the genotype at the resistance locus imparts no effect on mating competency.

Simulations

We used the modelling framework to run simulations investigating the effects of exposure probability (ϕ_{LLIN}/ϕ_{IRS}) (a more generic implementation of the effects of coverage and behavioural avoidance), resistance dominance (h_{RR}), fitness costs of resistance (c_{RR}), and the dominance of resistance costs (g_{RR}), on the resistance allele and adult female population dynamics. We also looked at the effect of initial resistance allele frequency (p_0) on the potential for resistance evolution under these scenarios. The effects of release frequency (κ_f) and ratio (κ_r) on the population and resistance management potential of release-only strategies, and the extent to which their efficacy is eroded by reductions in male mating competence and transgene penetrance, were also investigated.

Unless otherwise stated, we assumed a default LLIN-only and IRS-only exposure probability of 0.8 ($\phi_i = 0.8$), in line with WHO targets to achieve 80% coverage. The default release strategy involved biweekly releases ($\kappa_f = 2$) at a ratio of 1:1 transgenic males to wildtype males in the pre-intervention, equilibrial male population ($\kappa_r = 1$). The pest model was parameterised using, where available, estimates from the literature for *Anopheles stephensi*, an emerging threat to malaria control in Africa that already exhibits both resistance to all major insecticide classes, and ecological traits that could naturally reduce its exposure to LLINs / IRS.

The outputs from these simulations are designed to achieve three objectives:

1. Assess the extent to which reduced coverage, behavioural avoidance, and the evolution of resistance, can undermine the management of a theoretical anopheline vector using LLIN-only and IRS-only strategies

2. Identify scenarios in which LLINs and IRS deployment is most likely to require reinforcement with transgenic insect releases

3. Develop a baseline against which to assess the efficacy of strategies integrating releases with LLIN and IRS

The model was implemented using R version 4.2 (R Core Team, 2020). We modelled integer values for mosquito densities by removing fractional values for population sizes using the floor() function, avoiding the issue of defining a population extinction cut-off.

Results

Insecticide-only interventions (LLIN / IRS)

Effect of insecticide exposure probability (ϕ_{LLIN}/ϕ_{IRS})

Resistance proliferated when the exposure probability (ϕ_i) exceeded a threshold level dependent on both the dominance of resistance (h_{RR}) and its associated fitness penalty (g_{RR}) (Figs. 3.1-2, Figs. B.1-2). Above this threshold, the allele frequency increased monotonically to an equilibrium that increased with increasing exposure

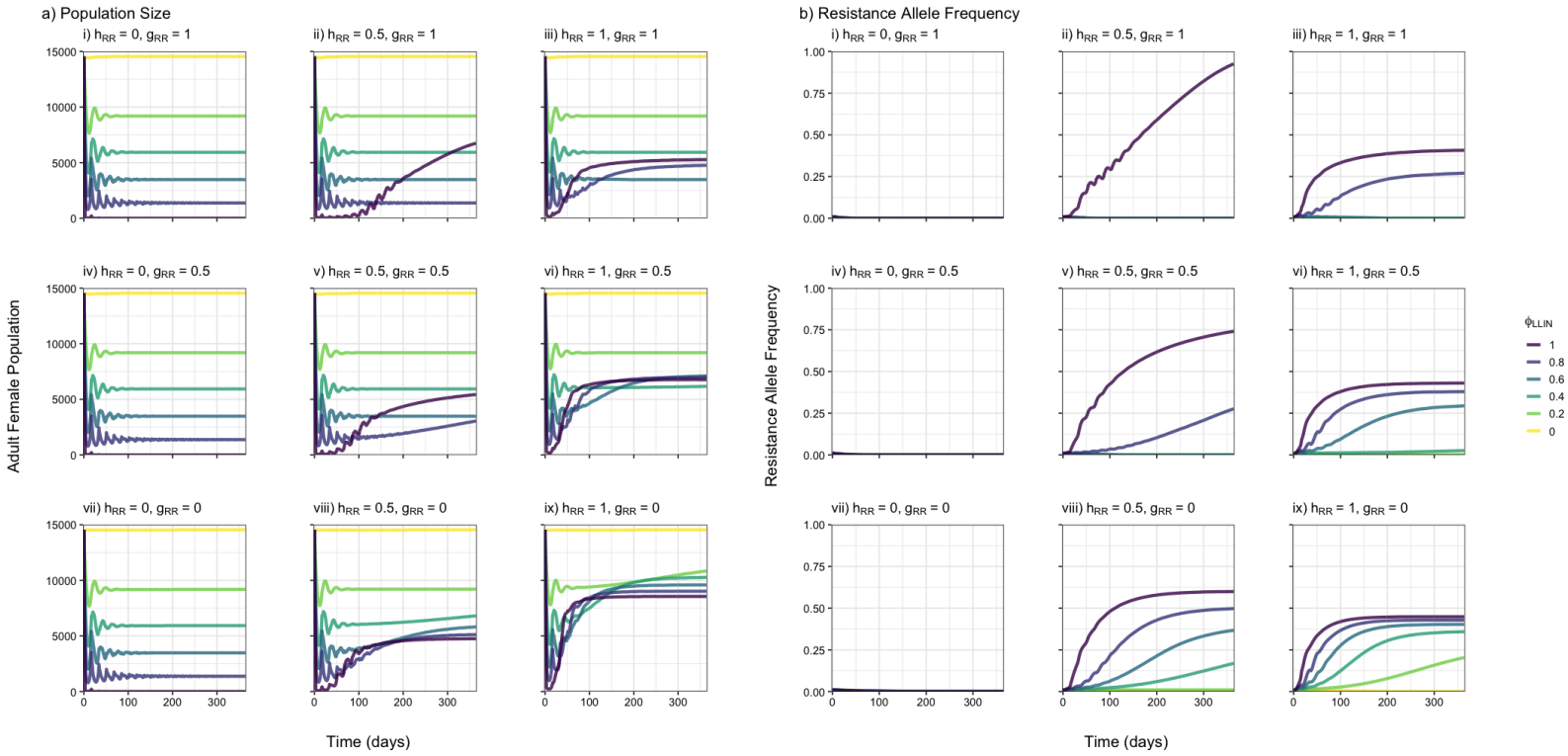


Figure 3.1: The effect of exposure probability, the dominance of resistance, and the dominance of costs of resistance on population and resistance management under LLIN-only strategies. Individual panels depict the a) adult female population and b) resistance allele dynamics observed under a range of LLIN-exposure probabilities (ϕ_{LLIN}, coloured lines) for a given combination of resistance dominance (h_{RR}) and dominance of costs of resistance (g_{RR}). The bottom left panels (*vii*) represent the case when both resistance and its costs are recessive ($h_{RR} = 0$, $g_{RR} = 0$), while the top right panel (*iii*) depicts the case when both are dominant ($h_{RR} = 1$, $g_{RR} = 1$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.01$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$.

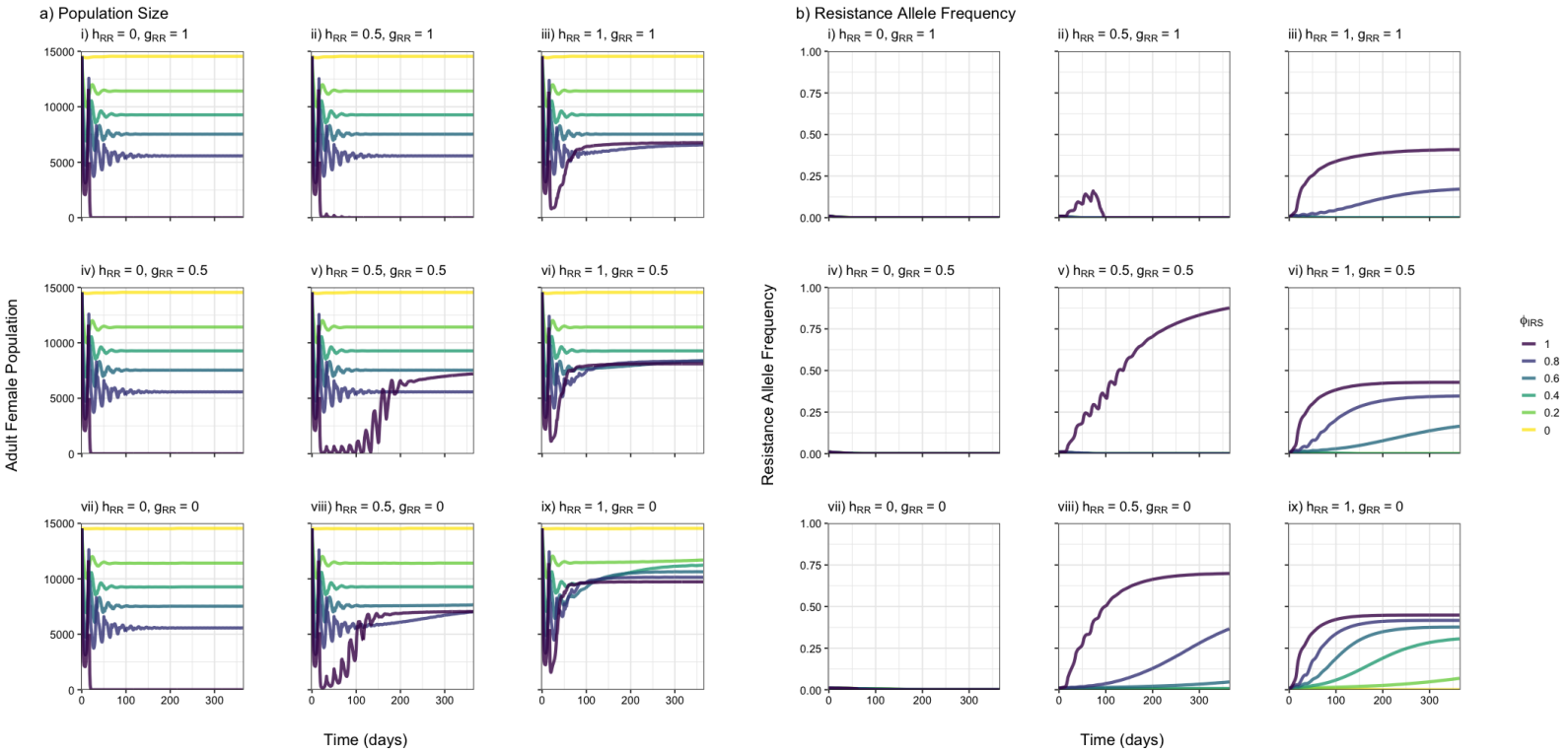


Figure 3.2: The effect of exposure probability, the dominance of resistance, and the dominance of costs of resistance on population and resistance management under IRS-only strategies. Individual panels depict the a) adult female population and b) resistance allele dynamics observed under a range of IRS-exposure probabilities (ϕ_{IRS}, coloured lines) for a given combination of resistance dominance (h_{RR}) and dominance of costs of resistance (g_{RR}). The bottom left panels (*vii*) represent the case when both resistance and its costs are recessive ($h_{RR} = 0$, $g_{RR} = 0$), while the top right panel (*iii*) depicts the case when both are dominant ($h_{RR} = 1$, $g_{RR} = 1$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.01$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$, $c_{RR} = 0.05$.

probability and fitness cost dominance and decreasing resistance dominance. Below this threshold, the allele frequency declined to extinction at a rate that increased with decreasing exposure probability and resistance dominance and increasing dominance of fitness costs. The rate of resistance decay was maximised in the absence of an insecticide challenge ($\phi_i = 0$), but this approach allowed the population size to return quickly to its pre-intervention equilibria.

Increasing the exposure probability always decreased the adult female population size under parameter combinations in which the resistance allele either went extinct (e.g. $h_{RR} = 0, g_{RR} = 1$) or remained extant across the full range of non-zero exposure probabilities (e.g. $h_{RR} = 1, g_{RR} = 0$). When resistance was driven to extinction, increasing the exposure probability increased the time taken for the population to reach equilibrium, whereas the population size stabilised more rapidly as the probability of exposure increased when resistance was extant. Under combinations of h_{RR} and g_{RR} for which the extinction status of the resistance allele depended on the exposure probability (phi), the evolution of resistance under high exposure probabilities enabled the population size to approach or exceed the sizes observed for lower exposure probabilities under which resistance went extinct (e.g. Fig. 3.2.a.v, Fig. 3.2.b.v).

Effect of the dominance of resistance (h_{RR}) and resistance cost (g_{RR})

The effects of the dominance of resistance (h_{RR}) and the dominance of fitness costs (g_{RR}) on the evolution of resistance and population control under LLIN-only and IRS-only are displayed in Figures 3.1-4. When resistance was initially rare ($p_0 = 0.01$), recessive resistance ($h_{RR} = 0$) failed to evolve under LLIN-only (Fig. 3.1b) or IRS-only control (Fig. 3.2b) irrespective of the probability of exposure (ϕ_i) or fitness cost dominance (g_{RR}). In the absence of resistance, the equilibrial adult female population size decreased with increasing ϕ_i, eliminating the pest when exposure was universal ($\phi_i = 1$).

Increasing the dominance of resistance (h_{RR}) and decreasing the dominance of costs (g_{RR}) enabled resistance to evolve from initial rarity ($p_0 = 0.01$) for increasingly

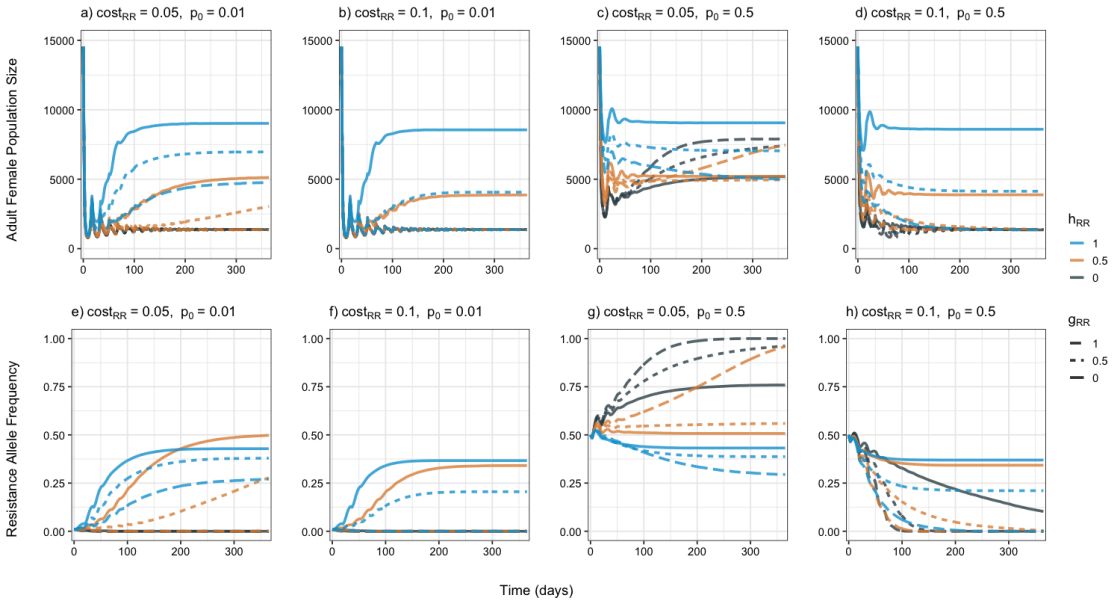


Figure 3.3: The effect of resistance costs and initial resistance frequency on population and resistance management under LLIN-only strategies. The top row of panels, a)-d), show the adult female population size and the bottom row of panels, e)-h), the resistance allele frequency over one year following the initiation of LLIN-only ($\phi_{LLIN} = 0.8$) control for resistance costs to fitness of 5% and 10%, and initial resistance frequencies of 1% and 50%. The dominance of resistance (h_{RR}) and dominance of costs of resistance (g_{RR}) are given by the line colour and line type respectively. All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\phi_{LLIN} = 0.8$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$.

lower exposure probabilities under both LLIN-only and IRS-only strategies, but decreased the equilibrial resistance allele frequencies; under dominant resistance ($h_{RR} = 1$) with recessive costs ($g_{RR} = 0$), the resistance allele frequency stabilised at approximately 0.5 (Fig. 3.1). The evolution of resistance eroded population control under LLIN-only or IRS-only strategies, enabling the population size to exceed that observed when resistance was extinct for equivalent values of ϕ_i, and, in some cases, those observed under lower values of ϕ_i.

When the resistance allele was initially widespread ($p_0 = 0.5$), recessive resistance evolved rapidly when $\phi_i \geq 0.8$, with the rate of evolution increasing as g_{RR} increased (Fig. B.1, Fig. B.2). This increased the equilibrial adult female population size, in some cases beyond that observed when resistance decayed to extinction under lower exposure probabilities. When $\phi_i \leq 0.6$ and $h_{RR} = 0$, the initially common

resistance allele decayed from the population at a rate that also increased with g_{RR}, with the long-term adult female population size again determined by the probability of exposure. Increasing the initial resistance frequency when the costs of resistance were dominant ($g_{RR} = 1$) changed the dynamic trajectory of the resistance allele and adult female population size, but did not affect the long-term outcomes (Fig B.1, Fig. B.2).

Effect of resistance cost (c_{RR})

Increasing the fitness cost associated with resistance (c_{RR}) reduced the range of dominance scenarios in which resistance evolved under both LLIN-only (Fig. 3.3) and IRS-only control (Fig. 3.4). For dominance combinations that facilitated the evolution of resistance under both the low ($c_{RR} = 0.05$) and high ($c_{RR} = 0.1$) fitness cost scenarios simulated, typically when resistance tended towards dominance ($h_{RR} \geq 0.5$) and the costs towards recessivity ($g_{RR} \leq 0.5$), the resistance allele stabilised at lower frequencies when the resistance cost was increased. By reducing the equilibrial resistance allele frequency or inducing resistance extinction, increasing the costs of resistance also reduced the equilibrial adult female population size.

Effect of initial resistance allele frequency (p_0)

Increasing the initial resistance allele frequency (p_0) enabled resistance to evolve under certain parameterisations in which small-population effects likely prevented its evolution when resistance was initially rarer (Figs. 3.1-4, Figs. B.1-2), most notably when resistance was completely recessive ($h_{RR} = 0$). Increasing the initial resistance frequency did not, however, protect the allele from extinction under parameterisations in which the selection pressure was insufficient to drive its evolution (e.g. $\phi_i \leq 0.4$). Where increasing the initial resistance frequency did prevent extinction, it had no effect on its frequency at equilibrium, but the time taken for resistance to equilibriate did typically differ with p_0. Increasing the initial resistance allele frequency increased the equilibrial population size in those cases in which it enabled resistance to avoid extinction, but did not otherwise affect the long-term population outcomes.

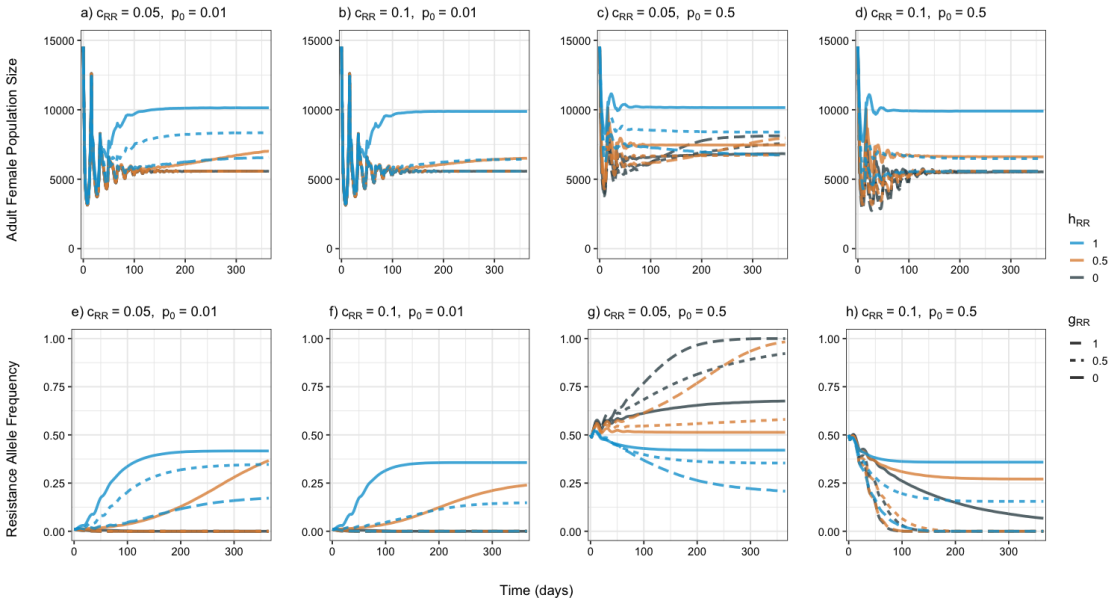


Figure 3.4: The effect of resistance costs and initial resistance frequency on population and resistance management under IRS-only strategies. The top row of panels, a)-d), show the adult female population size and the bottom row of panels, e)-h), the resistance allele frequency over one year following the initiation of IRS-only (ϕ_{IRS} = 0.8) control for resistance costs to fitness of 5% and 10%, and initial resistance frequencies of 1% and 50%. Costs of resistance were applied to the daily survival probability at each stage in the life cycle. The dominance of resistance (h_{RR}) and dominance of costs of resistance (g_{RR}) are given by line colour and line type respectively. All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\phi_{IRS} = 0.8$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$.

Effect of insecticidal intervention (LLINs vs. IRS)

In the absence of resistance, LLINs suppressed the adult female population size to lower equilibria than IRS under equivalent exposure probabilities (e.g. Fig. 3.1, Fig. 3.2). The resistance allele generally evolved faster, or decayed slower, and to higher equilibrial frequencies under LLIN control. LLINs also provided superior population suppression when resistance remained extant, but the degree of improvement for a given parameter set decreased relative to that observed when resistance was extinct (e.g. Fig. 3.1, Fig. 3.2).

One exception was when resistance was additive ($h_{RR} = 0.5$). Under LLIN-only control resistance evolved under a greater range of exposure probabilities, particularly as the costs of resistance tended to recessivity, but to lower equilibrial

frequencies than IRS-only control (Fig. 3.1, Fig. 3.2). However, LLINs still provided superior population suppression relative to IRS when $h_{RR} = 0.5$.

Releases-only interventions

Effects of release strategy (κ_f & κ_r) on population control and resistance management

Figure 3.5 and Figure B.3 depict the population and resistance allele dynamics after transgenic males are released into an equilibrial population with widespread initial resistance ($p_0 = 0.5$) in the absence of insecticide-based interventions. In the absence of either transgenic insect releases or an insecticide-based intervention ($\kappa_r = 0$), the population size exhibits an initial decline due to the increased daily mortality of resistance-bearing individuals relative to susceptible homozygotes, followed by a monotonic recovery to the pre-intervention equilibrial density (Fig. 3.5a) as the resistance allele declines to extinction (Fig. B.3a). Introducing releases yielded two population dynamic outcomes. For sufficiently aggressive release strategies, the transgene-induced mortality interrupts the recovery associated with the decline of the resistance allele and collapses the population to extinction (Fig. 3.5e-f). The minimum strategy required to achieve population extinction under the default pest parameterisation involved releasing at a rate of 5:1 ($\kappa_r = 5$) four days a week ($\kappa_f = 4$), which exterminated the population in 126 days (Fig. 3.5e). Less intensive release strategies provided strong initial suppression, but were unable to overcome the increased juvenile survival associated with the release from density-dependent regulation caused by transgene-induced larval mortality. Under such strategies, the decreased density-dependent regulation induced a limited recovery phase, before the population size re-stabilised at a level where increased density-dependent survival balanced transgene-induced mortality. Increasing the release rate and frequency both served to decrease this new equilibrial population size (Fig. 3.5). Transgenic releases also increased the speed of resistance extinction relative to the no-release strategy, with increases in both the release size and frequency reducing the time to resistance extinction by 10-54% (Fig. B.3).

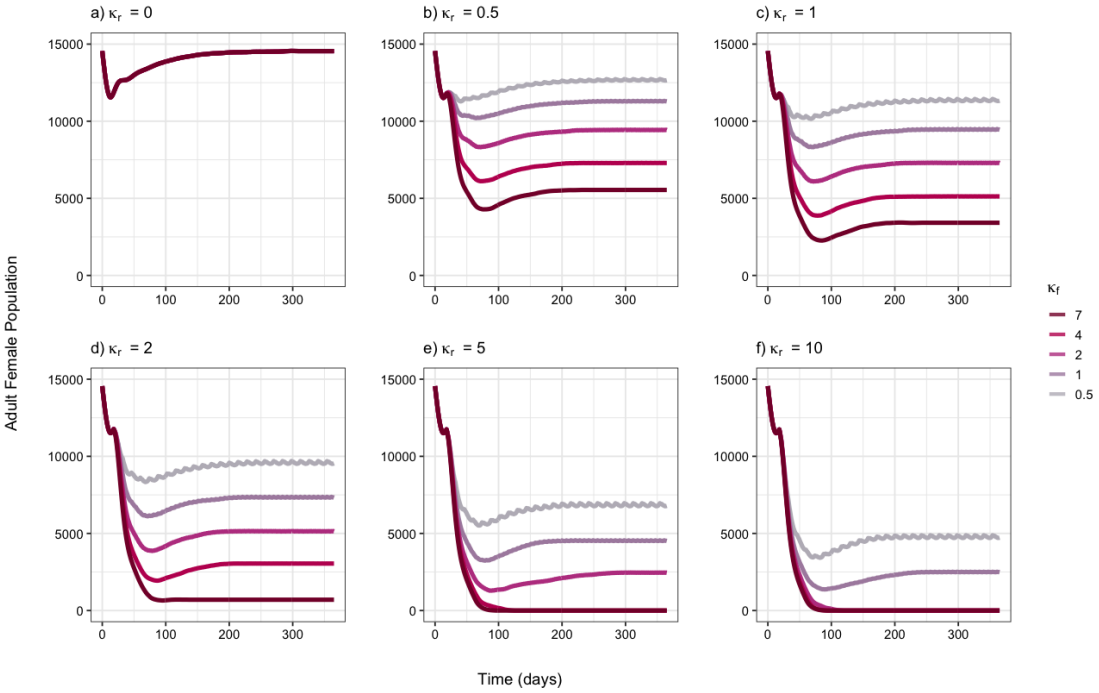


Figure 3.5: The effect of release strategy on the adult female population size under release-only management. Each panel depicts the adult female population dynamics under a different release ratio (κ_r), with panel a) showing the dynamics in the absence of releases, and individual lines depict release frequencies from fortnightly (κ_f = 0.5) to daily (κ_f = 7). The release ratio (κ_r) represents the number of transgenic male insects released per wildtype male in the pre-intervention, equilibrial population. The transgene was assumed to be fully penetrant ($\upsilon = 1$) and imposed an additive cost (g_{LL} = 0.5) to the mating competency of adult male homozygotes (j = –LL) of 50% (c_{LL} = 0.5). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, h_{RR} = 1

Effects of transgenic male mating competence (σ^{SSLL})

Releases of transgenic males provided the most effective population suppression and resistance management when no cost to mating competence was assumed ($\sigma^{SSLL} = 1$), with the default release strategy suppressing the equilibrial adult female population by ~60% and driving the resistance allele to extinction in 185 days (Fig. 3.6, Fig. B.4). Introducing a fitness penalty to the mating competence of transgene-bearing adult males eroded the efficacy of releases for both population and resistance allele management, with the equilibrial adult female population size and time taken for resistance to be eliminated increasing non-linearly with decreasing

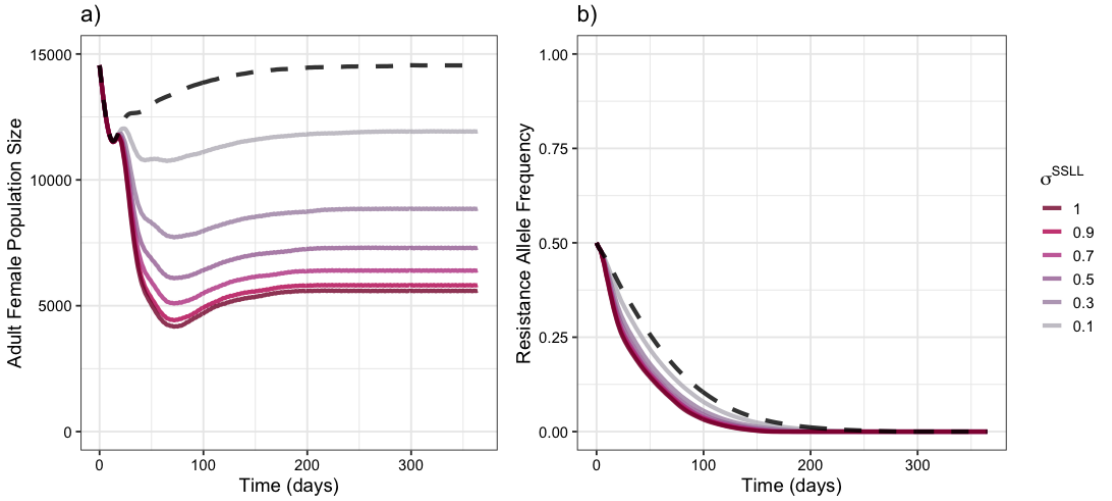


Figure 3.6: The effect of transgenic male mating competence on population and resistance management under transgenic insect release-only strategies. Panel a) depicts the adult female population size and panel b) the resistance allele frequency for one year following the onset of a release-only management programme of biweekly releases ($\kappa_f = 2$) at a ratio of 1:1 to the pre-intervention equilibrial male density ($\kappa_r = 1$). Coloured lines represent the dynamics observed under different released male mating competency values (σ^{SSLL}), and the black, two-dash lines show the dynamics observed in the absence of releases. The lethal transgene was assumed to be fully penetrant, terminating all female bearers late during the pupal stage ($\upsilon = 1$), and the cost imposed on transgene bearers to mating competence additive ($g_{LL} = 0.5$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, $h_{RR} = 1$.

mating competence (Fig. 3.6, Fig. B.4). Though the fitness cost associated with the resistance allele was sufficient for it to decline to extinction in the absence of insecticides, the release-only strategy provided superior population suppression and resistance management relative to the no-release case even when the costs to mating competence were severe ($\sigma^{SSLL} = 0.1$).

