MODELLING OPTIMAL STRATEGIES FOR NOVEL GENETICS-BASED PEST MANAGEMENT

Nina Alphey

St Anne's College, University of Oxford

Thesis submitted for the degree of Doctor of Philosophy, Trinity term 2009

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Abstract

Genetic transformation techniques for pest insects have enabled the development of novel methods to mitigate the enormous harm done by insects to human health (through transmission of diseases) and to agriculture (through damage to crops or livestock). I use mathematical modelling to analyse strategies using autocidal genetic constructs (dominant lethal genes that are repressible during mass-rearing); in parallel several research groups are developing the strains and the laboratory and field experimental work. Engineered insects would be released in large numbers and compete for mates, and their progeny would inherit one copy of a dominant lethal gene and die. The lethal mechanism can be made stage- or sex-specific. The aim is to reduce the number of pest insects in a population, suppressing numbers to a less harmful level or local elimination. I examine the evolutionary, ecological, and economic cost and benefit aspects of these novel interventions.

I consider application of this genetic technology against agricultural pest insects, combined with genetically modified crop plants engineered to produce insecticidal toxins, to which field-evolved resistance is emerging. Using a theoretical framework, I analyse the gene frequency evolution of resistant alleles and show that strategies using genetic constructs that are selectively lethal only to females could help to manage both pests and resistance.

I investigate potential resistance to the lethal mechanism of the genetic construct itself. I use population genetics and population dynamics models to explore the impact of heritable biochemically-based resistance on the effectiveness of genetic strategies for reducing populations of important pests in agriculture or public health. Released insects are homozygous for susceptibility to the lethal construct; this has an inherent element of resistance dilution.

Finally, I analyse genetic vector control methods to reduce the transmission of human disease. I combine vector population dynamics and epidemiological models with techniques for assessing cost-effectiveness of a genetic strategy for controlling a vector mosquito, and show that disease elimination is feasible on a practical timescale and economically beneficial.

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Table of contents

List of Figures

List of Tables

Statement of authorship

With approval from the Director of Graduate Studies, the following chapters of my thesis are published scientific papers, written in collaboration with my supervisors and others.

Chapter 2: **Nina Alphey**, Paul G. Coleman, Michael B. Bonsall and Luke Alphey (2008), Proportions of different habitat types are critical to the fate of a resistance allele, *Theoretical Ecology* 1(2):103-115. doi:10.1007/s12080-008-0010-8.
PGC devised the underlying population genetics equation and identified some of the steady states. NA formalised the framework, performed the full analysis and simulations, generated the results and figures, and wrote the paper. MBB and LA supervised the project.

Chapter 3: **Nina Alphey**,* Paul G. Coleman,* Christl A. Donnelly and Luke Alphey (2007) Managing insecticide resistance by mass-release of engineered insects, *Journal of Economic Entomology* 100(5):1642-1649. http://esa.publisher.ingentaconnect.com/content/esa/jee/2007/00000100/00000005/art00021 * Joint first author.
PGC created a preliminary version of the model, adapting an existing population genetics model of RIDL control by CAD to incorporate Bt resistance. NA developed and expanded the model, performed the simulations and analysis, and created the results and figures. LA and NA wrote the paper. LA and PGC supervised the project.

Chapter 4: **Nina Alphey**, Michael B. Bonsall and Luke Alphey (2009), Combining Pest Control and Resistance Management: Synergy of Engineered Insects with Bt Crops, *Journal of Economic Entomology* 102(2): 717-732. http://esa.publisher.ingentaconnect.com/content/esa/jee/2009/00000102/00000002/art00034
NA further developed and expanded the model from Chapter 3, including designing additional elements, performed the analysis and simulations, generated the results and figures and wrote the paper. LA and MBB supervised the project.

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CAD: Department of Infectious Disease Epidemiology, Imperial College London, Faculty of Medicine

Update: other publications arising from material in this thesis

Chapter 5: Nina Alphey, Michael B. Bonsall and Luke Alphey (2011) Modeling resistance to genetic control of insects, Journal of Theoretical Biology 270(1): 42-55. doi:10.1016/j.jtbi.2010.11.016

Chapter 6: Alphey N, Alphey L, Bonsall MB (2011) A model framework to estimate impact and cost of genetics-based sterile insect methods for dengue vector control. PLoS ONE 6(10): e25384. doi:10.1371/journal.pone.0025384

1 Introduction

1.1 Theme of my research

Pest insects do enormous damage to human health (through transmission of diseases such as dengue fever and malaria) and to agriculture (through damage to crops or livestock). Arthropods destroy about 18% of the world's annual crop production, contribute to nearly 20% losses of stored grains and cause damage of the order of US$100 billion annually (Nicholson 2007). There are hundreds of millions of cases of vector-borne diseases in humans each year, with a million deaths annually from malaria alone (Sachs and Malaney 2002, WHO 2008). Insecticide resistance has been reported in many important insects and against every chemical class of insecticide and some insecticidal crops (Nicholson 2007, Reynolds 2007). The recent development of genetic transformation techniques for pest insects has opened up the possibility of employing novel genetic methods to mitigate the harm done by insects.

My research presented in this thesis involves mathematical modelling to analyse the relative merits and sensitivities of these new biological approaches to controlling these pests. Two broad classes of genetic strategy have emerged (Terenius et al. 2008): (1) population reduction, in which the aim is to reduce the number of pest insects, for example using autocidal genetic constructs (Thomas et al. 2000); (2) "population replacement", in which the insect vector population is converted, by spreading a genetic construct through it, into a "refractory" strain that is unable (or less able) to transmit the disease (Sperança and Capurro 2007). I focus on the former, i.e. on approaches for population control, which term I use to indicate measures that aim to eliminate the pest locally or suppress its numbers to a less harmful level.

One existing strategy for pest management is the Sterile Insect Technique (SIT), an area-wide method of biological pest control (Dyck et al. 2005) (see §1.2 below). Large numbers of the pest insects are bred, sterilised (currently by irradiation) and then released. The sterile insects mate with the wild insects, but no viable offspring result. It has been suggested that SIT could be improved and made more widely applicable using modern genetic techniques (Thomas et al. 2000). A resulting patented technology, RIDL® (Release of Insects carrying a Dominant Lethal), was developed in Oxford, originally in the University's Department of Zoology and now at Oxitec Ltd, and is the main focus of my research. [RIDL® is a registered trademark of Oxitec Limited, UK, a biotechnology spin-out company.]

I develop theoretical frameworks and mathematical models to address questions about the effect of applying these RIDL techniques in various scenarios. I examine the economic costs and benefits of novel genetics-based interventions, either implicitly (e.g. using measures such as agricultural pest population size as a proxy for crop damage) or explicitly (e.g. using measures such as the direct or indirect costs per case of disease).

1.2 The Sterile Insect Technique (SIT)

The Sterile Insect Technique (SIT) is a species-specific method for insect population control with a low environmental impact that relies on the mass-rearing and release of sterile insects (Knipling 1955, Dyck et al. 2005). These released insects compete for mates with wild insects; a wild female mating with a released sterile male has no or fewer progeny, so the population tends to decline (Figure 1.1). If sufficient sterile insects are released for a sufficient period, the target population will be reduced or even locally eliminated. The SIT is a highly effective area-wide method of pest control. SIT has been used very successfully against a range of pest insects, including various tephritid fruit flies

(e.g. the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) "Medfly"), several moths (e.g. the pink bollworm *Pectinophora gossypiella* (Saunders)) and a small number of livestock pests (e.g. the New World screwworm *Cochliomyia hominivorax* (Coquerel)) (Dyck et al. 2005).

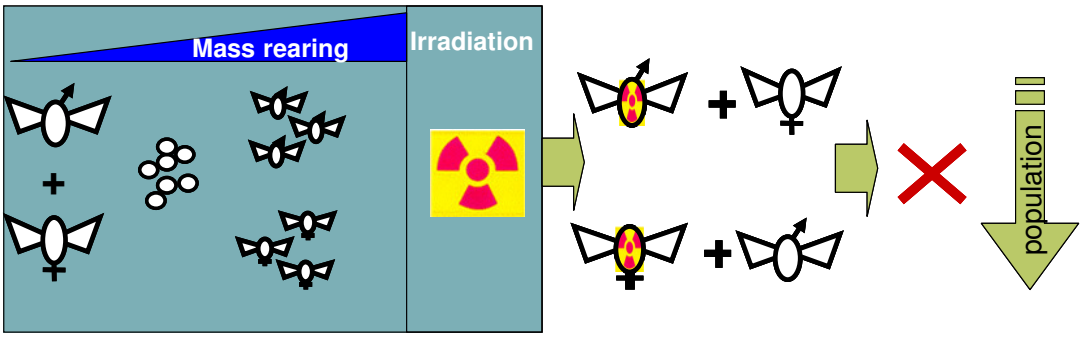

Figure 1.1 The Sterile Insect Technique
Pest insects are mass-reared in large quantities, then sterilised by irradiation and released into the target area where they compete for mates with wild insects. A wild female mating with a sterile male will have no (or fewer) progeny. Sufficient releases can cause the population to decline. [Diagram by Andrew McKemey, Oxitec Ltd, reproduced with permission]

However, modern genetics could potentially provide several improvements that would increase the cost-effectiveness of SIT, make it even safer and extend the range of suitable species. These include:

- an alternative to sterilisation by irradiation ("genetic sterilisation");
- improved identification of released individuals by incorporation of a stable, heritable, genetic marker;
- providing automated sex-separation prior to release ("genetic sexing");
- reduction of the hazard posed by non-irradiated accidental releases from mass-rearing facilities ("fail-safe").

None of these are necessarily unattainable by classical methods. However, the use of recombinant DNA methods may allow these benefits to be obtained in a shorter period and to be transferred more readily from one species to another than are the products of classical genetics.

1.3 Genetics-based strategies using the RIDL system

1.3.1 Autocidal control of insects

The idea of using genetically altered insects for autocidal control of insect populations was proposed independently in the 1940s, 1950s and 1960s (Gould and Schliekelman 2004). After the success of Knipling's programme at the United States Department of Agriculture (USDA) to eradicate the New World screwworm through massive releases of insects sterilised by irradiation (Knipling 1955), research began in earnest on a wide range of relevant issues. The conditional lethal release method uses insects carrying a dominant genetic trait that is only lethal under restrictive conditions, so that insects carrying that trait can be mass-reared for release. This method was first proposed in the 1960s but was not used for pest control, because of the difficulties of producing such insects using classical genetic methods (Schliekelman and Gould 2000a). An existing gene in *Drosophila melanogaster* was later shown to have suitable properties (Fryxell and Miller 1995).

The RIDL concept is a proposed modification to the SIT in which the released insects are not irradiated, but instead are homozygous for one or more conditional dominant lethal genes (Thomas et al. 2000, Alphey 2002, Alphey and Andreasen 2002, Alphey et al. 2007a, Alphey 2007). Progeny of such released insects inherit one copy of the dominant lethal gene and die (Figure 1.2). Like SIT using radiation, release of such insects in sufficient numbers reduces the reproductive potential of the insect population (Figure 1.3).

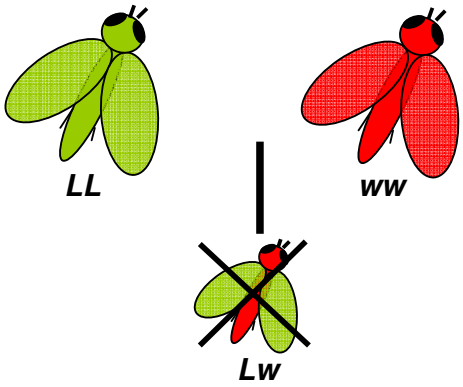

Figure 1.2 RIDL as genetic sterilisation
Released RIDL insects are homozygous for one or more dominant lethal genes (*LL*). Offspring of RIDL insects and wild mates (*ww*) are heterozygous for the RIDL construct *(Lw*), and therefore die.

An autocidal system that was always active would kill every individual that carried it and it would be impossible to breed and propagate a strain carrying such a system. Autocidal systems for SIT applications need to be conditional, so that they are switched on under certain conditions ("restrictive") and off under others ("permissive"). An engineered lethal system might be repressible or inducible. In an inducible system, the effect is only seen when a specific condition is applied, for example heat treatment. In a repressible system, the effect is seen unless a specific condition is applied. Use of radiation is an inducible sterility system; the insects are fertile unless and until a suitable sterilising dose of radiation is correctly applied. RIDL relies on a repressible lethal system; the system is repressed in the laboratory and mass-rearing facility by applying an artificial condition (dietary tetracycline), but elsewhere is not repressed and is therefore active. This has the beneficial consequence that the RIDL system is activated in escaped insects, or their progeny, providing a "fail-safe" to RIDL SIT releases that is not readily available or effective in inducible systems.

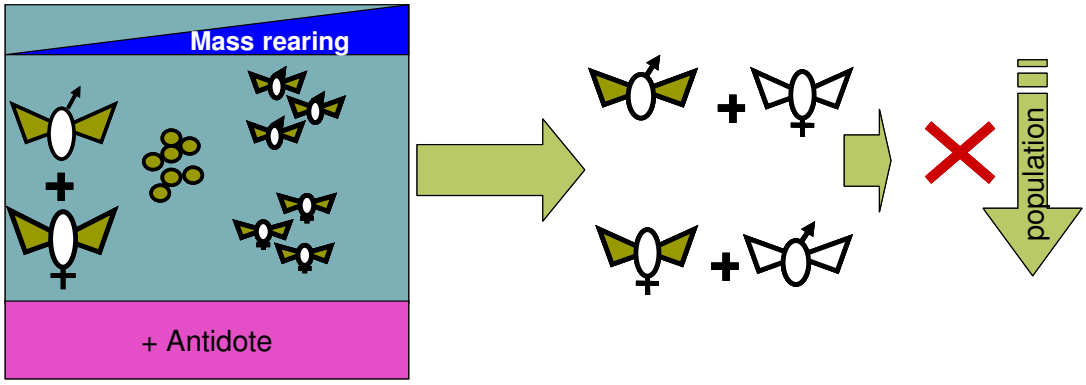

Figure 1.3 SIT with genetic sterilisation instead of irradiation
Homozygous genetically engineered strains of insects are mass-reared, repressing the lethal genetic construct using a dietary antidote. Insects are released into the target area, where they compete for mates with wild insects. The antidote is not available outside the facility, so progeny of a wild female mating with a released RIDL male are not viable.
[Diagram by Andrew McKemey, Oxitec Ltd, reproduced with permission]

Various existing methods for regulating gene expression could be adapted to make a conditional autocidal system. The conditionality of the RIDL system is based on the "tet-off" gene expression system (Gossen and Bujard 1992). In this system gene expression is silenced in the presence of tetracycline, or tetracycline-like chemicals, which can therefore be used like an "antidote" to switch off the lethal system. The tet-off expression system is based on a synthetic fusion protein called tTA (tetracycline-controlled transactivator) that binds to a short, specific DNA sequence, known as the tet operator (tetO). When bound by tTA, tetO acts as a transcriptional enhancer, stimulating gene expression from nearby receptive promoters (e.g. promoters driving expression of dominant lethal effector genes). tTA binds tetracycline and suitable tetracycline-like chemicals with high affinity, and in that bound form does not bind DNA and so does not bind tetO to act as a transcriptional enhancer. Further refinements have been made to the repression system, so that tTA can act as both transactivator and effector (Gong et al. 2005) (Figure 1.4).

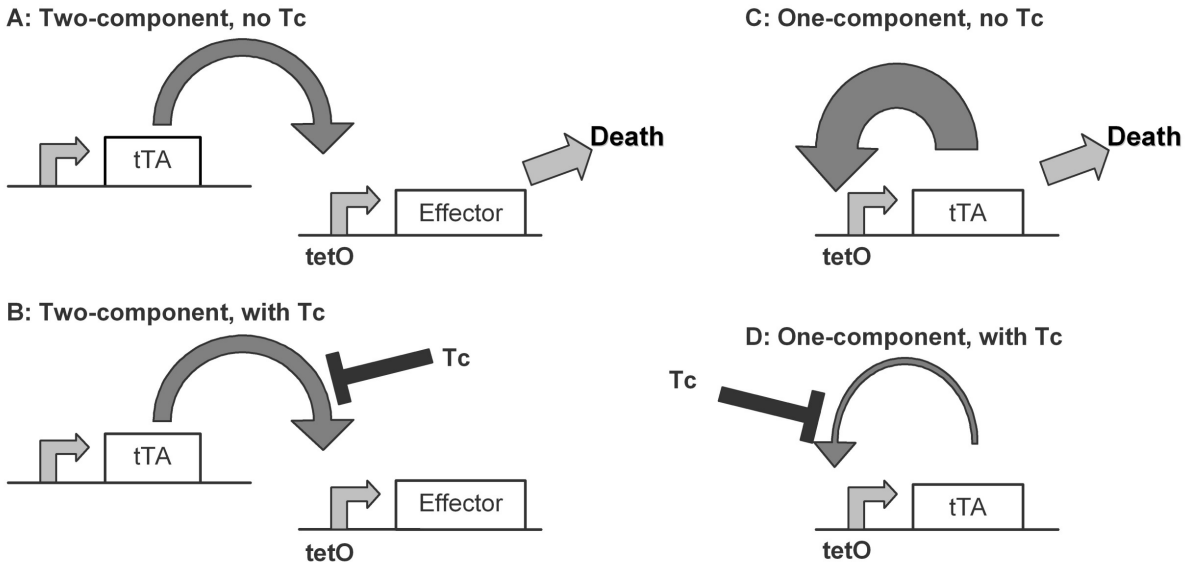

Figure 1.4 Tetracycline-repressible lethal systems
A, B: Two-component system (Heinrich and Scott 2000, Thomas et al. 2000, Horn and Wimmer 2003). tTA (Gossen and Bujard 1992) is placed under the control of a suitable promoter, e.g. constitutive, female-specific, embryo-specific, etc. A: In the absence of tetracycline (Tc) tTA binds tetO, drives expression of an effector molecule leading, in the case of a lethal effector, to death. B: In the presence of Tc, tTA binds Tc; the Tc-bound form does not bind DNA, therefore does not activate expression of the effector, and the system is inactivated. C, D: a simplified one-component system. C: In the absence of Tc, basal expression of tTA leads to the synthesis of more tTA, which accumulates to high level. This level can be regulated by modifying the stability and translational efficiency of the tTA mRNA. At the highest levels, expression is lethal, so tTA is both the driver and the effector. D: In the presence of Tc, tTA is inactivated by Tc and is therefore expressed only at basal levels. [Adapted from Fig 1. of Gong et al. (2005)]

Prototypes of RIDL insects were constructed in *Drosophila melanogaster* (Thomas et al. 2000, Alphey 2002, Horn and Wimmer 2003). Similar lethal systems have been used to create RIDL strains of pest insects, including Medfly (Gong et al. 2005), the yellow fever mosquito *Aedes (Stegomyia) aegypti* (L.) (Phuc et al. 2007) and the Mexican fruit fly *Anastrepha ludens* (Loew) ("Mexfly") (Condon et al. 2007).

By manipulating the construct, and choosing a suitable promoter (or in the two-component version, a suitable effector), effects specific to one sex or a particular life stage can be achieved. This makes different strategies potentially available for programmes deploying RIDL insects.

For example, a female-specific lethal effect can be produced (see §1.3.2 below). This could be used alone for genetic sexing or in conjunction with radiation replacement for population control.

Work on the mosquito *Ae. aegypti*, the principal vector of the dengue and yellow fever viruses, demonstrated a further advantage to genetic techniques, namely the ability to manipulate the stage of development at which death occurs (lethal phase) (Phuc et al. 2007). Phuc et al. investigated by mathematical modelling the effect of using late-acting lethality in an SIT programme against an insect population restricted by density dependent mortality at a larval stage. Late-acting lethality was predicted to be considerably more effective than early-acting lethality, as a result of competition between the "doomed" larvae and wild larvae. A strain of *Ae. aegypti* was constructed with the necessary properties of dominant, repressible, highly penetrant, late-acting lethality.

1.3.2 Female-specific lethality

One version of the RIDL system posits the release of male insects homozygous for one or more female-specific dominant lethals ("RIDL males"). These lethal genes would be repressed throughout mass-rearing, except in the generation intended for release (male-only release is generally desirable for SIT (Rendón et al. 2004, Marec et al. 2005)). Released RIDL males would mate with wild females; all of their F_1 progeny would be heterozygotes, inheriting a dominant female-specific lethal (see also Figure 3.1 in Chapter 3). The F_1 females would die, thereby reducing the reproductive potential of the wild population (much like the SIT as currently practised), but the males would live. Half of their (F_2) daughters would also inherit the RIDL gene(s) and die, so there would be some modest additional suppression.

Strains with the necessary genetic properties for this female-lethal version of RIDL have been constructed in several species, with proof-of-principle in *Drosophila melanogaster* (Heinrich and Scott 2000, Thomas et al. 2000) and success in two economically important tephritid fruit fly species, Medfly (Fu et al. 2007) and Mexfly (Stainton et al. unpublished data). There is active work and progress in Lepidopteran pests, especially pink bollworm, and *Aedes aegypti* (L. Alphey, personal communication).

The reproductive potential of an insect population depends primarily on the number of females – increasing the number of females will typically increase the number of progeny (unless there are insufficient males for all females to find mates), but increasing the number of males normally will not. It might therefore be conjectured that killing both sexes, or specifically killing females, could have equivalent effects on the size of the target population.

Mathematical models predict that a female-lethal genetic system can be more effective for population control than is conventional SIT, especially if the female-killing alleles are present at several loci (Schliekelman and Gould 2000b, Thomas et al. 2000). Figure 1.5 panels a and c illustrate marginal situations where conventional SIT and early-acting bisex-lethal RIDL releases do not control the population but early-acting female-lethal RIDL release strategies do. In the density dependent case (c) SIT can actually increase the equilibrium size of the adult female population (Rogers and Randolph 1984). Panels b and d show that, with a sufficiently high release ratio, SIT and the RIDL release strategies all control the population.

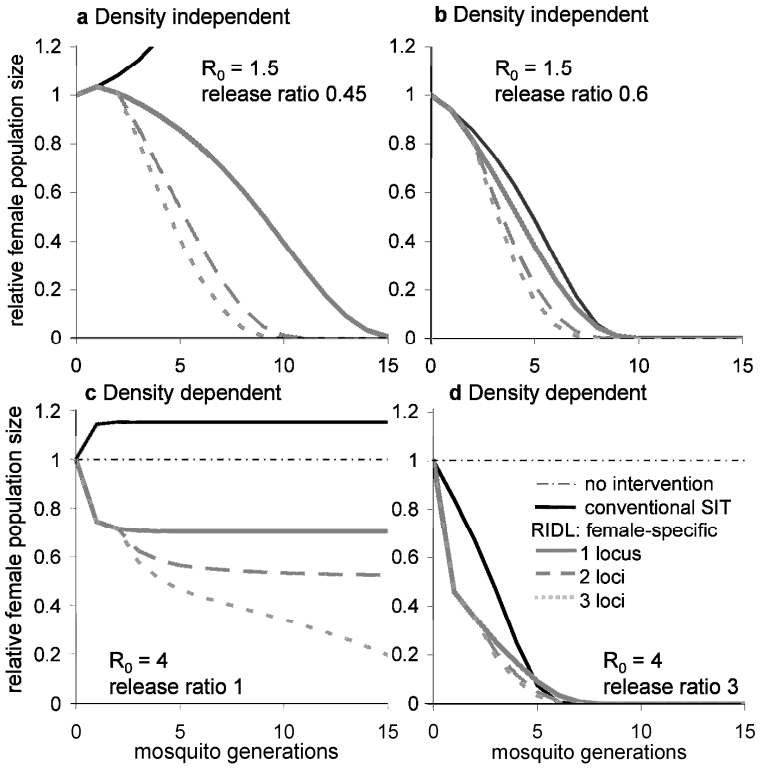

Figure 1.5 Female-specific lethality as genetic sterilisation
A deterministic discrete-generation population genetics model (based on that of Thomas et al. (2000)) is used here to predict the effects of releasing into a target population non-irradiated males homozygous at one or more loci for a female-specific lethal gene and to compare this with the effectiveness of conventional SIT. Assuming that the lethal gene acts on the developing insects before they reach reproductive maturity, for example at an embryonic or larval stage, a bisex-lethal version of the RIDL system that kills both males and females is equivalent in this model to conventional SIT; negative effects of irradiation or genetic engineering are ignored. The population is assumed to be closed and homogeneous, with a natural sex ratio of 1:1 and females selecting mates in proportion to their relative abundance.
The panels show the number of females in the population in each generation, relative to the initial number. The key in panel d applies to all panels. A constant number of adult males are released per insect generation; this "release ratio" is relative to the initial wild male population. R_0 denotes the number of female offspring produced by each female that would survive to adulthood if there were no intervention and no density dependent mortality.
Panels a and b assume no effects of pest density and $R_0 = 1.5$, and compare release ratios of 0.45 (a) and 0.6 (b). Without any intervention, the population would experience exponential growth (not shown).
Panels c and d assume density dependent mortality of larvae (using formulae from Maynard Smith and Slatkin (1973) and Rogers and Randolph (1984)) and $R_0 = 4$, and compare release ratios of 1 (a) and 3 (b). The population is assumed to start at carrying capacity and will therefore remain at the initial level if there is no intervention.

Male-only release is preferable for control programmes because single sex releases are more efficient and the harm done is often female-specific. For example, female mosquitoes take blood meals prior to oviposition and thereby provide the route for transmission of diseases between vector and host; males do not bite. (This is not true of all vectors; for example, Chagas disease (American Trypanosomiasis) is commonly acquired through contact with the faeces of either sex of infected blood-sucking triatomine bugs.) Released males may court released females instead of dispersing to mate with wild females, so a mixed sex release is less efficient and requires larger numbers of insects to be released for comparable outcomes. This mating between co-released males and females was shown to have a significant impact in large-scale field studies on Medfly (Rendón et al. 2004). Sterile males released without females were shown to be 3- to 5-fold more effective per male than sterile males co-released with sterile females. Genetic sexing mechanisms may make it easier, and in many cases make it possible at all, to separate males from females on the large scale needed for SIT and facilitate the preferred regime of male-only release.

1.4 Outline of thesis

I begin by considering the application of RIDL technology against agricultural pest insects in the context of another genetic control method, the use of genetically modified crop plants engineered to produce insecticidal toxins. Chapter 2 sets out a theoretical framework for analysing the gene frequency evolution of two alternative alleles (resistant or susceptible to plant-incorporated toxins) at a single genetic locus in a habitat comprising two environments in which the genotypes have different relative fitnesses (the engineered crops and a refuge of non-transgenic plants). Chapter 3 presents a mathematical model, based on the framework in Chapter 2, exploring whether specific RIDL strategies could form an effective component of a resistance management strategy for plant-incorporated

toxins. Chapter 4 extends and builds on the model in Chapter 3, relaxing assumptions and investigating the broader issue of synergy between engineered insects and engineered crops. This Chapter also incorporates recent data, as there is now evidence that some field-evolved resistance is beginning to emerge.

In Chapter 5 I assess hypothetical resistance to the lethal mechanism of the RIDL system itself. I use population genetics and population dynamics models to consider the possible impact of monogenic biochemically-based resistance on the effectiveness of RIDL strategies for population control of pests that are important in agriculture or public health.

Chapter 6 outlines a model framework with which to analyse the cost-effectiveness of genetic vector control methods to control the transmission of human diseases. Using the arthropod-borne virus (arbovirus) dengue as an example, I combine vector population dynamics and epidemiological models with techniques for assessing cost-effectiveness of a RIDL strategy for controlling the vector mosquito *Aedes aegypti*.

Chapter 7 further discusses key results and draws together themes from the core chapters. I set my research into a broader context and outline the potential for future research in this and related fields.

2 Proportions of different habitat types are critical to the fate of a resistance allele

Summary We describe a simple deterministic theoretical framework for analysing the gene frequency evolution of two alternative alleles at a single genetic locus in a habitat comprising two environments in which the genotypes have different relative fitnesses. We illustrate this for adaptation of pest insects, where one allele (resistance to toxins expressed in transgenic crops) is favoured in one environment (transgenic plants) and the other allele (susceptibility to toxins) is favoured in the other environment ("refuges" of non-transgenic plants). The evolution of allele frequencies depends on selection pressure because of relative sizes of the environments and relative fitnesses of the genotypes in each environment. We demonstrate that there are critical threshold proportions for habitat division that determine equilibrium allele frequencies. The stability of the system depends on relationships between the relative genotype fitnesses. In some cases, division of the habitat in exactly the threshold proportions removes selection pressure and maintains polymorphism at all allele frequencies.

2.1 Introduction

Natural populations live in heterogeneous habitats and the interactions between environment and genotype can lead to differential selection. Investigating how selection can maintain genetic variation in adaptive phenotypic traits is a classic and contemporary issue in evolutionary biology (Byers 2005). Originally, Levene (1953) showed, theoretically, that diversifying selection over different habitats could maintain polymorphism. Using a framework based on soft selection (whereby a fixed number of individuals survive in each niche) the conditions promoting polymorphism depend on the harmonic mean fitness of heterozygotes being larger than that for homozygotes (Levene

1953). From this and later models, we now know that variable selection over space generally results in broader conditions for maintenance of genetic polymorphism, although it does not necessarily always ensure it (Hedrick 2006).

An alternative approach to explaining how diversifying selection and habitat heterogeneity affect the maintenance of polymorphism was introduced by Dempster (1955). In contrast to Levene's model, this theoretical framework involves hard selection (by allowing a constant number of zygotes in each niche of which a variable number survive to adulthood). In this situation, changes in the allele frequency of the whole population depend on arithmetic mean fitness values. Real populations are probably somewhat intermediate in character, perhaps depending on whether interspecific or intraspecific competition is more important in limiting the number of fertile adults (Dempster 1955).

Levene (1953) noted that with preferential selection of niches and/or mating tending to occur within niches rather than at random across the whole population, conditions would be more favourable for polymorphic equilibria. Various authors have relaxed the model assumptions to investigate these effects (Hedrick 2006). For instance, Karlin (1977) determined conditions for the protection of a recessive allele (i.e. where its extinction is impossible from any initial polymorphic state) for almost any structure of migration between demes (as opposed to simple random mating and migration). This result holds true whether hard or soft selection is assumed. Further generalisations continue to be explored. For example, models of polymorphism for multiple alleles, with soft selection and arbitrary migration (Nagylaki and Lou 2001), have allowed the sufficient conditions for non-existence of a completely polymorphic equilibrium and the global loss of alleles to be deduced (Nagylaki and Lou 2006).

The gene frequency evolution of alternative alleles in a heterogeneous habitat is important to the broad questions of ecological generalism versus specialism. There are many examples of specialist species where at least one life stage develops in a particular environment. A rare allele or novel mutation might enable survival in a second environment. For example, a phytophagous insect whose larvae develop on a specific host plant might gain resistance to insecticides and so survive in a treated field. A parasite may extend its host range, by acquiring resistance to an antimicrobial drug and so be able to develop in treated individuals, or by adapting to a novel host species. Developing resistance can have fitness costs and these can be important in determining host range, with implications for public health, agriculture and conservation (Andersson and Levin 1999, Hastings and D'Alessandro 2000, Levin 2001, Coleman and Welburn 2004). Models can help predict or explain whether this resistance becomes predominant or disappears or some intermediate balance is maintained.

Some of the best documented examples of selection in heterogeneous environments involve recent human induced changes to the environment, such as the use of chemicals to control pests or pathogens (Hedrick 2006). Alternative environments may be created or manipulated deliberately, to influence the evolution of the undesirable trait (such as resistance to these chemicals). For example, strategies for delaying the evolution of drug resistance in parasites include applying chemotherapy selectively, treating only those individuals with high worm loads (macroparasitic infections) or high parasitaemias (microparasitic infections such as malaria), so that parasite populations in the untreated individuals dilute the parasite gene pool with genes for drug susceptibility (Anderson and May 1991). Similar approaches are used to delay resistance to conventional insecticides in

disease vectors and agricultural pests (e.g. the "stable zone strategy" where an area of critical size is left untreated (Lenormand and Raymond 1998)). Such strategies create a habitat sub-divided into two environments – one where the resistance allele is favoured (the treated area or hosts) and the other where it is not (untreated).

Population genetics models, such as those discussed above, typically allow parameters representing habitat variability to take arbitrary values and find conditions in terms of fitness parameters for stable equilibria that could maintain genetic polymorphism. Where the habitat is deliberately manipulated (or influenced), it could be more useful to express conditions for polymorphism in terms of habitat division, so that appropriate changes to the habitat proportions can be made to meet the objective of delaying or reversing resistance. Our aim in this study is to understand how habitat heterogeneity affects resistance evolution. Unlike many of the earlier studies, our interest is not in polymorphism *per se* but in understanding how the division of a habitat influences whether an undesirable trait (resistance) becomes universal, disappears or is able potentially to persist for a significant time at some intermediate level. We therefore consider all of the dynamical outcomes not just stable equilibria. We develop a population genetic framework that focuses on the relative sizes of two environments within a habitat and consider all potential steady states.

2.2 Biological motivation

One particular example of such a heterogeneous habitat that motivates our work arises in the context of transgenic crops that produce insecticidal toxins. These are now widely used to control insect pests (James 2006). We use this system, which has simple genetics and two habitat subtypes that differ in a single, important feature, as a model system to illustrate our framework for understanding resistance evolution.

Plants producing insecticidal proteins derived from *Bacillus thuringiensis* (Bt), principally maize and cotton, grew on 32 million ha worldwide during 2006 (James 2006). By reducing reliance on insecticidal sprays, decreasing production risk (insect damage) and/or improving yields, these can provide economic, health and environmental benefits (Shelton et al. 2002, Huang et al. 2003, Brookes and Barfoot 2006), which would be lost if resistance to the toxins spread to a significant proportion of the pest population. Although field resistance to Bt crops has not yet been documented, resistance to Bt sprays has been found in diamondback moth (*Plutella xylostella*) and cabbage looper (*Trichoplusia ni*), and strains of several pests that are substantially resistant to Bt toxins have been selected in the laboratory (Bates et al. 2005).

Strains of Bt produce crystalline (Cry) proteins with insecticidal properties. Each different protein is usually toxic to only a few species, and receptors on midgut epithelial cells are critical in determining Cry protein specificity. In Lepidoptera, the cadherin-like receptors are a major class of such receptors, several cadherin-like proteins confer susceptibility by binding to Cry toxins, and disruption in cadherin genes has been associated with resistance (Pigott and Ellar 2007). Different kinds of changes to cadherin genes imparting resistance have been identified, for example disruption by a retrotransposon (Gahan et al. 2001) or deletions (Morin et al. 2003), and genetic diversity of cadherin-specific genes of cotton bollworm (*Helicoverpa armigera*) populations in India differed over 100-fold in their susceptibility to Cry1Ac (Gujar et al. 2007a).

Laboratory-selected resistant strains have been investigated in numerous studies. A recessive mutation of a single autosomal gene in diamondback moth populations in Hawaii

and Pennsylvania, USA, confers high level resistance to four Bt toxins: Cry1Aa, Cry1Ab, Cry1Ac and Cry1F (Tabashnik et al. 1997a, Tabashnik et al. 1997b). Resistance to Bt Cry1Ab maize in sugarcane borer (*Diatraea saccharalis*) is determined by a nearly completely recessive allele at a single locus (Huang et al. 2007). *H. armigera* resistance to Cry2Ab toxin was found to be recessive (Mahon et al. 2007b). In several independent strains of pink bollworm (*Pectinophora gossypiella*), resistance to Cry1Ac toxin is associated with a single locus at which three mutant alleles of a cadherin gene confer resistance in larvae having any combination of two resistance alleles (Morin et al. 2003, Tabashnik et al. 2005); the assumption of two alternative alleles seems a reasonable simplification here. However, a few studies have identified resistance to Bt toxins that is not due to a single major gene. Resistance in diamondback moth populations in the Philippines shows multilocus control and for some toxins is not recessive (Tabashnik et al. 1997b). Two major Cry1Ac resistance genes (either of which confers resistance) have been identified in tobacco budworm (*Heliothis virescens*), a cotton pest (Gahan et al. 2001, Gahan et al. 2005); one of them, a cadherin mutation, was shown to be recessive and to account for 40-80% of resistance. However, one strain also exhibited resistance to Cry2Aa toxins, which is thought to be the cumulative result of several genes (Gahan et al. 2005). An analysis of inheritance of resistance in European corn borer (*Ostrinia nubilalis*) in lab-selected resistant strains showed that resistance was autosomal and suggested it was controlled by more than one locus, perhaps as many as 10-20 (Alves et al. 2006), with dominance of resistance decreasing as toxin concentration increased. Quantitative genetic variation has also been observed in resistance to both Cry1Ac and Cry2Ab toxins in bollworm (*Helicoverpa zea*) (Jackson et al. 2006).