Effects of initial resistance allele frequency (p_0)

The default release strategy ($\kappa_f = 2, \kappa_r = 1$) was sufficient to drive resistance to extinction irrespective of its initial frequency in the pre-intervention population (p_0), although increasing p_0 increased the time taken for resistance to be driven to extinction (Fig. 3.7b). The default release-only strategy achieved resistance elimination faster than the no intervention approach ($\kappa_r = 0$), with the relative

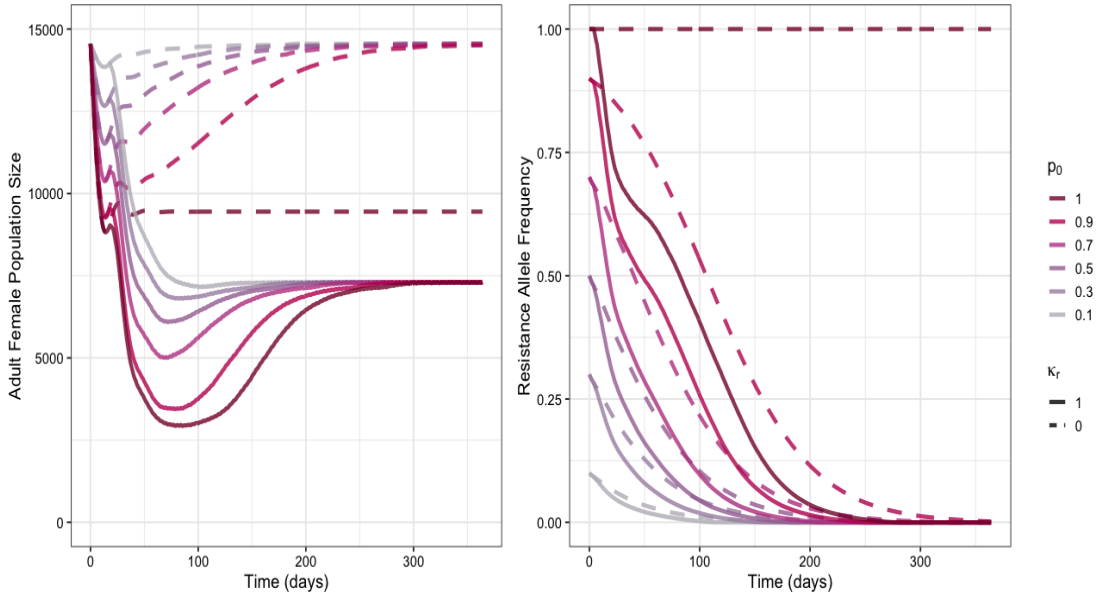


Figure 3.7: The effect of initial resistance frequency on population control and resistance management under trangenic insect release-only strategies. Panel a) shows the adult female population and b) the resistance allele frequency for one year following the onset of release-only management programme in which transgenic males are released biweekly ($\kappa_f = 2$) at a ratio of 1:1 to the pre-intervention equilibrial male density ($\kappa_r = 1$). Solid, coloured lines represent the dynamics observed under release-only management for different initial resistance allele frequencies (p_0). The coloured, two-dash lines show the population and resistance allele dynamics observed in the absence of releases for the equivalent initial resistance allele frequency. The lethal transgene was assumed to be dominant and fully penetrant ($\upsilon = 1$) and imposed a severe, additive ($g_{LL} = 0.5$) fitness cost on the mating competency of adult male bearers ($\sigma^{SSLL} = 0.5$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}. $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, $h_{RR} = 1$, $\upsilon = 1$, $c_{LL} = 0.5$, $g_{LL} = 0.5$.

reduction in time to extinction provided by transgenic releases increasing with initial resistance frequency (Fig. 3.7b, Fig. B.5).

Increasing the initial resistance allele frequency resulted in releases suppressing the adult female population to lower levels in the short term, particularly when $p_0 \geq 0.9$, and increased the time taken for the population size to stabilise, but had no effect on the equilibrial population size (Fig. 3.7a). In the absence of releases, increasing the initial resistance frequency also increased the time taken for the population size to stabilise, but had no effect on the equilibrial population size when $p_0 < 1$. When resistance was initially fixed $p_0 = 1$ and no intervention was

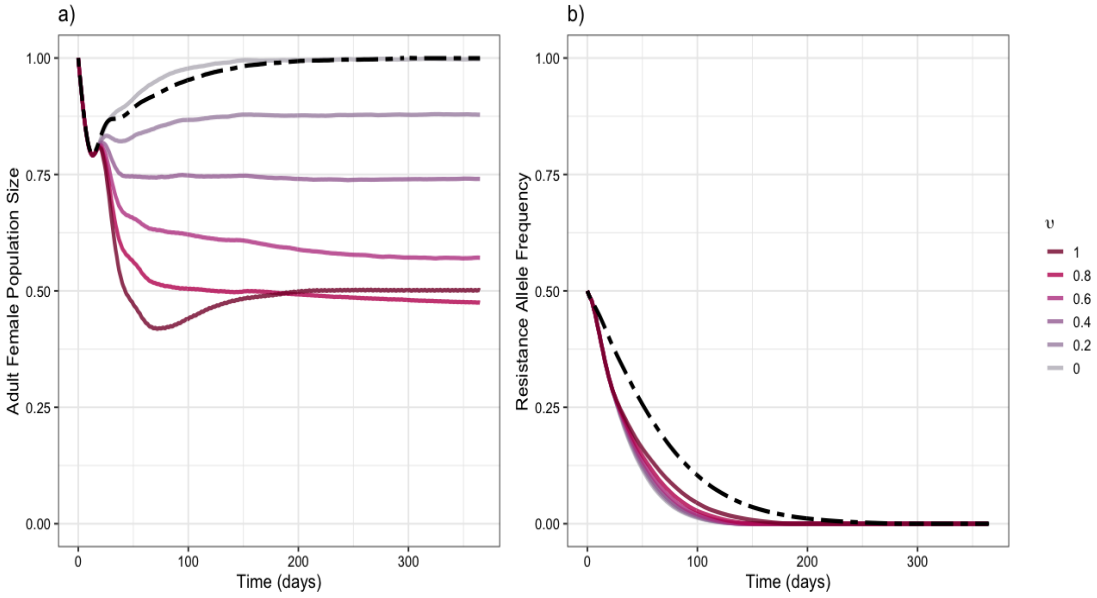


Figure 3.8: The effect of transgene penetrance on population control and resistance management under transgenic insect release-only strategies. Panel a) shows the adult female population and b) the resistance allele frequency for one year following the onset of release-only management programme in which transgenic males were released biweekly ($\kappa_f = 2$) at a ratio of 1:1 to the pre-intervention equilibrial male density ($\kappa_r = 1$). Solid, coloured lines represents the dynamics observed under different assumptions of transgene penetrance (υ). The black, two-dash lines depict the dynamics observed in the absence of releases for contrast. All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}. $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, $h_{RR} = 1$, $\upsilon = 1$, $c_{LL} = 0.5$, $g_{LL} = 0.5$

used, the resistance allele remained fixed and the adult female population size declined to an intermediate equilibria.

Effects of transgene penetrance (υ)

Figure 3.8 shows the effect of transgene penetrance (υ) on the population and resistance allele dynamics under the baseline release-only strategy of biweekly releases ($\kappa_f = 2$) at a ratio of 1:1 ($\kappa_r = 1$). Increasing transgene penetrance improved population control, providing more rapid suppression of the adult female population and stabilising it at a lower equilibria minimised when $\upsilon = 0.8$ (Fig. 3.8a, Fig. B.4a). Increasing transgene penetrance increased the time taken for resistance to be driven to extinction, although the resistance allele was eliminated for all

penetrance values simulated (Fig. 3.8b, Fig. B.4b). Resistance was eliminated most rapidly when $\upsilon = 0$, approximating the release of wildtype males, although this strategy provided no population suppression. Similarly, a no-intervention strategy also resulted in resistance extinction, albeit at a slower rate than observed for any simulated penetrance value, and without the benefits to population suppression (Fig. 3.8, dashed lines).

Discussion

We present an expansion of the stage-, age-, sex-, and genotype-structured demographic and genetic model, developed by Barbosa et al. (2018), capable of simulating anopheline population and resistance management strategies combining or replacing LLIN and IRS deployment with releases of self-limiting transgenic insects. We used the framework to demonstrate the detrimental effects of both the reduction in exposure and the evolution of resistance to insecticides on the continued efficacy of LLIN- or IRS-centred mosquito control strategies (Figs. 3.1-4, Figs B1-2). Releases of genetically modified insects carrying a female-specific, self-limiting transgene provided effective population suppression and resistance management, but large and/or frequent releases were required to eliminate the population in the absence of an insecticidal intervention, particularly if mating competence or transgene penetrance were low (Figs. 3.5-8, Figs. B3-6). Our work has identified scenarios in which reinforcing LLIN/IRS deployment with releases of self-limiting transgenic insects is likely to be beneficial and provided a benchmark against which to assess the efficacy of these combined strategies.

Decreasing the dominance of resistance (h_{RR}) and increasing the dominance of fitness costs (g_{RR}) facilitated the evolution of resistance by reducing the capacity of LLINS/IRS to eliminate heterozygous insects. When resistance was recessive and initially rare, and mating events between resistance homozygotes were therefore rare, the ability of LLINs and IRS to eliminate heterozygotes prevented resistance from evolving. However, as the dominance of resistance increased, resistance was increasingly able to evolve, particularly when the costs of resistance were recessive.

In these scenarios, increasing the coverage only served to increase the pace of resistance evolution. Removing selection for resistance, by removing the LLINs or IRS, allowed the costs associated with resistance to drive it to extinction, but at the cost of population control. One option to manage resistance in this scenario would be to rotate to an alternative insecticide, providing the resistance allele confers no cross resistance to the replacement, if this is logistically feasible. Another option could be to increase the concentration of the insecticide, or enhance its toxicity via synergists such as piperonyl butoxicde (PBO), to attempt to render resistance functionally recessive. If performed with an additional action to temporarily increase the source of susceptible alleles, perhaps by reducing LLIN/IRS coverage, or releasing transgenic insects, this could rapidly restore LLIN/IRS efficacy and re-establish population control.

Unsurprisingly, decreasing the probability of exposure to LLINs (ϕ_{LLIN}) and IRS (ϕ_{IRS}) improved resistance management outcomes, but at the expense of adult female population suppression. As insects avoid insecticides, whether due to low coverage and/or behavioural avoidance, insecticide-induced mortality declines, along with the selection pressure for resistance. Providing the dominance of fitness costs is non-recessive ($g_{RR} \geq 0$), susceptible homozygotes in the untreated population experience greater fitness than resistance carriers in the absence of insecticides due to the cost imposed by the resistance allele on daily survival (c_{RR}). The increased survival of untreated susceptible females relative to resistant individuals enhances the production of susceptible offspring, diluting resistance, but also increasing the population size. Low coverage was particularly problematic when resistance was dominant and the costs recessive. In this scenario, the fitness of heterozygotes in the presence or absence of insecticides is equivalent to the resistant and susceptible homozygotes respectively. These genetic "all-rounders" are thus selected for even under low insecticide exposure probabilities. If resistance is recessive and rare, increasing the coverage of LLINs and IRS is likely to be the best strategy for enhancing control. However, if resistance is widespread and/or non-recessive, integrating additional control tools may be required to re-establish control.

Decreasing the fitness cost associated with resistance (c_{RR}) increased the propensity for resistance to evolve and, therefore, for the suppressive effect of the insecticide to be diminished, particularly when this cost was experienced by heterozygotes ($g_{RR} > 0$) (Figs. 3.3-4). This was most notable when resistance was initially widespread ($p_0 = 0.5$); increasing the cost of resistance from 5% to 10% greatly contracted the number of scenarios in which resistance was retained in the population (Fig. 3.3g-h, Fig. 3.4g-h), reversing the fate of the resistance allele from fixation to extinction for recessive resistance ($h_{RR} = 0$) with non-recessive costs ($g_{RR} \geq 0$). This is consistent with previous work that demonstrated that fitness costs associated with resistance, along with the dominance of resistance and its fitness cost, shape the selection thresholds that govern the equilibrial resistance allele frequency (Alphey et al., 2008). In the presence of an insecticide, the selective pressure imposed must overcome the cost(s) associated with the resistance allele for resistance to confer sufficient advantage to drive its evolution, with this selective threshold increasing in tandem with the fitness cost of resistance. When the selective pressure is removed, the cost associated with resistance quickly selects against the resistance allele, which decays to extinction.

We opted to impose the fitness cost associated with resistance on the daily survival probability across the entire mosquito lifespan, but resistance costs can manifest themselves at different times in the life-cycle and on a range of ecological traits, including prolonged development time, reduced fecundity, impaired dispersal ability, and reduced over-wintering survival (Kliot & Ghanim, 2012). It is well established that increasing the cost of resistance can delay the evolution of resistance (Alphey et al., 2008). However, though the type of fitness cost assumed can alter the optimal strategy for managing pest insects and their resistance to insecticides (Hackett & Bonsall, 2016), this effect of the implementation of fitness costs is less well characterised. Given the inherent variation in ways in which fitness costs are realised, understanding how and where different types of fitness cost are likely to require alternative management approaches is another important objective for integrated population and resistance management.

LLINs generally provided superior population suppression to IRS, but also selected for higher resistance allele frequencies when resistance evolved. However, we limited the focus of our simulations to the effects of the resistance and exposure probability parameters and fixed those that governed the gonotrophic cycle, whereas the relative efficacy of LLINs and IRS is likely to depend on the average time spent in each gonotrophic cycle phase and the insecticide exposure and survival probabilities. Indeed, while our results are consistent with those of Barbosa et al. (2018), White et al. (2010) found that IRS provided significantly better suppression than LLINs under equivalent coverage. An analysis of the gonotrophic cycle parameters (cycle duration (τ), feeding success rate (H^j), phase-specific daily survival probabilities (ρ_s/ρ_r) and insecticide-survival probabilities (μ_{LLIN}/μ_{LLIN}), which govern the duration and severity of the selection pressure experienced in each phase, would be required to better understand the relative efficacies of LLIN-only and IRS-only strategies.

Increasing the frequency and size of releases of female-selecting, self-limiting releases reduced the equilibrial adult female population size (Fig. 3.5) and increased the rate at which the resistance allele was driven to extinction in the absence of selection (Fig. B3). Release strategies that equalled or surpassed releases of 20:1 transgenic males to wildtype males per week eliminated the pest population. As the number of insects released increases, the proportion of wild females mated to transgenic insects rises, increasing the proportion of total progeny that inherit the allele and therefore that are eliminated. Previous work modelling bisex-lethal releases found that smaller, more frequent releases provide better suppression than larger, less frequent releases (White et al., 2010). This was not immediately evident from our analyses (Fig. 3.5), but we confirmed this in separate simulations (Fig. B13). In the absence of a selective pressure for resistance, releases were able to drive fixed resistance to extinction. Given reports of widespread resistance across sub-Saharan Africa (Hancock et al., 2020), understanding the extent to which this can be replicated when insecticides are also present is important. Reducing the mating competence of released males or penetrance of the transgene both

decreased the efficacy of releases (Fig. 3.6, Fig. 3.8). The former reduces the proportion of females that mate with transgenic males for a given release strategy, and so reduces the suppression achieved. The latter reduces the proportion of larval female transgene bearers that are terminated by the allele, which also reduces the mortality imposed by the transgene.

Reducing the daily survival probabilities of each life-cycle stage improved the level of suppression achieved by the default insecticide-only ($\phi_i = 0.8$) and release only ($\kappa_f = 2, \kappa_r = 1$) strategies (Appendix B.4). Population dynamic outcomes were particularly sensitive to the larval survival probability (ρ_l), with reductions of $\geq 20\%$ resulting in extinction for each default intervention. We parameterised the model using, where available, estimates from laboratory studies of *Anopheles stephensi*. These values may, therefore, represent the upper limits of what a species can achieve, rather than that which they commonly achieve in natural settings, suggesting the control outcomes achieved in our simulations may be somewhat conservative. This also suggests, in common with previous work (Barbosa et al., 2018; White et al., 2010), that strategies would benefit greatly from integrating additional interventions that enhance larval mortality, such as larvicides or larval source management.

The results from our LLIN-only and IRS-only simulations are consistent with predictions from high-dose/refuge theory, which has formed the backbone of strategies that have been effective in maintaining susceptibility to Bt crops in agricultural settings (Tabashnik et al., 2013). This is unsurprising, as reducing the exposure probability below 100% creates an untreated population that, in the presence of a cost to resistance, serves as an effective refuge population. The size and, therefore, efficacy of this refuge population for resistance management increases as the exposure probability declines. Consistent with theory, this refuge provided the most effective population and resistance management outcomes when resistance was initially rare, recessive, and with dominant fitness costs. In the context of public health, willingly restricting access to interventions to create a refuge is unacceptable. However, understanding the effects of incidental refuges created by low coverage, behavioural avoidance, or, for zoophilic species, the presence of

unprotected non-human hosts (e.g. cattle), may help guide and enhance resistance management strategies. In our case, it highlighted scenarios in which LLIN-only and IRS-only strategies are likely to be overwhelmed and require the integration of additional control tools to retain control.

As the population genetic model was extended to track two independent loci, this framework can now also simulate the WHO recommended resistance management strategies that deploy two insecticides in sequences, mosaics, or combinations (World Health Organisation, 2012). Though the relative efficacy of different insecticide-based strategies for managing resistance, and their sensitivity to common resistance parameters, have been well characterised elsewhere (South & Hastings, 2018), a comparison of these strategies with those that deploy novel resistance management tools, such as self-limiting releases, would be of great interest, particularly if the costs associated with each strategy are also compared. Extending the population genetics to track a third locus would allow investigations of strategies that integrate releases with sequences, combinations, or mosaics, but at the cost of greatly increased model complexity.

The highly-structured and modular nature of this framework facilitate its adaptation to simulate alternative control measures in a way that captures both the genotypes and life-history stage(s) they target and any delay in their effect. Barbosa et al. (2018) showed that targeting the larval stages with insecticides provided the greatest suppression. If sufficient coverage with larvicides is achieved, which can be challenging when water sources are locally abundant, the reduced capacity for mosquitoes to exhibit behavioural avoidance to these interventions may recommend them as an effective supplement for controlling species that exhibit avoidance to LLINs and/or IRS (Derua et al., 2019; Killeen et al., 2002). These could easily be simulated using our framework by applying insecticide-induced mortality to the larval/pupal stages using the same approach used for the LLINs/IRS. The transgene component could also be modified to simulate alternative self-limiting strategies. For instance, late-acting, bisex-lethal releases have been demonstrated to provide superior suppression to female-specific phenotypes (Gentile et al., 2015),

and dampen the outbreaks of pests which exhibit cyclical population dynamics (White et al., 2010). Finally, self-sustaining approaches that bias gene transmission to achieve super-Mendelian inheritance are receiving significant attention for their potential as tools for both vector suppression (Eckhoff et al., 2017; Gantz et al., 2015) and resistance management (Collins, 2018). These could also be simulated using this framework by adapting the equations governing offspring production (Appendix B.1-2).

In light of the threats posed to the continued efficacy of vector management strategies centred on the deployment of LLINs and IRS by reduced coverage and bioefficacy, behavioural avoidance, and the evolution of insecticide resistance, prudence dictates that vector population and resistance management strategies that reinforce or replace insecticidal interventions with novel control tools be established. We developed a modelling framework capable of simulating mosquito population and resistance management strategies that can deploy LLINs, IRS and releases of self-limiting insects and used it to demonstrate that insecticide-only control can be compromised by the evolution of resistance when exposure probability is high and resistance is widespread and recessive, or resistance is dominant and the fitness costs of resistance are recessive. We also show that low exposure probability, as a proxy for limited coverage and/or behavioural avoidance, can also undermine population control. Finally, we show that self-limiting, female-specific releases could suppress or eliminate a highly-structured anopheline population with overlapping generations and, in the absence of insecticides, drive resistance to extinction.

4

Integrating transgenic releases with bed-nets and indoor spraying for mosquito population and resistance management

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Abstract

Background: The evolution of insecticide resistance, reduced bioefficacy, and behavioural avoidance all threaten the continued efficacy of anopheline control programmes centred on the deployment of insecticidal nets (LLIN) and indoor sprays (IRS). Reinforcing the deployment of LLINs/IRS with releases of self-limiting insects may overcome these threats by diluting insecticide resistance and providing an additional, active form of suppression.

Methods: Using a stage-, age-, sex-, and genotype structured model of an anopheline population, we investigated the capacity for releases to manage insecticide resistance under conditions which favour its evolution under insecticide-only control, and assessed the capacity for releases to compensate for reductions in insecticide exposure and efficacy.

Results: Reinforcing LLIN or IRS deployment with self-limiting releases maintained the population and resistance frequency at lower equilibria or drove them to extinction. When resistance was recessive, releases acted synergistically with the insecticidal intervention to dilute resistance and enhance suppression. Combined strategies were less effective as the dominance of resistance increased, but still enhanced population and resistance management relative to insecticide-only control. Combined strategies were more effective when the insecticide exposure probability and efficacy against susceptible insects were high, but could also compensate for reductions in LLIN/IRS exposure and efficacy.

Conclusions: This study is the first to investigate the insecticide resistance management potential of self-limiting releases in the context of vector control. Our simulations illustrate that combining the deployment of insecticidal-interventions with self-limiting releases has the potential to manage insecticide resistance and compensate for reduced bioefficacy and behavioural avoidance. Further work is required to determine how robust these strategies are to seasonal drivers of mosquito abundance and whether they are economically viable.

keywords: Mosquito, Insecticide, Resistance, Genetics, Modelling.

Introduction

Long-lasting insecticidal nets (LLINs) and indoor residual sprays (IRS) form the core of strategies to control anopheline vectors of malaria (World Health Organisation, 2019a). These insecticidal interventions, designed to exploit the indoor feeding (endophagic) and resting (endophilic) preferences characteristic of adult females of key *Anopheles* vectors, are estimated to have averted over 500 million cases of malaria between 2000-2015 (Bhatt et al., 2015). However, there is growing concern that the continued efficacy of these important tools may be threatened by insecticide resistance (Hemingway et al., 2016; World Health Organisation, 2012), behavioural avoidance (Pates & Curtis, 2005; Sanou et al., 2021), and reductions in bioefficacy and longevity (Lindsay et al., 2021).

The rapid and widespread increase in the use of LLINs and IRS, coupled with an over-reliance on a limited number of recommended insecticides, has predictably driven the evolution of insecticide resistance over large geographic areas in a short space of time (Hancock et al., 2020; Moyes et al., 2020). Monitoring efforts have identified resistance across all but the European WHO region, to all four of the main insecticide classes, and in all of the most problematic mosquito vectors (World Health Organization, 2020). There is concern that the recent stagnation of malaria control in Africa may be a result of the observed reduction in sensitivity to LLINs and IRS (Hemingway et al., 2016). The evidence explicitly linking the evolution of insecticide resistance to epidemiological control failures is currently limited (Kleinschmidt et al., 2018), with some citing insufficient coverage and low bioefficacy against susceptible insects resulting from inadequate funding as more likely causes (Lindsay et al., 2021). Nonetheless, in recognition of the urgent need to manage the spread of resistance, the WHO established a global plan for insecticide resistance management in malaria vectors (GPIRM), although the uptake of measures outlined in the GPIRM has been slow (Mnzava et al., 2015).

Resistance strategies recommended within the GPIRM centre on the deployment of multiple insecticides with non-overlapping modes of action in mixtures, temporal sequences, spatial mosaics, or combinations of insecticidal interventions with

different chemistries (e.g. pyrethroid LLINs with non-pyrethroid IRS) (World Health Organisation, 2012). These strategies are designed to increase sources of intra- and/or inter-generational mortality and prevent the build-up of resistance by ensuring that insect's resistance to one insecticide are quickly eliminated by another (REX Consortium, 2013). While guided by established evolutionary principles (Levick et al., 2017; South & Hastings, 2018), these strategies can be difficult to implement in practice, often exceeding the financial capacity of the authorities tasked with implementing them, and requiring an array of compatible insecticide chemistries that are in practice often unavailable (Mnzava et al., 2015). Multiply resistant species, so far detected in 45 countries (World Health Organization, 2020), also render these strategies unfeasible in some places. While these strategies should become more viable as the number and availability of insecticides expands, it would be prudent to develop novel, non-chemical alternatives that can be used in place of, or alongside, insecticides to manage the evolution of resistance and enhance suppression.

The continued efficacy of LLIN- and IRS-based vector control strategies may also be threatened by natural or learned behavioural avoidance (Gatton et al., 2013; Pates & Curtis, 2005). Insecticide avoidance can result from within-species adaptive shifts in mosquito behaviour away from the endophagy and endophily that promote their exposure to LLINs and IRS. A recent study of vector host choice and resting behaviour conducted over four years in Tanzania detected behavioural changes in populations of both *Anopheles funestus* and *Anopheles arabiensis* toward cattle feeding (zoophagy) and outdoor resting (exophily) (Kreppel et al., 2020). Shifts towards early-morning and outdoor feeding in *Anopheles funestus* were also observed over a three year period following the implementation of universal LLIN coverage in two Beninese villages (Moiroux et al., 2012). Changes in the composition of vector communities can also reduce the efficacy of LLINs and IRS if species whose feeding and resting behaviour naturally limit their exposure become more prevalent. In Burkina Faso, Sanou et al. (2021) linked a reduction in bites preventable by LLINs to changes in the species composition of vector communities within two years of the mass distribution of LLINs. If behavioural resistance undermines LLIN/IRS-based

control, introducing additional insecticidal interventions that can target exophillic species, such as outdoor sprays or the larviciding of breeding sites, will be required to maintain control. However, if the observed trends towards both reduced exposure due to avoidance and declining sensitivity caused by resistance continue, integrating additional, non-chemical control measures may become necessary.

An emerging alternative to insecticide-based suppression is the release of genetically modified insects bearing a late-acting, female-selecting, lethal transgene. Initially designed as a species-specific, environmentally-benign population suppression tool, non-biting male insects, homozygous for the lethal construct, are released into a target population to compete with wildtype males for mating opportunities. In the absence of a dietary repressor, the lethal transgene terminates female offspring resulting from these matings during late-larval development, enabling male carriers to survive and further propagate the transgene. The elimination of females erodes the reproductive capacity of the target population and, if releases are sustained over time, can achieve rapid suppression (Thomas et al., 2000). Furthermore, as self-limiting releases rely on the highly-evolved mate-seeking behaviour of the target species to actively deliver the lethal transgene into the population, they may serve to reinforce insecticidal interventions against populations exhibiting behavioural avoidance of LLINs and IRS. The capacity for releases to control insect populations has been demonstrated using theoretical modelling (Alphey, 2014; Gentile et al., 2015; White et al., 2011; White et al., 2010), laboratory assays (Harvey-Samuel et al., 2015; Leftwich et al., 2014) and field trials (Carvalho et al., 2015; Harris et al., 2012).

Female-specific self-limiting releases may also have utility as a tool for insecticide resistance management by introgressing insecticide-susceptibility alleles into the target population though the viable male offspring (Alphey et al., 2007). Theoretical and empirical work has demonstrated the capacity for self-limiting releases to slow or reverse the evolution of insecticide resistance in the context of agricultural pest management, particularly when used in combination with insecticide-free refuges (Alphey et al., 2009; Alphey et al., 2007; Zhou et al., 2018; Zhou et al., 2019). However, the use of releases for managing resistance in mosquitoes

has yet to be explored, and findings from agriculturally-focused studies are not necessarily generalisable. Agricultural insecticides target the larval stages of both sexes responsible for reductions in crop yields, whereas vector control programmes seek to minimise the adult female population whose bites are responsible for disease transmission. The reduced exposure of transgenic males to insecticides may therefore increase their potential to introgress susceptibility alleles and, therefore, the efficiency of releases. Furthermore, as mosquito larvae are not in themselves problematic, transgenes with a late-acting phenotype can be deployed which eliminate female carriers late during larval development to maximise the mortality imposed by density-dependent regulation.

In Chapter 3, we extended a population demographic and genetic model, originally developed by Barbosa et al. (2018), to model two genetic loci and facilitate investigations of integrated mosquito population and resistance management programmes that reinforce LLIN or IRS deployment with transgenic insect releases. The population genetics framework was also elaborated to capture the effects of resistance dominance, costs of resistance, and the dominance of resistance costs on resistance evolution and, therefore, population management. Using this framework, we demonstrated that resistance evolves quickly to undermine population control when the probability of exposure to LLINs or IRS is high and resistance is recessive and initially widespread, or resistance is dominant. We also showed that reducing the probability of exposure, which could result from low coverage and/or behavioural avoidance, greatly reduced the efficacy of insecticide-only control. Finally, we demonstrated that, when used in isolation, pulsed releases of female-specific, self-limiting males could suppress or eliminate an anopheline mosquito population, but their efficacy rapidly diminished if mating-competence or transgene penetrance were reduced.

Here, we build on this work to determine the extent to which releases of self-limiting insects can used in combination with LLINs or IRS to slow or reverse the evolution of resistance and compensate for reductions in insecticide bioefficacy and exposure probability. Specifically, we address the following questions:

1. Can reinforcing insecticides with transgenic insect releases slow or reverse the evolution of initially widespread, recessive resistance?

2. Can reinforcing insecticides with transgenic insect releases prevent, or slow, the evolution of dominant resistance?

3. To what extent does reducing the fitness cost of resistance impede the ability of releases to slow its evolution?

4. Can releases compensate for reductions in insecticide exposure probability and efficacy?

Methods

We adapted the demographic/genetic model described by (Barbosa et al., 2018) to investigate the effectiveness of mosquito population and resistance management strategies that reinforce LLIN/IRS deployment with genetic control under a range of control scenarios. This framework is a system of deterministic, discrete-time difference equations that capture an anopheline mosquito population with overlapping generations structured by age, stage, sex and genotype. The model is iterated through discrete, single-day time steps to capture daily changes in the number of individuals in each class. Though it yields a bulky system of equations, this model more precisely captures the stage-, sex- and genotype-specific nature of the mortality, and therefore selection pressure, associated with both the insecticidal and genetic interventions, as well as the potential costs to vital rate parameters experienced by insecticide-resistant or transgenic insects. The demographic nature of the model also allows the delayed effects of transgenic insect releases to be explicitly modelled. For reference, the ecological and operational parameters are summarised in Tables 4.1 and 4.2 respectively.

Table 4.1: Summary of the insect model parameters.

Symbol	Description	Value(s)
Indices		
a	Age of the individual	1:ζ
i	Insecticide Treatment	LLIN/IRS
j	Genotype	
k	Genotype of adult females' male mate	
p	Phase of the gonotrophic cycle	1:τ
s	Sex	f, m
t	time step (days)	
Juvenile Stages		
θ_e	Duration of egg stage (days)	2
θ_l	Duration of larval stage (days)	10
θ_p	Duration of pupal stage (days)	2
ζ	Total duration of juvenile stages (days)	14
ρ_e^j	Proportion of eggs of genotype j that survive per day	0.895
ρ_l^j	Proportion of larva of genotype j that survive per day	0.972
ρ_p^j	Proportion of pupa of genotype j that survive per day	0.975
α	Strength of density dependence	1×10^{-6}
β	Density-dependence shaping parameter	1
Adult Stages		
τ	Duration of the gonotrophic cycle (days)	3
ρ_m^j	Proportion of adult males of genotype j that survive per day	0.9
ρ_s^j	Proportion of host-seeking adult females of genotype j that survive per day	-
ρ_r^j	Proportion of resting adult females of genotype j that survive per day	0.9
σ^j	Mating competency of adult males of genotype j	1
H	Daily probability that a host-seeking female obtains a blood meal	0.67
ϵ^j	Number of eggs of of genotype j laid per female per gonotrophic cycle	100
γ	Sex ratio of eggs	0.5

Juvenile stages

Anopheline mosquitoes exhibit an aquatic juvenile phase in which eggs, oviposited into water by adult females, hatch and develop through a series of four larval instars

before pupating. We simplify this aquatic juvenile life cycle into egg, larval and pupal stages. The juvenile classes are represented by the notation $J_a^{sj}(t)$, which represents the number of juvenile individuals of sex s, genotype j, and age a, alive at the end of day t. The juvenile life cycle has a duration of ζ days, where $\zeta = \theta_e + \theta_l + \theta_p$, and θ_e, θ_l, and θ_p represent the length, in days, of the egg, larval and pupal stages respectively. A juvenile individual's lifecycle stage is therefore given by its age.

The number of newly oviposited eggs of sex s and genotype j that survive to the end of their first day ($a = 1$) on day t is given by:

$$J_1^{sj}(t) = \gamma \Lambda^j(t-1)\rho_e^j \tag{4.1}$$

where $\Lambda^j(t-1)$ is total number of eggs of genotype j oviposited at the end of the previous day, ρ_e^j is the daily survival proportion of eggs of genotype j, and γ is the egg sex ratio. The progression through the remaining $\theta_e - 1$ days in the egg stage is then given by:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\rho_e^j \tag{4.2}$$

for $2 \leq a \leq \theta_e$. Eggs hatch into larvae, which are assumed to be subject to density-dependent regulation for the duration of the larval stage. Density-dependence was implemented using the flexible two-parameter form described by (Maynard Smith & Slatkin, 1973) in which the strength and functional form are governed by a scaling constant α and shaping parameter β. Progression through the larval stages is described by:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^\beta}\right)\rho_l^j \tag{4.3}$$

for $(\theta_e + 1) \leq a \leq (\theta_e + \theta_l)$, where ρ_l^j is the daily, density-independent daily larval survival probability and $L(t-1)$ is the total larval density at time $t-1$. Larva then pupate and progress through the pupal stage for θ_p days:

$$J_a^{sj}(t) = J_{a-1}^{sj}(t-1)\rho_p^j \tag{4.4}$$

for $(\theta_e + \theta_l + 1) \leq a \leq \zeta$, where ρ_p^j is the daily pupal survival probability. We assume, for simplicity, that pupae are not subject to density-dependent regulation. Individuals that survive to the end of the pupal phase eclose into adult mosquitoes.