The main resistance management method currently used is the "high-dose/refuge" strategy. This is based on the assumption that resistance to Bt crops is functionally recessive (because of the high levels of Bt toxins expressed, i.e. the "high dose"), so that only those insects homozygous for a resistance allele can survive on Bt crops. Refuges are areas of non-Bt host plants in which susceptible insects can survive; if resistance has fitness costs (Bates et al. 2005), resistant insects would be at a selective disadvantage in the refuge. Susceptible homozygotes from the refugia mate with resistant insects from toxin-treated regions, producing heterozygous offspring that cannot survive on Bt crops, thereby tending to reduce the frequency of resistance alleles. Mathematical models predict that this strategy can slow or reverse the spread of resistance through wild pest populations (Alstad and Andow 1995, Gould 1998, Carrière and Tabashnik 2001, Tabashnik et al. 2005).

In this study we show that the evolution of resistance allele frequency depends on the relative sizes of the two environments (illustrated by Bt crop areas and refugia) and on the relative fitnesses of the genes in each environment. We demonstrate that there are critical threshold proportions for the division of the habitat that determine the equilibrium allele frequencies and examine the behaviour of the system at these thresholds.

2.3 Methods

2.3.1 Analytical model

We use a population genetic framework to examine the effects of refuges on the spread of resistance to Bt crops in an insect pest population (Hartl and Clark 1989, Tabashnik et al. 2005). We assume a closed homogeneous population, with random mating, and no immigration, emigration or mutation, and use a deterministic, discrete-generation model. We assume a 1:1 sex ratio. Larvae, the susceptible life stage, are assumed to spend their

whole developmental time either on Bt crops or in the refuge and we assume that migration occurs after emergence as adults and before mating (Figure 2.1).

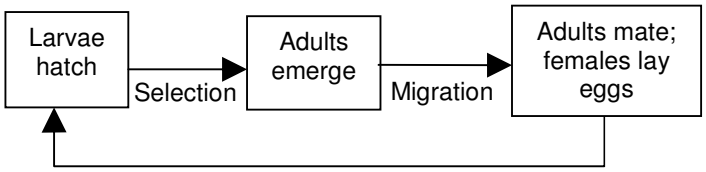

Figure 2.1 Model
Fitness costs associated with the *r* allele and with Bt toxins take effect during the larval stage. Larvae spend that entire life stage either on Bt plants or in a refuge. Adults migrate before mating at random and eggs are laid at random across Bt and non-Bt plants.

Our canonical set of parameters is listed in Table 2.1, together with the symbols used and the constraints placed on their values.

Table 2.1 Parameters and symbols

Symbol	Parameter	Constraints
r s	resistant allele susceptible allele	-
Φ $1-\Phi$	proportion of crops expressing Bt toxins refuge size	$0 < \Phi < 1$
i	genotype	*ss*, *sr* or *rr*
ω_i	relative fitness of larvae on Bt crops	$0 \le \omega_i \le 1$ $\omega_{rr} \ne \omega_{ss}$ $\omega_{ss} \le \omega_{sr} \le \omega_{rr}$
υ_i	relative fitness of larvae in non-Bt plants	$0 \le \upsilon_i \le 1$ $\upsilon_{rr} \le \upsilon_{sr} \le \upsilon_{ss} = 1$
Ω_i	average relative fitness of genotype i	
p q p_0	frequency of resistance allele r frequency of susceptible allele s in current adult generation initial r allele frequency	$0 \le p \le 1$ $0 \le q \le 1$ $p + q = 1$

We assume that the pest insect population is distributed at random across a fixed crop population, a proportion Φ of which expresses Bt toxins. The conventional (non-Bt) crop

refuge size is therefore 1-Φ. Hard selection (Wallace 1968) acts in these two environments, with the number of larvae surviving to reproductive maturity from each environment depending on the relative fitness values of the eggs laid there. Larval susceptibility to Bt toxins is assumed to be controlled by a single autosomal locus with two alternative alleles at that locus: resistant *r* (frequency *p* in adults) and susceptible *s* (frequency *q*, $p + q = 1$). There are three genotypes at this locus: *ss*, *sr* and *rr*.

We further assume that all fitness costs associated with the *s* and *r* alleles, in Bt and non-Bt crops, act during the larval stage (Figure 2.1). We use a subscript *i*, taking any of the three values *ss, sr* or *rr*, to indicate the genotype to which each larval fitness parameter applies. We denote the relative fitness of larvae on Bt crops by ω_i and υ_i is relative fitness of larvae on non-Bt crops. Fitnesses of these larvae are all relative to the *ss* genotype on non-Bt crops (set $\upsilon_{ss} = 1$). With random distribution of larvae across Bt and non-Bt crops, the mean relative fitness of larvae of genotype *i* (*i*: *ss, sr* or *rr*) is

$$\Omega_i = \omega_i \Phi + \upsilon_i (1 - \Phi) \qquad (1)$$

We assume that the resistance allele may have a cost (on non-Bt plants $\upsilon_{rr} \le \upsilon_{sr} \le \upsilon_{ss} = 1$), and that on Bt crops resistance is not over- or under-dominant ($\omega_{ss} \le \omega_{sr} \le \omega_{rr}$). By definition of resistance $\omega_{rr} > \omega_{ss}$, but the more general assumption that $\omega_{rr} \neq \omega_{ss}$ is sufficient in conjunction with the constraint set out in the previous sentence.

Dominance of alleles can be assessed in different ways and several measures have been used in insecticide studies (Bourguet et al. 2000). The most relevant to our model is in terms of the relative fitness of the three genotypes (rather than in terms of mortality levels, for example), either on Bt crops or in the refuge. The benefits of resistance affect fitness

on Bt plants and the costs of resistance affect fitness on both Bt and non-Bt plants. Special cases arise for our model when the resistance allele is dominant, recessive or co-dominant, in both environments, i.e. where the *sr* heterozygote has, respectively, the same fitness as the *rr* homozygote, the same fitness as the *ss* homozygote, or fitness midway between those of the homozygotes.

2.3.2 Assumptions and estimation of parameters

There are problems with validating these population genetic models. Ascertaining appropriate parameter values to use is difficult as field-evolved resistance to Bt crops has not yet been observed (Gressel 2005). However, there is a range of knowledge and experimental data available from which to create an appropriate parameter set.

As explained in our introduction (§2.1), in most known cases resistance to Bt toxins is monogenic (Ferré and Van Rie 2002, ffrench-Constant et al. 2004) and the assumption of two alternative alleles is a reasonable simplification. Most economically significant cases of conventional pesticide resistance are believed to be due to allelic variations at one or two gene loci. Consequently, one-locus two-allele models are commonly used (Tabashnik 1990). These assumptions are also thought to be realistic for many instances of resistance to drugs within diploid parasite populations or to pesticides in populations of insect or molluscan intermediate hosts of infectious diseases (Anderson and May 1991).

There are field data for estimated Bt resistance allele frequencies in several pests: tobacco budworm (Gould et al. 1997), cotton bollworm (*H. armigera*) (Li et al. 2004), pink bollworm (Tabashnik et al. 2005), European corn borer (Stodola et al. 2006) and sugarcane borer (Huang et al. 2007); broadly these estimates are of order of magnitude one in a thousand alleles (0.001), with one exception. Early results for pink bollworm in Arizona

estimated resistance allele frequencies at 0.13 or 0.16 in 1997 (Tabashnik et al. 2000); however, a later study estimated a mean frequency of 0.004 across 1998-2004 (95% confidence limits 0 to 0.01), with annual estimates varying approximately from 0 to 0.08 (Tabashnik et al. 2005).

In the particular context of resistance to Bt crops, it would be appropriate also to assume that no genotype is fitter on Bt crops than non-Bt plants ($\upsilon_i \geq \omega_i$). However, we do not impose this additional assumption in our model.

Where the *r* allele is an adaptation, representing a change from the wild type *s* allele, it is reasonable to assume that there may be a fitness cost that will be at least as great where two copies are present as with one copy ($\upsilon_{rr} \leq \upsilon_{sr} \leq \upsilon_{ss} = 1$). The assumptions about heterozygote fitness ($\upsilon_{rr} \leq \upsilon_{sr} \leq \upsilon_{ss} = 1$ and $\omega_{ss} \leq \omega_{sr} \leq \omega_{rr}$) determine the directions of some inequalities when analysing the model and ensure that some divisors are non-zero. In principle, the analysis could be repeated assuming the heterozygote form is superior, or inferior, to homozygotes in either or both environments.

The potential for the high-dose refuge strategy to delay the evolution of resistance increases as the magnitude and dominance of fitness costs increase (Carrière and Tabashnik 2001). Empirical methods for estimating relative fitness parameters in wild populations are problematic and estimated values vary considerably (Bourguet et al. 2000, Tabashnik et al. 2004, Tabashnik et al. 2005). Exceptionally, some extremely resistant strains of diamondback moth are able to develop on Bt crucifers with no ill effects (for our purposes $\omega_{rr} \approx 1$) (Ramachandran et al. 1998, Tang et al. 1999), but generally resistance is incomplete (in our model $\omega_{rr} < \upsilon_{rr}$) and fitness costs have been observed in resistant

strains of several species (Bates et al. 2005). In cotton bollworm (*H. armigera*), laboratory-selected resistance to Cry2Ab toxin was found to be recessive and to confer a very high level of resistance ($\omega_{rr} \approx 1$) (Mahon et al. 2007b). As noted earlier, most cases of resistance to Bt toxins have been shown to be recessive ($\omega_{sr} \approx \omega_{ss}$), especially at the high levels expressed in Bt crops. Fitness costs in pink bollworm resistant to Cry1Ac Bt cotton have been shown to be substantial, recessive and mainly affecting survival (Liu et al. 2001, Carrière et al. 2005b). One study found fitness costs reduced survival of resistant larvae on non-Bt cotton by an average of 51.5% compared to susceptible strains ($\upsilon_{rr} = 0.485$) (Carrière et al. 2001a). Another study measured their survival on Bt cotton at 46% relative to survival on non-Bt cotton ($\omega_{rr} = 0.46\upsilon_{rr}$) (Liu et al. 2001). An overwintering cost in pink bollworm appeared to be recessive to some extent and reduced emergence from diapause in spring was conservatively estimated at 71% (Carrière et al. 2001b). Fitness costs can affect traits other than survival; for example, paternity effect experiments showed that resistant pink bollworm males can have reduced success in competing for virgin females, and resistant males that mated first sired fewer offspring than first-mating susceptible males (Higginson et al. 2005). Resistance to Cry1Ac Bt cotton in *H. armigera* in Australia has been shown to have fitness costs whose dominance and magnitude vary with the host plant species deployed in refugia – cotton, pigeon pea or sorghum (Bird and Akhurst 2007). However, experimental evidence did not support a hypothesis that different cotton cultivars would affect the fitness costs of resistance to Bt cotton in pink bollworm (Carrière et al. 2005b). The presence of a nucleopolyhedrovirus can increase the fitness costs of resistance to Bt Cry1Ac sprays in diamondback moth (Raymond et al. 2007).

In our analysis, we considered the full range of theoretically possible values of fitness parameters and did not restrict ourselves to relative values that are plausible in the context of managing resistance to Bt crops. Similarly, we used a wide range of values for our simulations.

We assume that eggs are laid at random across the whole habitat. Clearly this would be a harmful strategy for a specialist able to survive only in a single habitat sub-type. Such a situation could occur, and persist, if two environment types are indistinguishable by the pest. This might be reasonable where the host plant has a form that is resistant to insects, giving two distinct plant phenotypes (rather than a spectrum of plant resistance levels). This could happen through genetic mutation in the plant, or where the alternate variety is an invader (e.g. one-off seeding from another location) or was deliberately planted (e.g. Bt crops or a variety selected for resistance through classical horticultural breeding methods).

It is common to assume that the organism is in only one of the environments throughout the life stage that is subject to differential selection (Hartl and Clark 1989). US refuge requirements for Bt maize or cotton stipulate placement as well as size, to minimise larval movement between Bt and refuge but allow adult dispersal (Bourguet et al. 2005, Carrière et al. 2005a), so each larva should remain on either Bt or non-Bt plants.

2.3.3 Population genetics

The change in resistance allele frequency in a generation, Δp, is given by (Hartl and Clark 1989, Gillespie 1998):

$$\begin{aligned}\Delta p &= \frac{p^2\Omega_{rr} + pq\Omega_{sr}}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}} - p \\ &= \frac{pq\big(p(\Omega_{rr} - \Omega_{sr}) + q(\Omega_{sr} - \Omega_{ss})\big)}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}}\end{aligned} \qquad (2)$$

Where $p, q \neq 0,1$ (resistance allele present but not at fixation), the sign of Δp is determined by the sign of $p(\Omega_{rr} - \Omega_{sr}) + q(\Omega_{sr} - \Omega_{ss})$. We assess stability using end-point analysis (Gillespie 1998).

2.3.4 Simulations

To confirm our analysis, we performed a series of simulations over a wide range of parameter values by iterating genotype frequencies using equation 2, reflecting the order of events illustrated in Figure 2.1, and calculating allele frequencies at each generation from the genotype frequencies. We selected parameter combinations sufficient to cover all classes of outcome, without regard to whether the values are plausible in the specific context of managing resistance to Bt crops. Values were chosen across the following ranges: p_0 0.1–0.9, υ_{sr} 0.4-1, υ_{rr} 0.4-0.7, ω_{ss} 0-0.2, ω_{sr} 0-0.4, ω_{rr} 0.1-0.6 and Φ 0.3-1. We ran further simulations with randomly generated values satisfying the model constraints, some of which fell outside the stated ranges.

2.4 Results

2.4.1 Equilibrium points

There are potentially three steady state points (Figure 2.2): $p^*=0$, $p^*=1$ and $p^*=\rho$ where

$$\rho = \frac{-\Omega_{ss} + \Omega_{sr}}{-\Omega_{ss} + 2\Omega_{sr} - \Omega_{rr}} = \frac{\left(-1 + \Phi - \omega_{ss}\Phi + \upsilon_{sr} - \upsilon_{sr}\Phi + \omega_{sr}\Phi\right)}{\left(-1 + \Phi - \omega_{ss}\Phi + 2\upsilon_{sr} - 2\upsilon_{sr}\Phi + 2\omega_{sr}\Phi - \upsilon_{rr} + \upsilon_{rr}\Phi - \omega_{rr}\Phi\right)} \quad (3)$$

Equation 3 is only biologically relevant if ρ exists between 0 and 1.

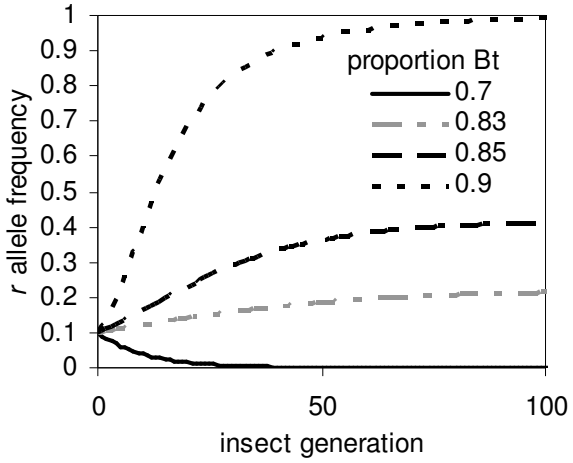

Figure 2.2 Division of habitat affects allele and phenotype frequency evolution The graph shows the frequency over time of the *r* allele for different divisions of the habitat and illustrates three possible outcomes: extinction, fixation or an intermediate steady state. The initial *r* allele frequency was 0.1. Relative fitnesses of the genotypes in the two environments were: $\upsilon_{ss} = 1$, $\upsilon_{sr} = 0.8$ and $\upsilon_{rr} = 0.4$ in the refuge and $\omega_{ss} = 0$, $\omega_{sr} = 0.05$ and $\omega_{rr} = 0.1$ on Bt crops. With these fitness parameters, the critical proportions are $\Phi_1 = 0.8$ and $\Phi_2 = 8/9 \approx 0.8889$. Where the proportion of Bt crops is below the lower threshold (Φ=0.7, solid line), the *r* allele goes to extinction. Above the higher threshold (Φ=0.9, dotted line), the *r* allele goes to fixation. In between the two (Φ=0.83, dashed grey line, or Φ=0.85, dashed black line), the *r* allele tends to an intermediate equilibrium (ρ=15/68 ≈0.2206 or ρ =5/12 ≈0.4167, respectively).

2.4.2 Threshold values for sizes of habitat sub-types (Bt crop area and refuge)

Special cases arise when the resistance allele is dominant, recessive or co-dominant, in both environments. These are dealt with separately below (§2.4.4).

In the three special cases there is no distinct third steady state ρ, so no internal equilibrium is possible. In other cases (partial dominance), there are two critical threshold values, Φ_1 and Φ_2, that determine whether a third steady state exists:

$$\Phi_1 = \frac{(1-\upsilon_{sr})}{(1-\upsilon_{sr}+\omega_{sr}-\omega_{ss})} \tag{4}$$

$$\Phi_2 = \frac{(\upsilon_{rr}-\upsilon_{sr})}{(\upsilon_{rr}-\upsilon_{sr}+\omega_{sr}-\omega_{rr})} \tag{5}$$

The third steady state ρ lies between 0 and 1 if and only if Φ lies between Φ_1 and Φ_2.

These thresholds Φ_1 and Φ_2 are calculated from the various fitness parameters, so the relative fitnesses of the three genotypes in the two environments determine the range of possible outcomes. The areas actually planted to Bt crops and refuge are important in determining which of those possible outcomes arises in practice.

Φ_1 is defined in terms of the fitness parameters of *sr* and *ss* genotypes. The proportion of crops expressing Bt toxins Φ relative to the threshold Φ_1 determines whether *sr* heterozygotes are fitter on average than *ss* individuals. Above that threshold ($\Phi>\Phi_1$), heterozygotes have higher average relative fitness than susceptible homozygotes ($\Omega_{sr}>\Omega_{ss}$). Below it ($\Phi<\Phi_1$), the heterozygotes are less fit on average ($\Omega_{sr}<\Omega_{ss}$) and at the threshold ($\Phi=\Phi_1$) they are equally fit ($\Omega_{sr}=\Omega_{ss}$).

Similarly, Φ_2 is defined in terms of the fitness of *sr* and *rr* genotypes. The Bt crop proportion Φ relative to the threshold Φ_2 determines whether *sr* heterozygotes are fitter on average than *rr* individuals. Above ($\Phi>\Phi_2$), below ($\Phi<\Phi_2$) or at ($\Phi=\Phi_2$) that threshold, *sr* individuals are, respectively, less fit on average ($\Omega_{sr}<\Omega_{rr}$), fitter ($\Omega_{sr}>\Omega_{rr}$) or equally fit ($\Omega_{sr}=\Omega_{rr}$).

These thresholds are determined by the interplay between the costs and benefits of resistance. If the difference in fitness between two genotypes is large on Bt crops compared to the difference between them in the refuge, the relevant threshold will be relatively small. Conversely, if the fitness difference is large in the refuge compared to the difference on Bt crops, the threshold will nearer to one. For example, if the *r* allele gives better resistance with two copies ($\omega_{sr} < \omega_{rr}$) and the fitness penalties of one or two copies are about the same ($\upsilon_{sr} \approx \upsilon_{rr}$), Φ_2 will be close to zero (eq. 5) and almost any planting of

Bt crops will mean that Φ is above Φ_2 and resistant homozygotes will be fitter on average than heterozygotes. If the fitness difference is the same in both environments, the relevant threshold is half of the habitat.

2.4.3 Eventual fate of resistance allele and stability of equilibria

In the general case (partial dominance), the stability of the system depends on the relative fitness of each genotype on Bt and non-Bt plants, in particular whether $\Phi_1 < \Phi_2$ or $\Phi_1 > \Phi_2$.

Where $\Phi_1 < \Phi_2$:

- if the proportion of Bt crops is below or equal to Φ_1, the resistance allele will decline to extinction (stable equilibrium $p^*=0$);
- if the proportion of Bt crops is above or equal to Φ_2, the resistance allele will go to fixation (stable equilibrium $p^*=1$);
- if the proportion of Bt crops lies between Φ_1 and Φ_2, the frequency of the resistance allele will settle at a stable internal equilibrium $p^*=\rho$ lying between 0 and 1.

Figure 2.2 illustrates these three possible patterns of frequency evolution of the resistance allele (extinction or fixation or intermediate equilibrium) for a given set of fitness parameters, and shows that the intermediate equilibrium frequency (ρ) depends on the value of Φ. Figure 2.3a shows the regions of outcomes on a plot of initial allele frequency (p_0) against the proportion of Bt crops (Φ). These equilibria are unique and globally stable over the range in question; for a given set of fitness values and a particular value of Φ, a unique equilibrium is determined and the *r* allele frequency will tend asymptotically toward that equilibrium from any value in the range $0<p<1$.

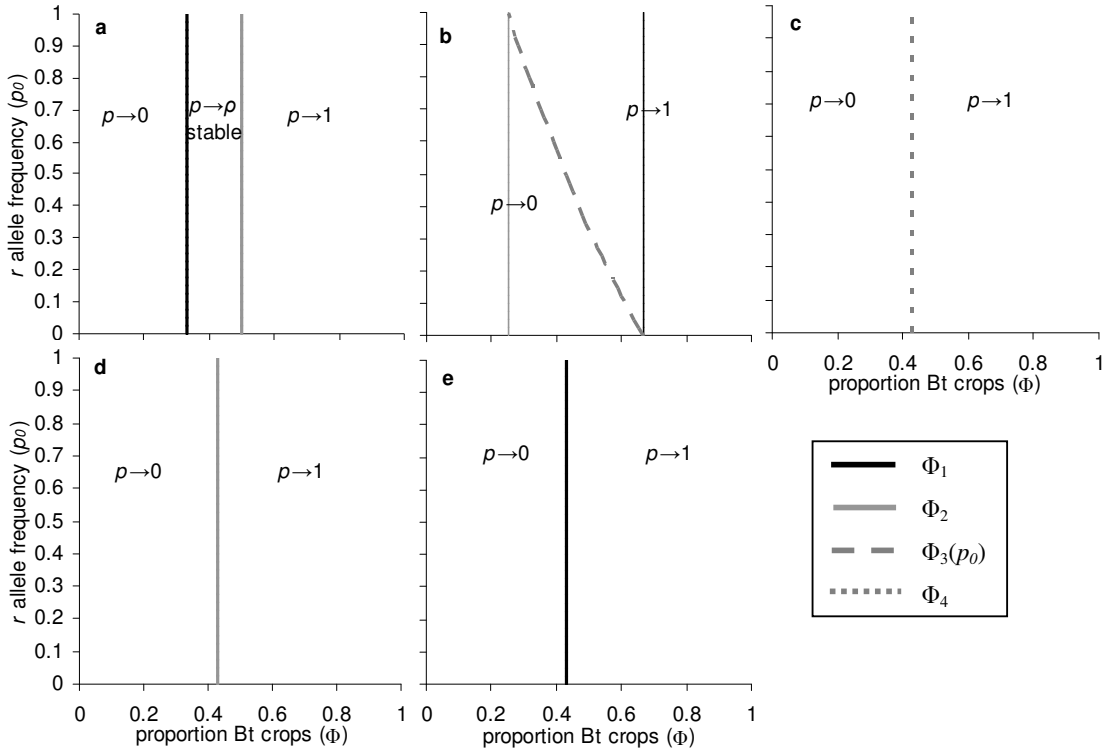

Figure 2.3 Critical thresholds determine allele fates and the nature of steady states The graphs show regions of outcomes for *r* allele frequency, plotting initial allele frequency (p_0) against the proportion of Bt crops (Φ). Resistant homozygotes have 0.7 relative fitness on non-Bt plants (υ_{rr}) and 0.4 fitness on Bt crops (ω_{rr}) and other genotypes are fully susceptible to the toxins ($\omega_{ss} = \omega_{sr} = 0$). The relative fitnesses of heterozygotes are varied between panels. (a) Incomplete dominance with $\Phi_1 < \Phi_2$ ($\upsilon_{sr} = 0.9$, $\omega_{sr} = 0.2$): with Φ at the thresholds the *r* allele goes to extinction ($\Phi = \Phi_1$) or fixation ($\Phi = \Phi_2$); ρ is a stable equilibrium. (b) Incomplete dominance with $\Phi_1 > \Phi_2$ ($\upsilon_{sr} = 0.8$, $\omega_{sr} = 0.1$): at the threshold ($\Phi = \Phi_3$) the *r* allele is at an unstable equilibrium (at frequency p_0). (c) Co-dominant ($\upsilon_{sr} = 0.85$, $\omega_{sr} = 0.2$). (d) Complete recessiveness ($\upsilon_{sr} = 1$, $\omega_{sr} = 0$). (e) Complete dominance ($\upsilon_{sr} = 0.7$, $\omega_{sr} = 0.4$). In (c)-(e), with Φ at the threshold, the *r* allele remains constant at any frequency. The only situation where the eventual *r* allele fate is dependent on p_0 is in case (b) where Φ lies between Φ_1 and Φ_2.

Where $\Phi_1 > \Phi_2$, the fixed point ρ is unstable (where it exists) and the resistance allele will eventually go to either extinction (stable equilibrium $p^*=0$) or fixation (stable $p^*=1$), depending on whether the proportion of Bt crops is, respectively, above or below a third threshold Φ_3, the value of which is dependent on the initial frequency of the resistance allele p_0 (Figure 2.3b):

$$\Phi_3 = \frac{\left(-1 + p_0 + \upsilon_{sr} - 2p_0\upsilon_{sr} + p_0\upsilon_{rr}\right)}{-1 + p_0 + \omega_{ss} - p_0\omega_{ss} + \upsilon_{sr} - \omega_{sr} - 2p_0\upsilon_{sr} + 2p_0\omega_{sr} + p_0\upsilon_{rr} - p_0\omega_{rr}} \qquad (6)$$

This threshold is a function of all the genotype fitness parameters and the initial *r* allele frequency. There is an unstable internal equilibrium ($p^* = \rho = p_0$), which is only achieved if the resistance allele frequency and proportion of Bt crops are at the appropriately related values from the start. If the proportion of Bt crops is equal to Φ_3, the frequency of the resistance allele will remain constant at p_0, but slight perturbations above or below that allele frequency (vertical movement away from the Φ_3 line on Figure 2.3b) will result in its fixation or extinction, respectively. As the value of the equilibrium ρ is a function of Φ, any perturbation in refuge size (horizontal disturbance on Figure 2.3b) will also lead to extinction or fixation. The equilibria $p^*= 0$ or 1 are locally stable; any perturbation that does not push the allele frequency to or beyond ρ (eq. 3, the allele frequency p at which $\Phi_3(p) = \Phi$) will not alter the *r* allele's ultimate fate.

The high-dose/refuge strategy aims to make the resistant phenotype functionally recessive ($\omega_{ss} = \omega_{sr}$) by having toxins expressed at sufficiently high levels. If the *r* allele is recessive to the *s* allele for resistance but the cost of resistance is not also recessive (so $\upsilon_{ss} > \upsilon_{sr}$), the threshold Φ_1 is equal to 1 (eq. 4). In these circumstances Φ_1 (=1) is always greater than Φ_2 and so an unstable steady state will exist at $\Phi=\Phi_3$ (which depends on the value of p_0) and the resistance allele will go to fixation or extinction according to whether the proportion of Bt crops is above or below this limit. If the *r* allele is initially rare ($p_0 \approx 0$), Φ_3 is also close to 1, so the allele will go extinct unless there is virtually no refuge.

Similarly, if the *r* allele is dominant to the *s* allele for resistance ($\omega_{rr} = \omega_{sr}$) but the cost of resistance is not dominant ($\upsilon_{sr} > \upsilon_{rr}$), the threshold Φ_2 is equal to 1 (eq. 5). This means $\Phi_1 < \Phi_2$ (=1) and so a stable internal equilibrium can exist. The resistance allele will decline to extinction or tend to the stable intermediate equilibrium ρ (defined by equation 3) according to whether the proportion of Bt crops is below or equal to Φ_1 (eq. 4), or between Φ_1 and 1, respectively. Such dominant resistance with non-dominant fitness penalties would never reach fixation. The outcome is independent of the initial frequency of the resistance allele, p_0.

Early tests on Bt cotton plants that achieved a high dose against tobacco budworm showed that the same plants only caused 75-90% relative mortality of susceptible bollworm (*H. zea*) larvae; a high dose was not achieved for that insect (Gould 1998). There are indications that cotton bollworm (*H. armigera*) is relatively more tolerant to Cry1Ac toxins than tobacco budworm, and inheritance of Cry1Ac resistance in *H. armigera* has been reported to vary from recessive to semi-dominant depending on the strain (Gujar et al. 2007b). If resistance to Bt cotton plants were non-recessive for such pests, one of Figure 2.3a & b would depict the relevant outcome. If relatively few heterozygous larvae could survive a life cycle on Bt cotton ($\omega_{sr} \approx 0$ and $\omega_{ss} = 0$), Φ_1 would be close to 1 (eq. 4) and so probably $\Phi_1 > \Phi_2$ and the situation shown in Figure 2.3b is likely to apply.

2.4.4 Special cases: co-dominant resistance, complete recessiveness or complete dominance

There are three special cases to consider, for which the outcomes are broadly similar.

In the case where both resistance and costs of resistance are co-dominant ($\omega_{sr} = ½(\omega_{ss} + \omega_{rr})$ and $\upsilon_{sr} = ½(\upsilon_{ss} + \upsilon_{rr})$, so $\Omega_{sr} = ½(\Omega_{ss} + \Omega_{rr})$), there is no third

steady state (eq. 3 denominator would be zero), and the resistance allele will eventually go to either extinction or fixation, depending on whether the proportion of Bt crops is above or below a fourth threshold Φ_4.

$$\Phi_4 = \frac{(1-\upsilon_{rr})}{(1-\upsilon_{rr}+\omega_{rr}-\omega_{ss})} \qquad (7)$$

This formula for Φ_4 is analogous to those for Φ_1 (eq. 4) and Φ_2 (eq. 5), but is defined in terms of the fitness parameters of the homozygous genotypes. For co-dominance (and for the completely recessive or dominant cases), the fitnesses of the heterozygotes in each environment are defined by the fitnesses of the homozygotes in that environment. The smaller the fitness difference between homozygotes on Bt plants ($\omega_{rr}-\omega_{ss}$) compared to the fitness difference in the refuge ($1-\upsilon_{rr}$), the nearer the critical threshold will be to 1.

Where the costs and benefits of resistance are fully recessive ($\upsilon_{ss}=\upsilon_{sr}=1$ and $\omega_{ss}=\omega_{sr}$, so $\Omega_{ss}=\Omega_{sr}$), equation 3 is equal to zero, so there is no distinct third steady state ρ, and Φ_1 does not exist. Φ_2 is the only critical threshold. Substituting $1=\upsilon_{sr}$ and $\omega_{ss}=\omega_{sr}$ into equation 5 shows that in this particular case Φ_2 is equal to Φ_4 as defined in equation 7. Similarly, Φ_2 also equals Φ_3 as defined in equation 6 and, in this case, Φ_3 is independent of p_0.

Where the costs and benefits of resistance are fully dominant ($\upsilon_{rr}=\upsilon_{sr}$ and $\omega_{rr}=\omega_{sr}$, so $\Omega_{rr}=\Omega_{sr}$), equation 3 is equal to one, there is no distinct third steady state ρ and Φ_2 does not exist. Again, the sole critical threshold, which here is Φ_1, is equal to Φ_4 (shown by substituting $\upsilon_{rr}=\upsilon_{sr}$ and $\omega_{rr}=\omega_{sr}$ into equation 4) and also equal to Φ_3, which is now independent of p_0.

In all three special cases (Figure 2.3c-e), if the proportion of Bt crops (Φ) is below the relevant threshold Φ_4, the resistance allele will decline to extinction (sole equilibrium $p^*=0$, globally stable over the range $0<p<1$). If Φ is above Φ_4, p will tend to fixation (sole globally stable equilibrium $p^*=1$). These results are consistent with Karlin's (1977) finding in a multi-deme model that, with a dominant or a recessive trait, fixations of the two alleles cannot both (simultaneously) be locally stable. If Φ is equal to Φ_4, the frequency of the resistance allele will remain constant regardless of its initial value, i.e. for <u>any</u> p_0.

At this critical threshold Φ_4, the steady state is not a conventional equilibrium value for the *r* allele frequency. At a stable (or unstable) equilibrium point the frequency would not change, but at values of *p* either side of the equilibrium the frequency would move towards (or away from) the equilibrium value. Here, if $\Phi=\Phi_4$ the frequency *p* does not change, whatever its value ($\Delta p = 0$ for <u>all</u> *p*). The relative fitness of the various genotypes on conventional and Bt crops (υ_i, ω_i) and the refuge size ($1-\Phi$) are such that the average fitnesses of all three genotypes are equal ($\Omega_{ss} = \Omega_{sr} = \Omega_{rr}$) and so there is no selective pressure and the frequency of the *r* allele will remain constant at any value. Any perturbation in allele frequency (assuming no change in refuge size) will merely result in the allele frequency staying constant at the new value. Any perturbation in refuge size will lead to extinction or fixation of the *r* allele. So this steady state is unstable with respect to the proportion of Bt crops (Φ), but not with respect to the frequency of the resistance allele (*p*).

As noted above, resistance to Bt toxins is thought to be recessive for many pests so if fitness costs are also recessive (as observed in pink bollworm, for example) the situation represented by Fig 3d is the most likely in practice.

2.4.5 Dependence on initial conditions

Only in the case of partial dominance where $\Phi_1 > \Phi_2$, is the critical threshold proportion of Bt crops, Φ_3, dependent on the initial frequency of the resistance allele p_0. However, Φ_3 always lies between Φ_2 and Φ_1 (Figure 2.3b). In both the general cases of partial dominance (Figure 2.3a&b), a sufficiently large or sufficiently small refuge will determine the ultimate fate of the resistance allele independently of initial conditions. If Φ is below the lower of Φ_1 and Φ_2, the resistance allele will go to extinction regardless of p_0. If it exceeds the higher of Φ_1 and Φ_2, the *r* allele will go to fixation irrespective of its initial frequency.

Where resistance is co-dominant, completely recessive or completely dominant the threshold proportion of Bt crops (Φ_4) is independent of the initial frequency of the resistance allele. Thus, the eventual fate of the *r* allele is not sensitive to its initial frequency.

2.4.6 Comparison of completely dominant and completely recessive resistance

Note that the thresholds for extinction or fixation are the same for completely recessive resistance and completely dominant resistance (see §2.4.4 above). For any particular homozygote fitnesses (*rr* and *ss*), a given refuge size will lead to the same eventual frequency of the resistance allele (i.e. fixation or extinction according to whether the proportion of Bt crops is above or below Φ_4) for both (Figure 2.3d&e). However, the rate of approach to fixation or extinction, which depends on the initial resistance allele

frequency p_0 and the magnitude of Φ, would be different (compare values of Δp in equation 2 with either Ω_{rr} or Ω_{ss} substituted for Ω_{sr}). Either the recessive version or the dominant version can be faster to extinction or faster to fixation, depending on the parameter values and initial conditions.

2.5 Discussion

Here we have shown from analysis and simulations of a simple population genetic model that there are critical threshold proportions for dividing a habitat which will determine the equilibrium allele frequencies. We also identified the way in which the nature of the steady states at these thresholds depends on the dominance of resistance and of fitness costs. Our motivation for this was to understand how insect resistance might evolve in response to areas planted with transgenic (Bt) and conventional crops.

Arthropods are well-known to cause major devastation of crops, destroying around 18% of the world annual crop production, and act as vectors for many human and veterinary diseases (Nicholson 2007). Widespread resistance to insecticides among arthropod populations limits the efficacy of pest control. Resistance has now been reported in a wide variety of important insects, and against every chemical class of insecticide, including microbial drugs and insect growth regulators (Nicholson 2007).

A recent study (Brookes and Barfoot 2006) highlights global benefits attributed to GM crop technology used by millions of farmers worldwide, most of them resource-poor farmers in developing countries. Insect resistant crops have had positive economic effects, increasing global agricultural income, achieving environmental gains, improving health and safety and increasing crop quality. The evolution of resistance to plant-incorporated insecticidal toxins would be of economic and environmental concern.