Adult life stages

Adult female dynamics

To enable the differential effects of LLINs and IRS, which target adult females at different phases of the gonotrophic cycle, to be captured, the adult female stage is divided into three behavioural classes that indicate their current phase in the gonotrophic cycle (p): host-seeking and feeding ($p = 1$); resting and egg maturation ($2 \leq p \leq (\tau - 1)$); and oviposition ($p = \tau$). The average duration (days) of a single gonotrophic cycle is given by τ and, after ovipositing and completing a cycle, females start another the following day. The female population compartments are denoted $A_p^{fjk}(t)$, which represents the number of adult females of genotype j in the p-th phase of the gonotrophic cycle that mated with males of genotype k alive at the end of day t.

Mating

For simplicity, adult females are assumed to eclose and mate immediately and singly, storing sufficient sperm to produce offspring for the duration of their lives. Females mate with males of each genotype with a probability M^j, which is a function of the relative frequency of the genotype in the adult male population and the mating competency of adult males of that genotype σ^j:

$$M^j(t) = \frac{\sigma^j(J_\zeta^{mj}(t-1) + A^{mj}(t-1))\rho_m^j}{\sum_j \sigma^j(J_\zeta^{mj}(t-1) + A^{mj}(t-1))\rho_m^j}. \tag{4.5}$$

Gonotrophic cycle: host-seeking and feeding phase

In the first phase of the gonotrophic cycle ($p = 1$), adult females seek a host and attempt to obtain a blood meal, which they achieve with daily probability H. Females enter the host-seeking phase following eclosion, if they failed to obtain a blood meal the previous day $(1 - H)$, or if they finished a previous cycle the

day before, and survive each day with probability ρ_s^j. To capture the effects of LLIN-induced mortality, the host-seeking adult female population is subdivided into unexposed (A_1^{fjk}) and exposed ($\hat{A}_1^{fjk}$) classes:

$$A_1^{fjk}(t) = \Bigg(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg) \rho_s^j (1-\phi_{LLIN}) \tag{4.6}$$

$$\hat{A}_1^{fjk}(t) = \Bigg(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg) \rho_s^j \phi_{LLIN} \mu_{LLIN}^j \tag{4.7}$$

where ϕ_{LLIN} represents the probability of exposure to an LLIN and μ_{LLIN}^j is the genotype-dependent probability of surviving exposure to an LLIN.

Gonotrophic cycle: resting and oviposition

Host-seeking females progress to the resting phase if they successfully obtained a blood meal (H) and survived the previous day. Indoor residual sprays target adult female mosquitoes resting on surfaces and thus IRS-induced mortality is applied to adult females during the resting phase of the gonotrophic cycle ($2 \leq p \leq \tau$). Assuming females rest on IRS treated surfaces with probability ϕ_{IRS}, progression from the host-seeking to the resting phase is divided into unexposed and exposed classes:

$$A_2^{fjk}(t) = \left(A_1^{fjk}(t-1) + \hat{A}_1^{fjk}(t-1)\right) H^j \rho_r^j (1-\phi_{IRS}) \tag{4.8}$$

$$\hat{A}_2^{fjk}(t) = \left(A_1^{fjk}(t-1) + \hat{A}_1^{fjk}(t-1)\right) H^j \rho_r^j \phi_{IRS} \mu_{IRS}^j \tag{4.9}$$

where $\hat{A}_2^{jfk}(t)$ and $A_2^{jfk}(t)$ represent the number of newly resting females in exposed and unexposed populations, ρ_r^j, is the daily probability of survival while resting, and μ_{IRS}^j is the genotype-dependent probability of surviving IRS exposure. In

practice, we did not model strategies combining LLINs and IRS, so equations 8 and 9 simplified to:

$$A_2^{fjk}(t) = A_1^{fjk}(t-1)H^j\rho_r^j(1-\phi_{IRS}) \tag{4.10}$$

$$\hat{A}_2^{fjk}(t) = A_1^{fjk}(t-1)H^j\rho_r^j\phi_{IRS}\mu_{IRS}^j \tag{4.11}$$

when simulating strategies involving IRS deployment. Mosquitoes exposed while resting remain exposed for the duration of the phase (no transition between the exposed and unexposed states):

$$A_p^{fjk}(t) = A_{p-1}^{fjk}(t-1)\rho_r^j. \tag{4.12}$$

$$\hat{A}_p^{fjk}(t) = \hat{A}_{p-1}^{fjk}(t-1)\rho_r^j\mu_{IRS}^j \tag{4.13}$$

for $2 \leq p \leq \tau$. At the end of the gonotrophic cycle females cease resting and seek a suitable water source into which they each oviposit ϵ^j eggs. The number of eggs off each genotype laid in each time step depends on the genotypic composition of the population of females in the egg-laying phase at time t, and the equations governing offspring genotype proportions and the number of eggs of each genotype laid per day ($\Lambda^j(t)$) are provided in Appendix B.1 and B.2. We assumed that ovipositing females from exposed and unexposed areas use oviposition sites with uniform probability and, therefore, the eggs laid by females in treated and untreated populations were, in practice, combined into a single first-day egg class:

$$J_1^{sj}(t) = \gamma(\Lambda^j(t-1) + \hat{\Lambda}^j(t-1))\rho_e^j \tag{4.14}$$

where $\Lambda^j(t-1)$ and $\hat{\Lambda}^j(t-1)$ represent the number of eggs of each genotype laid by ovipositing females in the unexposed and exposed classes respectively. Following oviposition, surviving adult females start a new gonotrophic cycle the following day:

$$A_1^{fjk}(t) = \Bigg(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1) + \hat{A}_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg) \rho_s^j (1-\phi_{LLIN}) \tag{4.15}$$

$$\hat{A}_1^{fjk}(t) = \Bigg(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{\Big[\hat{A}_1^{fjk}(t-1) + A_1^{fjk}(t-1)\Big](1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1) + \hat{A}_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \Bigg) \rho_s^j \phi_{LLIN} \mu_{LLIN}^j \tag{4.16}$$

However, as we did not model strategies combining LLIN and IRS deployment, females beginning new gonotrophic cycles in simulations involving IRS were pooled into a single, untreated host-seeking class:

$$A_1^{fjk}(t) = \left(\underbrace{J_\zeta^{fj}(t-1)M^j(t)}_{New\ Emergers} + \underbrace{A_1^{fjk}(t-1)(1-H^j)}_{Repeat\ Feeders} + \underbrace{A_\tau^{fjk}(t-1) + \hat{A}_\tau^{fjk}(t-1)}_{Repeat\ Cyclers} \right) \rho_s^j \tag{4.17}$$

Adult male dynamics

Anopheline adult males do not seek blood meals and are therefore assumed to avoid exposure to LLINs and IRS. Instead, it is assumed that they spend the majority of their lives seeking mating opportunities. Their dynamics therefore simplify to:

$$A^{mj}(t) = \Big(J_\zeta^{mj}(t-1) + A^{mj}(t-1) \Big) \rho_m^j \tag{4.18}$$

which represents the number of newly emerged males, $J_\zeta^{mj}(t-1)$, and males surviving from the previous day, $A^{mj}(t-1)$, that survive the current day with probability ρ_m^j. For simplicity, we assume no effect of age on survival or mating probability and, therefore, only segregate the adult male classes by genotype.

Releases of self-limiting insects

We simulated pulsed releases of $\kappa_r A^{m*}$ males with a per-week frequency κ_f, where A^{m*} is the pre-intervention equilibrial adult male population size and κ_r is the ratio of released males to wildtype males. Transgenic males ($j = SSLL$) are released directly into the adult male population class:

$$A^{mSSLL}(t) = (\underbrace{J_{\zeta}^{mj}(t-1)}_{New-emergers} + \underbrace{A^{mSSLL}(t-1)}_{SurvivingMales} + \underbrace{\kappa_r A^{m*}}_{Releases})\rho_m^{SSLL} \tag{4.19}$$

We modelled a transgene construct with a dominant, late-acting, female-specific action that terminates both wL and LL female larvae on the final day of larval development ($a = \theta_l$), but has no effect on male carriers:

$$J_{\theta_l}^{f--wL}(t) = J_{\theta_l-1}^{f--wL}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^{\beta}}\right)\rho_l^{f--wL}\mu_{LL} \tag{4.20}$$

$$J_{\theta_l}^{f--LL}(t) = J_{\theta_l-1}^{f--LL}(t-1)\left(\frac{1}{1+(\alpha L(t-1))^{\beta}}\right)\rho_l^{f--LL}\mu_{LL} \tag{4.21}$$

where μ_{LL} is the probability of surviving the transgene-induced mortality. The lethal phenotype of the transgene is assumed to be completely dominant and fully penetrant, such that no female carriers survive to pupate ($\mu_{LL} = 0$).

Population genetics

Dominance of genetic resistance

The resistance allele increases the probability that bearers survive exposure to insecticides such that $\mu_i^{RR--} \geq \mu_i^{RS--} \geq \mu_i^{SS--}$. The probability that resistant heterozygotes survive exposure is determined by the dominance of resistance, h_{RR}:

$$\mu_i^{RS--} = (1 - h_{RR--})\mu_i^{SS--} + h_{RR}\mu_i^{RR--} \tag{4.22}$$

where $h_{RR} = 1$ represents dominant resistance ($\mu_i^{RS--} = \mu_i^{RR--}$), and $h_{RR} = 0$ recessive resistance ($\mu_i^{RS--} = \mu_i^{SS--}$).

Costs of genetic resistance

The resistance allele is assumed to impose an energetic fitness cost, c_{RR}, to carriers relative to homozygous wildtype individuals ($j = SSww$). Though this fitness penalty has been linked to changes in a number of key biological traits, including developmental rate, size, and fecundity, for simplicity we only impose it on the daily survival probability:

$$\rho_s^{RR--} = (1 - c_{RR})\rho_s^{SS--} \tag{4.23}$$

The degree to which heterozygotes experience the fitness costs associated with the resistance allele is determined by the dominance of costs of resistance, g_{RR}:

$$\rho_s^{RS--} = (1 - g_{RR})\rho_s^{SS--} + g_{RR}\rho_s^{RR--} \tag{4.24}$$

Table 4.2: Summary of the intervention parameters and their default values.

Symbol	Description	Base Value(s)
Insecticides		
ϕ_i	Probability of exposure to LLIN/IRS	0.8
μ_i^{SS--}	Proportion of homozygous susceptible individuals that survive exposure to insecticide intervention i	0.01
μ_i^{RR--}	Proportion of homozygous resistant individuals that survive exposure to insecticide intervention i	0.9
c_{RR}	Fitness cost of carrying two copies of resistance allele	0.05
g_{RR}	Dominance of costs of resistance	0, 1
h_{RR}	Dominance of resistance	0, 1
p_0	Initial resistance frequency	0.01, 0.5
Releases		
υ	Penetrance of the lethal transgene	1
μ_L	Probability of surviving the lethal transgene phenotype	0
κ_r	Release ratio of transgenic males	1
κ_f	Frequency of releases (per week) of transgenic males	2
c_{LL}	Fitness cost of carrying two copies of the transgene construct	0.5
g_{LL}	Dominance of costs of the transgene construct	0.5

where $g_{RR} = 1$ represents dominant costs ($\rho_s^{RS--} = \rho_s^{RR--}$) and $g_{RR} = 0$ represents recessive costs ($\rho_s^{RS--} = \rho_s^{SS--}$). We assume that the lethal allele does not affect daily survival beyond the effect intended by the transgene construct, such that the values for wL and LL genotypes are given by their wildtype ($j = --ww$) equivalents (e.g. $\rho_s^{SSww} = \rho_s^{SSwL} = \rho_s^{SSLL}$).

Fitness costs of the lethal transgene

Transgenic males reportedly display a reduced ability to obtain a mate. We therefore imposed a fitness cost, c_{LL}, with a dominance g_{LL}, on the mating competency (σ^j) of adult male carriers of the transgene, where the mating competency of transgenic homozygotes is calculated as:

$$\sigma^{--LL} = (1 - c_{LL})\sigma^{--ww} \tag{4.25}$$

and the mating competency of insects bearing a single copy of the transgene is:

$$\sigma^{--wL} = (1 - g_{LL})\sigma^{--ww} + g_{LL}\sigma^{--LL}. \tag{4.26}$$

It is assumed that the genotype at the resistance loci imparts no effect on mating competency.

Simulations

The model was used to simulate the control of an anopheline population and its resistance to insecticides under management strategies that combine self-limiting releases with LLINs or IRS deployment. We simulated a series of scenarios to assess the efficacy of integrated management strategies under the following, challenging conditions:

1. widespread ($p_0 = 0.5$) , recessive resistance ($h = 0$)
2. dominant resistance ($h_{RR} = 0$) with recessive costs ($g_{RR} = 0$)
3. lowered costs of resistance (c_{RR})

4. reduced probability of exposure (ϕ_{LLIN}/ϕ_{IRS}) and/or efficacy against susceptible insects (μ_{SSww}) of LLINs/IRS

The population model was parameterised using point estimates for ecological parameters from the literature for, where possible, *Anopheles stephensi*, which currently poses a significant management challenge in east Africa (Sinka et al., 2020). In each simulation, a mosquito population was initiated by introducing 500 individuals to each homozygous wildtype class (j = SSww) and running the model for a 2-year burn-in period in the absence of a control intervention to generate a pre-intervention, baseline population. Resistance was then introduced at an initial frequency p_0 dependent on the scenario being simulated, with the genotype frequencies in Hardy-Weinberg equilibrium and the population naive to the lethal transgene. We modelled integer values for mosquito densities by removing fractional values for population sizes using the floor() function, avoiding the issue of defining a population extinction cut-off. All simulations were performed using R version 4.0.2 (R Core Team, 2020).

Results

Reinforcing LLIN/IRS deployment with self-limiting releases slows or reverses the evolution of widespread, recessive resistance

Supplementing widespread LLIN ($\phi_{LLIN} = 0.8$, Fig. 4.1) or IRS ($\phi_{IRS} = 0.8$, Fig. 4.2) deployment with releases of males bearing a self-limiting transgene slowed or reversed the evolution of initially widespread ($p_0 = 0.5$) and recessive ($h_{RR} = 0$) resistance and enhanced the suppression of the adult female population.

Reinforcing LLIN or IRS deployment with pulsed releases reduced both the equilibrial resistance allele frequency (Fig. 4.1a-d, Fig. 4.2a-d) and adult population size (Fig. 4.1e-h, Fig. 4.2e-h) relative to an insecticide-only approach ($\kappa_r = 0$) for all release frequencies (κ_f) and release ratios (κ_r) simulated (Figs. 4.1-2). Release programmes that achieved a minimum intensity of approximately two transgenic

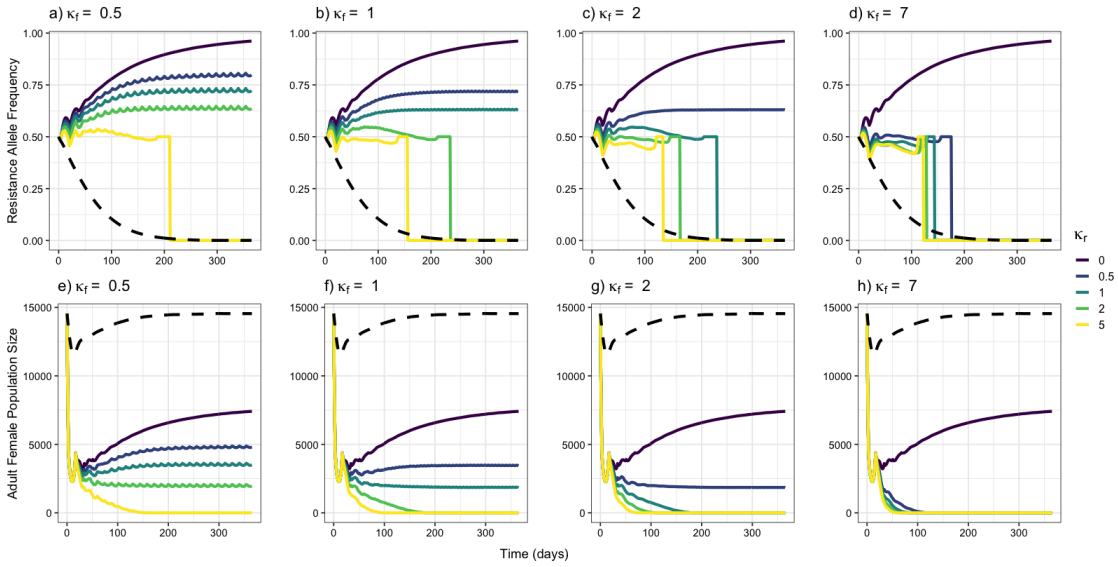


Figure 4.1: Supplementing effective LLIN-deployment with releases of transgenic insects enhances population and resistance management. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of LLIN exposure ρ_{LLIN} was fixed at 0.8 and combined with release strategies from fortnightly ($\kappa_f = 0.5$) to daily ($\kappa_f = 7$) releases at release ratios from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially widespread ($p_0 = 0.5$), fully recessive ($h_{RR} = 0$), and with additive fitness costs ($g_{RR} = 0.5$). LLIN-only ($\kappa_r = 0$, purple line) and no-intervention ($\kappa_r = 0$, ϕ_{LLIN}, black dashed line) were included for comparison. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$.

males released per wildtype male per week prevented the evolution of resistance and, in combination with the insecticidal intervention, eliminated the mosquito population for both LLINs and IRS (Figs. 4.1-2). The characteristic resistance extinction pattern, in which the allele frequency plateaued at ∼0.5 before collapsing to extinction, is produced because the differential mortality imposed by the management interventions eliminates the adult female population before the adult male population which, due to high frequencies of homozygous resistant females and homozygous susceptible released males, is predominantly heterozygous (see Appendix C.1), with the final crash in the resistance allele frequency representing the death of the final adult male(s).

Combining LLINs or IRS with less intensive release programmes failed to

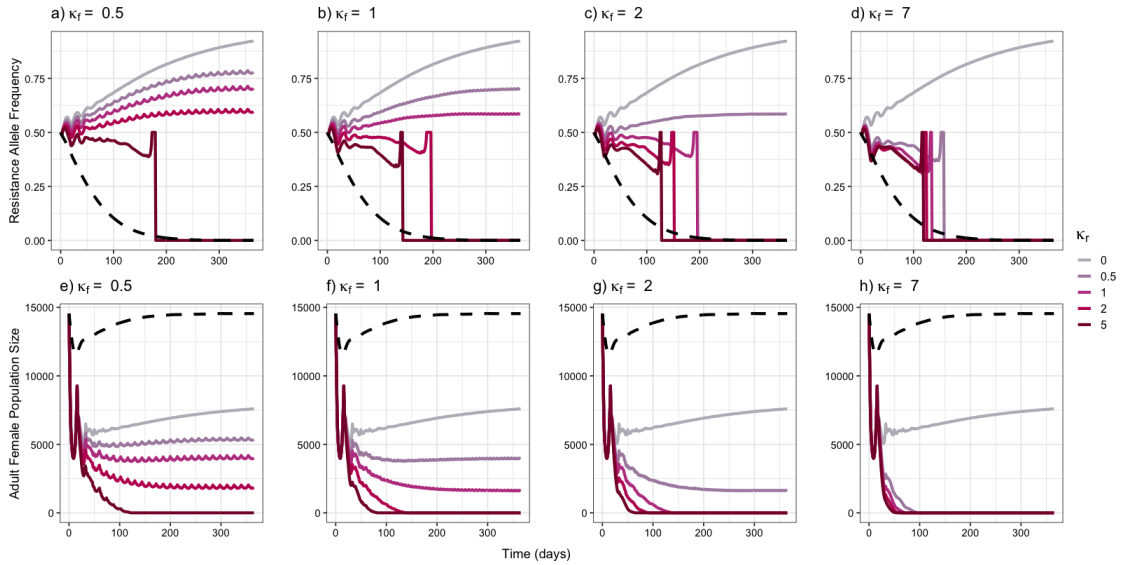


Figure 4.2: Supplementing effective IRS-deployment with releases of transgenic insects enhances population and resistance management. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of IRS exposure ρ_{IRS} was fixed at 0.8 and combined with release strategies from fortnightly ($\kappa_f = 0.5$) to daily ($\kappa_f = 7$) releases at release ratios from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially widespread ($p_0 = 0.5$), fully recessive ($h_{RR} = 0$), and with additive fitness costs ($g_{RR} = 0.5$). IRS-only ($\kappa_r = 0$, purple line) and no-intervention ($\kappa_r = 0$, ϕ_{IRS}, black dashed line) were included for comparison. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$, $c_{RR} = 0.05$.

eliminate resistance or the adult female population but maintained both at levels that decreased with increasing release frequency (κ_f) and/or ratio (κ_r). Where extinction was not achieved, removing the insecticidal-intervention more effectively managed resistance than combined strategies, but also enabled the adult female population to recover rapidly to its pre-intervention equilibrium (Figs. 4.1-2, black dashed lines).

Strategies reinforcing LLIN and IRS deployment with transgenic insect releases are less effective when resistance is dominant with recessive costs

Integrating transgenic insect releases with LLINs or IRS deployment was less effective when resistance was dominant ($h_{RR} = 1$) and the associated fitness costs recessive ($g_{RR} = 0$), but did maintain both the resistance allele frequency and adult

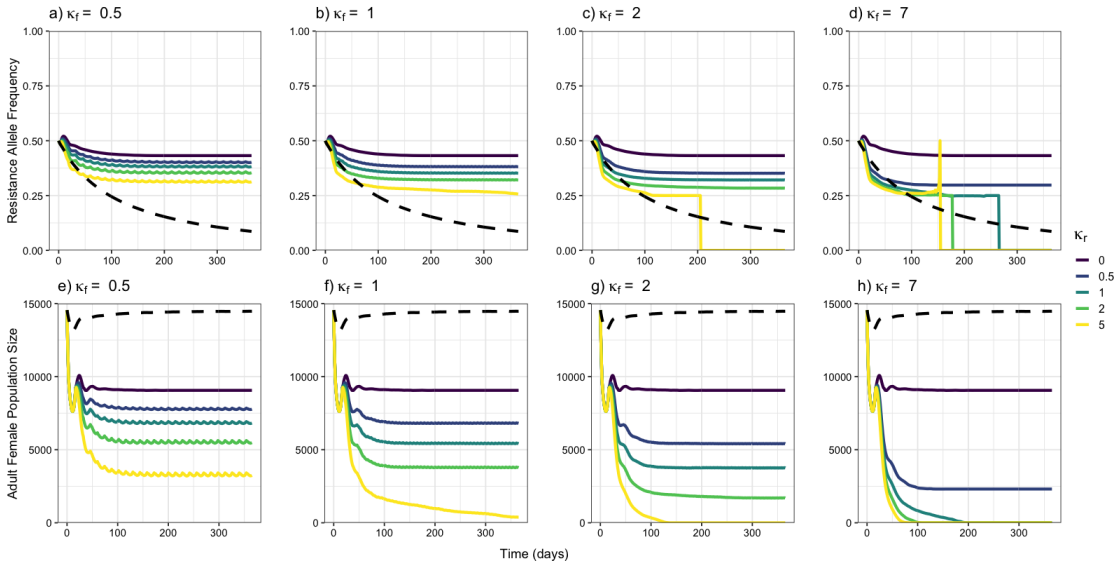


Figure 4.3: Strategies supplementing LLIN deployment with transgenic insect releases are less effective when resistance is dominant with recessive fitness costs. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of LLIN exposure ρ_{LLIN} was fixed at 0.8 and combined with release strategies from fortnightly ($\kappa_f = 0.5$) to daily ($\kappa_f = 7$) releases at release ratios from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially widespread ($p_0 = 0.5$), completely dominant ($h_{RR} = 1$), and with recessive fitness costs ($g_{RR} = 0$). LLIN-only ($\kappa_r = 0$ purple line) and no-intervention ($\kappa_r = 0$, ϕ_{LLIN}, black dashed line) were included for comparison. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$.

female population at lower equilibria relative to insecticide-only strategies (Figs. 4.3-4). The release intensity threshold required to achieve extinction when resistance was dominant with recessive costs increased to ∼5 transgenic males per wildtype male per week (Figs. 4.3-4) and extinction took longer to achieve for equivalent parameterisations than when deployed against a population with recessive resistance ($h_{RR} = 0$, Figs. 4.1-2). When dominant resistance was driven to extinction, the resistance frequency typically declined to ∼0.25 before being eliminated as the adult male population was composed equally of heterozygote and homozygote susceptible individuals.

When resistance remained extant, increasing the release frequency (κ_f) and/or ratio (κ_r) provided marginal reductions in the equilibrial resistance frequency but

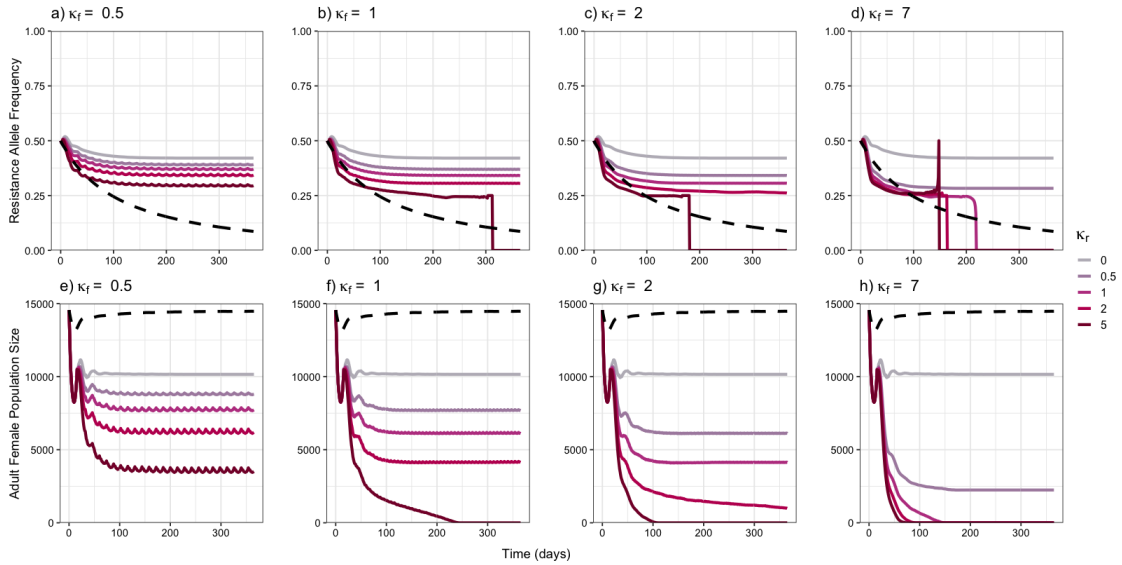


Figure 4.4: Strategies supplementing IRS deployment with transgenic insect releases are less effective when resistance is dominant with recessive fitness costs. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of IRS exposure ρ_{IRS} was fixed at 0.8 and combined with release strategies from fortnightly ($\kappa_f = 0.5$) to daily ($\kappa_f = 7$) releases at release ratios from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially widespread ($p_0 = 0.5$), completely dominant ($h_{RR} = 1$), and with recessive fitness costs ($g_{RR} = 0$). IRS-only ($\kappa_r = 0$, purple line) and no-intervention ($\kappa_r = 0$, ϕ_{IRS}, black dashed line) were included for comparison. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$, $c_{RR} = 0.05$.

greatly enhanced the suppression of the adult female population. Reducing the initial resistance frequency ($p_0 = 0.01$) had no effect on the population dynamic or genetic outcomes when resistance was dominant and the costs recessive, as combined strategies failed to prevent resistance evolving from rarity (Figs. C4-5). Combined strategies maintained the resistance allele at lower frequencies when resistance was dominant, but the equilibrial adult female population size was significantly higher under equivalent parameterisations when resistance was recessive ($h_{RR} = 0$) (Figs. 4.1-4).

For both recessive ($h_{RR} = 0$) and dominant ($h_{RR} = 1$) resistance, the insecticidal intervention combined with releases did not affect the resistance extinction outcome, but strategies combining releases with IRS achieved resistance allele and population

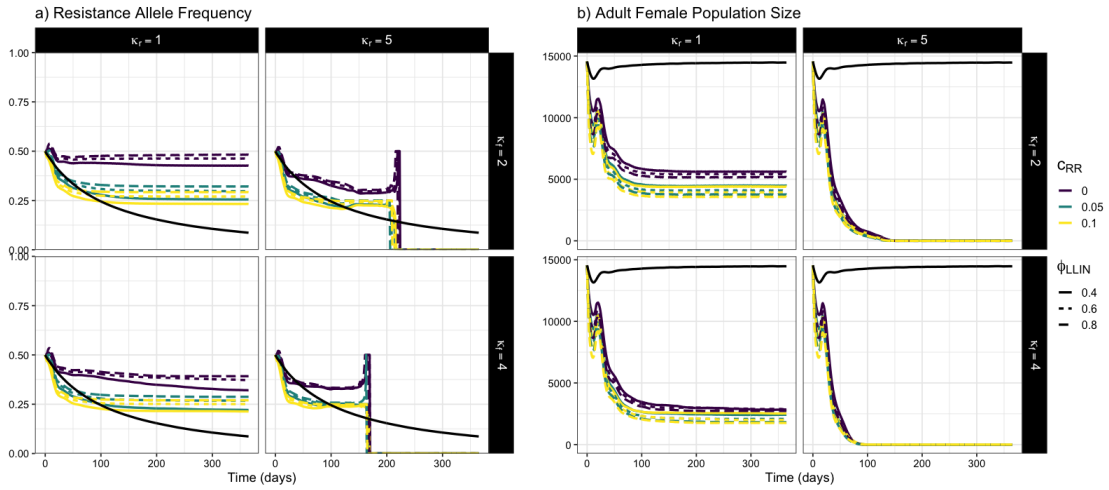


Figure 4.5: The effect of reducing the fitness cost associated with the resistance allele on the capacity of combined LLIN and release strategies of different intensities to manage a) resistance and the b) adult female population. Resistance was initially widespread ($p_0 = 0.5$), dominant ($h_{RR} = 1$), and associated with a recessive fitness cost ($g_{RR} = 0$). The line colours and styles represent different costs of resistance (c_{RR}) and LLIN-exposure probabilities (ϕ_{LLIN}) respectively, while the panel rows and columns depict different release ratios (κ_r) and frequencies (κ_f). Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$.

elimination in fewer days. Under operational parameterisations that did not yield extinction, combining releases with IRS maintained resistance at marginally lower frequencies but provided slightly weaker long-term population suppression relative to LLIN and release strategies (Figs. 4.1-4, Figs. C4-5).

The effect of resistance costs (c_{RR}) on strategies combining insecticidal interventions with transgenic insect releases

When resistance was dominant ($h_{RR} = 1$) and the fitness costs recessive ($g_{RR} = 0$), decreasing the fitness cost of resistance (c_{RR}) had little effect when LLINs or IRS were coupled with large scale releases ($\kappa_r = 5$), which eliminated the mosquito population for all exposure probabilities simulated (Figs. 4.5-6). Reducing the c_{RR} also had no effect on the time taken to eliminate the population, but did facilitate higher resistance allele frequencies while the population remained extant (Fig. 4.5a, Fig. 4.6a).