Analysis of our population genetic model illustrates that the evolution of resistance allele frequency depends on the relative sizes of Bt crop areas and refugia and on the relative fitnesses of the genotypes in each environment. The relative advantages of the genotypes are dictated by the two threshold values Φ_1 and Φ_2 (which depend on the genetic parameters) and the proportion of the habitat that is planted with Bt crops (Φ) relative to those thresholds. A necessary and sufficient condition for stable polymorphism is that $\Phi_1 < \Phi < \Phi_2$. This is equivalent to heterozygotes having the highest average fitness across the entire habitat, i.e. "marginal overdominance" (Wallace 1968). Similarly, the condition for unstable polymorphism, $\Phi_2 < \Phi < \Phi_1$, is equivalent to heterozygotes having lower average fitness than both homozygotes, i.e. "marginal underdominance". The potential for existence of a stable intermediate equilibrium decreases if the *r* allele is increasingly recessive to the *s* allele for resistance (as ω_{sr} approaches ω_{ss}), or with increasingly dominant costs of resistance (as υ_{sr} approaches υ_{rr}).

For all parameter combinations except where there is marginal underdominance, the outcome is not sensitive to the initial allele frequency. Under the model assumptions, for any set of genetic parameter values, resistance can be reversed, even from very high initial frequencies, by arranging for a sufficiently large refuge to exist for a sufficiently long time (although this may be an impractical or uneconomic solution to managing an actual resistance event). The key assumption here is that resistance has fitness costs so the *r* allele is always at a disadvantage in untreated areas (or in untreated hosts, in the case of resistant pathogens).

Where both resistance and fitness costs are recessive, dominant or co-dominant, a habitat divided in exactly the critical threshold proportions will maintain allele frequencies whatever their initial prevalence (i.e. not just at a single equilibrium allele frequency but at all allele frequencies). Habitats are usually dynamic systems, so the proportions into which the habitat is divided may fluctuate over time and the allele frequencies would not be expected to remain constant indefinitely. However, where the mix of environments is at or near a critical threshold proportion, or fluctuates around such a threshold, apparent stability might be observed for a number of generations. In resistance management, the wrong size of refuge, untreated area or group could effectively maintain the frequency of resistance alleles, at least for the short term.

The extent to which habitat proportions are amenable to control or influence, or even to measurement, varies with context. Planting of Bt or non-Bt crops can be controlled with reasonable accuracy, although less so if the pest is a more generalist species and alternative host plants persist nearby. However, the level of drug usage in a population might vary between areas of different transmission intensity and a policy to limit a new drug to small fraction of cases can be impossible to implement because of preferred use by health professionals and self-treatment by the general public (Hastings and D'Alessandro 2000). Nevertheless, if fitness values can be estimated, a model such as this can indicate the likely limitations on the relative size of the untreated group or area, even if that division cannot be implemented precisely in practice.

Models such as the one presented here identify factors that should be considered in the design of resistance management programmes but that remain unknown and need to be measured in the field or laboratory. It can be difficult, expensive and inaccurate to

measure crucial parameters such as baseline frequencies of resistance alleles or small fitness differences between genotypes; rather than attempting to measure these factors directly, practitioners are often limited to considering their implications in principle when designing a control or surveillance project (Hastings and D'Alessandro 2000). Mathematical models provide a powerful tool, setting out key relationships between factors that can assist in the planning and management of insect resistance.

The central assumptions in any mathematical model need careful evaluation. For instance, our assumption of random mating and egg-laying might break down over time. In the long term, genetic traits might arise that limit gene flow and potential consequences include isolation leading to speciation (Templeton 1981). In the context of resistance to Bt crops, there is no isolating mechanism, but there might be selection for mating barriers between resistant and susceptible forms if the combination of fitness parameters and refuge size makes heterozygotes the inferior form on average (Kirkpatrick and Ravigné 2002). However, the potential for speciation in this manner is likely to be low, as the conditions under which it can arise are restrictive and indirect selection (as here, where an assortment trait would probably be influenced by other genes that are only genetically correlated with the resistance gene, on which direct selection is acting) is a weaker force than direct selection (Templeton 1981, Kirkpatrick and Ravigné 2002). Note that habitat divergence is not an issue in these circumstances, because it is unlikely that the resistant phenotype would evolve to be fitter on the toxic crops than in the refuge (for example), so all phenotypes would prefer the same habitat if they were able to identify it.

Although numerous models predict that refugia can delay the evolution of resistance to high-dose Bt crops (Alstad and Andow 1995, Gould 1998, Carrière and Tabashnik 2001,

Tabashnik et al. 2005), few of them (Vacher et al. 2003, Tabashnik et al. 2005, Mohammed-Awel et al. 2007) attempt to find a threshold value for refuge size. Typically, a small number of discrete values for refuge size are simulated and, of course, experimental evidence supporting the prediction was likewise restricted to a small number of refuge sizes. Most published models on Bt resistance management are sophisticated simulations, often of specific crops and their target pest insects; the underlying models are too complicated to allow mathematical analysis so that a threshold refuge size could be expressed in terms of fitness parameters and habitat division, as we have done.

Our model provides a framework that is useful for gaining overall insight into complex allele dynamics in heterogeneous habitats. Key conclusions may extend to more sophisticated models. Other numerical simulation models that take account of spatial structure also indicate that there is a critical size of refuge or untreated area determining whether resistance evolves against pesticides (Lenormand and Raymond 1998) and, in a stochastic framework, against Bt crops (Vacher et al. 2003). A simple model including constant immigration of susceptible pests, emigration and density dependent population growth also identified critical refuge size thresholds, although it showed that in some circumstances with intermediate dominance values for the resistance allele, a refuge could spoil the resistance management benefit from immigration of susceptible insects (Mohammed-Awel et al. 2007). Although we have examined the case of a diploid organism reproducing sexually, population genetics models of insecticide resistance in haplodiploid insects have found results similar to those for diploids (Crowder et al. 2006), and other models suggest that bacteria resistant to antibiotics can only become established when the rate of treatment or prophylaxis exceeds a threshold value (Levin 2001). In the

case of pathogens, a fuller understanding would require consideration of epidemiological factors as well as a population genetics approach.

We have presented a model that provides a generic framework with which to gain insights into the evolution of allele frequency in heterogeneous habitats that can be applied in a range of ecological and public health settings. In principle, it could be broadened further by extension to more than two alternative alleles and more than two environments within the habitat.

3 Managing insecticide resistance by mass-release of engineered insects

Summary Transgenic crops producing insecticidal toxins are now widely used to control insect pests. The benefits of this method would be lost if resistance to the toxins spread to a significant proportion of the pest population. The primary resistance management method, mandatory in the US, is the high-dose/refuge strategy, requiring toxin-free crops as refuges near the insecticidal crops, and the use of toxin doses sufficiently high to kill insects heterozygous for a resistance allele, thereby rendering resistance functionally recessive. We propose that mass-release of harmless susceptible (toxin-sensitive) insects could substantially delay or even reverse the spread of resistance. Mass-release of such insects is an integral part of RIDL® (Release of Insects carrying a Dominant Lethal), a method of pest control related to the Sterile Insect Technique (SIT). We show by mathematical modelling that specific RIDL strategies could form an effective component of a resistance management strategy for plant-incorporated protectants (PIPs) and other toxins.

3.1 Introduction

Transgenic plants, principally maize and cotton, producing insecticidal proteins derived from *Bacillus thuringiensis* Berliner (Bt), grew on 32 million ha worldwide during 2006 (James 2006). The use of such plant-incorporated protectants (PIPs) can reduce reliance on insecticidal sprays, thereby providing economic, health and environmental benefits (Shelton et al. 2002). However, such benefits would be reduced or even eliminated if resistance to PIPs became widespread in pest populations. For many pest species, strains substantially resistant to Bt toxins have been selected in the laboratory, though resistance to Bt crops has not yet been found in the field (Tabashnik et al. 2003, Bates et al. 2005,

Tabashnik et al. 2005, Ferry et al. 2006). However, field resistance to Bt sprays has been found in diamondback moth *Plutella xylostella* (L.) (Ferré and Van Rie 2002) and greenhouse populations of cabbage looper *Trichoplusia ni* (Hübner) (Janmaat and Myers 2003). Considerable attention has therefore been devoted to methods to prevent or reverse the spread of resistance through wild pest populations.

Mathematical models predict that the high-dose/refuge strategy slows or prevents the spread of resistance (Alstad and Andow 1995, Gould 1998, Carrière and Tabashnik 2001, Tabashnik et al. 2003, Vacher et al. 2003, Tabashnik et al. 2004, Tabashnik et al. 2005). Resistance to Bt toxins is thought to be partially dominant, with a strong recessive component (Ferré and Van Rie 2002, Tabashnik et al. 2003). The high-dose part of the strategy aims to reduce this to purely recessive resistance by using sufficiently high doses of the toxin that heterozygotes are not functionally resistant (i.e. do not survive). Refuges are a crucial component – they provide an area of non-Bt host plants and thereby provide a source of susceptible (toxin-sensitive) genotypes that, by mating with insects from toxin-treated regions, tend to reduce the frequency of resistance alleles.

In principle, an alternate source of susceptibility alleles could be provided by mass-rearing susceptible insects and deliberately releasing them into the environment. We first investigated the simple case of the release of wild type, susceptible insects, before exploring the potential use of more sophisticated, non-wild type strains.

The mass-release of normal, fertile pest insects is unlikely to be acceptable in practice. In the Sterile Insect Technique (SIT), pest insects are indeed mass-reared and released, but only after they have been sterilized by irradiation (Knipling 1955, Krafsur 1998, Dyck et

al. 2005). In this case, no viable hybrids are formed between the released insects and the wild population, so there is no introgression of susceptibility alleles into the wild population. However, we have previously proposed a modification to the SIT in which the released insects are not irradiated, but instead are homozygous for one or more dominant lethal genes (Release of Insects carrying a Dominant Lethal, RIDL) (Thomas et al. 2000, Alphey 2002, Alphey and Andreasen 2002, Gong et al. 2005, Alphey 2007). One version of this system posits the release of male insects homozygous for one or more female-specific dominant lethals (RIDL males) (Figure 3.1). These lethal genes would be repressed throughout mass-rearing, except in the generation intended for release (male-only release is generally desirable for SIT (Rendón et al. 2004, Marec et al. 2005)). Released RIDL males would mate with wild females; all of their F_1 progeny would inherit a dominant female-specific lethal. The F_1 females would die, thereby reducing the reproductive potential of the wild population (much like the SIT), but the males would live. Half of their (F_2) daughters would also inherit the RIDL gene(s) and die, so there would be some modest additional suppression. Strains with the necessary genetic properties for this female-lethal version of RIDL have been constructed in several species, with proof-of-principle in *Drosophila melanogaster* (Heinrich and Scott 2000, Thomas et al. 2000) and recent success in a major pest insect, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Fu et al. 2007). No such strains have yet been described in Lepidoptera, but there is active work and progress in this area.

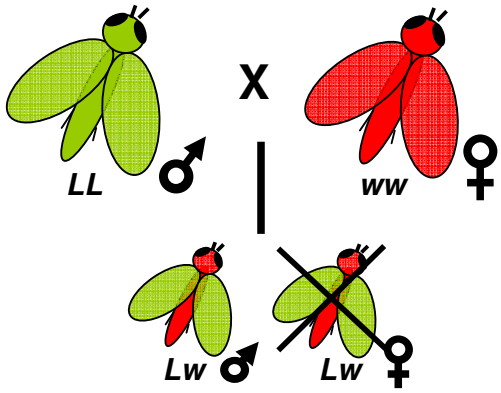

Figure 3.1 Female-lethal RIDL
Released RIDL males are homozygous for one or more female-specific dominant lethal genes (*LL*). Offspring of RIDL males and wild females (*ww*) are heterozygous for the RIDL construct (*Lw*). *Lw* females die but *Lw* males are unaffected, inheriting one copy to pass on to half their offspring.

The potential utility of such systems for control of target pest populations has been explored by mathematical modelling, which indicated several advantages over conventional SIT (Schliekelman and Gould 2000b, Thomas et al. 2000, Alphey 2002, Alphey and Andreasen 2002). A previously unidentified and novel feature of such systems is that mating between released males and wild females will produce viable, fertile, male progeny, leading to introgression of alleles from the mass-reared strain into the wild population. This has significant benefits for any control programme combining the use of female-specific RIDL with the use of toxins, such as PIPs.

The high-dose/refuge strategy is underpinned by a body of theoretical analysis and some years of field experience (Alstad and Andow 1995, Gould 1998, Carrière and Tabashnik 2001, Shelton et al. 2002, Glaser and Matten 2003, Tabashnik et al. 2003, Bates et al. 2005, Tabashnik et al. 2005). Assuming random mating and completely (or nearly) recessive resistance, the factors favouring prevention or reversal of resistance are thought to be: non-recessive costs of resistance, low initial frequency of the resistance allele (*r*), large refuges, and incomplete resistance (*rr* genotype fitness on transgenic crops less than on non-transgenics). We explored the effects of the release of RIDL males on the spread

of resistance, and investigated the relationship between the ratio of released RIDL males to males in the wild, the refuge size and the fitness parameters of the resistant insects.

3.2 Methods

3.2.1 Use of wild type insects

We used a deterministic, discrete-generation population genetic model (Carrière and Tabashnik 2001, Tabashnik et al. 2005) and assume a closed homogeneous population, with random mating, and no immigration, emigration or mutation. We assume that eggs are male and female in equal proportions (sex ratio 1:1). Larvae, the Bt-susceptible life stage, are assumed to spend their whole development either on Bt crops or in the refuge; migration occurs after emergence as adults and prior to mating.

We assume a fixed crop population, a proportion Φ of which expresses Bt toxins, so the conventional (non-Bt) crop refuge size is 1-Φ. Larval susceptibility to Bt toxins is assumed to be controlled by a single autosomal locus with two alternative alleles: resistant *r* (frequency *p*) and susceptible *s* (frequency *q*, $p + q = 1$). There are three genotypes at this locus: *ss*, *sr* and *rr*.

All insects are affected by Bt toxins. Resistant insects are expected to be at a selective disadvantage on non-Bt host plants. We assume that all fitness costs associated with the *s* and *r* alleles take effect during the larval stage. Assuming that larvae are distributed at random across Bt and non-Bt crops, the average relative fitness of larvae of genotype *i* (*i*: *ss, sr* or *rr*) is

$$\Omega_i = \omega_i \Phi + \upsilon_i (1 - \Phi) \qquad (1)$$

where ω_i is the relative fitness of larvae on Bt crops and υ_i is relative fitness of larvae in non-Bt crops. Fitness is measured relative to *ss* homozygotes on non-Bt crops (so υ_{ss} =1).

With no release of insects, the change in *r* allele frequency in the next generation is given by

$$\Delta p = \frac{pq\left(p\Omega_{rr} + (q-p)\Omega_{sr} - q\Omega_{ss}\right)}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}} \qquad (2)$$

With release of homozygote susceptible adults (*ss* wild type males and females in equal numbers) in ratio *d*:1 to adults in the wild, the change in *r* allele frequency is given by

$$\Delta p = \frac{p\left(pq\Omega_{rr} + (q+d)(q-p)\Omega_{sr} - (q+d)^2\Omega_{ss}\right)}{(q+d)^2\Omega_{ss} + 2p(q+d)\Omega_{sr} + p^2\Omega_{rr}} \qquad (3)$$

3.2.2 Use of engineered insects

We extended the basic model to incorporate the effects of release of RIDL males. We assume that at each generation, as adults emerge prior to mating, mass-reared adult RIDL males are released at a fixed ratio to the number of males in the wild population. The RIDL release ratio is defined as the number of RIDL males released per insect generation, as a proportion of the number of males in the wild (of any genotype) in that generation that survived to reproductive maturity. Females mate with wild or released males at random.

All male, engineered released males are assumed to be homozygous susceptible to Bt toxins (*ss*), and homozygous for the RIDL construct. There are two possible alleles at the insertion site of the RIDL construct: *L*, representing insertion of the construct, and the wild type allele *w*, representing absence of the construct. We assume the RIDL insertion and the locus controlling resistance to Bt are not linked and neither of these is sex-linked.

We assume the RIDL construct imposes a sex-specific fitness cost ε during the larval stage. The average relative fitness of larvae of genotype *i* is then

$$\Omega_i = (1-\varepsilon_i)[\omega_i\Phi + \upsilon_i(1-\Phi)] \qquad (4)$$

where ε_i is the sex-specific, relative fitness cost imposed by RIDL.

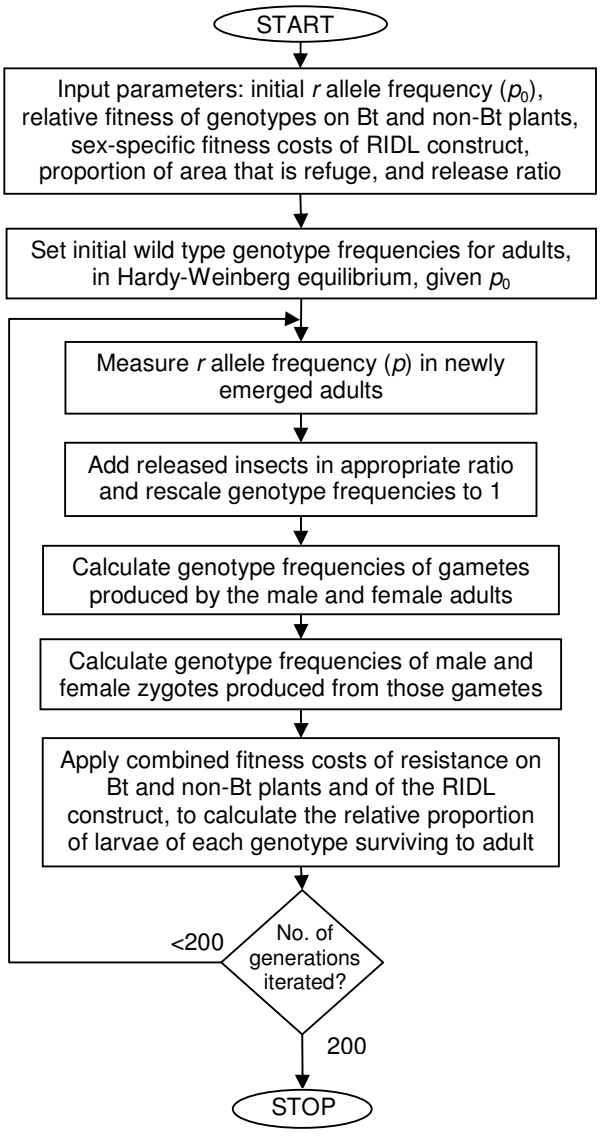

Figure 3.2 Simulation

A simulation approach was adopted (Figure 3.2) because the resulting system of difference equations is unwieldy (they involve eighteen potential genotypes and cannot be reduced to expressions in terms of *p*) and there are no analytical solutions for allele or genotype frequencies or for the critical release ratios or critical refuge sizes.

We assume RIDL-induced lethality is fully dominant and penetrant, so for *Lw* females $\varepsilon_{\text{female}} = 1$ and average fitness $\Omega_i = 0$, irrespective of Φ, ω_i and υ_i. Under these conditions, *LL* homozygotes do not arise in the field and there are 7 male and 3 female viable genotypes defined at the *L/w* autosome and *s/r* locus. The *LLss* male genotype is the engineered RIDL release male, and six male genotypes (*Lwss*, *Lwsr*, *Lwrr*, *wwss*, *wwsr* and *wwrr*) and three female genotypes (*wwss*, *wwsr* and *wwrr*) arise from matings in the field. In these simulations, $\varepsilon_{\text{male}}$ was set to 0.1 for *Lw* male larvae, to allow for the modest fitness penalty likely to be associated with the RIDL insertion (for example, due to insertional mutagenesis and/or expression of the marker gene (Moreira et al. 2004, Marrelli et al. 2006)).

We explored the effects of release of homozygous RIDL males on the spread of resistance by simulating the frequencies of all genotypes under the above assumptions over 200 generations. We simulated outcomes for different combinations of υ and ω representing fully recessive costs and benefits of resistance ($\omega_{rr} = 0.1$, 0.2 or 0.4, $\upsilon_{rr} = 0.7$ or 0.4, $\upsilon_{sr} = \upsilon_{ss} = 1$, $\omega_{ss} = \omega_{sr} = 0$) (Carrière and Tabashnik 2001), i.e. six different combinations of ω_{rr} and υ_{rr}, over a range of refuge sizes (0 to 0.6) and initial *r* allele frequencies p_0 (0.1, 0.01 or 0.001). The range of p_0 values used reflects field data: 0.0015 estimated for tobacco budworm *Heliothis virescens* (F.) (Gould et al. 1997), 0.13 and 0.16 in 1997 and a mean of 0.004 across 1998-2004 (95% confidence limits 0 to 0.01, annual estimates vary

approximately from 0 to 0.08) for pink bollworm *Pectinophora gossypiella* (Saunders) in Arizona (Tabashnik et al. 2000, Tabashnik et al. 2005), 0.0015 (95% credibility interval 0 to 0.0044) for European corn borer *Ostrinia nubilalis* (Hübner) in the US southern corn belt in 2000-2003 (Stodola et al. 2006) and 0.0023 (95% confidence interval 0.0003-0.0064) for sugarcane borer *Diatraea saccharalis* (F.) in Louisiana in 2004 (Huang et al. 2007). We explored release ratios from 1:1000 (one RIDL male per thousand males in the wild) to 100:1.

3.3 Results

3.3.1 Use of wild type insects

The average relative fitnesses of each genotype, determined from the parameters, dictate whether resistance spreads and threshold conditions for decrease of *r* allele frequency can be deduced. We found that, if *ss* homozygotes are released each generation at a constant ratio to the wild population, these thresholds increase, allowing for the same level of control to be provided by a smaller refuge.

3.3.2 Use of engineered insects

We found that, in all cases, release of RIDL males slowed the spread of resistance. A significant effect was observed, in some cases reversing the spread of resistance, at release ratios considerably lower than those typical for SIT programmes providing effective suppression of the target population. Figure 3.3 shows an example, in which *p* (the frequency of the *r* allele) reaches 0.5 in 36 generations with no release of insects (Carrière and Tabashnik 2001). Release of RIDL insects, at a ratio of only 1:5, reverses the spread of resistance. Figure 3.3 also illustrates the potential for rapid increase in the frequency of resistant individuals (*rr* homozygotes) after a long lag phase. This is a consequence of the

non-linear relationship between the frequency of the resistance allele (p) and the frequency of resistant individuals.

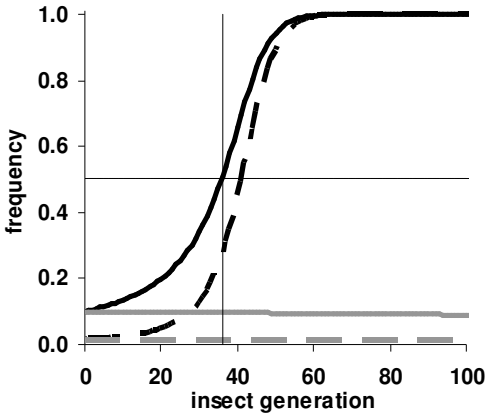

Figure 3.3 Releasing RIDL males can reverse the spread of resistance
The graph shows the frequency over time of the *r* allele (solid lines) and the *rr* resistant phenotype (dashed lines), for no release of insects (black lines) and for a RIDL release ratio of 1:5, i.e. one RIDL male per 5 males in the wild (grey lines). The initial *r* allele frequency was 0.1, the refuge size was 10%. The costs and benefits of resistance were fully recessive ($\upsilon_{sr} = \upsilon_{ss} = 1$), with resistant homozygotes having relative fitness of 0.1 on Bt crops (ω_{rr}) and 0.4 fitness on non-Bt crops (υ_{rr}). Other genotypes are fully susceptible to the Bt toxins ($\omega_{ss} = \omega_{sr} = 0$). With these parameters the resistance allele frequency p increases over time, reaching 0.5 in 36 generations (Carrière and Tabashnik 2001). However, release of RIDL males at a ratio of only 1:5 was sufficient to reverse this.

We explored the effect of the RIDL release ratio on the change over time of the frequency of the resistance allele, under various assumptions about the fitness of *rr* homozygotes. This is illustrated in Figure 3.4 for a particular set of fitness values. In each case, sufficient release of RIDL males can prevent the spread of the resistance allele. Reducing the refuge size increases the rate at which the resistance allele will spread, and correspondingly increases the number of RIDL males required to be released to prevent the spread of resistance (Fig. 3.4, compare panels (a) and (c) with (b) and (d) respectively), though any RIDL release will always slow the spread relative to no release. As well as refuge size, another critical parameter is p_0, the initial allele frequency of *r*. For fully recessive resistance, the fitness advantage of the resistance allele is strongly frequency-dependent, as the frequency of the homozygote, the only form to gain significant advantage, is approximately the square of the allele frequency; with no RIDL release, at equilibrium, this is p^2. For given fitness parameters of *r*, a higher value of p_0 (Fig. 3.4,

compare panels (a) and (b) with (c) and (d) respectively) or a smaller refuge mean that a higher RIDL release ratio is required to prevent the resistance allele from spreading.

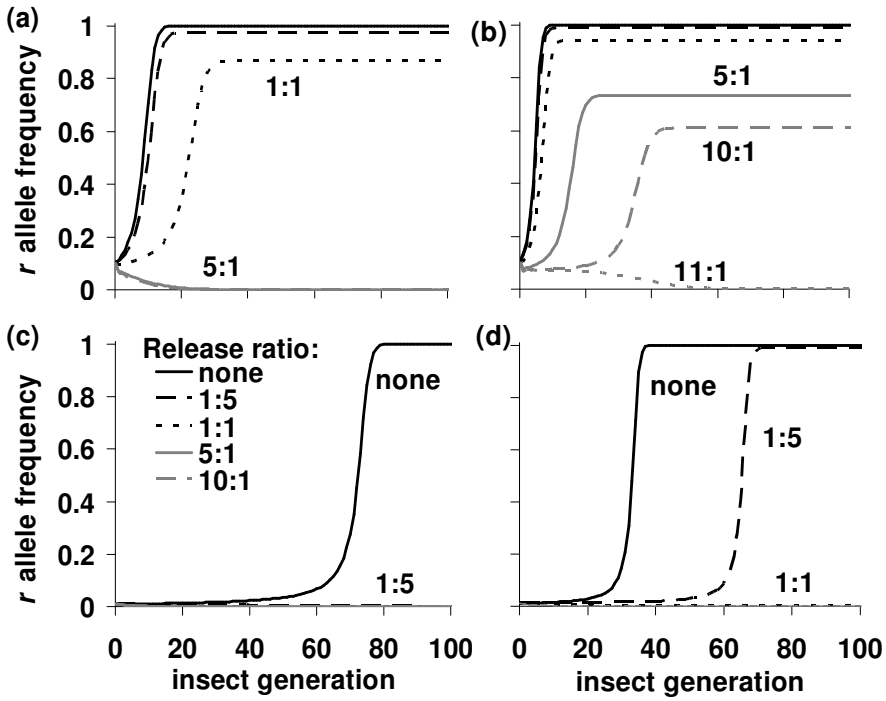

Figure 3.4 Effect of release ratio on the spread of resistance, for different refuge sizes and initial allele frequencies
The graphs show the frequency over time of the *r* allele for no release and for RIDL release ratios 1:5, 1:1, 5:1, 10:1 and, in panel b, 11:1. Fitness assumptions were as in Figure 3.3 ($\upsilon_{sr} = \upsilon_{ss} = 1$ and $\omega_{ss} = \omega_{sr} = 0$) except those of resistant homozygotes were 0.2 on Bt crops ($\omega_{rr} = 0.2$) and 0.7 on non-Bt plants ($\upsilon_{rr} = 0.7$). The initial *r* allele frequency (p_0) was 0.1 (panels a and b) or 0.01 (c and d), and the refuge size was 10% (a and c) or 5% (b and d). In all cases, release slows the spread of the *r* allele. Above a critical threshold release ratio the release reverses the spread of resistance. Below this threshold, the *r* allele increases to an equilibrium frequency which may be less than 1. Note the potential for a long lag phase followed by rapid increase of *r* allele frequency, where there is no, or insufficient, RIDL release.

We investigated the relationship between the minimum refuge size required to prevent the spread of resistance and the RIDL release ratio, for various fitness parameters of *r* and initial allele frequencies of *r* (Figure 3.5). For all parameter values examined, a considerable reduction in the minimum refuge size required to prevent spread can be achieved by release of RIDL males.

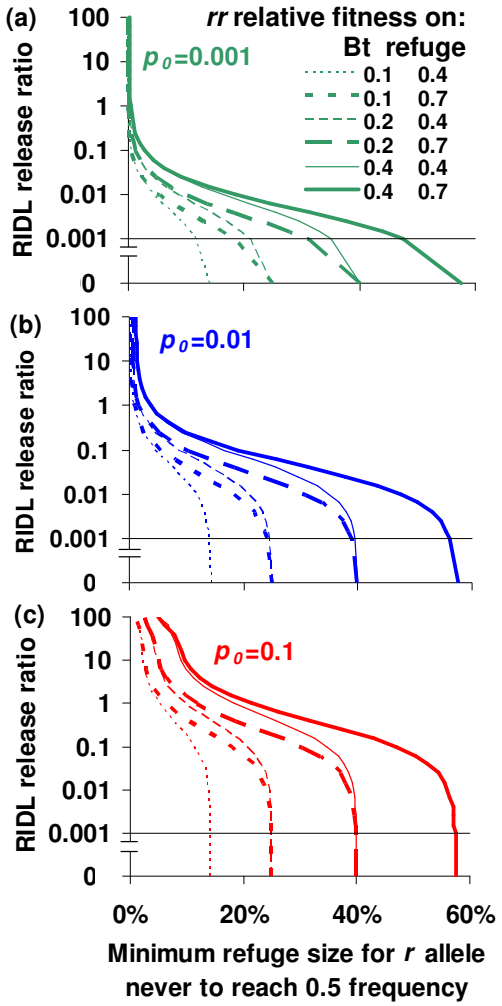

Figure 3.5 Effect of RIDL release on the minimum refuge size needed to stop the spread of resistance

Critical refuge sizes at which p neither increases nor decreases were calculated for the 'no release' baseline case and were closely approximated for a range of RIDL release ratios from 1:1000 to 100:1 by determining the minimum refuge size for which resistance allele frequency will not reach 0.5 (i.e. will either decrease or reach a non-zero equilibrium frequency below 0.5). These are shown for three initial r allele frequencies (p_0); a: 0.001, b: 0.01 and c: 0.1 and for various fitness parameters. The RIDL release ratio axis is on a log scale except that the values for 0 (no release) are plotted and joined to those for 0.001 (1:1000) by straight lines. As in Figures 3.3 and 3.4, resistance and costs of resistance are fully recessive. Fitness of resistant homozygotes on Bt crops (ω_{rr}) is 0.1, 0.2 or 0.4 and fitness on non-Bt crops (υ_{rr}) is 0.4 or 0.7, as indicated in the key in panel a, which applies to all panels.

Regulators have to establish advisory or mandatory refuge sizes before the fitness parameters of hypothetical resistance alleles are known. We therefore examined the equivalence between refuge size and RIDL release ratio in terms of the trade-off between refuge size and release ratio to achieve an equivalent outcome. We calculated the proportional reduction in refuge size that could be made, at a given release ratio, while still just preventing the spread of a given resistance allele (Figure 3.6). The initial allele frequency (p_0) appears critical, whereas the precise values of υ_{rr} and ω_{rr} have relatively little effect, even where they are close to 1 (e.g. black line in Figure 3.6).

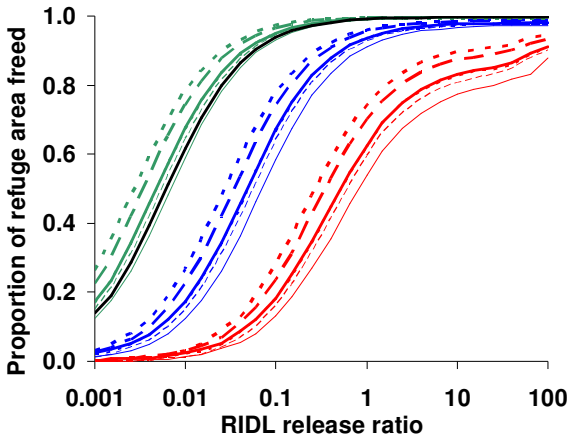

Figure 3.6 RIDL release reduces the minimum refuge size needed to stop the spread of resistance
We calculated the reduction in minimum refuge size needed to stop the spread of resistance as a proportion of the equivalent minimum refuge size with no release. This proportion of refuge potentially freed to be planted with Bt crops is shown against RIDL release ratio for the same values of p_0 and combinations of resistant homozygote fitness as in Figure 3.5. Line colours indicate values of p_0: green 0.001, blue 0.01, red 0.1, just as in Figure 3.5 panels a-c. Fitness of resistant homozygotes on Bt crops (ω_{rr}) are 0.1, 0.2 or 0.4 and on non-Bt plants (υ_{rr}) are 0.4 or 0.7, as indicated in the key in Figure 3.5a. In addition, the black line shows the outcomes for a rare resistance allele (p_0 = 0.001) with higher homozygote fitness values: 0.8 on Bt crops and 0.9 on non-Bt plants. Although these fitness values are much higher, it nonetheless falls within the range of other outcomes with the same p_0 (green lines).

3.4 Discussion

We have explored the effects of the release of RIDL male insects and predict that such release would slow or reverse the spread of resistance to PIPs. Our investigation of the relationship between the ratio of released RIDL males to males in the wild, the refuge size and the fitness parameters of the resistant insects showed that RIDL release could achieve a considerable reduction in the minimum refuge size required to prevent the spread of resistance. Current SIT programmes use release rates at the upper end of the range we modelled; in many cases we found that even much lower release rates would be sufficient to justify substantial reductions in refuge size (Fig. 3.6). This suggests that the necessary release rates would be feasible in many cases.

For conventional SIT against a density-independent population, with no immigration or emigration, the minimum required release ratio of fully competitive males efficiently dispersed into the population is (R_0-1):1, where R_0 is the average number of progeny produced per adult pest insect that survive to adulthood (Knipling 1955). Above this threshold ratio the population will tend to decline, while at lower release ratios the population will increase, albeit more slowly than it would otherwise have done. R_0 might range from 2 to >10 for typical agricultural pests under field conditions. Actual release ratios used are higher, at least 10:1 and often 60:1 or higher (Bloem et al. 2005); this is to compensate for the reduced lifespan and competitive mating ability of mass-reared, irradiated insects, for imperfect distribution, and to achieve good suppression of the pest population, rather than just marginal control. Release ratios in these models are not exactly equivalent to the release ratios typical of SIT-based population control programmes, because we do not account for reduced competitiveness and imperfect distribution. However, in a successful SIT programme the release rate must be equivalent to rates of 1:1 to 10:1 or higher in our models, based on the (R_0-1):1 argument above. Our models therefore demonstrate that useful resistance management can be achieved at release ratios equivalent to, or lower than, those currently used for SIT (Figure 3.6), especially at low p_0.

For p_0 = 0.001, approximately as estimated for tobacco budworm (Gould et al. 1997) and European corn borer (Stodola et al. 2006), a release ratio as low as 1:4 could rationally justify a reduction in refuge size to as low as 2-3% of the value required without RIDL release (Figure 3.6). In many cases, such a low refuge size of perhaps 0.2-0.3% of the crop area would automatically be provided by alternative host plants or less than universal planting of the insecticidal variety. Reducing the size of the refuge, while maintaining

equivalent resistance management, would allow higher average crop yields and more efficient use of valuable land.

It is reassuring that the proportional reductions in minimum refuge sizes are not very sensitive to differing relative fitness estimates (Figure 3.6), as empirical methods for estimating these in field populations are problematic and estimated values vary considerably (Bourguet et al. 2000, Tabashnik et al. 2004, Tabashnik et al. 2005). A higher p_0 requires either a higher RIDL release ratio or a smaller reduction in the minimum refuge size to prevent resistance spreading. This sensitivity to p_0 indicates a potential advantage to implementing such a release strategy as a preventative measure, preferably before resistance reaches a detectable level, rather than as a reactive measure once resistance has reached relatively high levels.

We have focused on the idea of using engineered insects to manage resistance to Bt crops and identified several key features of the potential effect on resistance evolution of using such a strategy. In order to do so we used a population genetics approach. A population dynamics approach would be needed in addition, in order to assess the effect of RIDL release on the size of the pest population and, therefore, on crop damage. Male release acts by substituting released males for some of the wild males with which females would otherwise have mated. Assuming that reproduction is not limited by the availability of males, no greater numbers of progeny arise and survive than would otherwise have done so, and the female-lethal effect of the RIDL construct would therefore be expected to have a population suppression effect synergistic with that provided by the Bt crops. Empirical (Carrière et al. 2003) and modelling studies (Peck et al. 1999, Caprio 2001, Sisterson et al. 2004) have shown that population sizes of susceptible insects are greatly reduced by the

use of Bt crops; the additional suppression provided by the RIDL strategy should at least to some extent improve this and might in some cases result in local extinction of the pest.