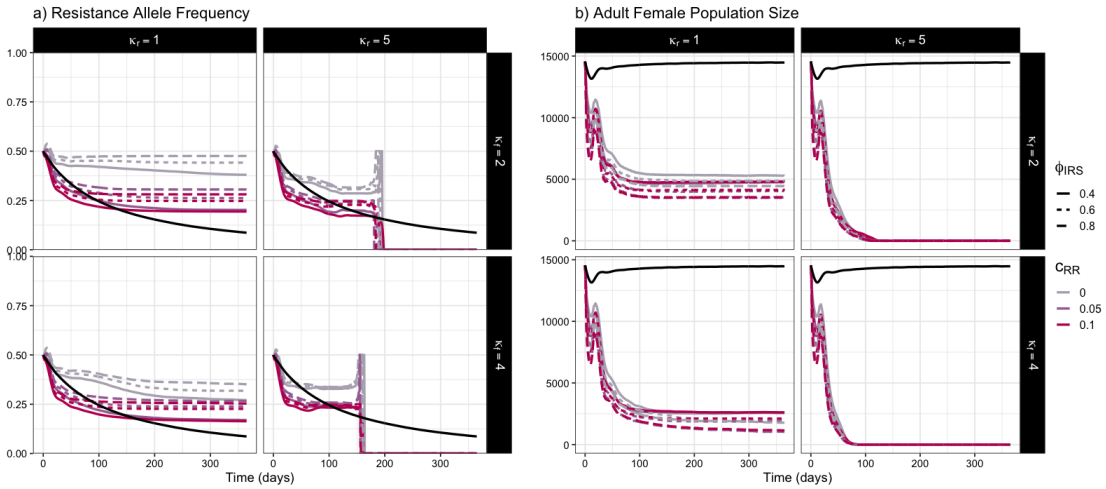


Figure 4.6: The effect of reducing the fitness cost associated with the resistance allele on the capacity of combined IRS and release strategies of different intensities to manage a) resistance and the b) adult female population. Resistance was initially widespread ($p_0 = 0.5$), dominant ($h_{RR} = 1$), and associated with a recessive fitness cost ($g_{RR} = 0$). The line colours and styles represent different costs of resistance (c_{RR}) and IRS-exposure probabilities (ϕ_{IRS}) respectively, while the panel rows and columns depict different release ratios (κ_r) and frequencies (κ_f). Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$.

Under insecticide and release strategies that failed to eliminate the population, decreasing both the cost of resistance (c_{RR}) and the probability of insecticide exposure (ϕ_i) decreased the equilibrial resistance frequency. Decreasing the cost of resistance yielded larger increases in resistance frequency than reductions in exposure probability, but the effect that either had on the equilibrial adult female population size was modest relative to the effects of the release ratio and frequency.

The effects of decreasing the cost of resistance were more pronounced when resistance was recessive ($h_{RR} = 0$, Figs. C.6-7). When LLINs or IRS were reinforced with small and/or infrequent releases ($\kappa_r = 1, \kappa_f \geq 2$), lowering the cost of resistance from 0.1 to 0.05 increased the time taken for resistance to go extinct, particularly under higher insecticide exposure probabilities. Imposing no fitness penalty enabled resistance to escape extinction and even increase in frequency (Fig. C.6a, Fig. C.7a), which substantially increased the equilibrial adult female population size

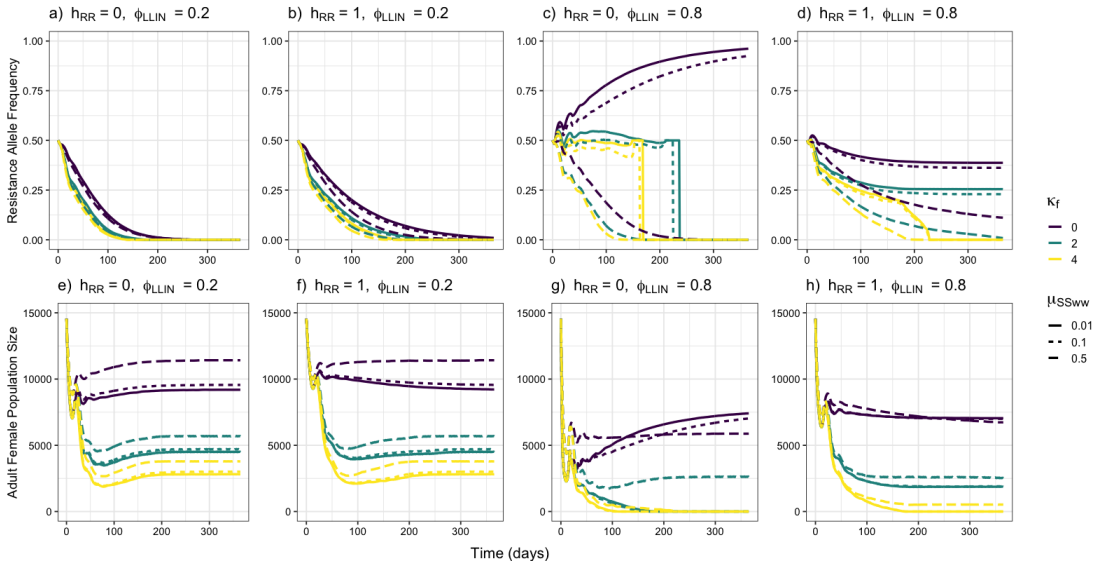


Figure 4.7: Reinforcing LLIN deployment with transgenic insect releases compensates for low coverage and reduced efficacy of nets. Panels a-d) and e-h) show the whole-population resistance allele frequency and adult female population size dynamics under management strategies combining LLINs with releases of female-specific, self-limiting insects under different scenarios of LLIN exposure probability (ϕ_{LLIN}), resistance dominance (h_{RR}), release frequencies (κ_f), and insecticide efficacy (μ_{SSww}). The release ratio was fixed at 1:1 transgenic males to pre-intervention wildtype males ($\kappa_r = 1$) and the release frequency is represented by line colour, with purple lines showing dynamics under an LLIN-only strategy ($\kappa_f = 0$). The probability that a susceptible homozygote survives exposure to an LLIN (μ_{SSww}) is given by line type. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, $\mu_{LLIN}^{RRww} = 0.9$.

(Fig. C.6b, Fig. C.74b). Increasing the release ratio to 5:1 overcame the effects of decreasing the resistance cost.

Releases of transgenic insects can compensate for low or ineffective LLIN/IRS implementation

In the absence of releases ($\kappa_f = 0$), limited exposure to LLINs or IRS ($\phi_i = 0.2$) reversed the evolution of resistance but yielded a substantial increase in equilibrial population size. Increasing the probability that susceptible homozygotes survive exposure to LLINs or IRS (μ_{SSww}) delayed the evolution of resistance. This was most notable under high exposure probability, as increasing μ_{SSww} to 0.5 when

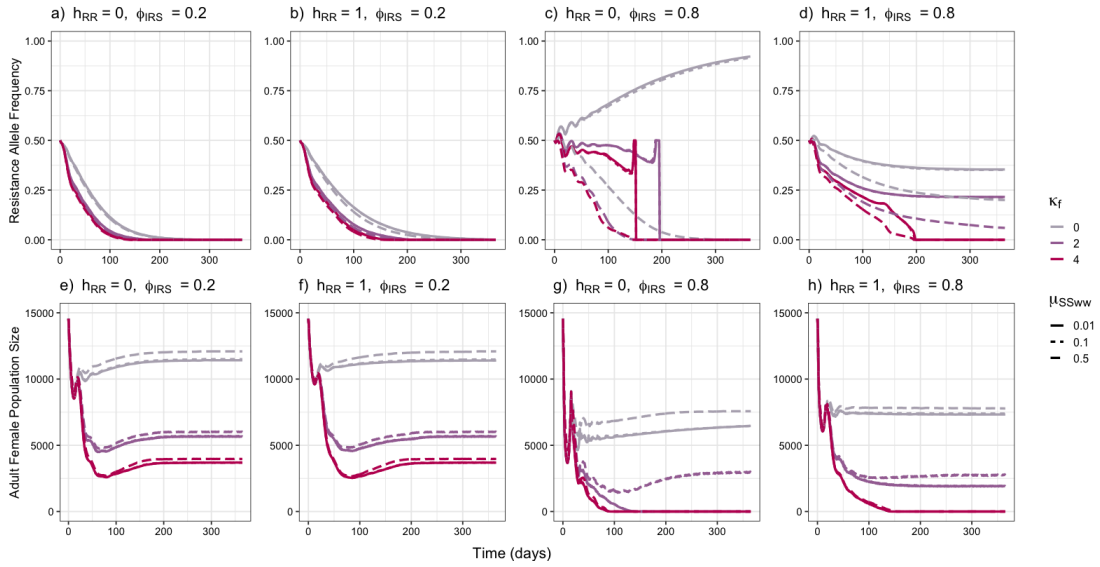


Figure 4.8: Reinforcing IRS deployment with transgenic insect releases compensates for low coverage and reduced efficacy of nets. Panels a-d) and e-h) show the whole-population resistance allele frequency and adult female population size dynamics under management strategies combining LLINs with releases of female-specific, self-limiting insects under different scenarios of IRS exposure probability (ϕ_{IRS}), resistance dominance (h_{RR}), release frequencies (κ_f), and insecticide efficacy (μ_{SSww}). The release ratio was fixed at 1:1 transgenic males to pre-intervention wildtype males ($\kappa_r = 1$) and the release frequency is represented by line colour, with purple lines showing dynamics under an IRS-only strategy ($\kappa_f = 0$). The probability that a susceptible homozygote survives exposure to an IRS (μ_{SSww}) is given by line type. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $c_{RR} = 0.05$, $g_{RR} = 0.5$, $\mu_{IRS}^{RRww} = 0.9$.

$\phi_i = 0.8$ prevented resistance from fixing in the population (Fig. 4.7c-d, Fig. 4.8c-d). The effect of increasing μ_{SSww} on the population dynamics depended on the exposure probability. When $\phi_i = 0.2$, increasing μ_{SSww} reduced the level of suppression achieved by both insecticide-only and combined strategies (Fig. 7e-f, Fig. 8e-f), whereas the equilibrial population size decreased with with increasing μ_{SSww} when $\phi_i = 0.8$.

Supporting LLINs and IRS with transgenic insect releases enhanced population and resistance management when the probability of exposure to insecticides (ϕ_i), or the efficacy of insecticides against susceptible homozygotes, was reduced (Fig. 4.7, Fig. 4.8). When the exposure probability was low, supplementing both LLINs

and IRS with releases decreased the time taken to achieve resistance extinction and provided greater suppression than insecticide-only strategies when ϕ_i was increased to 0.8. Increasing μ_{SSww} reduced the suppressive power of combined strategies, but could be compensated for by increasing the release frequency (κ_f). Integrating releases with LLIN/IRS programmes with high exposure probabilities eliminated the mosquito population when $\mu_{SSww} \leq 0.1$ and resistance was recessive ($h_{RR} = 1$), but failed when $\mu_{SSww} = 0.5$, even following the extinction of resistance. Increasing μ_{SSww} under high exposure probabilities prevented combined strategies from eliminating either the resistance allele or the mosquito population when resistance was dominant ($h_{RR} = 1$), but releases greatly reduced both the equilibrial resistance allele frequency and population size.

Discussion

We used a population demographic and genetics framework to determine the extent to which the evolution of resistance to insecticide-based LLINs and IRS can be managed using self-limiting releases to enhance the control of an anopheline vector population. Previous studies have investigated the impact of LLINs and IRS on mosquito population dynamics (Griffin et al., 2010; White et al., 2011), the management of mosquito populations using genetic control tools (Khamis et al., 2018; North et al., 2019; White et al., 2010), and the resistance management potential of self-limiting releases in agricultural settings (Alphey et al., 2009; Alphey et al., 2007; Brewer & Bonsall, 2021). The work presented here represents, to our knowledge, the first to model strategies supplementing LLIN and IRS deployment with transgenic insect releases for the management of an anopheline population and its resistance to insecticides. We show that integrating self-limiting releases with LLINs or IRS can slow or reverse the evolution of resistance and enhance the suppression of an anopheline population under scenarios that would favour the evolution of resistance under insecticide-only control. Our findings contribute to the growing body of work suggesting that self-limiting releases with a dominant,

late-acting, female-specific phenotype could represent a valuable addition to both the integrated vector and resistance management toolkits.

Combining LLIN or IRS deployment with releases was substantially more effective when resistance was recessive. Increasing the dominance of resistance increased the equilibrial population size achieved under equivalent insecticide and release regimes, as well as the threshold release-intensity required to eliminate the population (Figs. 4.1-4). This is consistent with previous modelling work which first investigated the utility of transgenic insect releases for resistance management in agricultural settings (Alphey et al., 2009; Alphey et al., 2007). When resistance is recessive, releases act synergistically with LLINs/IRS, introducing an additional source of mortality through the female-lethal effect of the transgene and enhancing insecticide-induced mortality through the production of offspring with genotypes that render them vulnerable (SS/RS). As resistance becomes increasingly dominant, this synergistic effect on mortality diminishes as heterozygote-mortality declines, with the mortality achieved by combined strategies becoming increasingly additive. As we demonstrate, larger and/or more frequent releases can overcome this disadvantage by increasing the total transgene-induced mortality. However, a more effective approach might be to attempt to render resistance functionally recessive by increasing its concentration and/or toxicity (e.g. using a synergist like piperonyl butoxide (PBO)), or to rotate to an insecticide against which resistance has not developed.

Our simulations suggest that integrating self-limiting releases with LLIN/IRS deployment is likely to be an effective option for maintaining population suppression when the probability that adult females encounter LLINs/IRS is low and/or their efficacy against susceptible insects is reduced. This is because the lethal transgene provides an additional source of suppression that compensates for the reduction in insecticide-induced mortality to a degree that increases with increased release ratio and frequency and reduced dominance (Figs 4.7-8). Exposure probability and insecticidal efficacy represent real challenges to current vector control programmes. In a recent assessment of LLIN-based vector management, net availability, bioefficacy and longevity were all raised as threats to continued malaria control in Africa

(Lindsay et al., 2021). Furthermore, *Anopheles stephensi*, a competent vector of both *Plasmodium vivax* and *Plasmodium falciparum* whose ecology naturally limits their exposure to LLINs and IRS, which already exhibits reduced sensitivity to multiple insecticide classes, and which has begun to establish in urban areas where LLIN/IRS deployment has historically been difficult to implement (Sinka et al., 2020), has recently expanded into east Africa. While increasing the efficacy and distribution of LLIN/IRS will both be essential to sustain vector control (Bhatt et al., 2015), integrating new control tools with novel modes of action are likely to be necessary to overcome these operational and ecological challenges to LLIN/IRS-based vector and resistance management. While experimental work will be required to validate the theoretical results, our findings provide further evidence that genetic control has significant potential to be used to reinforce insecticidal interventions for both integrated vector and resistance management strategies.

While we have demonstrated that strategies combining releases with single-insecticide interventions can provide effective management when resistance has evolved to high frequencies, how these strategies compare with the rotations, mosaics and combinations that are currently recommended by the WHO for IRM (World Health Organisation, 2012) in delaying its evolution from rarity requires further investigation. By replacing the transgene locus with a second resistance/susceptibility locus, the framework presented here could readily be adapted to simulate strategies that rotate two different insecticides in space and/or time, or use them in combination, facilitating comparison with the strategies investigated here. Given the current challenges involved with implementing insecticide-based resistance management strategies, particularly the limited number of complimentary insecticides, the logistical challenges involved in implementing them, and the affordability of these strategies, in the short term it seems more likely that release-based strategies would be used instead of, rather than alongside, these existing approaches. However, as these challenges are overcome and our understanding of resistance management improves, a logical next step would be to assess the feasibility of strategies integrating releases with insecticide mosaics, rotations

and combinations. Our model could simulate such strategies by extending the population genetic framework to include a third locus, although this would greatly increase the model complexity.

Studies investigating the impact of control interventions on population and/or resistance dynamics, including this one, often focus on identifying strategies that provide the fastest or most effective population or resistance suppression whereas, in practice, the most effective vector control strategy is likely to be that which suppresses the vector population below the density required to sustain disease transmission in the most resource efficient manner. For instance, strategies that eliminate a vector population have no practical value if they are too expensive to employ. Similarly, those which eliminate resistance are ineffective if they fail to suppress the population size below the density required to sustain transmission. This is illustrated in our own work, where the most effective strategy for managing resistance was often to remove the insecticide and allow the fitness costs associated with resistance to remove it from the population, despite this approach allowing the population size to rapidly recover to its pre-intervention carrying capacity (e.g. Fig. 4.1). Releasing susceptible wildtype insects into the population would provide even more effective resistance management (Alphey et al., 2007), but may risk increasing the size, and therefore transmission potential, of the population if the number of females that find mates increases.

There are notable exceptions. In their recent study of the effects of resistance evolution on the control of mosquito populations using LLINs and IRS, Barbosa et al. (2018) linked their population dynamic model to a Ross-Macdonald model to determine the conditions under which resistance evolution would undermine control and enable disease transmission to resume. Lacy et al. (2021) included an economic assessment of malaria control strategies using genetically engineered mosquitoes. Hackett and Bonsall (2019) explored the ability of approximate dynamic programming to identify resistance management strategies that balanced the short term costs to pest damage against the longer term demands of maintaining susceptibility to an insecticide. Given that economic viability is often one of, if not,

the major obstacle to effective population and resistance management strategies (e.g. global funding for malaria control has remained fixed at approximately $3 billion, despite increases in both the unit cost of interventions and the size of the populations they are used to protect (Lindsay et al., 2021)), and that vector control efforts are often centred in nations with limited resources, further effort to link population and resistance dynamic outcomes with transmission and/or economic modules should be encouraged.

To develop a system that captured the stage, sex and genotypic structure of an anopheline population, we made a series of simplifying assumptions to limit the complexity of the model that are important to consider when interpreting our results. Firstly, we imposed a density-dependence structure that yielded stable, long-term equilibria. In reality, mosquito populations are highly, and variably, responsive to biotic and abiotic conditions and can exhibit high degrees of spatiotemporal variability (Whittaker et al., 2021), particularly over intra-annual periods, with implications for their control (White et al., 2011). When levied against populations that exhibit cyclical dynamics, the success of release campaigns is highly dependent on their timing in the cycle (White et al., 2010). In modelling a closed population, we also ignored the potentially important effects of immigration, which could pose important additional complications for both population and resistance management strategies (e.g. if resistant homozygotes are immigrating from outside the area under management) (Watkinson-Powell & Alphey, 2017). The parameter estimates we used were obtained from populations reared under stable laboratory conditions which, particularly for daily survival probability and fecundity, likely represent the upper limits of what the species can achieve rather than that which they typically display in the field. In Chapter 3, we demonstrated that reducing the daily survival probabilities, particularly of larvae, greatly increases the efficacy of both insecticide- and release-only strategies, suggesting that the control outcomes presented here may be conservative. Future work that simulated a more limited set of control tools and/or resistance scenarios using an ecologically richer population dynamics model could be of great interest and utility.

Conclusions

Vector control strategies based on traditional LLIN and IRS deployment are increasingly threatened by the expansion of vector populations in response to anthropogenic climate and land-use change (Hertig, 2019), the emergence of widespread resistance to the limited number of recommended insecticide classes (Hancock et al., 2020; Hancock et al., 2018), and expansion of vectors whose behavioural ecology limits their exposure to nets and/or sprays (Sinka et al., 2020; Yared et al., 2020), exacerbating calls for the development of novel vector and resistance management tools that can be integrated into existing control strategies (Wilson et al., 2020). Releases of genetically modified insects are a potential option that could be used in synergy with insecticide-based interventions to enhance population suppression and, potentially, manage resistance. We have shown, using an ecologically detailed model parameterised using published estimates for *An. stephensi*, that reinforcing LLINs and IRS with plausible self-limiting release strategies can slow or reverse the evolution of resistance and enhance population suppression. The efficacy of these integrated strategies is greatly enhanced when resistance is functionally recessive, but remain superior to insecticide-only approaches when resistance is dominant. While our model serves as a useful proof-of-concept, investigating the influence of seasonality in population dynamics on these combined approaches, analysing their effects on the transmission potential of the target population, and estimating the relative costs of different strategies would all help to better understand their utility in more realistic control scenarios.

5
Discussion

The evolution of resistance represents a very real threat to the long-term efficacy of the insecticide-based interventions that have, for decades, formed the backbone of successful efforts to manage both agricultural pests and disease vectors. There is an increasingly urgent need to develop both alternative pest population suppression tools and strategies with which to manage resistance to them. Releases of genetically-modified male insects bearing a dominant, female-specific, self-limiting transgene have the exciting potential to both enhance pest insect suppression and manage the evolution of insecticide resistance. Building on the foundational, proof-of-principle studies that first highlighted the resistance management potential of self-limiting releases, and integrating ideas from the wider resistance management literature, the work presented in this thesis has sought to expand our understanding of the utility of release-based strategies to consider new implementations, and to develop additional mathematical tools with which to study them. In the discussion to follow, I summarise the methods and key findings presented in the research chapters and suggest directions in which the ideas presented might fruitfully be taken in the future.

Landscape level considerations of strategies combining refuges with self-limiting releases

The use of self-limiting releases in a resistance management capacity was conceptualised using spatially-implicit population genetics (Alphey et al., 2007) and population dynamics and genetics (Alphey et al., 2009) models over a decade ago. These studies demonstrated that, by introgressing genetic susceptibility to a given insecticide, self-limiting releases could be deployed alongside the high-dose/refuge (HDR) strategy to delay or reverse the evolution of resistance and reduce the refuge size required to maintain resistance to insecticidal crops at trivial frequencies. However, genetic control programmes are generally envisaged as a landscape-level tool for area-wide (Dyck et al., 2006) or localised (Zhou et al., 2019) management, and the efficacy of release-based suppression is sensitive to spatial population structure (Legros et al., 2012) and processes such as dispersal (Yakob & Bonsall, 2009). Furthermore, spatial heterogeneity in Bt crop and refuge planting is known to influence the evolution of resistance (Huang et al., 2017; Peck et al., 1999; Sisterson et al., 2004; Sisterson et al., 2005) and cases of field-evolved resistance are generally linked to refuge non-compliance and non-recessive resistance due to reduced crop bioefficacy, both of which would introduce further heterogeneity in the selection pressure experienced by the pest (Tabashnik & Carrière, 2017).

In chapter 2, I developed a spatially-explicit extension of the population dynamic and genetics framework described by Alphey et al. (2009) to facilitate landscape-level investigations of population and resistance management strategies supplementing the HDR strategy with self-limiting releases. This model is highly adaptable and capable of addressing a suite of pertinent questions relating to control strategies, pest ecologies, landscape contexts and intended management outcome(s). The model was used to assess whether strategies supplementing refuge planting with self-limiting releases could enhance population and resistance management under conditions known to facilitate field-evolved resistance, and determine whether strategies integrating the HDR strategy with localised rather than area-wide releases could provide effective population and resistance suppression.

Supplementing the HDR strategy with releases compensated for violations of HDR principles to provide superior population and resistance management outcomes relative to HDR-only strategies. Releases acted synergistically with the Bt crop to provide additional transgene-induced mortality and enhance insecticide-induced mortality through the increased production of susceptible (SS/RS) offspring (Alphey et al., 2009). Consistent with the experimental findings of Zhou et al. (2019), area-wide releases were required to reliably eliminate the pest population. However, localised releases targeted at population hotspots were sufficient to extinguish resistance when the threshold for releasing was low (approximately 5% of the equilibrial population size). If the landscape was dominated by insecticidal crops (either due to large refuges or low refuge compliance) and/or the release ratio was high (10:1), localised releases were also able to exterminate the pest population. Where localised releases were insufficient to eliminate the pest, they slowed the rate of landscape colonisation, maintained the pest at substantially lower densities, and prevented resistance from evolving to fixation.

Previous work has demonstrated the utility of self-limiting releases for landscape-level suppression (Legros et al., 2012; Yakob & Bonsall, 2009). Ours represents, to our knowledge, the first to investigate their effect on landscape-level insecticide resistance dynamics. While the HDR strategy has proved highly effective for preserving susceptibility to Bt crops (Tabashnik et al., 2004), field-evolved resistance has been detected when assumptions of the strategy are breached (Tabashnik & Carrière, 2017, 2019). The findings from chapter 2 provide further evidence that self-limiting releases can be used to reinforce the HDR strategy when its efficacy is undermined by violations of its assumptions (Alphey et al., 2009). Replacing refuge planting with prophylactic area-wide SIT has been used successfully to prevent the evolution of resistance to Bt cotton in pink bollworm (Tabashnik et al., 2010), but such strategies are expensive and require significant coordination among growers (Dyck et al., 2006). If suppression rather than eradication is an acceptable outcome, smaller scale, responsive releases could be used to maintain the population below tolerable levels, potentially allowing time to address the

issue(s) undermining HDR-based management (e.g. switching to varieties yielding higher toxin doses, planting larger refuges).

Using their more detailed Skeeter Buster model, Legros et al. (2012) investigated the effect of spatial population structure on the management of mosquito populations using genetic control strategies. The strategies they simulated included homogeneous (area-wide) releases, and either uniform or random point releases across 10% of the control area. While homogeneous releases could eliminate the population reliably within 3 years with an approximate release ratio of 2.5:1, significantly greater release ratios were required to achieve extinction using uniform (20:1) and random (60:1) point releases. They also showed that spatial heterogeneity in mosquito density can frustrate efforts to achieve extinction using area-wide releases unless the ratio used is sufficient to eliminate even the most dense local populations. The findings from chapter 2 suggest that a fourth strategy that used threshold-based point releases might reduce the release-ratio required to achieve extinction with a high degree of confidence. As noted by the authors, however, the cost of such a strategy would need to be balanced carefully against the potential benefits to suppression, particularly if the population monitoring programme required to implement the strategy imposed significant costs.

Managing resistance to vector control tools using self-limiting releases

In chapter 3, I extended considerations of the use of self-limiting releases for integrated population and resistance management into the realm of vector control. Building on an existing population genetic/demographic model (Barbosa et al., 2018), I set out a theoretical framework for simulating vector control strategies that reinforce the deployment of insecticidal nets and sprays with self-limiting releases. Parameterising the model using published estimates for *Anopheles stephensi*, an emerging threat to malaria control in East Africa (Balkew et al., 2021; Seyfarth et al., 2019), I assessed the sensitivity of single-intervention strategies to operational and ecological parameters. Simulations showed that insecticide-based control was

quickly undermined by both the evolution of resistance, particularly when resistance was dominant, or when resistance was recessive and initially widespread, and when the probability of exposure declined, which could result from low coverage or behavioural avoidance, was reduced. In the absence of insecticides, releasing self-limiting insects at plausible ratios and/or frequencies greatly reduced or eliminated the pest population, and could drive resistance from fixation to extinction.

Building on these results, in chapter 4 the demographic and genetics model was used to assess whether self-limiting releases could be used to reinforce insecticidal bed nets and sprays under conditions that limited the efficacy of insecticide-only approaches. I showed that these integrated strategies could be used to reverse the evolution of insecticide resistance, suppressing the allele frequency, and therefore the population size, to lower equilibria, or eliminating the pest population. Lower intensity release regimes were required to achieve extinction when resistance was recessive, as releases served to both contribute an additional source of mortality and enhance that imposed by the insecticide. However, extinction could be achieved when resistance was dominant by enhancing the release intensity. Combined strategies remained effective even as the probability of exposure to, and bioefficacy of, the insecticide declined. This study represents the first to demonstrate that female-specific, self-limiting releases can be used alongside the bed nets and indoor sprays that underpin malaria control to reverse the evolution of insecticide resistance and enhance the suppression of a vector population.

The role of mathematical modelling in developing population and resistance management strategies

Experimentally investigating the efficacy of pest suppression and resistance management strategies, particularly those that integrate multiple tools and/or take place in the field, is time-consuming, expensive, logistically-demanding and, particularly in the case of strategies involving genetically modified material, can involve significant social and regulatory barriers. In light of these difficulties, mathematical models have made significant contributions to the development of pest population and

resistance management strategies. Indeed, the design of strategies to manage resistance to Bt crops following their commercialisation was, reportedly, guided exclusively by the outputs of mathematical models (Tabashnik & Carrière, 2017), while the early theoretical work performed by Alphey et al. (2009) provided testable resistance management predictions for laboratory cage experiments to validate (Harvey-Samuel et al., 2014; Zhou et al., 2018).

The complexity of the models used varies, with different theoretical approaches serving different purposes that reflect both the nature of the question(s) being asked and the interests and/or competencies of the modeller and intended audience. For instance, basic models that ignore many of the ecological and operational complexities of a management problem can serve as effective proof-of-principles, helping to assess the plausibility of various approaches and give an idea of the kinds of dynamic behaviours the system under study may exhibit (e.g. Alphey et al. (2007)). Modelling can also be used to guide the design and interpretation of the experimental work required to verify the outputs of simpler models (e.g. Zhou et al. (2018)). More detailed frameworks which explicitly model many ecological and operational processes, for example the Skeeter Buster model (Magori et al., 2009), get closer to informing real-world management strategies by addressing more detailed questions, such as the role of population and genetic structure, or environmental heterogeneity in resource availability on population control. However, the complexity of such models often precludes analytical solutions identifiable with simpler models and makes determining the sensitivity of model outputs to individual parameters more challenging.

It is important to recognise that the models presented in this thesis, while bulky, are still relatively simple with respect to this spectrum of model complexity. The outputs from these models do not represent accurate projections of pest population sizes and allele frequencies through time under different control measures, but instead serve to illustrate the relative efficacy of various strategies under different conditions, and the types of transient and long-term equilibrium dynamics that might be observed. The results presented in this thesis build incrementally on

previous work to provide additional evidence of the potential value of self-limiting releases for resistance management, but there remains much work to be done to confirm the operational value of these approaches. Given the increasing demand for the development of novel, integrated pest and resistance management strategies, mathematical modelling will continue to play an important role in the development of pest population and resistance management strategies.

Avenues for future research

The nascent nature of considerations of self-limiting releases for resistance management, coupled with the novel and flexible nature of the mathematical models developed in this thesis, leave substantial scope for future work.

Firstly, an interesting extension to the work presented in chapter 2 would be to assess more nuanced strategies for localised releases. Population-based thresholds provided effective resistance management when the release threshold was low, but the improvement relative to area-wide strategies diminished quickly as the threshold increased. A simple improvement could be to release if the population size or resistance allele frequency exceed problematic thresholds. To better contain localised outbreaks, threshold-based approaches could also be combined with hybrid release strategies that release into fields within a defined radius (Yakob & Bonsall, 2009). Finally, by assigning costs to control interventions, strategies could be identified that minimised the inputs required to maintain the pest population or resistance allele frequency below a user-selected critical value (Hackett & Bonsall, 2019).

In chapters 3 and 4, strategies deploying self-limiting releases resulted either in the extinction of the resistance allele and/or mosquito, or its suppression to a lower equilibrium, depending on the combination of release size and frequency used. This indicates the presence of a threshold release intensity sufficient to eradicate the resistance allele and/or mosquito population. While the simulations performed allowed for broad observations about the nature of this threshold (e.g. it decreased when releases are combined with LLINs/IRS, but increased with resistance dominance), a more complete characterisation of the exact release strategy required

under different control scenarios would represent a significant improvement on the existing analysis. A simple, albeit computationally expensive, solution would be to simulate over a larger set of release strategies. A more advanced approach would be to apply techniques designed for the analysis of nonlinear matrix models to identify the critical release ratio/frequency and assess its sensitivity to other ecological (e.g. daily survival probability) and operational parameters (e.g. net coverage) (Caswell, 2000, 2019; Ezard et al., 2010).

In the work chapters presented in this thesis, I have analysed the relative effects of different management strategies on pest population and resistance allele dynamics. However, in practice the most effective strategies are those that optimally use the available, usually budgetary, resources to maintain the pest population below densities that are economically damaging (agriculture) or are required to sustain disease transmission (vector control). More meaningful comparisons between strategies integrating releases with insecticides would be facilitated by feeding the population dynamic outputs into crop injury or epidemiological models (e.g. Barbosa et al. (2018) and Hackett and Bonsall (2019)). This would allow the modeller to determine the conditions under which the evolution of resistance (physical or behavioural) resulted in pest densities exceeding agricultural or epidemiologically-important thresholds to highlight where integrated approaches are likely to be required to manage resistance and enhance suppression. Estimating the relative costs of different control strategies would also be an important next step.

Finally, in each of our models we used simple population models, parameterised using point estimates, that yielded highly stable, long-term equilibrial dynamics. In reality, pest population are highly dynamic, exhibiting great intra-annual variability linked to seasonal changes in abiotic (e.g. temperature, precipitation, insecticide titre) and biotic (e.g. crop planting regimes, host availability) variables. This variation in abundance is known to have profound implications for genetic pest population management. For instance, White et al. (2010) showed that the efficacy of releases used to suppress populations with cyclical dynamics depended critically on their timing. However, our understanding of temporal changes in resistance in

wild populations is limited (although improving, see Hancock et al. (2020), Hancock et al. (2018)), and even less is therefore known about the effect of population variation on strategies to manage resistance. A difficult, albeit rewarding, next step could be to simulate resistance management strategies in seasonal, perhaps even seasonally-forced, models of population dynamics.

Conclusions

Pest insect populations subject to control measures are, in evolutionary terms, a moving target. While insecticidal applications have revolutionised pest insect suppression in both agricultural and public health settings (Bhatt et al., 2015; Tabashnik et al., 2013), our continued ability to manage pest populations will inevitably depend on our ability to develop novel control tools and evolutionarily-enlightened approaches with which to preserve susceptibility to them (REX Consortium, 2013). This thesis has expanded on early theoretical considerations of the implementation of female-specific, self-limiting releases to demonstrate their potential for both landscape-level agricultural pest population and resistance management and for integrated vector and resistance management. In doing so, many more questions have inevitably been left open than have been addressed, some of which I have suggested in this discussion. In light of concerns surrounding the evolution of resistance and the negative off-target effects of insecticides, agricultural and public health pest insect management is increasingly turning towards ecologically and evolutionarily sustainable strategies that integrate chemical and non-chemical control tools (European Union, 2020; IVCC, 2021). It is my hope that the work presented in this thesis sheds new light on the potential of self-limiting releases as an one such environmentally-friendly population and resistance management tool, and provides useful, adaptable mathematical models with which others interested in the application of releases might further explore their utility.

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Appendices

A

Supplementary Material for Chapter 2

Contents

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A.1: Implementation of system as an individual-based model

The model described in the main text was implemented as an individual based-model. The model was implemented using scripts written in R, and the process is outlined below.

Each population in the landscape ($N_{xy,t}$) is represented by an n x 2 (rows x columns) dataframe, with the columns representing biological sex and genotype, and each row representing an individual pest insect. In this way, all individuals of each sex (male and female) and genotype (*ij = SSww, RSww, RRww, SSwL, RSwL, RRwL, SSLL, RSLL, RRLL*) in each field were explicitly tracked. The dataframes are stored in a list, with their index representing their position in the landscape.

The population life cycle was imagined as a step-by-step process in which the following processes occur and were applied algorithmically to each population in the landscape:

1. Density-**independent** mortality (as a function of genotype and natal crop type)
2. Density-**dependent** survival (genotype independent)
3. Transgenic insect releases (if $N_{xy,t} > N_{thresh}$)
4. Mating (stochastic)
5. Dispersal
6. Oviposition

1. Density-independent mortality

In our model there are two sources of density-independent mortality. The first is determined by the genotype of the individual and crop type it develops on (Bt crop or non-toxic refuge). The second is the lethal phenotype of the female-specific,

early-acting, self-limiting transgene (early-acting because it occurs early during larval development, before density-dependent mortality).

The proportion of individuals of a given genotype survive the density-independent mortality phase is determined by it's average, relative fitness ($\Omega_{xy,t,ij}$). This is a function of an individuals sex, genotype, and the proportions of Bt crop ($F^{Bt}_{xy,t}$), the proportion of non-toxic refuge crop ($F^{Ref}_{xy,t}$) in it's natal field, as well as the relative fitness of it's genotype (at the R/S locus) on Bt crop (ω_i) and refuge crop (υ_i), and it's genotype at the w/L locus. The fitness of each genotype is relative to that of homozygous susceptible individuals ($i = SS$) on refuge crop, which is fixed at 1 ($\upsilon_{SS} = 1$). The average relative fitness of each genotype in the *xy-th* field is calculated as:

given by:

$$\Omega_{xy,t,ij} = (1 - \epsilon_j)[F^{Bt}_{xy,t}\omega_i + F^{Ref}_{xy,t}\upsilon_i]. \tag{A.1}$$

,

This is implemented in practice by calculating the average relative fitness for each cell at the start of the simulation. This proportion is then used to calculate the number of individuals of each genotype that survive density-independent mortality, by multiplying it by the number of individuals of that genotype ($N_{xy,t}$) in the field in the current time step (rounded down to the nearest integer). All female carriers of the lethal transgene, heterozygous or homozygous, are removed from the population in each field at this stage, as we assume $\epsilon_j = 1$ for all lethal allele carriers. The remaining populations then progress to the density-dependent mortality stage.

2. Density-dependent mortality

Once the density-independent mortality has been applied, the surviving populations experience density-dependent selection. The probability of surviving density-dependence is assumed to be independent of sex ans genotype, with the proportion of each population in each field given by:

$$N''_{xy,t} = \frac{N'_{xy,t}}{1 + (\alpha N'_{xy,t})^{\beta}}, \tag{A.2}$$

where $N'_{xy,t}$ is the number of individuals in the *xy-th* field in the current time step that survived the density-independent mortality step, α is a scaling constant proportional to a theoretical, within-field/cellular carrying-capacity, and β is a shaping parameter. $N''_{xy,t}$ represents the number of individuals that have escaped density-independent and density-dependent mortality to survive to adulthood, and progress to the mating phase.

Density-dependent mortality is imposed by determining the number of individuals that must be removed from the population $N'_{xy,t} - N''_{xy,t}$, rounding the number down to the nearest integer, and then selecting that many individuals, at random, from the dataframe to remove.

3. Transgenic insect releases

After density-dependent mortality has been applied to each population dataframe in landscape, $T_{xy,t}$ transgenic males ($ij = SSLL$) are added to each dataframe for which $N_{xy,t} > N_{thresh}$.

4. Mating

After the release phase, all females in the dataframe are paired with a male from the dataframe at random such that all females are guaranteed to mate. If the number of females exceeds the number of males. If the female population is smaller than the male population, some males go without mates. If the number of females exceeds the number of males, some males are chosen at random mate multiply. After each female has been assigned a mate, the genotype of the mate is stored and the males are removed from the dataframe as they play no further role in the current time step.

5. Dispersal

After mating, a proportion κ of the females from each population in the landscape are selected at random to disperse. For each dispersing female, a dispersal angle

(θ, where $0 \leq \theta \leq 2\pi$) is drawn from a uniform distribution and a dispersal distance is drawn from an inverse Gaussian distribution, ($\mu = 0.4, \lambda^2 = 0.2$). A set of intracellular coordinates is also drawn for each dispersing female within their pre-dispersal cell/field. Using the intracellular coordinates, the dispersal angle, and dispersal distance, the post-dispersal cell/field is then calculated. For each dispersing female, dispersal is enacted by removing their row for the pre-dispersal dataframe and appending it to the dataframe for the post-dispersal field/cell.

6. Oviposition

Following the dispersal phase, adult females from the current time step are assumed to oviposit $2R_0$ eggs and die. The new offspring are stored in a new dataframe, with a unique dataframe for each cell/field in the landscape. The sex of each offspring is assigned using the sample function, with an equal probability of assigning an individual to male or female. The genotype of each offspring depends on the genotype of their mother and the male with which she mated. For each genotype combination, the potential genotypes and their expected frequencies were calculated, and these used to assign genotypes to offspring.

For instance, if the adult female genotype was *RSww* and the male genotype *SSLL*, the offspring produced can be either *RSwl* or *SSwL*, with equal probability. So the $2R_0$ offspring produced by such a mating would be assigned male or female with a 50:50 probability, and *RSwl* or *SSwL* with equal probability. The resulting dataframes of eggs, with sex and genotype, become the starting point for the next generation, beginning again with the density-independent mortality phase.

Summary

In implementing the model in this way in the codebase, the mathematical model presented in Chapter 2 is used to run individual-based simulations, with whole-numbered populations of pest insects that can go extinct and re-colonise and avoiding issues around what fraction of a mosquito represents an extant/extinct population. It also introduces stochasticity through the way in which females find mates, and in which offspring of each genotype are produced, which produces the large variation

between simulations apparent in the figures presented in Chapter 1. The final thing to note is that the order of events presented here is also likely to affect the dynamics, perhaps quite strongly, observed in the simulations. While I did not in the present work investigate the effect of the different events/stages, this model/codebase could easily be adapted to say, look at the effects of post-dispersal mating.

A.2: Supplementary figures for Chapter 2

Below are versions of figures presented in the main text with the results for the complete set of parameter combinations simulated. Note that, in Supp. Figs 3 - 6, a different colour scheme is used to enhance the legibility of the figures, but the line colours still represent the release ratios.

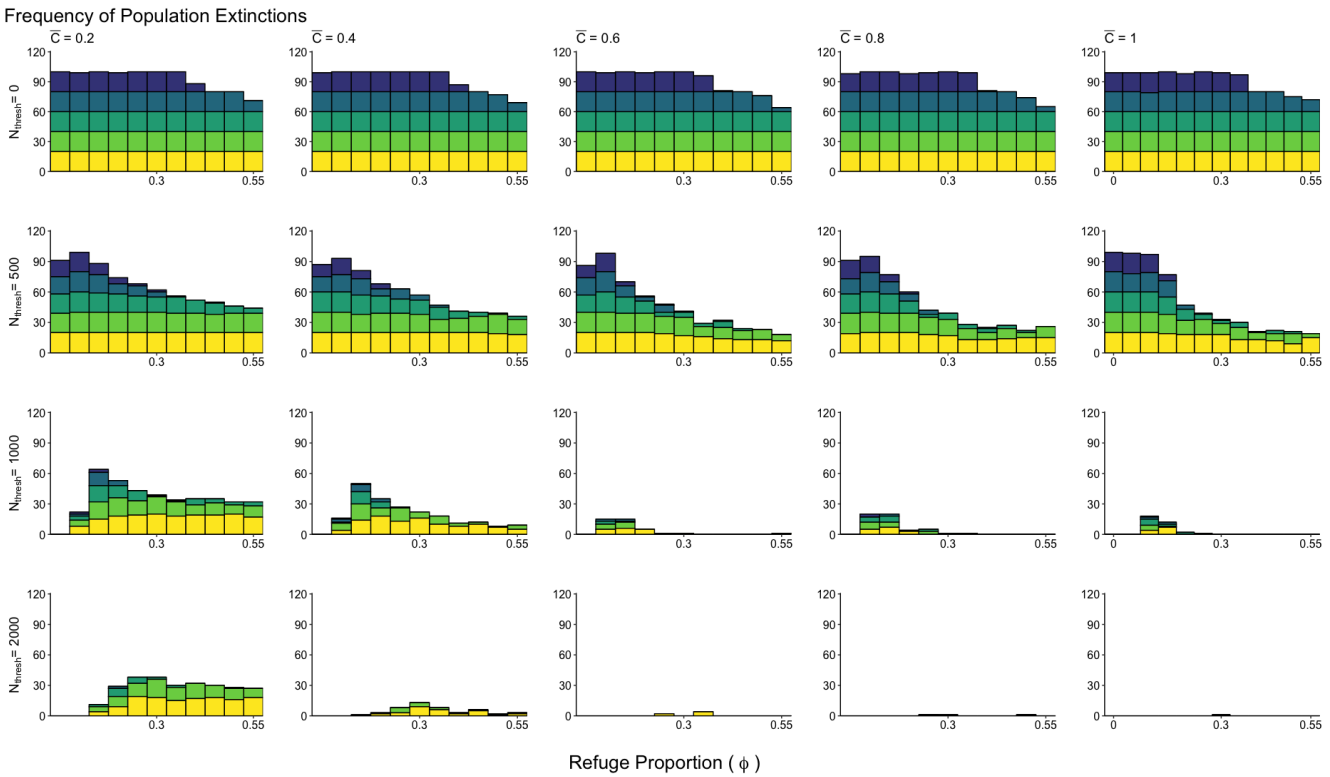


Figure A.1: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level pest population extinction. Individual plots depict the frequency of landscape-level population extinctions under the full combinatorial range of refuge proportions (ϕ), refuge compliances ($\bar{C}$), release thresholds (N_{thresh}), and release ratios (ρ) modelled. Each parameter combination was replicated 20 times on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

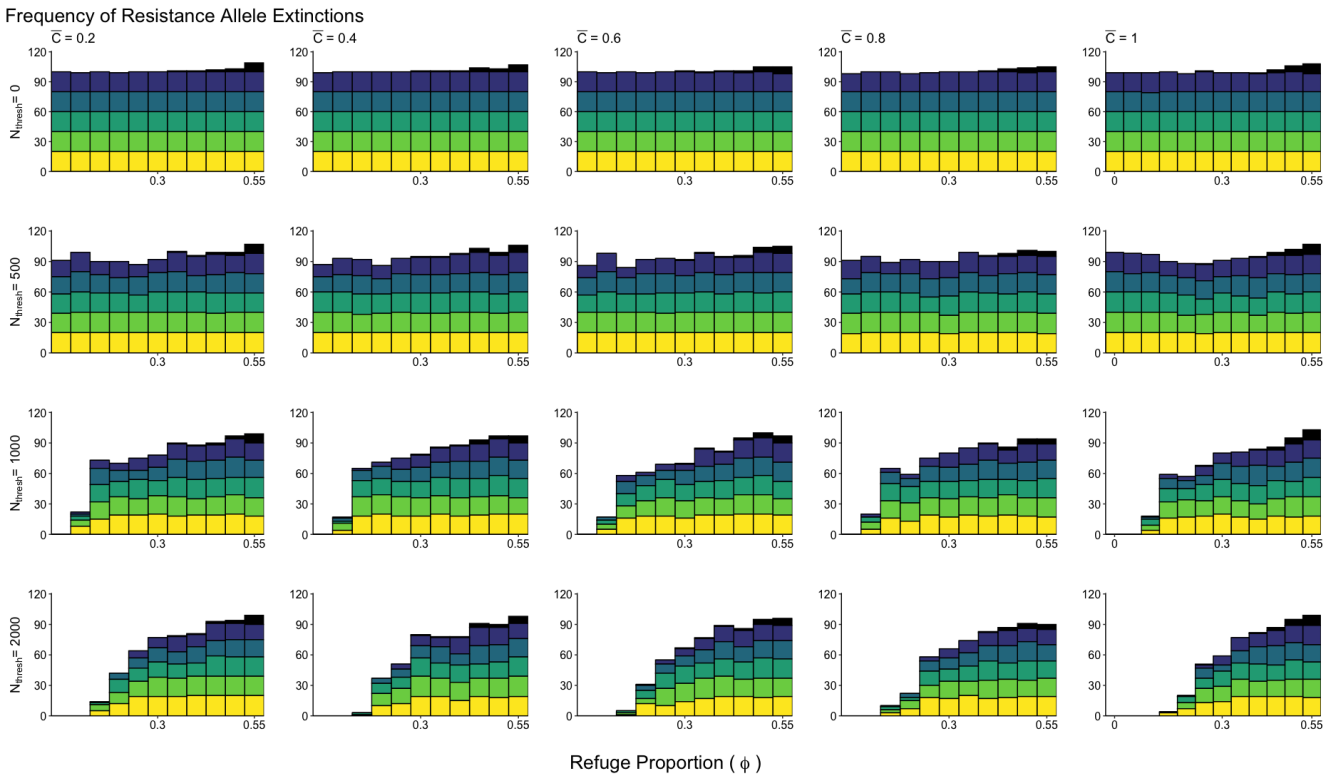


Figure A.2: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level resistance allele extinction. Individual plots depict the frequency of landscape-level resistance allele extinctions under the full combinatorial range of refuge proportions (ϕ), refuge compliances ($\bar{C}$), release thresholds (N_{thresh}), and release ratios (ρ) modelled. Each parameter combination was replicated 20 times on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

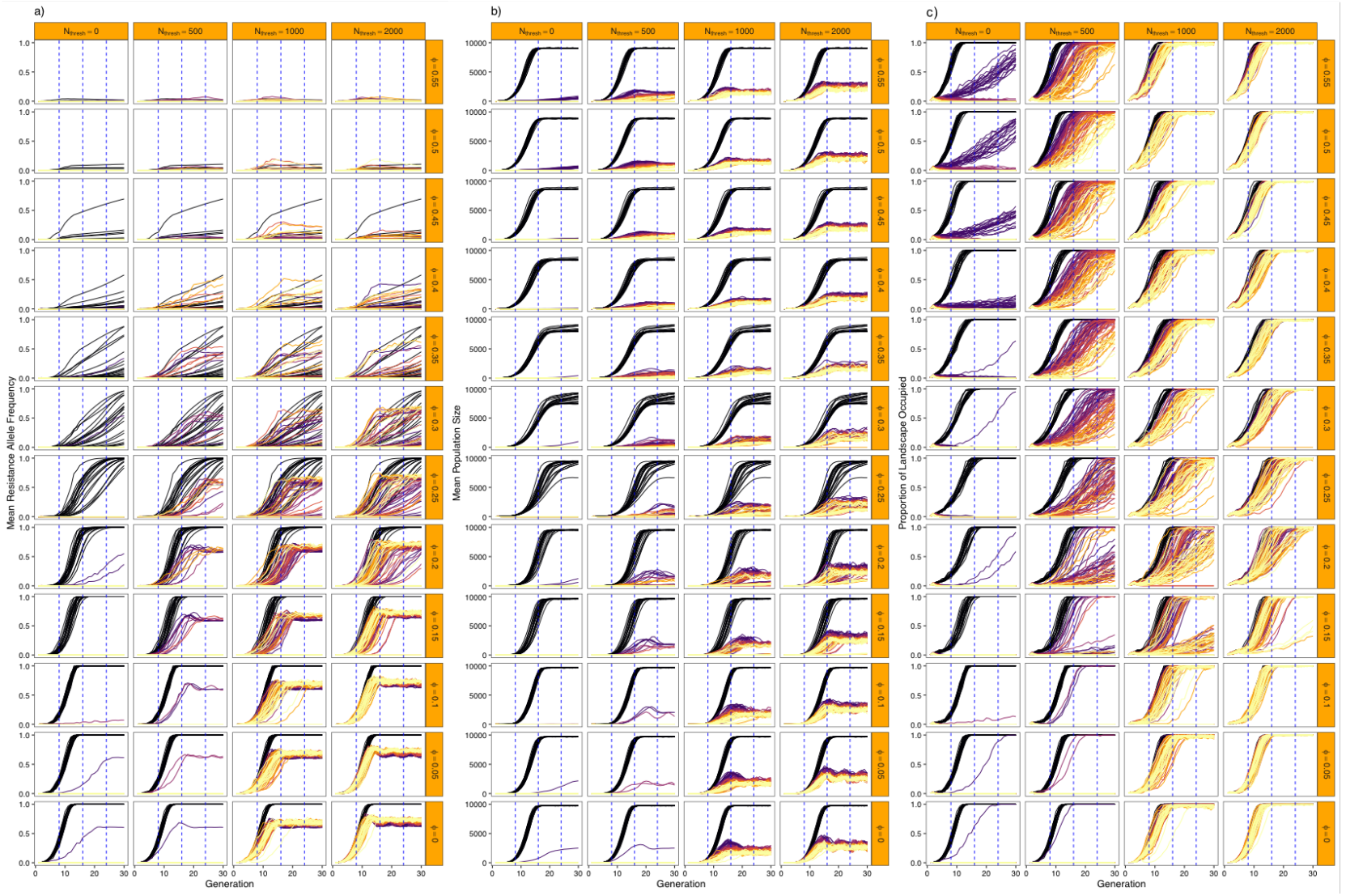


Figure A.3: Pest population and resistance allele dynamics under a range of refuge and transgenic insect release management strategies in fully-compliant agricultural landscapes. Individual panels show the dynamics of a) the across-field mean resistance allele frequency, b) the across-field mean population size, and c) the proportion of the landscape occupied. Plots within panels present the dynamics observed for the complete set of release threshold (N_{thresh}), release ratio (ρ), and refuge proportion (ϕ) combinations simulated in fully compliant landscapes ($\overline{C} = 1$). Line colour is used to represent the release ratio employed, with black lines showing the dynamics observed under refuge-only management strategies ($\rho = 0$). Blue dashed lines, denoting generations 8, 16 and 24, are included to aid visual comparison. 20 realisations were generated per combination of release threshold, release ratio and refuge proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

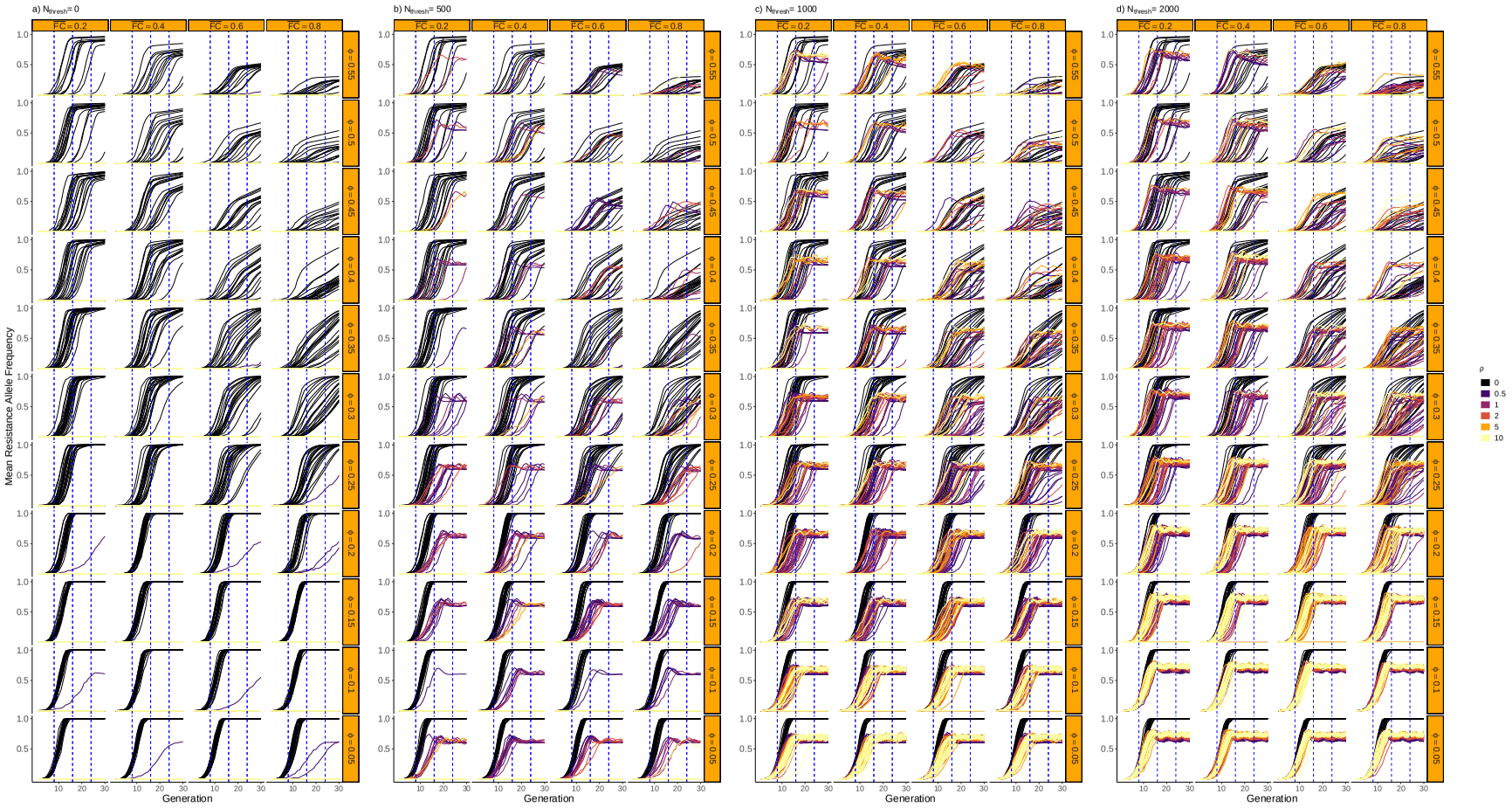


Figure A.4: Across-field mean resistance allele frequency dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels present the response of mean resistance allele frequency under a) area-wide releases ($N_{thresh} = 0$) and b)-d) localised releases ($N_{thresh} > 0$). Plots within panels present the dynamics observed for the complete set of release ratio (ρ) and refuge proportion (ϕ) combinations simulated in incompletely compliant landscapes ($\overline{C} < 1$). Each line represents the landscape-level mean resistance allele frequency for a single simulation run. Black lines represent the mean resistance allele dynamics observed under refuge-only management strategies ($\rho = 0$). Blue dashed lines, denoting generations 8, 16 and 24, are included to aid visual comparison. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

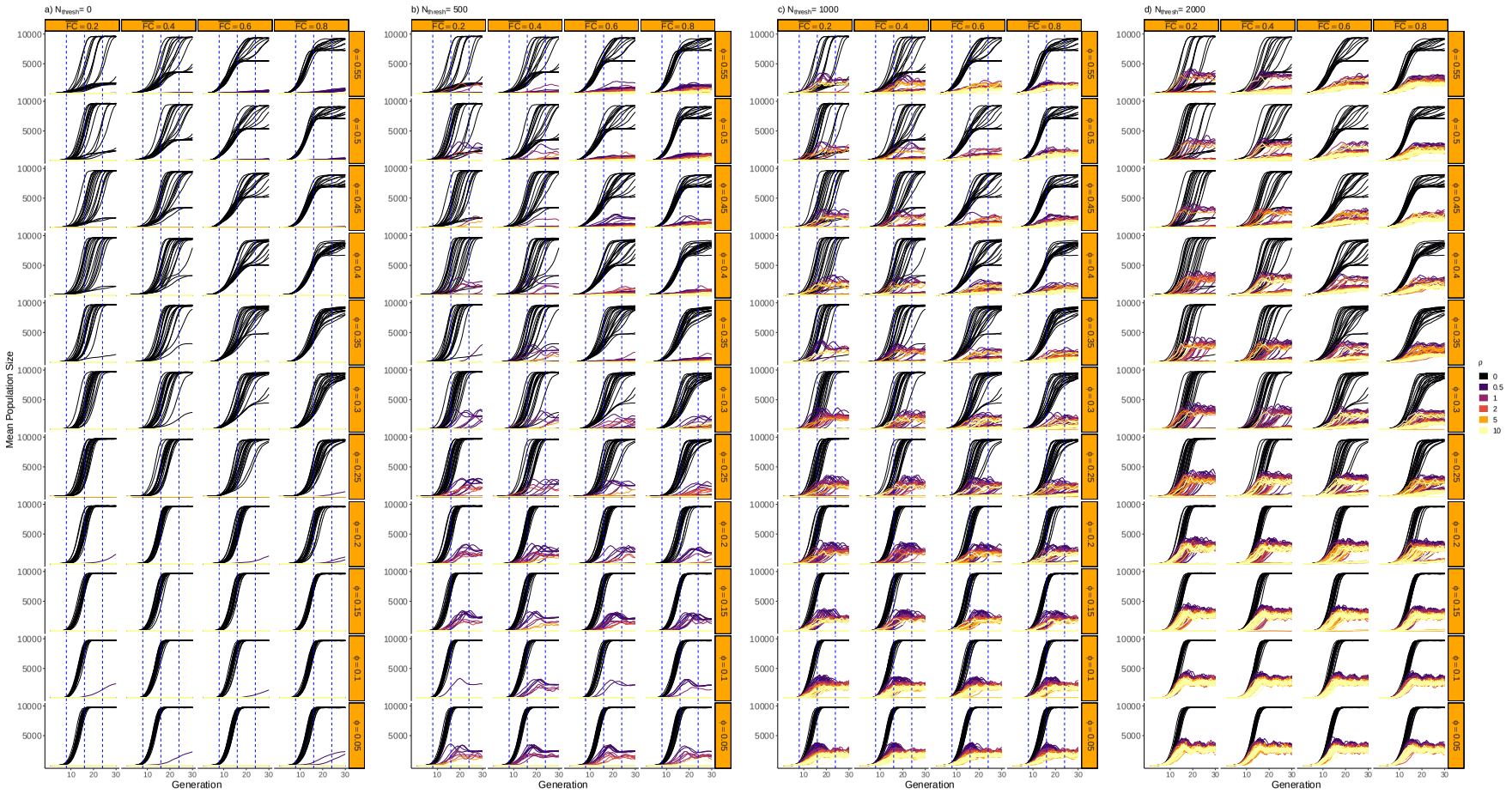


Figure A.5: Across-field mean population size dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels present the response of mean population size under a) area-wide releases ($N_{thresh} = 0$) and b)-d) localised releases ($N_{thresh} > 0$). Plots within panels present the dynamics observed for the complete set of release ratio (ρ) and refuge proportion (ϕ) combinations simulated in incompletely compliant landscapes ($\overline{C} < 1$). Each line represents the landscape-level mean resistance allele frequency for a single simulation run. Black lines represent the mean population size dynamics observed under refuge-only management strategies ($\rho = 0$). Blue dashed lines, denoting generations 8, 16 and 24, are included to aid visual comparison. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

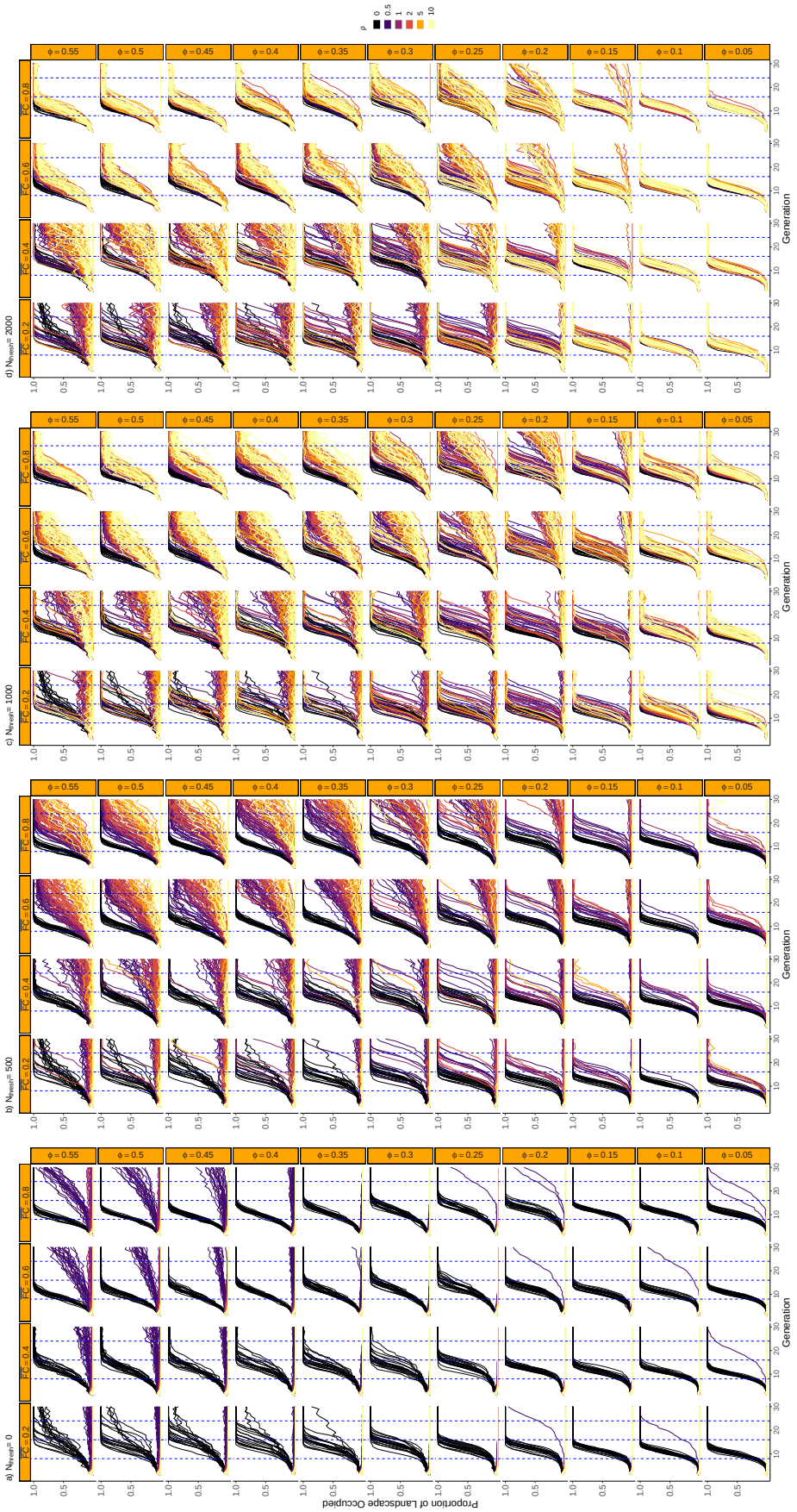


Figure A.6: Landscape colonisation dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels show the proportion of the fields in the landscape that are colonised through time under a) area-wide releases ($N_{thresh} = 0$) and b)-d) localised releases ($N_{thresh} > 0$). Plots within panels present the dynamics observed for the complete set of release ratio (ρ) and refuge proportion (ϕ) combinations simulated in incompletely compliant landscapes ($\overline{C} < 1$). Black lines show the dynamics observed under refuge-only management strategies ($\rho = 0$). Blue dashed lines, denoting generations 8, 16 and 24, are included to aid visual comparison. 20 realisations were generated per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