Our analysis suggests that the release of female-lethal RIDL male insects could allow a very significant reduction in refuge size while maintaining equivalent resistance management. The economic attractiveness of this approach would depend on the costs of such a release programme compared with the value of increased yields to be obtained by replacing refuge plants with Bt crops (net of the premium on Bt seeds). An assessment of relative cost would require an extension of our model to include population dynamics as well as genetics, because the number of insects released each generation, and therefore the cost of the programme, varies with the level of population suppression.

Our analysis has primarily focused on RIDL release and refuge strategies aimed at preventing the spread of resistance from a relatively low initial level. An additional advantage of the RIDL strategy stems from the effect that increasing release ratios above the critical threshold speeds the reversal of resistance. A RIDL release programme could be deployed as remedial action to counter a resistance event, by using higher release ratios more typical of SIT population suppression programmes to achieve a much more rapid reduction in resistance allele frequency than would be possible using only refuges (even under a complete ban on Bt crops, i.e. 100% refuge, the rate of recovery would otherwise be constrained by the fitness cost of resistant insects on non-Bt plants).

Given that SIT is already operated on an industrial and area-wide scale against pink bollworm and codling moth *Cydia pomonella* (L.) (Dyck et al. 2005), rearing and distributing lepidopteran pests on a suitable scale is not infeasible. If the relatively low

numbers that would be required for routine use in resistance management programmes could be produced from spare capacity in existing facilities, the marginal cost of such programmes would be smaller than if dedicated facilities were needed.

This model is not sufficiently sophisticated alone to determine an appropriate release schedule. In any case, as the numbers of males released in the model are proportional to the numbers of males in the wild each generation, some estimation would be required. Population sizes would generally be small, but if resistance did evolve this would tend to increase the population size (Peck et al. 1999, Sisterson et al. 2004) and consequently the numbers of RIDL males to be released. For implementation purposes, we would envisage starting with a prudent approximation for release numbers, based on survey data and any available estimates of resistance allele frequency, and using sample data over time to obtain estimates of the numbers of homozygous RIDL males relative to males of other genotypes and adjust the release rate accordingly.

One of the attractive features of this method for managing the spread of resistance is that no prior knowledge of the mechanism of resistance is required. This approach therefore provides, in principle, a new and general method for managing the spread of pest resistance to toxins such as engineered insecticidal crop plants.

4 Combining Pest Control and Resistance Management: Synergy of Engineered Insects with Bt Crops

Summary Transgenic crops producing insecticidal toxins are widely used to control insect pests. Their benefits would be lost if resistance to the toxins became widespread in pest populations. The most widely used resistance management method is the high-dose / refuge strategy. This requires toxin-free host plants as refuges near insecticidal crops, and toxin doses intended to be sufficiently high to kill insects heterozygous for a resistant allele, thereby rendering resistance functionally recessive. We have previously shown by mathematical modelling that mass-release of harmless susceptible (toxin-sensitive) insects engineered with repressible female-specific lethality using RIDL® (Release of Insects carrying a Dominant Lethal) technology could substantially delay or reverse the spread of resistance and reduce refuge sizes. Here we explore this proposal in depth, studying a wide range of scenarios, considering impacts on population dynamics as well as evolution of allele frequencies, comparing with releases of natural fertile susceptible insects, and examining the effect of seasonality. We investigate the outcome for pest control for which the plant-incorporated toxins are not necessarily at a high dose (i.e., they might not kill all homozygous susceptible and all heterozygous insects). We demonstrate that a RIDL-based approach could form an effective component of a resistance management strategy in a wide range of genetic and ecological circumstances. As there are significant threshold effects for several variables, we expect that a margin of error would be advisable in setting release ratios and refuge sizes, especially as the frequency and properties of resistant alleles may be difficult to measure accurately in the field.

4.1 Introduction

Transgenic plants, mainly maize and cotton, producing insecticidal proteins derived from *Bacillus thuringiensis* (Berliner) (Bt), are widely used to control insect pests (James 2007). They can provide economic, health and environmental benefits (Shelton et al. 2002, Huang et al. 2003, Brookes and Barfoot 2006), so attention has focused on methods to prevent or reverse the spread of resistance to these toxins.

One approach is the high-dose/refuge strategy. This is a common tool for managing resistance to Bt crops and is mandatory in several countries. This aims to render any resistance functionally recessive by using such high toxin concentrations that heterozygotes do not survive. Refuges of host plants that produce no toxins (non-Bt), provide a source of susceptible genotypes to dilute the frequency of resistant (*r*) alleles. Theoretical models, now supported by empirical evidence from monitoring, predict that this strategy slows or prevents the spread of resistance (Alstad and Andow 1995, Gould 1998, Carrière and Tabashnik 2001, Tabashnik et al. 2005, Tabashnik et al. 2008).

In principle, instead of refuges an alternative source of susceptible (*s*) alleles could be provided by mass-rearing susceptible insects and releasing them into the environment. In the Sterile Insect Technique (SIT), a control method used to eliminate or suppress pest populations, insects are mass-reared, sterilized by irradiation and released (Dyck et al. 2005); however, no viable hybrids are formed so there is no introgression of alleles into the wild population. Releasing natural fertile susceptible insects would allow such introgression, but released fertile females would contribute to population growth (very few reliable sex-separation methods have been developed for large-scale use in SIT, so generally both sexes would be released). Genetic technology (Release of Insects carrying

a Dominant Lethal, RIDL) could modify the SIT by releasing insects that are not irradiated, but instead are homozygous for one or more dominant lethal genetic constructs that are repressible during mass-rearing (Thomas et al. 2000, Alphey et al. 2007a, Alphey 2007). A female-lethal version of RIDL, with male insects homozygous for one or more female-specific dominant lethals ('RIDL males'), has been constructed in several species including the major pests, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Fu et al. 2007) and Mexican fruit fly *Anastrepha ludens* (Loew) (Stainton et al. unpublished data). F_1 progeny of RIDL males and wild females inherit a dominant female-specific lethal; the F_1 females die, thereby reducing the reproductive potential of the wild population, but the F_1 males are viable and fertile, so alleles from the mass-reared strain might be introgressed into the wild population. We previously proposed that control programmes could combine the release of such engineered insects with plants incorporating Bt or similar toxins to manage pests with fully recessive resistance where the toxins are fully lethal to susceptible genotypes (Alphey et al. 2007b). Here we investigate applying this approach to a wider range of ecological and genetic circumstances.

The evolution of allele frequencies depends on selection pressure due to relative areas of Bt crop and refuge, and relative fitnesses of the genotypes in each. There are critical threshold proportions for habitat division that determine equilibrium allele frequencies (Alphey et al. 2008). If susceptible (*ss*) homozygotes are released each generation at a constant ratio to the wild population, these thresholds increase, allowing for equivalent resistance management to be provided by a smaller refuge (Alphey et al. 2007b), which could increase crop yields.

Our previous work, in which we explored fully recessive resistance (where the *r* allele is recessive on Bt and non-Bt plants so that both the effects and fitness costs are recessive), found that release of RIDL males slows the spread of resistance and sufficient releases can prevent or reverse the spread of the *r* allele (Alphey et al. 2007b). This analysis suggested that release of RIDL male insects, at release ratios considerably lower than those typical for effective SIT pest suppression programmes, could allow a significant reduction in refuge size while maintaining equivalent resistance management benefits.

There is a tension between the benefit of using refuges to delay or prevent the spread of resistance and the benefit foregone by not planting insecticidal plants there (which would reduce crop damage by killing pest insects). In contrast, RIDL release could contribute simultaneously to reducing pest densities and managing resistance. The presence of released males will lead to some females mating with those instead of with wild males, so assuming that reproduction was not limited by the availability of males, release of males only does not increase the number of progeny (the cause of crop damage). The female-lethal effect of the RIDL construct would therefore be expected to have a population suppression effect synergistic with that provided by the Bt crops. Bt crops greatly reduce population sizes of susceptible insects (Caprio 2001, Carrière et al. 2003, Sisterson et al. 2004); the additional suppression provided by the RIDL strategy should improve this and might in some cases result in local elimination of the pest. To explore this potential synergy, which would cause desirable effects on population size as well as on resistant allele frequency, we must combine a population dynamics approach within the population genetics framework.

Whatever the approach used, resistance management analyses typically focus on preventing the spread of resistance from a very low initial level. A RIDL release programme potentially could also be deployed as remedial action where resistance has become widespread. Higher release ratios, more typical of SIT population suppression programmes, could achieve a much more rapid reduction in *r* allele frequency than would be possible using only refuges (even with no Bt crops, i.e. 100% refuge, the rate of recovery would otherwise be driven only by the fitness cost of resistant insects on non-Bt plants).

As well as a low initial *r* allele frequency, it is common for modelling studies to assume that Bt crops kill all susceptible larvae and that resistance is recessive. These assumptions are consistent with available evidence for some pests but not for others; there is a need also to understand the evolution of resistance in pests for which the toxins are not high-dose. For example, Cry1Ac cotton does not kill all susceptible larvae of the economically important pests bollworm *Helicoverpa zea* (Boddie) and cotton bollworm *Helicoverpa armigera* (Hübner), and some laboratory-selected resistance is not recessive (EPA 1998, Gould 1998, Akhurst et al. 2003, Gujar et al. 2007b, Luttrell and Ali 2007) . It is often also assumed that fitness costs exist and are recessive. However, fitness costs observed in resistant strains range from undetectable to substantial, and recessive to partially-dominant, and may be environment-dependent (Ramachandran et al. 1998, Tang et al. 1999, Liu et al. 2001, Bates et al. 2005, Carrière et al. 2005b, Raymond et al. 2005).

The resistance trait in some species has been shown to have further fitness costs for overwintering (in affected regions), observed as reduced emergence from diapause (Alyokhin and Ferro 1999, Carrière et al. 2001b, Bird and Akhurst 2004). Conversely,

continuous crop cultivation throughout the year is thought to be one key factor responsible for significant damage to Cry1F Bt corn (maize) by larvae of fall army worm *Spodoptera frugiperda* (J.E. Smith) in Puerto Rico in 2006 (Reynolds 2007). We investigate the impact of seasonality on our results and conclusions.

Here, through mathematical modelling, we explore these issues for the proposed RIDL strategy for resistance management. We examine the relationship between critical release ratio, initial allele frequency and equilibrium allele frequency, first considering the evolution of resistant allele frequency for recessive resistance with non-recessive fitness costs. We then explore the interaction between pest control and resistance management by modelling relative population size over time (and compare this with release of wild type, fertile, susceptible insects instead of RIDL males), and then consider the effect of seasonality on our conclusions about fully recessive resistance. Finally we investigate scenarios where Bt crops are not high dose and either resistance or fitness costs vary in dominance.

4.2 Materials and Methods

4.2.1 Underlying population genetics model

We used a deterministic, discrete-generation population genetic model (Carrière and Tabashnik 2001, Alphey et al. 2008) and assume a closed homogeneous population, with random mating, and no immigration, emigration or mutation. We assume a 1:1 sex ratio. Larvae, the Bt-susceptible life stage, are assumed to spend their whole development either on Bt crops or in the refuge; dispersal occurs after emergence as adults and prior to mating.

We assume a fixed crop population, a proportion Φ of which are Bt plants, so the (non-Bt) refuge size is $1-\Phi$. Hard selection (Wallace 1968) acts in these two environments, with the

number of larvae surviving to reproductive maturity from each environment depending on the relative fitness values of the eggs laid there. Larval susceptibility to Bt toxins is assumed to be controlled by a single autosomal locus with two alternative alleles: resistant *r* (frequency *p*) and susceptible *s* (frequency *q*, $p + q = 1$). There are three possible genotypes at this locus: *ss*, *sr* and *rr*.

All insects are affected by Bt toxins. We assume that all fitness costs associated with the *s* and *r* alleles take effect during the larval stage. Assuming that larvae are distributed at random across Bt and non-Bt crops, the average relative fitness of larvae of genotype *i* (*i*: *ss, sr* or *rr*) across the area is

$$\Omega_i = \omega_i \Phi + \upsilon_i (1 - \Phi) \qquad (1)$$

where ω_i is the relative fitness of larvae on Bt crops and υ_i is relative fitness of larvae in non-Bt crops. Fitness is measured relative to *ss* homozygotes on non-Bt plants (so $\upsilon_{ss} = 1$). By definition of resistance $\omega_{rr} > \omega_{ss}$. We assume that no genotype is fitter on Bt crops than on non-Bt plants ($\upsilon_i \geq \omega_i$). We assume that the resistant allele may have a fitness cost (on non-Bt plants $\upsilon_{rr} \leq \upsilon_{sr} \leq \upsilon_{ss} = 1$), and that on Bt crops resistance is not over- or under-dominant ($\omega_{ss} \leq \omega_{sr} \leq \omega_{rr}$). Resistance may be incomplete ($\omega_{rr} \leq \upsilon_{rr}$).

With no release of insects, the change in *r* allele frequency is given by

$$\Delta p = \frac{pq\left(p\Omega_{rr} + (q - p)\Omega_{sr} - q\Omega_{ss}\right)}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}} \qquad (2)$$

Using eq. (2) we have demonstrated that there are two critical threshold values, Φ_1 and Φ_2, resulting from the interplay between the costs and benefits of resistance, that determine the possible outcomes for the *r* allele (Alphey et al. 2008). The outcome is determined by the

proportion of Bt crops (Φ) in relation to these thresholds and, in some cases, by the allele's initial frequency (p_0).

$$\Phi_1 = \frac{(1 - \upsilon_{sr})}{(1 - \upsilon_{sr} + \omega_{sr} - \omega_{ss})} \qquad (3)$$

$$\Phi_2 = \frac{(\upsilon_{rr} - \upsilon_{sr})}{(\upsilon_{rr} - \upsilon_{sr} + \omega_{sr} - \omega_{rr})} \qquad (4)$$

Where $\Phi_1 < \Phi_2$, if the proportion of Bt crops is below or equal to Φ_1 the resistant allele will decline to extinction; if Φ is above or equal to Φ_2, the resistant allele will go to fixation; if Φ lies between Φ_1 and Φ_2, the frequency of the resistant allele will settle at a stable internal equilibrium value (i.e., between 0 and 1) that can be calculated.

Where $\Phi_1 > \Phi_2$, the resistant allele will go to either extinction or fixation, depending on whether the proportion of Bt crops is below or above a third threshold Φ_3. The value of Φ_3 is dependent on the initial frequency of the resistant allele p_0. Φ_3 lies between Φ_1 (the two are equal when $p_0 = 0$) and Φ_2 (they are equal when p_0=1), and decreases as p_0 increases. In special cases, where the *r* allele is recessive on both Bt and non-Bt plants, dominant on both or co-dominant on both, one of equations (3) or (4) is not defined or they are equal, and there is a single threshold determining whether the *r* allele goes to fixation or extinction.

To explore resistance with a range of genetic parameter values, it is helpful to define the dominance of resistance and the dominance of the fitness costs of resistance. We then vary the relative fitness values by changing those parameters. We define the dominance of resistance h ($0 \le h \le 1$) as (Tabashnik et al. 2008)

$$h = \frac{\omega_{sr} - \omega_{ss}}{\omega_{rr} - \omega_{ss}} \qquad (5)$$

(so $h = 0$ for recessive resistance and $h = 1$ for dominant resistance) and, where appropriate, calculate heterozygote relative fitness on Bt plants using h

$$\omega_{sr} = (1-h)\omega_{ss} + h\omega_{rr} \qquad (6)$$

Similarly, we define the dominance of the fitness costs of resistance g ($0 \le g \le 1$, $g = 0$ for recessive costs and $g = 1$ for dominant costs) as

$$g = \frac{\upsilon_{sr} - \upsilon_{ss}}{\upsilon_{rr} - \upsilon_{ss}} = \frac{1-\upsilon_{sr}}{1-\upsilon_{rr}} \qquad (7)$$

so that heterozygote relative fitness in the refuge becomes

$$\upsilon_{sr} = (1-g)\upsilon_{ss} + g\upsilon_{rr} = 1 - g(1-\upsilon_{rr}) \qquad (8)$$

4.2.2 Release of wild type insects

To extend this basic model to incorporate the effects of the release of fertile wild type insects, we assume that at each generation, as adults emerge prior to mating, mass-reared adult insects are released at a fixed ratio (the 'release ratio') to the number of males in the wild population in that generation that survived to reproductive maturity. All released adults are assumed to be homozygous susceptible to Bt toxins (*ss*). Males and females are released in equal numbers. Wild and released adults mix homogeneously and mate at random.

With release of homozygote susceptible adults (*ss* wild type) in ratio *d*:1 to naturally emerging males in the wild, the change in *r* allele frequency is given by

$$\Delta p = \frac{p\left(pq\Omega_{rr} + (q + \tfrac{1}{2}d)(q-p)\Omega_{sr} - (q+\tfrac{1}{2}d)^2\Omega_{ss}\right)}{(q+\tfrac{1}{2}d)^2\Omega_{ss} + 2p(q+\tfrac{1}{2}d)\Omega_{sr} + p^2\Omega_{rr}} \qquad (9)$$

In practice, rather than determining the *r* allele frequency by calculating Δp from eq. (9), we used a simulation approach that involved calculating the frequencies of every genotype (Alphey et al. 2007b). This approach allowed us to analyze the model in the same way for

both release of wild type insects and the more complicated case of release of engineered insects.

4.2.3 Release of engineered insects

We further extend the model to reflect the release of males carrying a dominant female-lethal RIDL construct, which renders female progeny inviable but allows male progeny to survive. For this purpose, we assume that at each generation, as adults emerge prior to mating, mass-reared adult RIDL males are released at a fixed ratio (the 'RIDL release ratio') to the number of males in the wild population (of any genotype) that survived to reproductive maturity. Females mate with wild or released males at random.

All engineered released males are assumed to be homozygous susceptible to Bt toxins (*ss*), and homozygous for the RIDL construct. There are two possible alleles at the insertion site of the RIDL construct: *L*, representing insertion of the construct, and the wild type allele *w*, representing absence of the construct. We assume the RIDL insertion and the locus controlling resistance to Bt are not linked and neither of these is sex-linked.