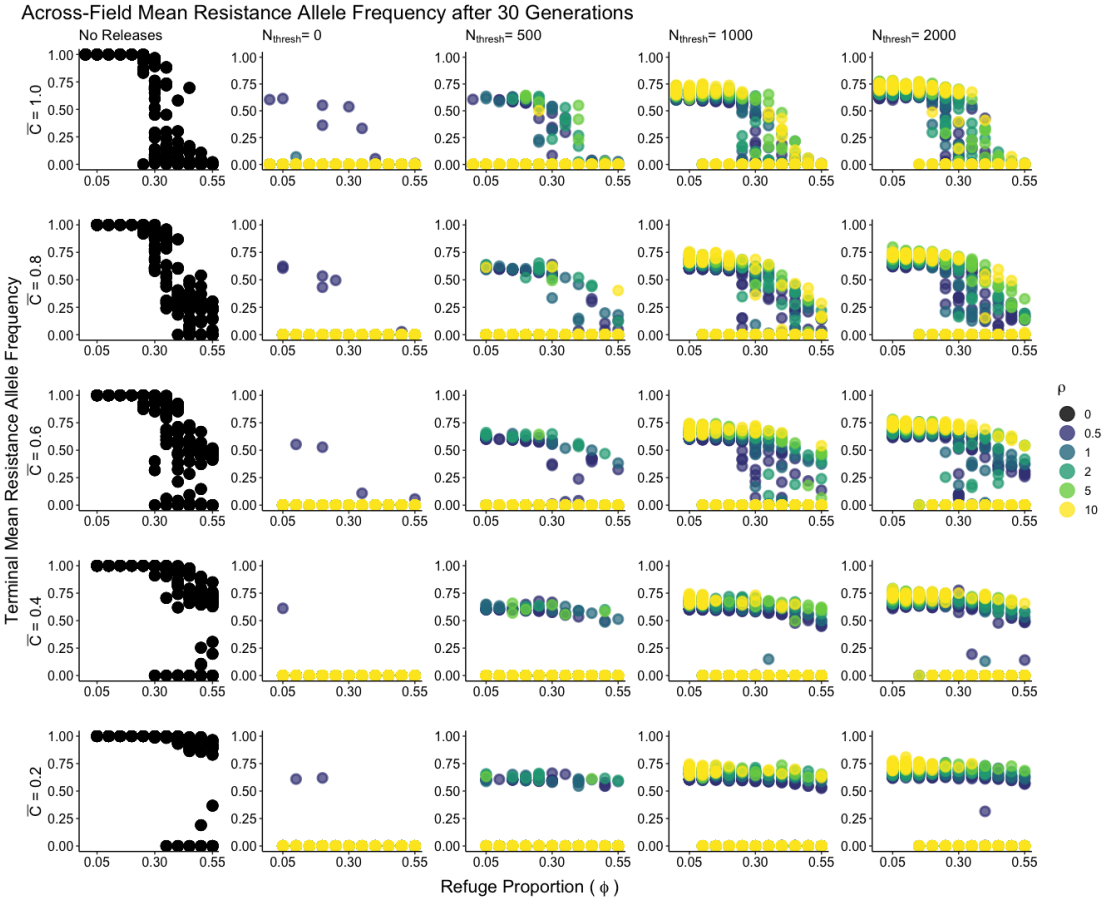


Figure A.7: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean resistance allele frequency. Individual panels present the across-field mean resistance allele frequencies after 30 generations for the full combinatorial range of refuge proportions (ϕ), refuge compliance proportions ($\bar{C}$), release thresholds (N_{thresh}), and release ratios (ρ) simulated. Each point represents a single model realisation, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

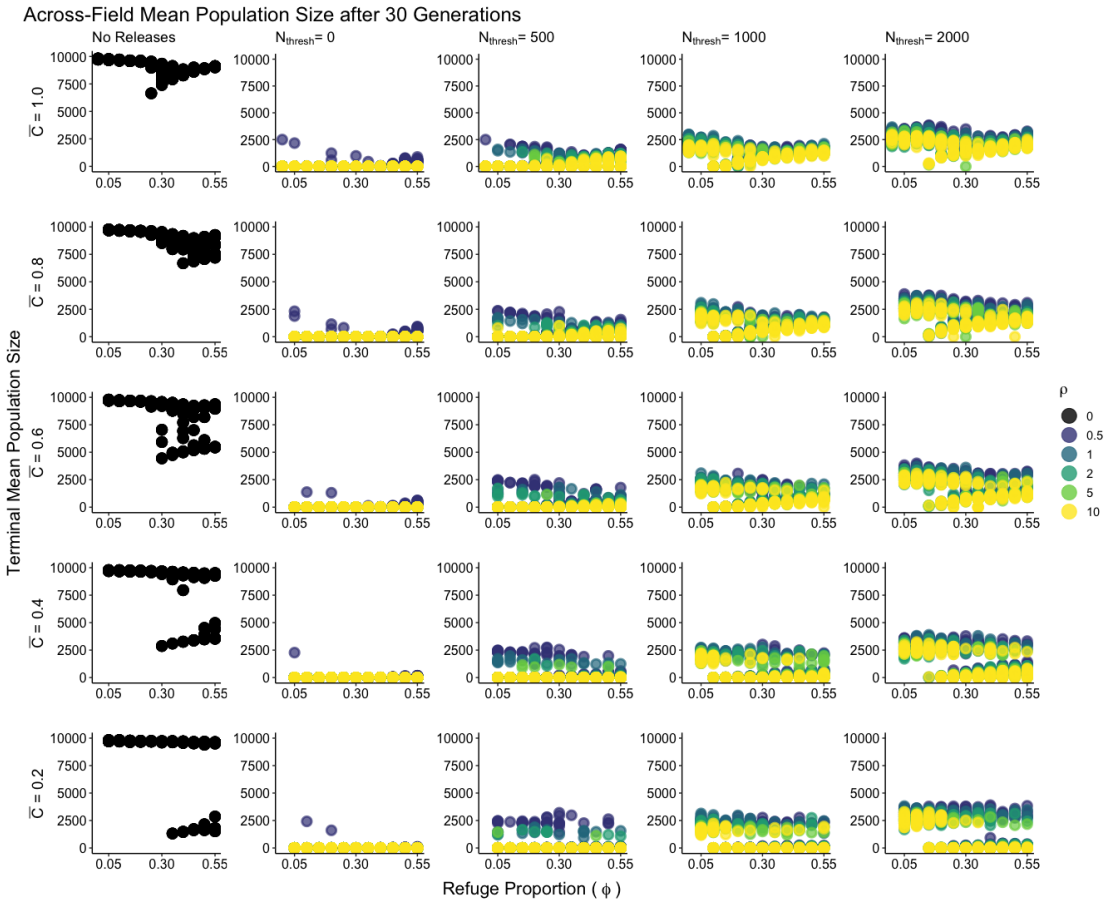


Figure A.8: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean population size. Individual panels present the across-field mean population size after 30 generations for the full combinatorial range of refuge proportions (ϕ), refuge compliance proportions ($\bar{C}$), release thresholds (N_{thresh}), and release ratios (ρ) simulated. Each point represents a single model realisation, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

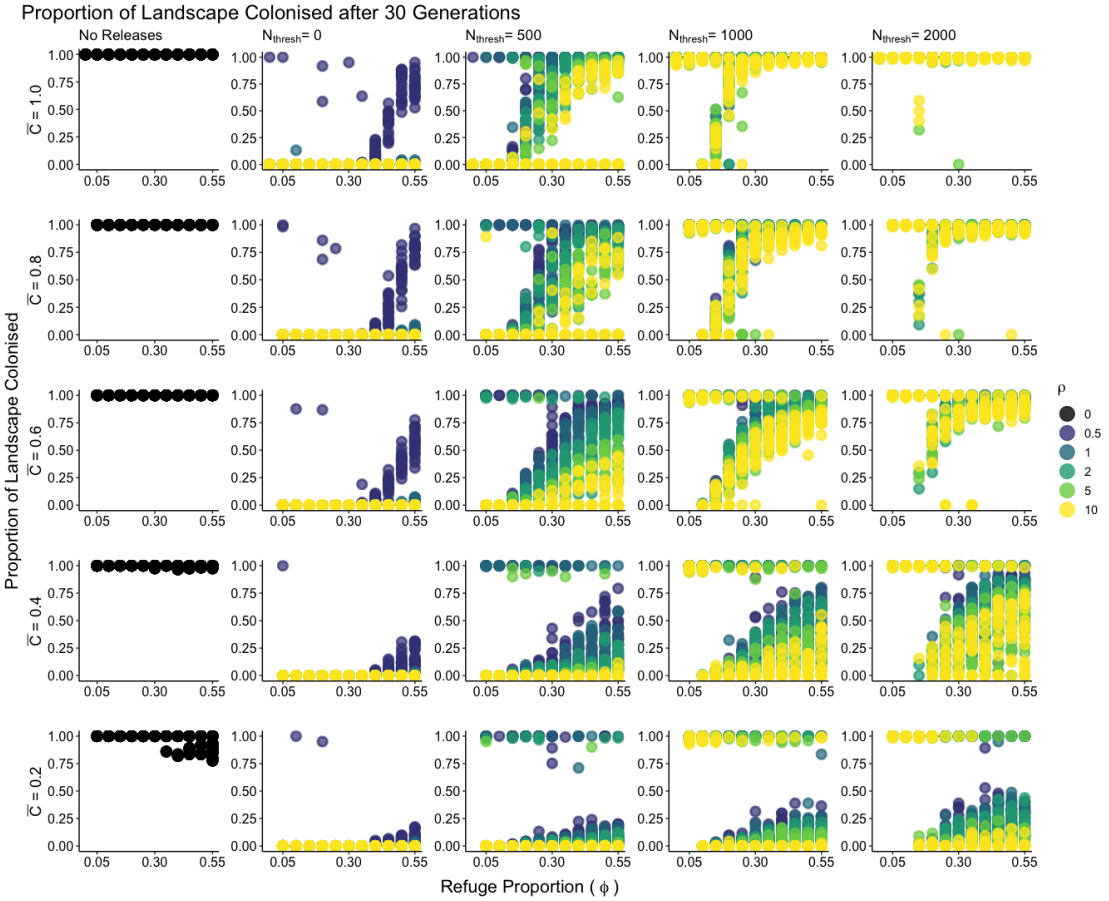


Figure A.9: The effect of refuge implementation and transgenic insect release regime on the terminal proportion of the landscape colonised. Individual panels present the proportion of the landscape colonised after 30 generations for the full combinatorial range of refuge proportions (ϕ), refuge compliance proportions ($\bar{C}$), release thresholds (N_{thresh}), and release ratios (ρ) simulated. Each point represents a single model realisation, with 20 realisations generated per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

B

Supplementary Material for Chapter 3

Contents

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B.1: Genotype-specific egg production

In each time step, the number of eggs laid is the total number of females in the egg-laying phase of the gonotrophic cycle multiplied by the number of eggs laid per female per gonotrophic cycle (ϵ). The number of eggs of each genotype is dependent on the genotypic composition of the egg-laying female population. The equations governing the number of eggs of each genotype laid on each day, given the number of females of genotype j that have mated with a male with genotype k ($A_\tau^{fjk}(t)$ for all combinations of j and k) in the ovipositing phase of the gonotrophic cycle ($p = \tau$) alive at the end of day t. In the equations below, $\Lambda^j(t)$ represents the number of eggs of genotype j laid on the t-th day, while $f_\tau^{j\cdot k}(t)$ gives the number of ovipositing females of genotype j that mated with males of genotype k. The number of eggs laid per ovipositing female is denoted ϵ^j. It is assumed that the two genetic loci are independently segregating and follow Mendelian patterns of inheritance. There are 81 combinations (45 unique) of parental genotypes, the complete set of which, along with their offspring proportions, are outlined in Supplementary Materials 2.

$$
\begin{aligned}
&\Lambda^{SSww}(t) = \\
&\epsilon^{SSww}\left(f_\tau^{SSww\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{SSww\cdot RSwL}(t)\right) \\
&+ \epsilon^{RSww}\left(\tfrac{1}{2}f_\tau^{RSww\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RSww\cdot RSwL}(t)\right) \\
&+ \epsilon^{SSwL}\left(\tfrac{1}{2}f_\tau^{SSwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{SSwL\cdot RSwL}(t)\right) \\
&+ \epsilon^{RSwL}\left(\tfrac{1}{4}f_\tau^{RSwL\cdot SSww}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot SSwL}(t) + \tfrac{1}{16}f_\tau^{RSwL\cdot RSwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RRww}(t) = \\
&\epsilon^{RSww}\left(\tfrac{1}{4}f_\tau^{RSww\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{RSww\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RRwL}(t)\right) \\
&+ \epsilon^{RRww}\left(\tfrac{1}{2}f_\tau^{RRww\cdot RSww}(t) + f_\tau^{RRww\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RRww\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot RRwL}(t)\right) \\
&+ \epsilon^{RSwL}\left(\tfrac{1}{8}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRww}(t) + \tfrac{1}{16}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RRwL}(t)\right) \\
&+ \epsilon^{RRwL}\left(\tfrac{1}{4}f_\tau^{RRwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{RRwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RRwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RSww}(t) = \\
&\epsilon^{SSww}\left(\tfrac{1}{2}f_\tau^{SSww\cdot RSww}(t) + f_\tau^{SSww\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{SSww\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot RRwL}(t)\right) \\
&+ \epsilon^{RSww}\Bigg(\tfrac{1}{2}f_\tau^{RSww\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot SSwL}(t) \\
&+ \tfrac{1}{4}f_\tau^{RSww\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RRwL}(t)\Bigg) \\
&+ \epsilon^{RRww}\left(f_\tau^{RRww\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RRww\cdot RSwL}(t)\right) \\
&+ \epsilon^{SSwL}\left(\tfrac{1}{4}f_\tau^{SSwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{SSwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RRwL}(t)\right) \\
&+ \epsilon^{RSwL}\Bigg(\tfrac{1}{4}f_\tau^{RSwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot SSwL}(t) \\
&+ \tfrac{1}{8}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RRwL}(t)\Bigg) \\
&+ \epsilon^{RRwL}\left(\tfrac{1}{2}f_\tau^{RRwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RRwL\cdot RSwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{SSwL}(t) = \\
&\epsilon^{SSww}\left(\tfrac{1}{2}f_\tau^{SSww\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{SSww\cdot RSwL}(t) + f_\tau^{SSww\cdot SSLL}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot RSLL}(t)\right) \\
&+ \epsilon^{RSww}\left(\tfrac{1}{4}f_\tau^{RSww\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RSww\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RSLL}(t)\right) \\
&+ \epsilon^{SSwL}\Bigg(\tfrac{1}{2}f_\tau^{SSwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSwL}(t) \\
&+ \tfrac{1}{2}f_\tau^{SSwL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSLL}(t)\Bigg) \\
&+ \epsilon^{RSwL}\Bigg(\tfrac{1}{4}f_\tau^{RSwL\cdot SSww}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSwL}(t) \\
&+ \tfrac{1}{4}f_\tau^{RSwL\cdot SSLL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSLL}(t)\Bigg) \\
&+ \epsilon^{SSLL}\left(f_\tau^{SSLL\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{SSLL\cdot RSwL}(t)\right) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{2}f_\tau^{RSLL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{8RSLL\cdot RSwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RSwL}(t) = \\
&\epsilon^{SSww}\left(\tfrac{1}{4}f_\tau^{SSww\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{SSww\cdot RSLL}(t) + f_\tau^{SSww\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSww}\left(\tfrac{1}{4}f_\tau^{RSww\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot SSLL}(t)\right. \\
&\left. + \tfrac{1}{2}f_\tau^{RSww\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRww}\left(\tfrac{1}{2}f_\tau^{RRww\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RRww\cdot RSwL}(t) + f_\tau^{RRww\cdot SSLL}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot RSLL}(t)\right) \\
&+ \epsilon^{SSwL}\left(\tfrac{1}{4}f_\tau^{SSwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot RRwL}(t)\right. \\
&\left. + \tfrac{1}{4}f_\tau^{SSwL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSwL}\left(\tfrac{1}{4}f_\tau^{RSwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot SSwL}(t)\right. \\
&\left. + \tfrac{1}{4}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RSLL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRwL}\left(\tfrac{1}{2}f_\tau^{RRwL\cdot SSww}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSwL}(t)\right. \\
&\left. + \tfrac{1}{2}f_\tau^{RRwL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSLL}(t)\right) \\
&+ \epsilon^{SSLL}\left(\tfrac{1}{2}f_\tau^{SSLL\cdot RSww}(t) + f_\tau^{SSLL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{SSLL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot RRwL}(t)\right) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{2}f_\tau^{RSLL\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot SSwL}(t)\right. \\
&\left. + \tfrac{1}{4}f_\tau^{RSLL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RRwL}(t)\right) \\
&+ \epsilon^{RRLL}\left(f_\tau^{RRLL\cdot SSww}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RRLL\cdot RSwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RRwL}(t) = \\
&\epsilon^{RSww}\left(\tfrac{1}{8}f_\tau^{RSww\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{RSww\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RSww\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRww}\left(\tfrac{1}{4}f_\tau^{RRww\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{RRww\cdot RSLL}(t) + f_\tau^{RRww\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSwL}\Bigg(\tfrac{1}{8}f_\tau^{RSwL\cdot RSww}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRwL}(t) \\
&+ \tfrac{1}{8}f_\tau^{RSwL\cdot RSLL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRLL}(t)\Bigg) \\
&+ \epsilon^{RRwL}\Bigg(\tfrac{1}{4}f_\tau^{RRwL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot RRwL}(t) \\
&+ \tfrac{1}{4}f_\tau^{RRwL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot RRLL}(t)\Bigg) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{4}f_\tau^{RSLL\cdot RSww}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot RRww}(t) + \tfrac{1}{8}f_\tau^{RSLL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RRwL}(t)\right) \\
&+ \epsilon^{RRLL}\left(\tfrac{1}{2}f_\tau^{RRLL\cdot RSww}(t) + f_\tau^{RRLL\cdot RRww}(t) + \tfrac{1}{4}f_\tau^{RRLL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot RRwL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{SSLL}(t) = \\
&\epsilon^{SSwL}\left(\tfrac{1}{4}f_\tau^{SSwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{SSwL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSLL}(t)\right) \\
&+ \epsilon^{RSwL}\left(\tfrac{1}{8}f_\tau^{RSwL\cdot SSwL}(t) + \tfrac{1}{16}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot SSLL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSLL}(t)\right) \\
&+ \epsilon^{SSLL}\left(\tfrac{1}{2}f_\tau^{SSLL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{SSLL\cdot RSwL}(t) + f_\tau^{SSLL\cdot SSLL}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot RSLL}(t)\right) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{4}f_\tau^{RSLL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RSLL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RSLL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RRLL}(t) = \\
&\epsilon^{RSwL}\left(\tfrac{1}{16}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RRwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSLL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRwL}\left(\tfrac{1}{8}f_\tau^{RRwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{8}f_\tau^{RSLL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRLL}\left(\tfrac{1}{4}f_\tau^{RRLL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot RSLL}(t) + f_\tau^{RRLL\cdot RRLL}(t)\right)
\end{aligned}
$$

$$
\begin{aligned}
&\Lambda^{RSLL}(t) = \\
&\epsilon^{SSwL}\left(\tfrac{1}{8}f_\tau^{SSwL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{SSwL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{SSwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSwL}\left(\tfrac{1}{8}f_\tau^{RSwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RSwL}(t) + \tfrac{1}{8}f_\tau^{RSwL\cdot RRwL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot SSLL}(t)\right. \\
&\left. + \tfrac{1}{4}f_\tau^{RSwL\cdot RSLL}(t) + \tfrac{1}{4}f_\tau^{RSwL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRwL}\left(\tfrac{1}{4}f_\tau^{RRwL\cdot SSwL}(t) + \tfrac{1}{8}f_\tau^{RRwL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{RRwL\cdot SSLL}(t) + \tfrac{1}{4}f_\tau^{RRwL\cdot RSLL}(t)\right) \\
&+ \epsilon^{SSLL}\left(\tfrac{1}{4}f_\tau^{SSLL\cdot RSwL}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{SSLL\cdot RSLL}(t) + f_\tau^{SSLL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RSLL}\left(\tfrac{1}{4}f_\tau^{RSLL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RSwL}(t) + \tfrac{1}{4}f_\tau^{RSLL\cdot RRwL}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot SSLL}(t)\right. \\
&\left. + \tfrac{1}{2}f_\tau^{RSLL\cdot RSLL}(t) + \tfrac{1}{2}f_\tau^{RSLL\cdot RRLL}(t)\right) \\
&+ \epsilon^{RRLL}\left(\tfrac{1}{2}f_\tau^{RRLL\cdot SSwL}(t) + \tfrac{1}{4}f_\tau^{RRLL\cdot RSwL}(t) + f_\tau^{RRLL\cdot SSLL}(t) + \tfrac{1}{2}f_\tau^{RRLL\cdot RSLL}(t)\right)
\end{aligned}
$$

Note that this system of equations can be adapted to model two combined insecticide based interventions (LLIN + IRS) by replacing the w/L locus with a second S/R locus.

B.2: Offspring genotype proportions for all unique parental genotype mating combinations

Outlined below are the offspring genotypes and their Mendelian ratios that arise from matings between the 81 unique combinations of adult male and adult female genotypes in the dilocus system. These offspring genotype frequencies form the basis for calculating the number of eggs of each genotype that are laid in each time step, Λ^j.

Female genotype, j, = SSww:

1) $A^{f.SSww.SSww} : J_1^{SSww}$

2) $A^{f.SSww.RSww} : \frac{1}{2}J_1^{SSww}$ & $\frac{1}{2}J_1^{RSww}$

3) $A^{f.SSww.RRww} : J_1^{RSww}$

4) $A^{f.SSww.SSwL} : \frac{1}{2}J_1^{SSww}$ & $\frac{1}{2}J_1^{SSwL}$

5) $A^{f.SSww.RSwL} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$

6) $A^{f.SSww.RRwL} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$

7) $A^{f.SSww.SSLL} : J_1^{SSwL}$

8) $A^{f.SSww.RSLL} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$

9) $A^{f.SSww.RRLL} : J_1^{RSwL}$

Female genotype, j, = RSww:

10) $A^{f.RSww.SSww} : \frac{1}{2}J_1^{SSww}$ & $\frac{1}{2}J_1^{RSww}$

11) $A^{f.RSww.RSww} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{2}J_1^{RSww}$ & $\frac{1}{4}J_1^{RRww}$

12) $A^{f.RSww.RRww} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RRww}$

13) $A^{f.RSww.SSwL} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$

14) $A^{f.RSww.RSwL} : \frac{1}{8}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{8}J_1^{RRww}$ & $\frac{1}{8}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{RRwL}$

15) $A^{f.RSww.RRwL} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

16) $A^{f.RSww.SSLL} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$

17) $A^{f.RSww.RSLL} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

18) $A^{f.RSww.RRLL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RRwL}$

Female genotype, j, = RRww:

19) $A^{f.RRww.SSww} : J_1^{RSww}$

20) $A^{f.RRww.RSww} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RRww}$

21) $A^{f.RRww.RRww} : J_1^{RRww}$

22) $A^{f.RRww.SSwL} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$

23) $A^{f.RRww.RSwL} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

24) $A^{f.RRww.RRwL} : \frac{1}{2}J_1^{RRww}$ & $\frac{1}{2}J_1^{RRwL}$

25) $A^{f.RRww.SSLL} : J_1^{RSwL}$

26) $A^{f.RRww.RSLL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RRwL}$

27) $A^{f.RRww.RRLL} : J_1^{RRwL}$

Female genotype, j, = SSwL:

28) $A^{f.SSwL.SSww} : \frac{1}{2}J_1^{SSww}$ & $\frac{1}{2}J_1^{SSwL}$

29) $A^{f.SSwL.RSww} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$

30) $A^{f.SSwL.RRww} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$

31) $A^{f.SSwL.SSwL} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{2}J_1^{SSwL}$ & $\frac{1}{4}J_1^{SSLL}$

32) $A^{f.SSwL.RSwL} : \frac{1}{8}J_1^{SSww}$ & $\frac{1}{8}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{SSLL}$ & $\frac{1}{8}J_1^{RSLL}$

33) $A^{f.SSwL.RRwL} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RSLL}$

34) $A^{f.SSwL.SSLL} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{SSLL}$

35) $A^{f.SSwL.RSLL} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$

36) $A^{f.SSwL.RRLL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RSLL}$

Female genotype, j, = RSwL:

37) $A^{f.RSwL.SSww} : \frac{1}{4}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$

38) $A^{f.RSwL.RSww} : \frac{1}{8}J_1^{SSww}$ & $\frac{1}{4}J_1^{RSww}$ & $\frac{1}{8}J_1^{RRww}$ & $\frac{1}{8}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{RRwL}$

39) $A^{f.RSwL.RRww} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

40) $A^{f.RSwL.SSwL} : \frac{1}{8}J_1^{SSww}$ & $\frac{1}{8}J_1^{RSww}$ & $\frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{SSLL}$ & $\frac{1}{8}J_1^{RSLL}$

41) $A^{f.RSwL.RSwL} : \frac{1}{16}J_1^{SSww}$ & $\frac{1}{8}J_1^{RSww}$ & $\frac{1}{16}J_1^{RRww}$ & $\frac{1}{8}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{RRwL}$ & $\frac{1}{16}J_1^{SSLL}$ & $\frac{1}{8}J_1^{RSLL}$ & $\frac{1}{16}J_1^{RRLL}$

42) $A^{f.RSwL.RRwL} : \frac{1}{8}J_1^{RSww}$ & $\frac{1}{8}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{8}J_1^{RSLL}$ & $\frac{1}{8}J_1^{RRLL}$

43) $A^{f.RSwL.SSLL} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$

44) $A^{f.RSwL.RSLL} : \frac{1}{8}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{RRwL}$ & $\frac{1}{8}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{8}J_1^{RRLL}$

45) $A^{f.RSwL.RRLL} : \frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{4}J_1^{RRLL}$

Female genotype, *j*, = RRwL:

46) $A^{f.RRwL.SSww} : \frac{1}{2}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$

47) $A^{f.RRwL.RSww} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{4}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

48) $A^{f.RRwL.RRww} : \frac{1}{2}J_1^{RRww}$ & $\frac{1}{2}J_1^{RRwL}$

49) $A^{f.RRwL.SSwL} : \frac{1}{4}J_1^{RSww}$ & $\frac{1}{2}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RSLL}$

50) $A^{f.RRwL.RSwL} : \frac{1}{8}J_1^{RSww}$ & $\frac{1}{8}J_1^{RRww}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{8}J_1^{RSLL}$ & $\frac{1}{8}J_1^{RRLL}$

51) $A^{f.RRwL.RRwL} : \frac{1}{4}J_1^{RRww}$ & $\frac{1}{2}J_1^{RRwL}$ & $\frac{1}{4}J_1^{RRLL}$

52) $A^{f.RRwL.SSLL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RSLL}$

53) $A^{f.RRwL.RSLL} : \frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{4}J_1^{RRLL}$

54) $A^{f.RRwL.RRLL} : \frac{1}{2}J_1^{RRwL}$ & $\frac{1}{2}J_1^{RRLL}$

Female genotype, *j*, = SSLL:

55) $A^{f.SSLL.SSww} : J_1^{SSwL}$

56) $A^{f.SSLL.RSww} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$

57) $A^{f.SSLL.RRww} : J_1^{RSwL}$

58) $A^{f.SSLL.SSwL} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{SSLL}$

59) $A^{f.SSLL.RSwL} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$

60) $A^{f.SSLL.RRwL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RSLL}$

61) $A^{f.SSLL.SSLL} : J_1^{SSLL}$

62) $A^{f.SSLL.RSLL} : \frac{1}{2}J_1^{SSLL}$ & $\frac{1}{2}J_1^{RSLL}$

63) $A^{f.SSLL.RRLL} : J_1^{RSLL}$

Female genotype, j, = RSLL:

64) $A^{f.RSLL.SSww} : \frac{1}{2}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$

65) $A^{f.RSLL.RSww} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{2}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$

66) $A^{f.RSLL.RRww} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RRwL}$

67) $A^{f.RSLL.SSwL} : \frac{1}{4}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$

68) $A^{f.RSLL.RSwL} : \frac{1}{8}J_1^{SSwL}$ & $\frac{1}{4}J_1^{RSwL}$ & $\frac{1}{8}J_1^{RRwL}$ & $\frac{1}{8}J_1^{SSLL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{8}J_1^{RRLL}$

69) $A^{f.RSLL.RRwL} : \frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{4}J_1^{RRLL}$

70) $A^{f.RSLL.SSLL} : \frac{1}{2}J_1^{SSLL}$ & $\frac{1}{2}J_1^{RSLL}$

71) $A^{f.RSLL.RSLL} : \frac{1}{4}J_1^{SSLL}$ & $\frac{1}{2}J_1^{RSLL}$ & $\frac{1}{4}J_1^{RRLL}$

72) $A^{f.RSLL.RRLL} : \frac{1}{2}J_1^{RSLL}$ & $\frac{1}{2}J_1^{RRLL}$

Female genotype, j, = RRLL:

73) $A^{f.RRLL.SSww} : J_1^{RSwL}$

74) $A^{f.RRLL.RSww} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RRwL}$

75) $A^{f.RRLL.RRww} : J_1^{RRwL}$

76) $A^{f.RRLL.SSwL} : \frac{1}{2}J_1^{RSwL}$ & $\frac{1}{2}J_1^{RSLL}$

77) $A^{f.RRLL.RSwL} : \frac{1}{4}J_1^{RSwL}$ & $\frac{1}{4}J_1^{RRwL}$ & $\frac{1}{4}J_1^{RSLL}$ & $\frac{1}{4}J_1^{RRLL}$

78) $A^{f.RRLL.RRwL} : \frac{1}{2}J_1^{RRwL}$ & $\frac{1}{2}J_1^{RRLL}$

79) $A^{f.RRLL.SSLL} : J_1^{RSLL}$

80) $A^{f.RRLL.RSLL} : \frac{1}{2}J_1^{RSLL}$ & $\frac{1}{2}J_1^{RRLL}$

81) $A^{f.RRLL.RRLL} : J_1^{RRLL}$

B.3: Supplementary figures

This section contains figures supplementary to those presented in the main text. Consistent with the figures presented in the main text, all simulations in the supplementary figures presented here were initially run to equilibrium in the absence of any control intervention, initiated with an initial resistance allele frequency, and then run for a 5-year simulation period. Unless otherwise specified, the simulations presented in the figures were run using the default parameter values given in Table 3.1 and Table 3.2.

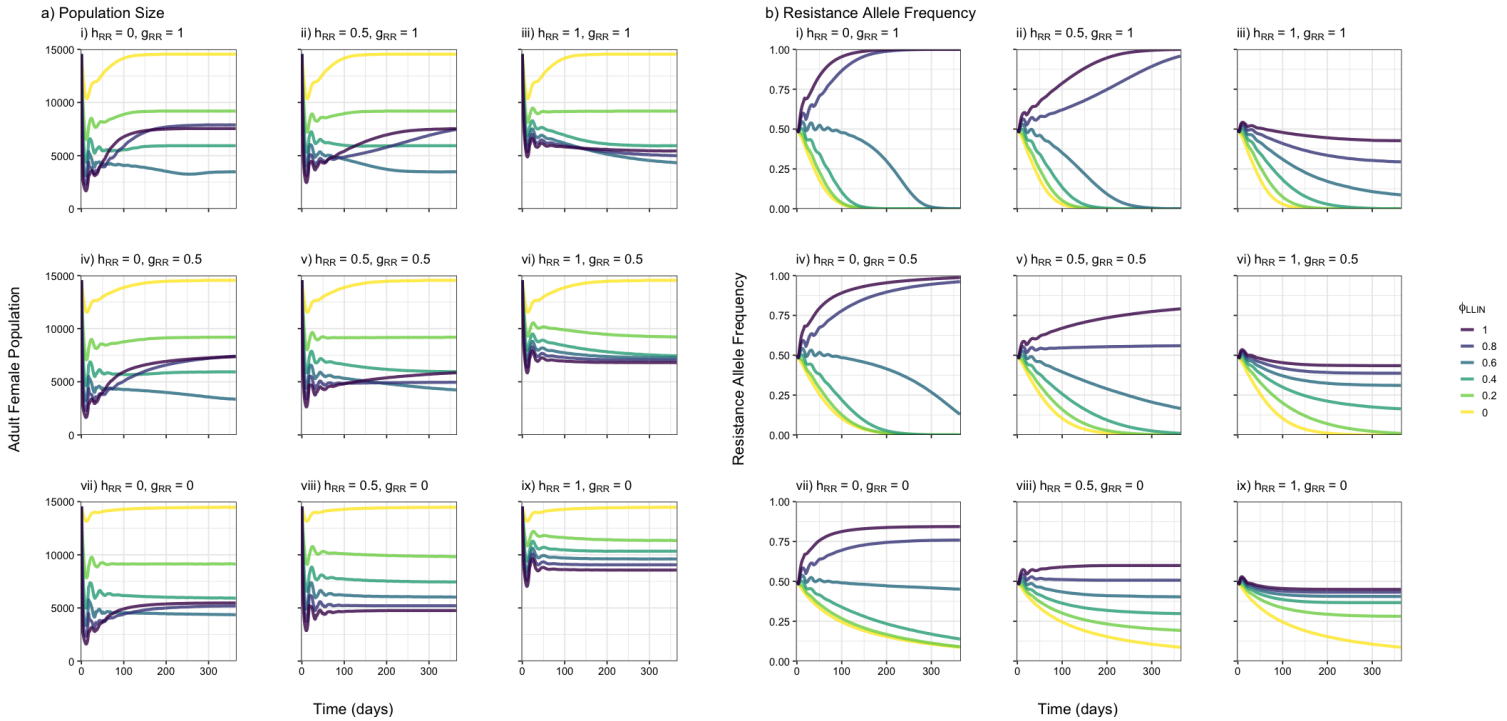


Figure B.1: The effect of exposure probability, the dominance of resistance, and the dominance of costs of resistance on population and resistance management under LLIN-only strategies when resistance is initially rare. Individual panels depict the a) adult female population and b) resistance allele dynamics observed under a range of LLIN-exposure probabilities (ϕ_{LLIN}, coloured lines) for a given combination of resistance dominance (h_{RR}) and dominance of costs of resistance (g_{RR}). The bottom left panels (*vii*) represent the case when both resistance and its costs are recessive ($h_{RR} = 0$, $g_{RR} = 0$), while the top right panel (*iii*) depicts the case when both are dominant ($h_{RR} = 1$, $g_{RR} = 1$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$.

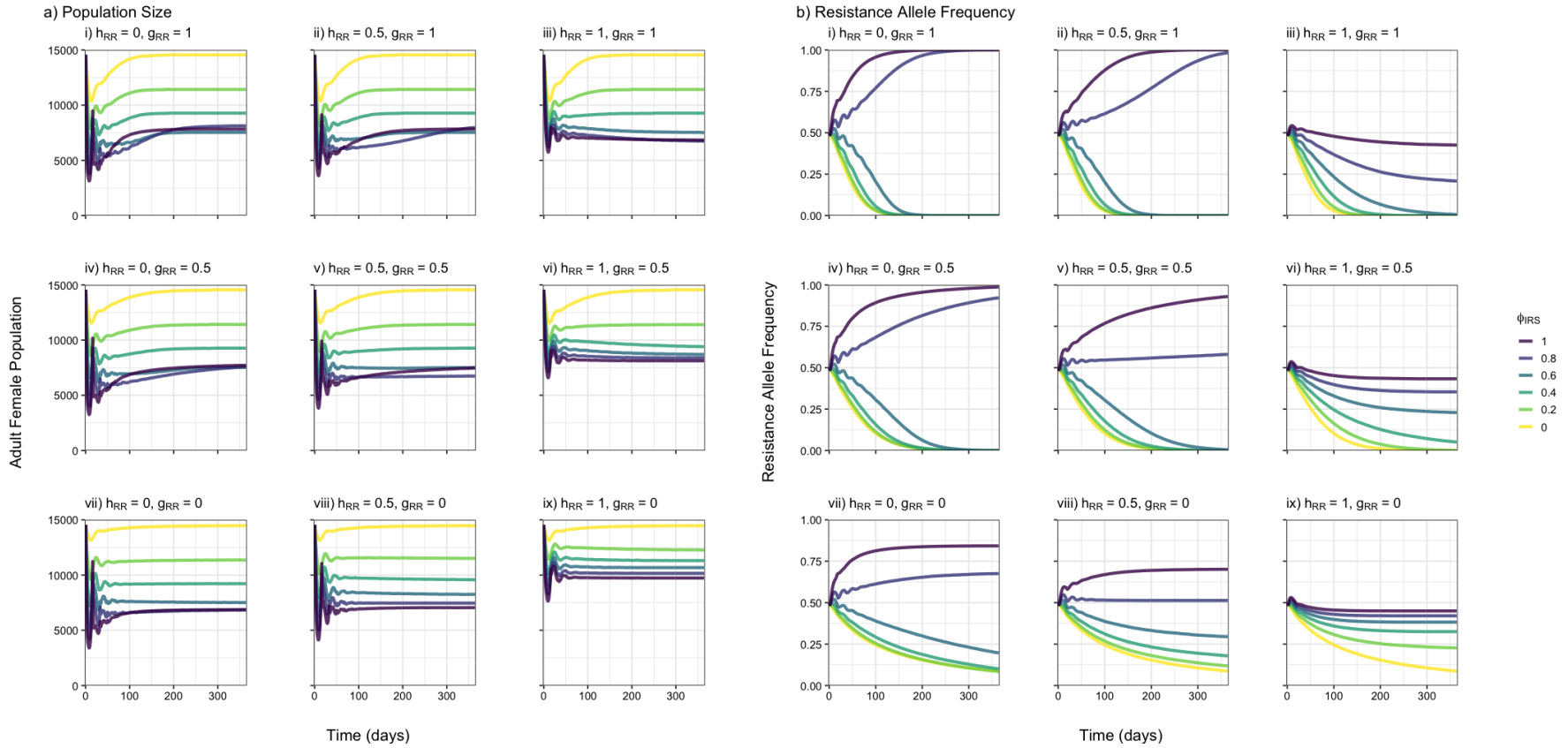


Figure B.2: The effect of exposure probability, the dominance of resistance, and the dominance of costs of resistance on population and resistance management under IRS-only strategies when resistance is initially rare. Individual panels depict the a) adult female population and b) resistance allele dynamics observed under a range of LLIN-exposure probabilities (ϕ_{IRS}, coloured lines) for a given combination of resistance dominance (h_{RR}) and dominance of costs of resistance (g_{RR}). The bottom left panels (*vii*) represent the case when both resistance and its costs are recessive ($h_{RR} = 0$, $g_{RR} = 0$), while the top right panel (*iii*) depicts the case when both are dominant ($h_{RR} = 1$, $g_{RR} = 1$). All simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$, $c_{RR} = 0.05$.

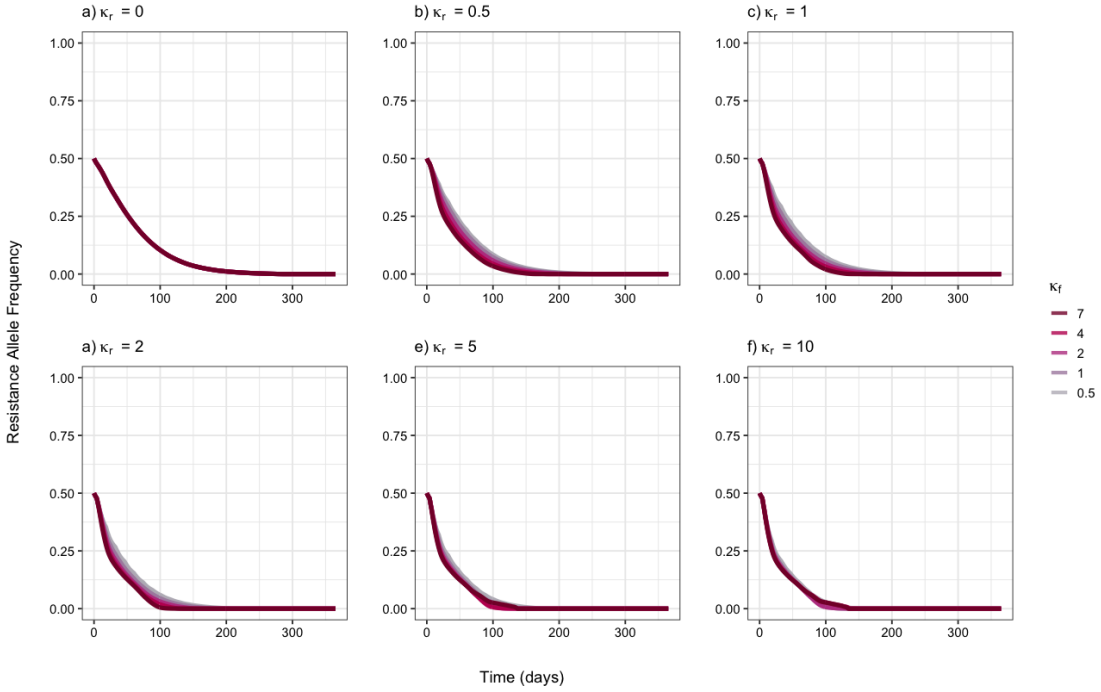


Figure B.3: The effect of release strategy on the resistance allele frequency. Each panel depicts the resistance allele dynamics under a different release ratio (κ_r), with panel a) showing the dynamics in the absence of releases, and individual lines depict release frequencies from fortnightly ($\kappa_f = 0.5$) to daily ($\kappa_f = 7$).

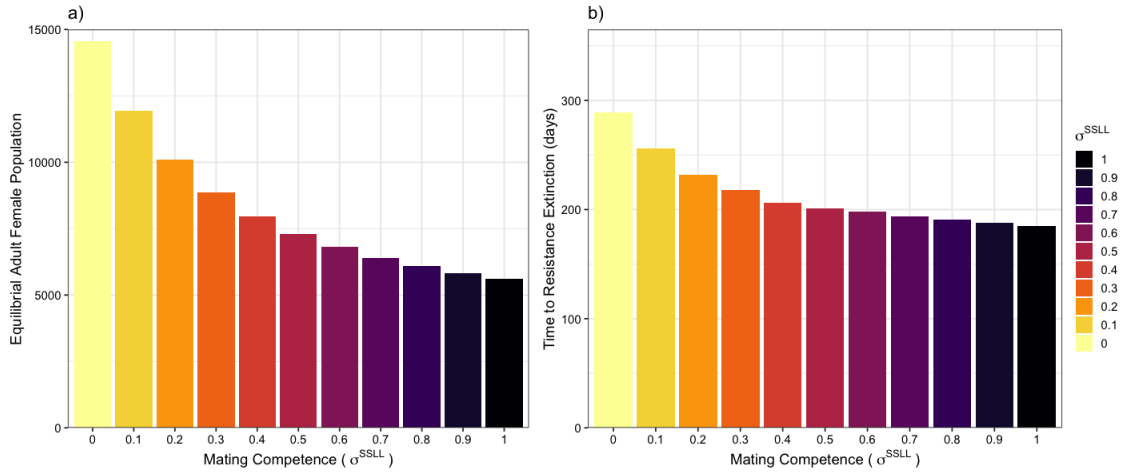


Figure B.4: The effect of transgenic male mating competence on the equilibrial adult female population size and the resistance elimination time. Panel a) depicts the equilibrial adult female population size under different assumptions of transgenic male mating competence (σ^{SSLL}). Panel b) shows the effect of transgenic male mating competence on the time (days) taken to drive resistance to extinction.

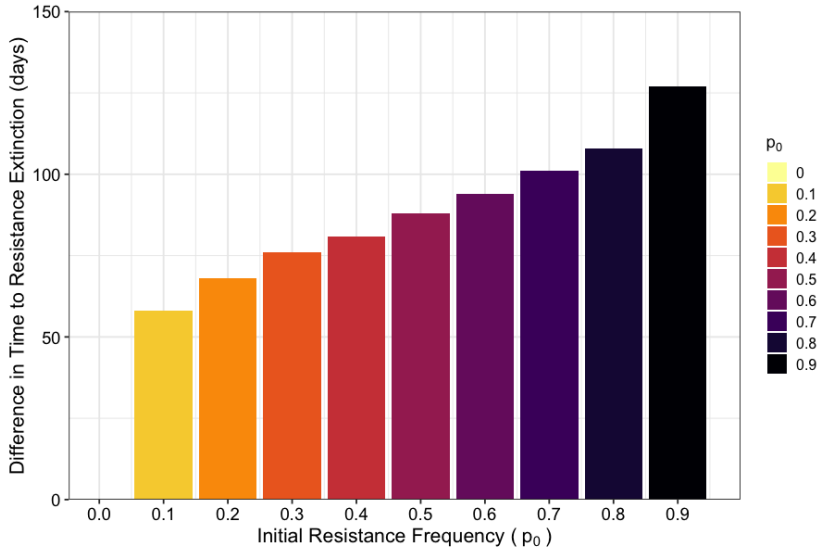


Figure B.5: The additional time (days) required for resistance to be removed from the population under a no-intervention strategy relative to the default release-only strategy. Each bar represents the difference, in number days, between the time taken to achieve resistance extinction under the default release-only ($\kappa_f = 2, \kappa_r = 1$) and no-intervention strategies.

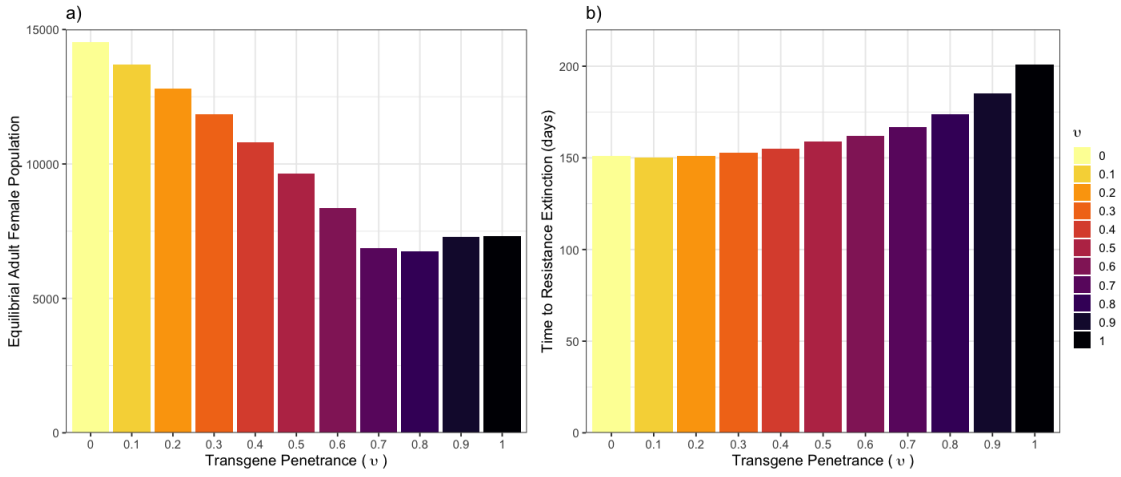


Figure B.6: The effect of transgene penetrance on the a) equilibrial adult female population size and b) number of days required to eliminate resistance. Panel a) depicts the equilibrial number of adult females in the population and panel b) gives the number of days required to achieve extinction under a release-only management programme in which transgenic males were released biweekly ($\kappa_f = 2$) at a ratio of 1:1 to the pre-intervention equilibrial male density ($\kappa_r = 1$). Each line represents the dynamics observed under different assumptions of transgene penetrance (υ).

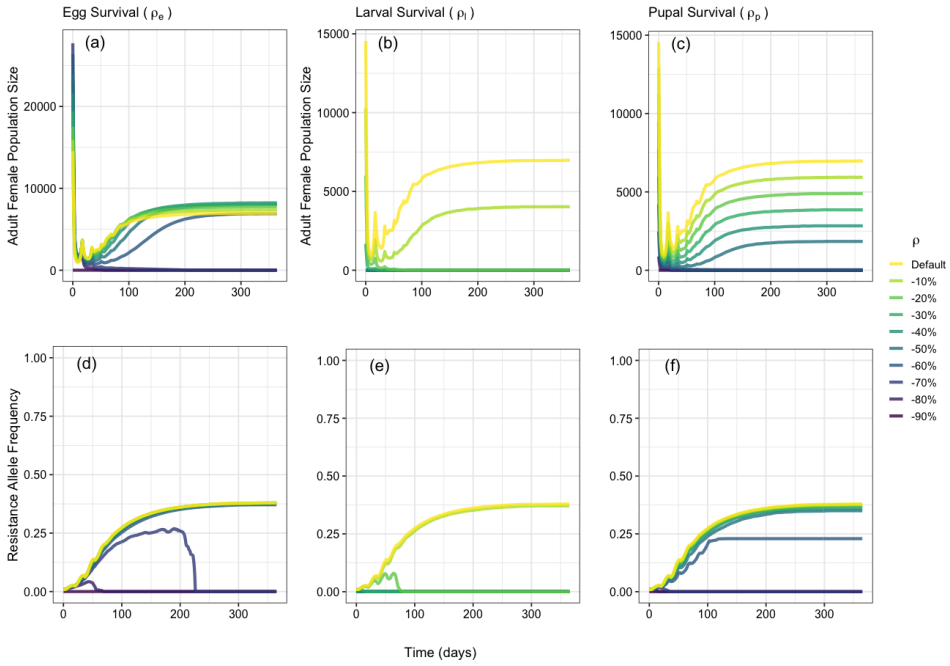


Figure B.7: The effect of juvenile stage daily survival probabilities on the population dynamics and resistance evolution under LLIN-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each juvenile stage ($\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

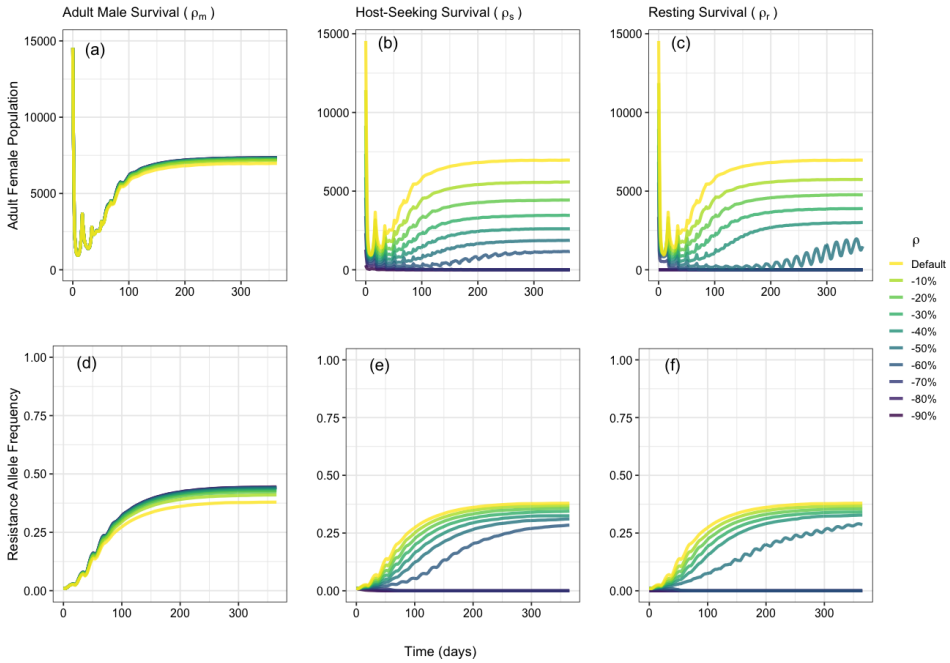


Figure B.8: The effect of adult daily survival probabilities on the population dynamics and resistance evolution under LLIN-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each adult stage ($\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

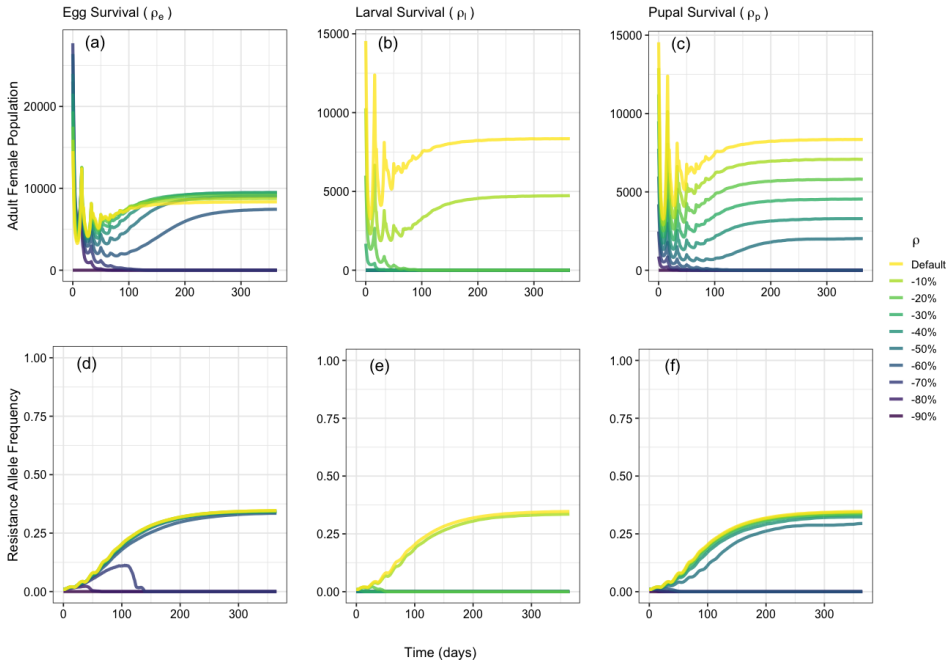


Figure B.9: The effect of juvenile stage daily survival probabilities on the population dynamics and resistance evolution under IRS-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each juvenile stage ($\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

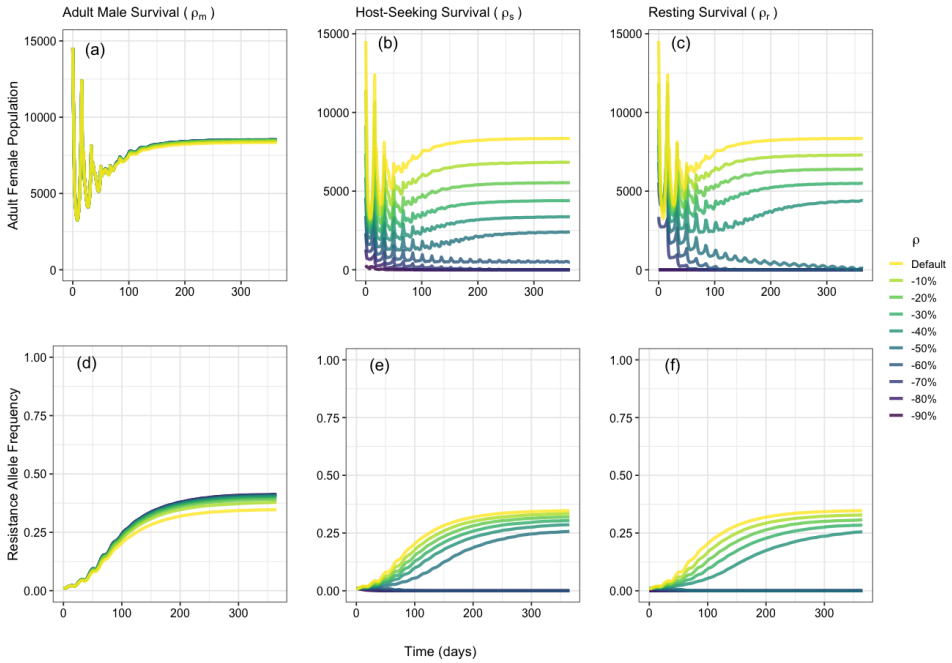


Figure B.10: The effect of adult stage daily survival probabilities on the population dynamics and resistance evolution under IRS-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each adult stage ($\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

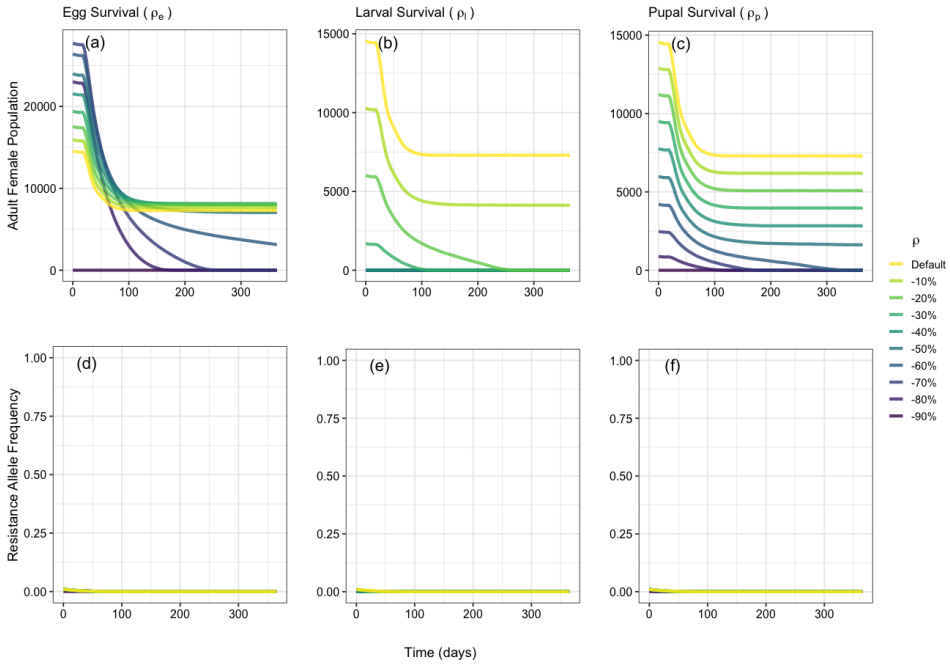


Figure B.11: The effect of adult stage daily survival probabilities on the population dynamics and resistance evolution under releases-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each adult stage ($\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

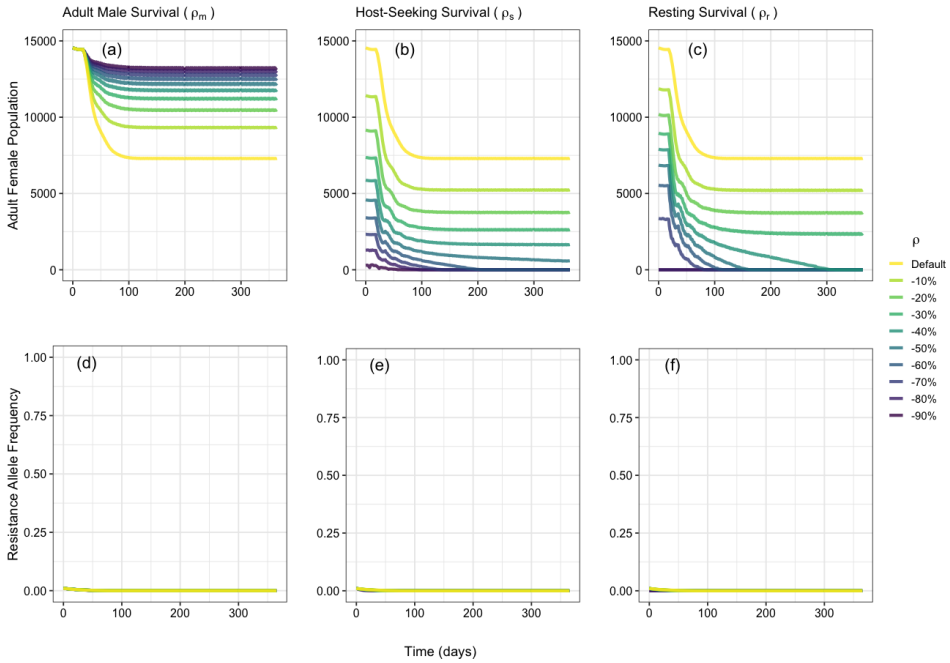


Figure B.12: The effect of adult stage daily survival probabilities on the population dynamics and resistance evolution under releases-only control. Yellow lines represent the dynamics observed under the default daily survival probability for each adult stage ($\rho_m = 0.9$, $\rho_s = 0.9$, $\rho_r = 0.9$). The remaining colours depict the effect of scaling the default value by -10% to -90%.

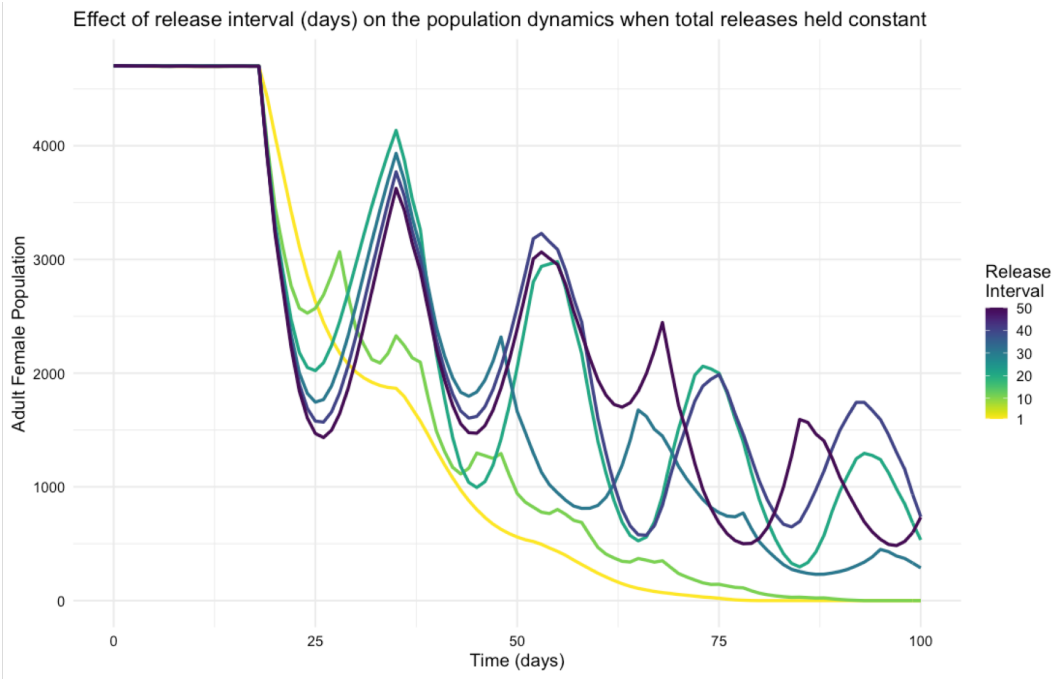


Figure B.13: Smaller, more frequent releases provide superior suppression than larger, less frequent releases. Each coloured line represents a release interval, in days, between successive releases. The number of transgenic males introduced into the target population per release increased with the release interval and was calculated such that each strategy released an equal number of males over the simulated period. All simulation were run using the following base parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.777$, $\rho_p = 0.975$, $\rho_m = 0.693$, $\rho_s = 0.691$, $\rho_r = 0.691$, $\alpha = 1$ x 10^{-6}. $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $p_0 = 0$, $c_{RR} = 0.05$, $\phi_i = 0.8$, $g_{RR} = 0.5$, $h_{RR} = 0.5$, $\upsilon = 1$, $c_{LL} = 0.5$, $g_{LL} = 0.5$.

B.4: Effect of daily survival probabilities (ρ^{SSww})

Barbosa et al. (2018) showed that the adult female population size, and therefore transmission potential, was sensitive to the stage-specific daily survival probabilities (ρ^{SSww}), particularly during the larval, pupal and adult female stages. Estimates in the literature vary widely between studies and are sensitive to environmental factors (e.g. temperature, precipitation) and species. These estimates are typically generated from laboratory-based cage experiments under fixed environmental conditions likely to be more forgiving than those to which they are exposed naturally. Given this variability, when selecting values for these parameters we elected for conservatively high estimates ($\rho_e^{SSww} = 0.895, \rho_l^{SSww} = 0.972, \rho_p^{SSww} = 0.975, \rho_m^{SSww} = 0.9, \rho_s^{SSww} = 0.9, \rho_r^{SSww} = 0.9$) from laboratory studies of *Anopheles stephensi* to model the efficacy of control-interventions under what could be considered a 'worst-case' assumption of high background survivorship.

We assessed the sensitivity of the population and resistance allele dynamics to the daily survival probability of each stage by running simulations in which these probabilities were varied while all other parameters were fixed at their default values. Simulations were run for a 2-year burn-in period in the absence of an intervention to determine the effect of these parameters on the equilibrial adult female population size (in the absence of resistance, $p_0 = 0$) under the no-intervention scenario ($\phi_{LLIN} = 0, \phi_{IRS} = 0, \kappa_f = 0$). We then assessed their effect under each single-intervention strategy. Rare initial resistance was added to each equilibrial population and simulations were run for each intervention under their default settings: LLIN/IRS-only ($\phi_i = 0.8, \mu_i^{SSww} = 0.01, \mu_i^{RRww} = 0.9, h_{RR} = 0.5, c_{RR} = 0.05, g_{RR} = 0.5, p_0 = 0.01$) and release-only ($\kappa_f = 2, \kappa_r = 1, \mu_{LL} = 0, \upsilon = 1, c_{LL} = 0.5, g_{LL} = 0.5$).

Figures B.7-12 depict the population and resistance allele dynamics observed under the default parameterisations for each single-intervention approach when the daily survival probability of each life-history stage is reduced sequentially from its baseline value (see Table 3.1) to 10% of that value, in 10% increments. This approach

was taken to facilitate comparison between changes in parameter with different initial values. The effect of reductions in the daily survival probabilities on the equilibrial adult female population size in the absence of any control interventions can be observed in Figs B.11-12.

Effect of reducing daily survival rates in the absence of control interventions

With the exception of the egg and adult male stages, reducing stage-specific daily survival probabilities (ρ_{-}^{SSww}) when no control interventions were applied decreased the equilibrial adult female population size (Fig B.12-13). Decreasing egg daily survival (ρ_e^{SSww}) by up to 70% of the default value increased the adult female population size (Fig. B.11a), while decreasing the daily survival of adult males had no effect (Fig. B.12a). The equilibrial adult female population size was most sensitive to reductions in larval daily survival (ρ_l^{SSww}); reducing ρ_l^{SSww} by more than 30% yielded non-viable populations, whereas the population was resilient to 60% reductions to the daily survival of pupae ρ_p^{SSww} and host-seeking females ρ_s^{SSww}, and a 50% reduction to resting female ρ_r^{SSww} survival.

Effect of reducing daily survival rates under insecticide-based interventions (LLIN-only/IRS-only)

Implementing LLIN-only or IRS-only strategies with the default exposure probability ($\phi_i = 0.8$) increased the minimum daily survival probability required to eliminate the mosquito population in all but the adult male stage (Fig. B.7-10). Indeed, reducing adult male daily survival had little effect under either strategy, producing only marginal increases in the equilibrial resistance allele (Fig. B.8d, Fig. B.10d) and adult female population size (Fig. B.8a, Fig. B.10a). Where reducing daily survival did not induce population extinction under the insecticide-based strategies, decreasing daily survival both increased the time taken for the population and resistance allele frequencies to stabilise, and decreased their equilibrial levels. The only exception to this pattern was observed in the egg-phase, for which reductions in daily survival increased both the terminal resistance allele frequency

and number of adult females (Fig. B.8, Fig. B.10). While LLINs generally provided greater population suppression for equivalent daily survival probabilities when the population was not eliminated, the IRS-only strategy increased the range of daily survival probabilities in which extinction was achieved for the pupal, host-seeking, and resting stages.

Effect of reducing daily survival rates under genetic pest management (release-only)

The baseline implementation of transgenic insect releases ($\kappa_f = 2, \kappa_r = 1$) prevented resistance from evolving ($p_0 = 0.01$) for all values of daily survival probability simulated across all life-stages (Figs B.11-12). Reductions in the daily survival probabilities of all but the adult male stage (ρ_m^{SSww}) introduced scenarios in which the default release strategy eliminated the mosquito population. This effect was most notable for host-seeking adult female daily survival (ρ_s^{SSww}); in the absence of control interventions even reductions of 90% yielded a viable (if tiny) population, whereas reductions greater than 50% enabled releases to eliminate the population (Fig. B.12b). Where population eradication was not achieved, reductions in the daily survival probabilities of all but the egg stage enhanced the suppressive power of the default release-only strategy, reducing the equilibrial adult female population size. Finally, while reducing the daily survival of adult males had little effect on the equilibrial adult female population size under LLIN-only (Fig. B.8a) or IRS-only (Fig. B.10a) control, the suppressive effect of the default release only-strategy declined non-linearly with adult male daily survival (Fig. B.12a).

C

Supplementary Material for Chapter 4

Contents

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C1: Population and resistance allele extinction dynamics

- The population and resistance dynamic profiles observed under different management strategies can differ substantially based on the inclusion/exclusion of different subsections of the population
- Under management strategies sufficient to eliminate the mosquito population, while the dynamics are qualitatively similar, the adult female population goes extinct substantially earlier than the total population
- Conversely, the resistance allele dynamics look significantly different when released males are included/excluded from the calculation, but the time to extinction is unaffected
- These differences are explained by a staggered population collapse, with the adult female population dying off first, followed by the juveniles, and finally the adult males
- Understanding these differences helps to improve understanding of the events driving the dynamics therefore the management of mosquito populations and resistance management

Throughout our analysis of this model, we have focused on the effects of control interventions on i) the adult female population dynamics, and ii) the resistance allele frequency across the whole population, excluding released adult males (A_m^{SSLL}). The former was chosen because adult female mosquitoes are responsible for disease transmission and is the primary target of insecticide-based interventions and some genetic control strategies (e.g. fs-RIDL). The latter was chosen because we assumed it important to understand the dynamics of resistance across the entire target population rather than just the adult female population. Furthermore, while resistance only offers protection to the adult females exposed to insecticides, through its associated fitness penalty it also affects the population dynamics in the wider

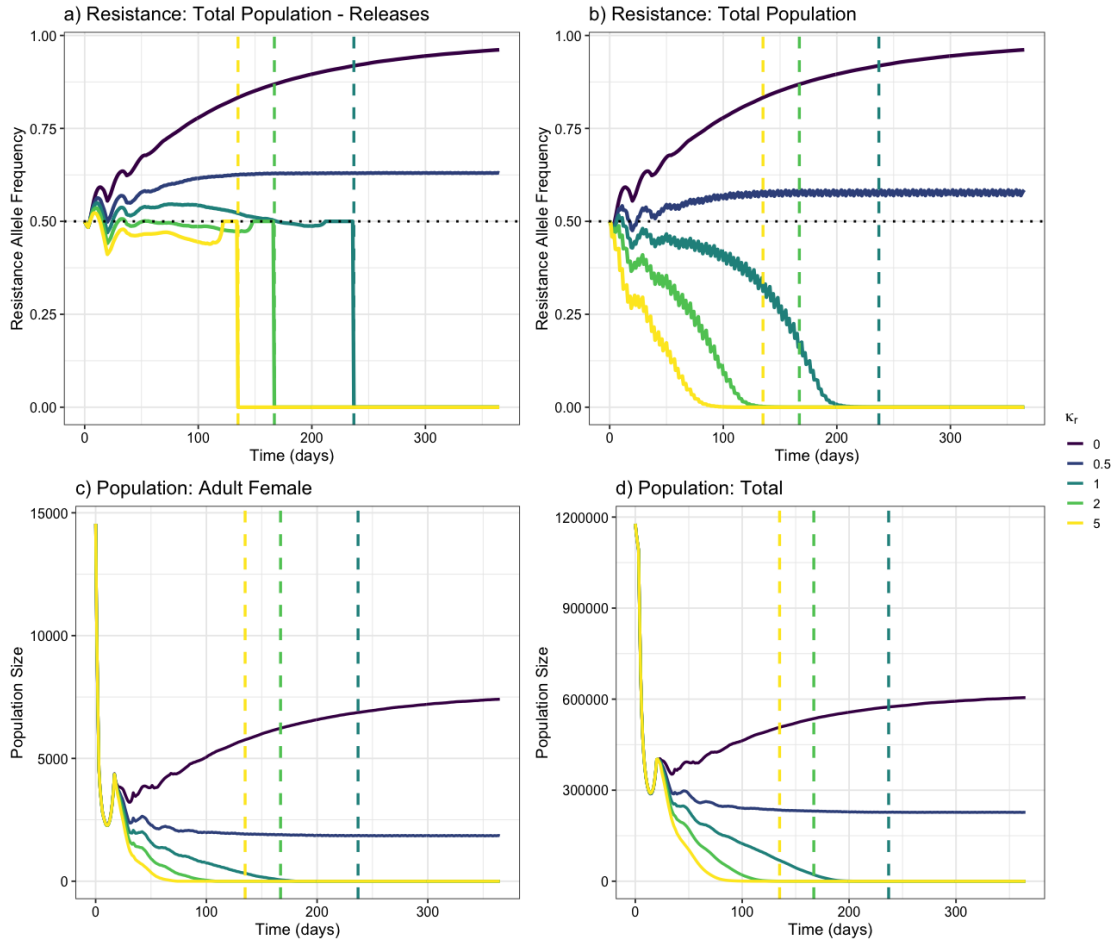


Figure C.1: Subsetting population size and resistance allele calculations affects resistance dynamics but not extinction times. The top row shows the resistance frequency through time in a) the total population, and b) the total population excluding released males (A_m^{SSLL}). The bottom row depicts the population dynamics for c) the adult female classes, and d) the total population, excluding released insects (A_m^{SSLL}). Each panel shows the dynamics observed under a control strategy with an LLIN exposure probability ρ_{LLIN} of 0.8 and biweekly releases ($\kappa_f = 2$). Solid lines represent release ratios from 0-5 per adult male in the equilibrial pre-intervention population, with $\kappa_r = 0$ giving the no-release case (LLIN-only). Where resistance extinction was achieved, the day on which this is occurred is indicated by the dashed line corresponding to the release ratio that achieved it. Resistance was initially widespread ($p_0 = 0.5$), fully recessive ($h_{RR} = 0$), and with additive fitness costs ($g_{RR} = 0.5$).

population. However, the part of the population visualised, or used to calculate the resistance allele frequency can, naturally, affect the observed dynamics.

This is illustrated in Figure S1 for both population and resistance allele frequency dynamics. The top two panels compare the resistance allele frequency dynamics for the population when released males are excluded (Fig. S1a) and the total population

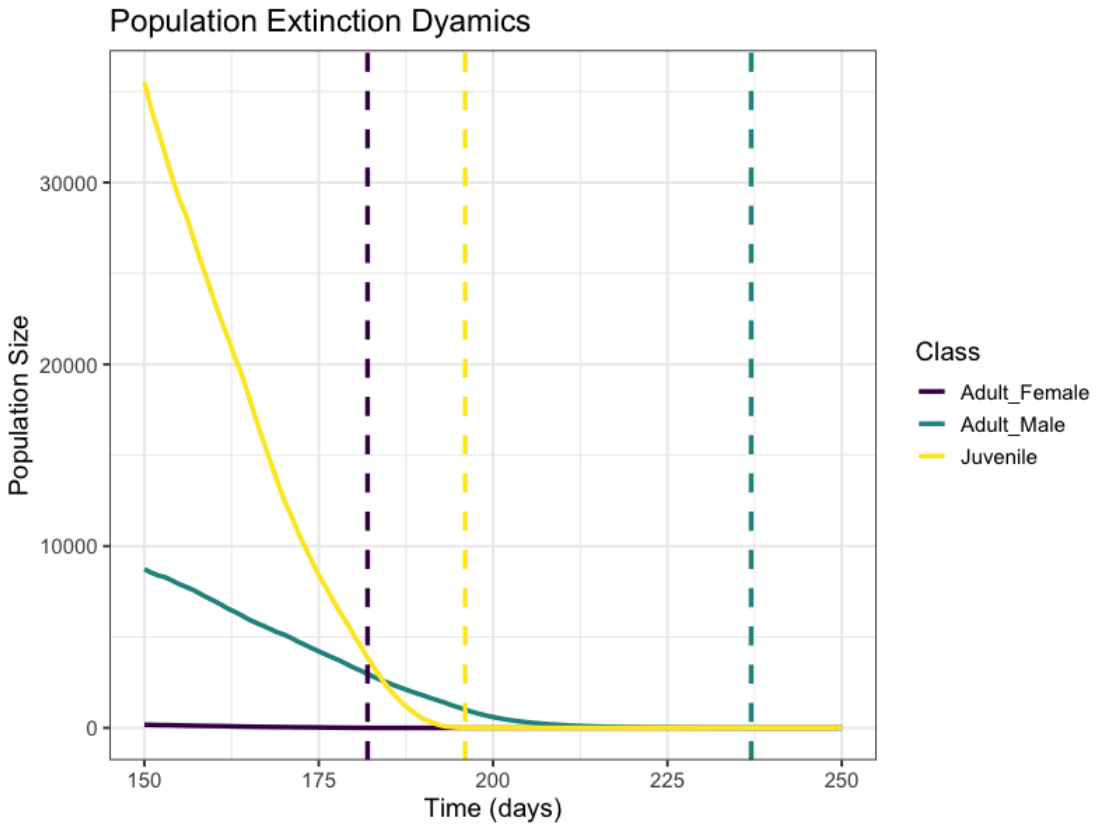


Figure C.2: The population extinction is staggered by life-history stages / classes. The population was managed using strategies combining LLIN deployment ($\phi_{LLIN} = 0.8$) with biweekly releases ($\kappa_f = 2$) of transgenic males at a rate of 1:1 ($\kappa_r = 1$). Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$

(Fig. S1b), from the same simulation(s). Overlayed in dashed lines are the days on which the resistance allele went extinct, which do not differ. The resistance allele frequency, calculated across the whole population rather than just the adult female class, exhibits very different dynamics depending on whether the allele frequency calculation includes released males (A_m^{SSLL}). When A_m^{SSLL} are included in the calculation, the resistance allele frequency declines to extinction at a rate that increases, and that exhibits fewer fluctuations, with increasing release frequency (κ_f) and ratio κ_r. When A_m^{SSLL} are excluded, however, the initially declines, then exhibits a recovery, before plummeting precipitously to extinction. Despite the observable differences in dynamics, the time to extinction is the same for each and

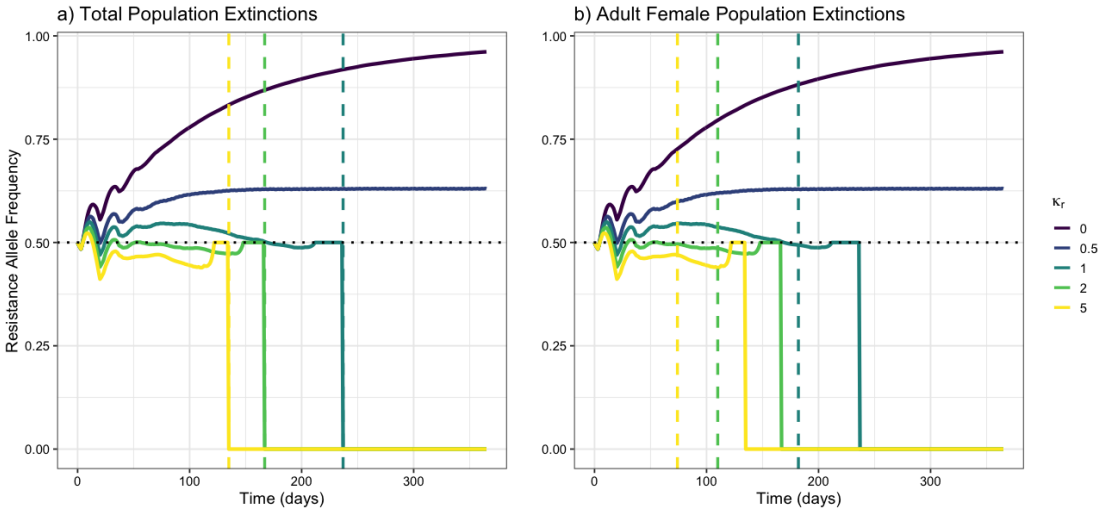


Figure C.3: The adult female population is eliminated prior to the total population and/or the resistance allele. The number of days required to achieve a) total population and b) adult female population extinction are overlaid as dotted lines on the resistance allele frequency dynamic curves. The population was managed using strategies combining LLIN deployment ($\phi_{LLIN} = 0.8$) with biweekly releases ($\kappa_f = 2$) of transgenic males at various release rates. The dashed lines represent the times at which population extinction was achieved for the corresponding population-type and release ratio. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$

is also equivalent to the time taken to eliminate the entire population.

Conversely, comparing the dynamics of the adult female population with the total population reveal qualitatively identical dynamics. However, the time to extinction is reduced in the adult female population. Furthermore, Figure S2 shows that the adult female population goes extinct before the resistance allele, which fails at the same time as the total population size. The adult female population is eliminated first because it is the only class in the model that experiences insecticide-induced mortality. Following the extinction of the adult female class, egg production ceases and the juvenile class therefore dries up after a period of time equivalent to the combined duration of the egg, larval and pupal stages (τ), due to a combination of juvenile mortality and maturation. The adult male class is the last to die off and this occurs approximately 40-50 days after the maturation of the last cohort of pupae.

This time period represents the tail end of the exponentially distributed longevity generated by assuming a daily survival probability of 90% ($\rho_m = 0.9$)(Fig. S2).

Figure S3 further illustrates that the adult female population goes extinct before the total population, by overlaying the days on which the a) total population and b) adult female population go extinct on a plot of the resistance allele dynamics (excluding SSLL males). The total population goes extinct on the same day as the resistance allele, whereas the adult female is eliminated significantly earlier.

C2: Supplementary figures for Chapter 4

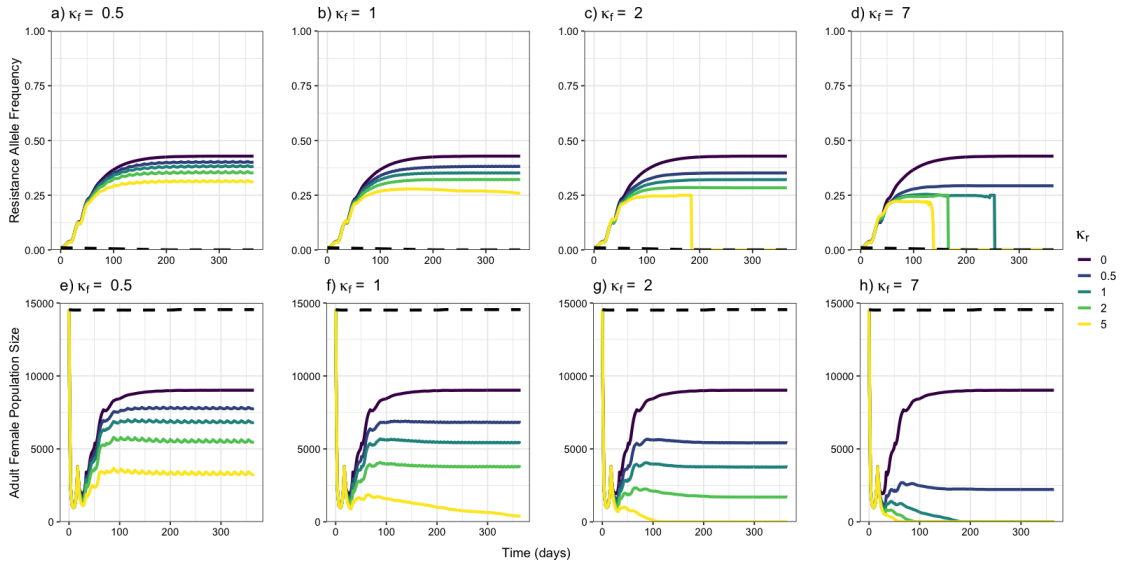


Figure C.4: Intensive release programmes required to protect LLIN deployment from the evolution of dominant resistance with recessive costs even when resistance is initially rare. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of LLIN exposure ρ_{LLIN} was fixed at 0.8 and combined with release strategies from fortnightly to daily ($\kappa_f = 0.5 - 7$) releases at release ratios (κ_r) from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially rare ($p_0 = 0.01$), completely dominant ($h_{RR} = 1$), and with recessive fitness costs ($g_{RR} = 0$). LLIN-only ($\kappa_r = 0$, purple line) and no-intervention ($\kappa_r = 0$, $\phi_{LLIN} = 0$, black dashed line) scenarios were included for reference. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$, $c_{RR} = 0.05$.

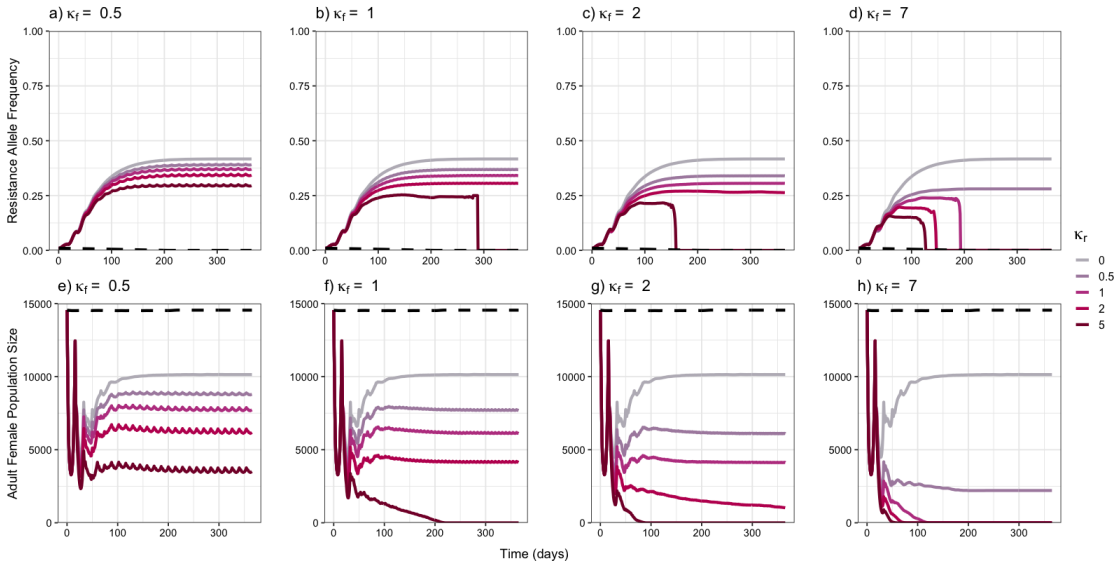


Figure C.5: Intensive release programmes required to protect IRS deployment from the evolution of dominant resistance with recessive costs even when resistance is initially rare. Panels show the target population resistance allele frequency (a-d) and adult female population dynamics (e-h). The probability of IRS exposure ρ_{IRS} was fixed at 0.8 and combined with release strategies from fortnightly to daily ($\kappa_f = 0.5-7$) releases at release ratios (κ_r) from 0.5-5 per adult male in the equilibrial pre-intervention population. Resistance was initially rare ($p_0 = 0.01$), completely dominant ($h_{RR} = 1$), and with recessive fitness costs ($g_{RR} = 0$). IRS-only ($\kappa_r = 0$, purple line) and no-intervention ($\kappa_r = 0$, $\phi_{IRS} = 0$, black dashed line) scenarios were included for reference. Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$, $c_{RR} = 0.05$.

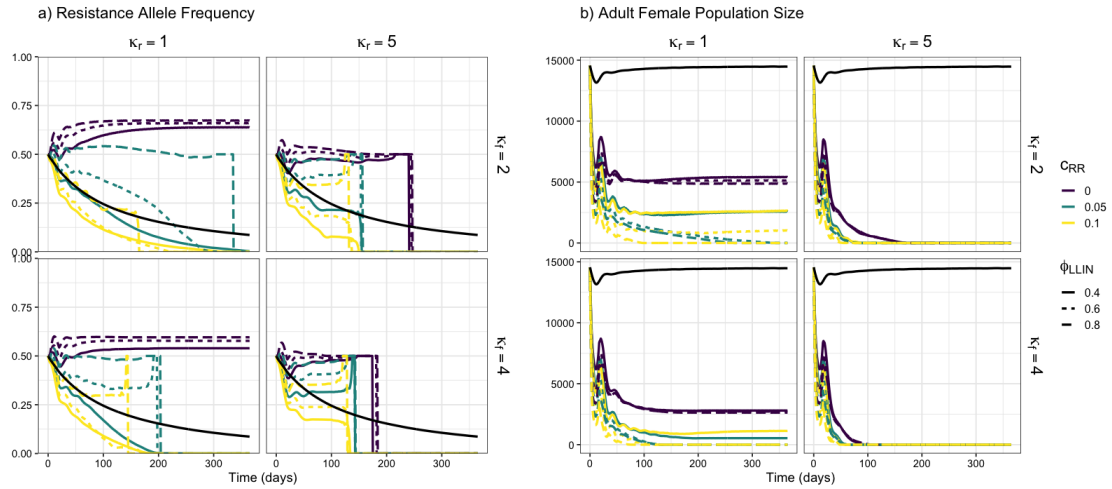


Figure C.6: The efficacy of management strategies combining LLIN deployment with transgenic insect releases is sensitive to the cost associated with resistance. Panels a) and b) show the effects of the cost of resistance (c_{RR}) on the resistance and population dynamics respectively under LLIN (ϕ_{LLIN}) and transgenic insect release (κ_f, κ_r) implementations of different intensities. Resistance was initially widespread ($p_0 = 0.5$), recessive ($h_{RR} = 0$), and associated with a recessive fitness cost ($g_{RR} = 0$). The line colours and styles represent different costs of resistance (c_{RR}) and LLIN-exposure probabilities (ϕ_{LLIN}) respectively, while the panel rows and columns depict different release ratios (κ_r) and frequencies (κ_f). Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{LLIN}^{SSww} = 0.01$, $\mu_{LLIN}^{RRww} = 0.9$.

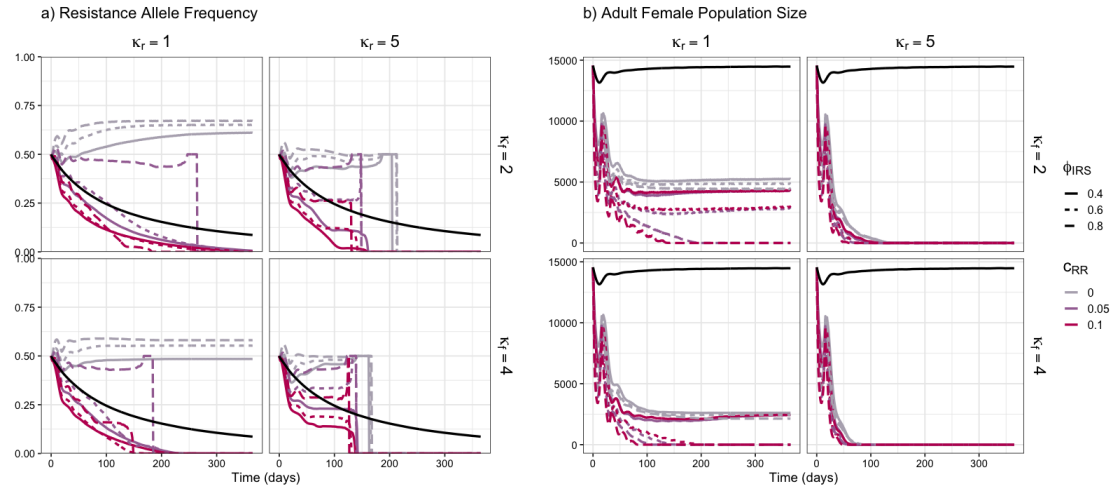


Figure C.7: The efficacy of management strategies combining IRS deployment with transgenic insect releases is sensitive to the cost associated with resistance. Panels a) and b) show the effects of the cost of resistance (c_{RR}) on the resistance and population dynamics respectively under IRS (ϕ_{IRS}) and transgenic insect release (κ_f, κ_r) implementations of different intensities. Resistance was initially widespread ($p_0 = 0.5$), recessive ($h_{RR} = 0$), and associated with a recessive fitness cost ($g_{RR} = 0$). The line colours and styles represent different costs of resistance (c_{RR}) and IRS-exposure probabilities (ϕ_{IRS}) respectively, while the panel rows and columns depict different release ratios (κ_r) and frequencies (κ_f). Simulations were initiated with an equilibrial population generated from a 2-year, no-intervention, burn-in period and then run with the following default parameter values: $\theta_e = 2$, $\theta_l = 10$, $\theta_p = 2$, $\rho_e = 0.895$, $\rho_l = 0.972$, $\rho_p = 0.975$, $\rho_s = 0.9$, $\rho_r = 0.9$, $\alpha = 1$ x 10^{-6}, $\beta = 1$, $\tau = 3$, $H = 0.67$, $\epsilon = 100$, $\gamma = 0.5$, $\mu_{IRS}^{SSww} = 0.01$, $\mu_{IRS}^{RRww} = 0.9$.