We assume the RIDL construct imposes a sex-specific fitness cost ε during the larval stage. The average relative fitness of larvae of genotype *i* is then

$$\Omega_i = (1-\varepsilon_i)[\omega_i \Phi + \upsilon_i (1-\Phi)] \qquad (10)$$

where ε_i is the sex-specific, relative fitness cost imposed by RIDL.

This results in a complex system of 18 difference equations that cannot be reduced to expressions in terms of *p*. There are no analytical solutions for allele or genotype frequencies or for the critical release ratios or critical refuge sizes.

4.2.4 Population dynamics

R_0 is the average number of progeny produced per adult pest insect that survive to adulthood in a density-independent population, commonly expressed as female offspring per adult female. For agricultural pests under field conditions, after taking into account factors such as egg hatch rates, predation and pathogens that are common to all genotypes throughout the habitat, R_0 might typically range from 5 to10 but can be much higher (Dyck et al. 2005). The population size is determined by

$$N_{t+1} = 2R_0 F_t \sigma_t \qquad (11)$$

where N_t is the population size of mature adults at generation t relative to the initial pest density (set $N_0 = 1$), of which F_t are female. The growth rate is $2R_0$ (each adult female contributes R_0 male and R_0 female larvae to the next generation) and σ_t is the proportion of offspring surviving to maturity (simulated by applying the fitness costs of *r* alleles, Bt toxins and the RIDL construct as appropriate to each genotype). N_t is indicative of the level of crop damage, because every individual that survives to reproductive maturity has completed larval development on plants.

Equation (11) is unrealistic at very high densities (where limited resources would cause density-dependent mortality) or very low densities (where rare insects could fail to find mates and stochastic effects would be observed, and our model can yield fractions of insects rather than whole numbers). However, this measure of relative population size is useful because it illustrates trends in pest densities.

4.2.5 Seasonality

It has been observed that the resistance trait can reduce emergence from diapause (Alyokhin and Ferro 1999, Carrière et al. 2001b, Bird and Akhurst 2004). To represent the effects of winter on a pest in which resistance is fully recessive, we apply further fitness

penalties to larvae in every *n*th generation, where the insect goes through *n* generations in a year. One factor reduces survival of all genotypes, and another factor further reduces survival only of resistant *rr* genotypes to represent an overwintering cost associated with the resistant phenotype (e.g., impairing the ability to enter and emerge from diapause or the timing of those). The average relative fitness of larvae of genotype *i* in winter generations is then

$$\begin{cases} \Omega_i \text{ x [overwinter survival]} & \text{for } i \neq rr \\ \Omega_{rr} \text{ x [overwinter survival] x [relative overwinter survival of } rr\text{]} & \text{for } rr \end{cases} \quad (12)$$

4.2.6 Simulations

We assume the RIDL construct is fully dominant and completely lethal to females (but not to males), so for *Lw* females $\varepsilon_{\text{female}} = 1$ and average fitness $\Omega_i = 0$, irrespective of Φ, ω_i and υ_i. Under these conditions, *LL* homozygotes do not arise in the field and there are seven male and three female viable genotypes defined at the *L*/*w* and *s*/*r* loci. The *LLss* male genotype is the engineered released RIDL male, and the other six male genotypes (*Lwss*, *Lwsr*, *Lwrr*, *wwss*, *wwsr* and *wwrr*) and three female genotypes (*wwss*, *wwsr* and *wwrr*) arise from matings in the field. In these simulations, $\varepsilon_{\text{male}}$ was set to 0.1 for *Lw* male larvae, to allow for a modest fitness penalty that might be associated with the RIDL insertion (for example, due to insertional mutagenesis and/or expression of the marker gene (Marrelli et al. 2006)).

We explore the effects of release of RIDL males by simulating different combinations of υ and ω representing varying strength and fitness costs of resistance, over a range of refuge sizes (0 to 1 or a subset thereof) and release ratios from 1:1000 (one RIDL male per

thousand males in the wild, 0.001) to 25:1 (or a subset thereof). We simulate the frequencies of all genotypes over:

2500 generations when estimating the value of an equilibrium allele frequency

200 generations when exploring population dynamics and seasonality

500 generations when estimating minimum refuge sizes for the *r* allele not to reach 0.5 frequency in the period simulated, for pests for which Bt crops are not high dose

These durations are far beyond the realistic practical lifetime of any single insecticidal crop product, but we use them to illustrate the direction of change of *r* allele frequency (which can take a long time to become apparent especially where it is initially very rare) and to facilitate estimation of long-term results such as an equilibrium value towards which the *r* allele frequency will tend.

A standard genetic criterion for the time to evolve resistance is the number of generations taken for *r* allele frequency to exceed 0.5 (Comins 1977, Tabashnik et al. 2008); this is deemed to indicate a 'resistance event'. In some circumstances, the allele can tend to an internal equilibrium frequency (between 0 and 1) and so this criterion can make a seemingly arbitrary distinction between a resistance management regime deemed unsuccessful because the *r* allele settles just above 0.5 and one deemed adequate because the *r* allele settles just below 0.5. However, there seems to be a strong connection between the effect on population size and the *r* allele exceeding 0.5 (see Results including Fig. 4.1), so the distinction is relevant. For any given set of parameter values, trial and error can be used to ascertain the minimum refuge size for the *r* allele not to reach 0.5 frequency. For multiple datasets, the approach was automated to approximate the minimum refuge size needed to prevent the *r* allele frequency reaching 0.5 during the generations simulated. (Alternative cut-off points such as frequency 0.3 give the same conclusions on relative

times to resistance when comparing scenarios; we use 0.5 because of its common acceptance and the clear impact on population size shortly after that value is reached).

Table 4.1 Empirical data used to parameterize the model, for pests of Bt Cry1Ac cotton.

Parameter	Pest species	Values estimated from empirical data	References
Relative fitness on non-Bt plants (υ) and Bt crops (ω)	Pink bollworm *Pectinophora gossypiella*	$\upsilon_{ss} = \upsilon_{sr} = 1$ $\upsilon_{rr} = 0.485$ $\omega_{ss} = \omega_{sr} = 0$ $\omega_{rr} = 0.46\upsilon_{rr} = 0.2231$	(Tabashnik et al. 2000, Carrière et al. 2001a, Liu et al. 2001, Carrière et al. 2005b)
	Helicoverpa zea	$\upsilon_{ss} = \upsilon_{sr} = \upsilon_{rr} = 1$ $\omega_{ss} = 0.1$ $\omega_{sr} = 0.351$ $\omega_{rr} = 0.404$	(Tabashnik et al. 2008)
	Helicoverpa armigera	$\upsilon_{sr} = 0.925$ $\upsilon_{rr} = 0.515$ $\omega_{ss} = 0.047$ $\omega_{sr} = 0.121$ $\omega_{rr} = 0.293$	(Tabashnik et al. 2008)
Dominance of resistance (h)	*H. zea*	0.826	Calculated from the fitness values above (Tabashnik et al. 2008)
Dominance of fitness costs of resistance (g)	*H. armigera*	0.155	Calculated from the fitness values above
Reproductive number (R_0)	pink bollworm	8.6 (we use $2R_0 = 17$)	(Liu et al. 2001)
Generations per year	pink bollworm	5	(Tabashnik et al. 2008)
Overwinter survival	pink bollworm	0.05	(Gutierrez and Ponsard 2006)
Relative overwinter survival of resistant (*rr*) individuals	pink bollworm	0.29	(Carrière et al. 2001b)

We use data on pink bollworm to parameterize our model when comparing the evolution of *r* allele frequency and population size under alternative assumptions of no seasonality or overwintering. We use *H. zea* data to investigate the effect on the evolution of *r* allele frequency of varying dominance of resistance. We use *H. armigera* data to assess the effect on *r* allele frequency of varying the dominance of fitness costs of resistance.

Conditions favouring resistance (for example, high *r* allele frequency, non-recessive resistance and low fitness costs) are more likely to warrant extra measures such as RIDL to delay resistance. For such situations, we parameterized the model using published data for pest insects (Table 4.1), adapting values as appropriate to the aspect under investigation.

We modelled initial allele frequency p_0 of 0.01 or 0.001. Although for many pests, *r* allele frequencies are of the order of 10^{-3} based on field data (Gould et al. 1997, Tabashnik et al. 2005, Stodola et al. 2006, Huang et al. 2007, Mahon et al. 2007a), the frequency has increased substantially in some field populations of *Helicoverpa zea* (Ali et al. 2006, Tabashnik et al. 2008) and *Helicoverpa armigera* (Liu et al. 2008) and resistant fall armyworm have caused Bt corn crop failure (Reynolds 2007), so higher values could be relevant to situations where a RIDL strategy might be implemented. Overestimating the p_0 value is likely to underestimate potential benefits of RIDL releases implemented at lower allele frequencies; for fully recessive resistance with given fitness parameters, we previously showed that a higher value of p_0 would need a higher RIDL release ratio to prevent the *r* allele from spreading (Alphey et al. 2007b).

For fully recessive resistance and p_0 0.001 (reflecting field data), we previously showed that a release ratio as low as 1:4 could justify a refuge as small as 2-3% of the size required without RIDL release (Alphey et al. 2007b). In this study, we generally simulated release ratios (5:1 and below) lower than those typically employed in an SIT programme aimed at pest suppression or elimination. As we were interested in assessing RIDL release combined with smaller refuges, for most purposes we modelled refuge sizes lower than are typically observed (for example, the state-wide mean refuge size for Bt cotton in Arizona is about 50%, varying from 14% to 78% per county (Carrière et al. 2005a)). We generally

used 4% or 5% refuge, noting that these were the minimum options for mandatory Bt cotton refuge in the USA pre- and post- 2001, respectively. When addressing questions of dominance, we used empirical data for pests with relevant resistance characteristics and considered refuge sizes appropriate to those. For dominance of resistance, we modelled 39% refuge, as estimated for Arkansas and Mississippi where field-evolved resistance to Cry1Ac cotton has been detected in *H. zea* (Tabashnik et al. 2008), 20% refuge, one of the options under US requirements, and 5% refuge. Decreased *H. armigera* susceptibility to Cry1Ac has not been observed in the field, likely due to large effective refuge sizes (70% or more in Australia and 87-95% in China (Tabashnik et al. 2008)), except for one report where the effective refuge size is 32% (Liu et al. 2008). Unsurprisingly, when we modelled 70% refuge with even a modest RIDL release, we found that the *r* allele was driven extinct in most cases. To explore fully the effects of varying fitness costs, and for consistency, we again modelled 39%, 20% and 5% refuge.

4.3 Results

The *r* allele never reaches fixation with RIDL release because of the dilution effect of adding new *s* alleles at each generation, but the equilibrium frequency can be very close to 1. Simulations reveal that for any given set of parameter values there is a critical release ratio, above which the *r* allele tends to extinction. This critical value depends on the genotypes' relative fitness values, the initial allele frequency and the refuge size. We conclude that extending the model to RIDL releases alters the critical refuge sizes (eqs. 3-4) by the inclusion of terms incorporating the RIDL release ratio and p_0. If we were able to express these modified formulae explicitly, we could in principle rearrange them to show critical values for any element while keeping the others constant. Instead of determining critical Φ keeping all else constant, we might thus determine the critical value for RIDL release ratio (for given p_0, refuge size and fitness values) or derive a critical

value for initial frequency p_0 (for given RIDL release ratio, refuge size and fitness values). We therefore begin by considering the critical release ratio and its relationship with refuge size and initial *r* allele frequency. Resistance is generally thought to be recessive for most pests, so we start by analyzing that situation. Later we consider the full range of dominance of resistance or of fitness costs.

Where resistance is recessive ($\Phi_1 = 1$, eq.3) but fitness costs are not ($\Phi_2 < 1$, eq.4), without releases the *r* allele will go to extinction or fixation depending on the refuge size and initial allele frequency ($\Phi_2 < \Phi_1$ and there is one critical refuge size at $1-\Phi_3$, which depends on the value of p_0). We considered such cases where resistance would not go extinct with no release, and assessed which factors influence the evolution of *r* allele frequency.

The equilibrium *r* allele frequency decreases with increasing RIDL release ratio (see example in Table 4.2). The release ratio affects the equilibrium value because it affects the level of dilution by *s* alleles from released insects that prevents the *r* allele reaching fixation. This value does not decrease smoothly from close to 1 (for a very small RIDL release ratio) down to 0 (at or above the critical release ratio) but jumps discontinuously from a positive level to zero. This threshold non-zero equilibrium value could not be determined analytically. With parameters as in Table 4.2, the equilibrium allele frequency drops from approximately 0.999 to zero on passing a critical RIDL release ratio just below 0.083:1. An allele that is heading to extinction will decline more quickly with a higher RIDL release ratio. If the outcome is a non-zero equilibrium, increasing the RIDL release ratio slows the approach to that. With parameters as in Table 4.2, the *r* allele frequency would reach 0.5 in 27 generations (from p_0 0.01) with no release, but would become less

than 0.5 x 10^{-3} in that period (and negligible $<10^{-5}$ within 51 generations) with RIDL release of 1:2.

Table 4.2 Resistant allele equilibrium frequency varies with RIDL release ratio, with a large discontinuity at the threshold.

These results were obtained from simulations with the following parameter values:
Relative fitness on non-Bt plants $\upsilon_{ss} = 1$, $\upsilon_{sr} = 0.9$, $\upsilon_{rr} = 0.7$
Relative fitness on Bt plants $\omega_{ss} = \omega_{sr} = 0$, $\omega_{rr} = 0.6$
Refuge size $1-\Phi = 0.05$ (5% refuge)
Initial *r* allele frequency $p_0 = 0.01$

RIDL release ratio (released RIDL males per male in the wild)[a]	Equilibrium *r* allele frequency (to 5 d.p.)	Time to exceed 0.5 frequency (generations)	Time to below 10^{-5} frequency (generations)
0 (no release)	1	27	N/A
0.01	0.99982	28	N/A
0.05	0.99911	35	N/A
0.08269972	0.99853	188	N/A
0.08269973	0	N/A	239
0.1	0	N/A	94
0.2	0	N/A	70
0.5	0	N/A	51

[a] expressed as a decimal, e.g., 0.5 released males per male in the wild is equivalent to ratio 1:2 and 0.01 is equivalent to 1:100

The value of the equilibrium does not vary with p_0 (see example in Table 4.3). There is a threshold switch effect; all else being equal, for a given level of RIDL release the value of p_0 determines whether the *r* allele goes to extinction or towards a fixed equilibrium value, which can be close to 1. Lowering p_0 without passing that threshold slows the approach to the non-zero equilibrium if that is the outcome. If the allele is going extinct, lowering p_0 hastens its demise. Starting a release programme sooner (at lower *r* allele frequency) therefore gives a better resistance management outcome. It seems intuitively reasonable that modest changes in this initial condition do not change the value of the outcome but do affect the time it takes to get close to it.

Table 4.3 Initial *r* allele frequency acts as a switch between two possible outcomes but does not determine the value of a non-zero equilibrium frequency.

These results were obtained from simulations with the following parameter values:
Relative fitness on non-Bt plants $\upsilon_{ss} = 1,\ \upsilon_{sr} = 0.9,\ \upsilon_{rr} = 0.7$
Relative fitness on Bt plants $\omega_{ss} = \omega_{sr} = 0,\ \omega_{rr} = 0.6$
Refuge size $1-\Phi = 0.05$ (5% refuge)
RIDL release ratio 0.2 (1:5 released RIDL males to males in the wild)

Initial *r* allele frequency p_0	Equilibrium *r* allele frequency (to 5 d.p.)	Time to exceed 0.5 frequency (generations)	Time to below 10^{-5} frequency (generations)
0.2	0.99645	2	N/A
0.1	0.99645	3	N/A
0.05	0.99645	5	N/A
0.01153776	0.99645	137	N/A
0.01153775	0	N/A	178
0.01	0	N/A	70
0.005	0	N/A	53
0.001	0	N/A	36

The effect of the refuge size is qualitatively similar to that of the RIDL release ratio. There is a critical refuge size above which the *r* allele will tend towards extinction, with the allele approaching an internal equilibrium if the refuge is too small, and a potentially large discontinuity at that threshold. A larger refuge increases the rate of approach to extinction or slows the approach to an internal equilibrium (see example in Table 4.4).

The frequency and fitness properties of resistant alleles are impossible to predict in advance and difficult to measure accurately in the field even when present. The significant threshold effects mean that a comfortable margin of error would be advisable in setting release ratios and refuge sizes.

Table 4.4 Resistant allele equilibrium frequency varies with refuge size, with a large discontinuity at the threshold.

These results were obtained from simulations with the following parameter values:
Relative fitness on non-Bt plants $\upsilon_{ss} = 1$, $\upsilon_{sr} = 0.9$, $\upsilon_{rr} = 0.7$
Relative fitness on Bt plants $\omega_{ss} = \omega_{sr} = 0$, $\omega_{rr} = 0.6$
Initial *r* allele frequency $p_0 = 0.01$
RIDL release ratio 0.2 (1:5 released RIDL males to males in the wild)

Refuge size (1-Φ)	Equilibrium *r* allele frequency (to 5 d.p.)	Time to exceed 0.5 frequency (generations)	Time to below 10^{-5} frequency (generations)
0% (all plants are Bt)	1	1	N/A
2%	0.99862	9	N/A
4%	0.99719	25	N/A
4.368768%	0.99692	137	N/A
4.368769%	0	N/A	212
5%	0	N/A	70
10%	0	N/A	57
20%	0	N/A	55

4.3.1 Population control and resistance management

The frequency of a spreading *r* allele follows a classic sigmoid pattern: a relatively long period with little change (the length depending on the starting point p_0), followed by a period of rapid spread, then levelling off approaching the equilibrium frequency, which is usually fixation (Fig. 4.1). The decline of an *r* allele due to selection pressure tends to be slower, again following classic resistance evolution patterns, with monotonic decrease (and little apparent change if starting from a low frequency). These general patterns are also seen in the presence of insect releases (Fig. 4.1). Although the *r* allele cannot go to fixation, it might get very close to it.

RIDL release introgresses *s* alleles into the population and contributes to reducing the population size through the female-lethal effect, so it is always more effective at both managing resistance and reducing the pest population than the no release strategy (Bt crops and refuge alone) for any given refuge size. The relative performance of wild type (WT)

and RIDL strategies depends on whether both (Fig. 4.1a), WT only (Fig. 4.1b) or neither (not shown) control resistance.

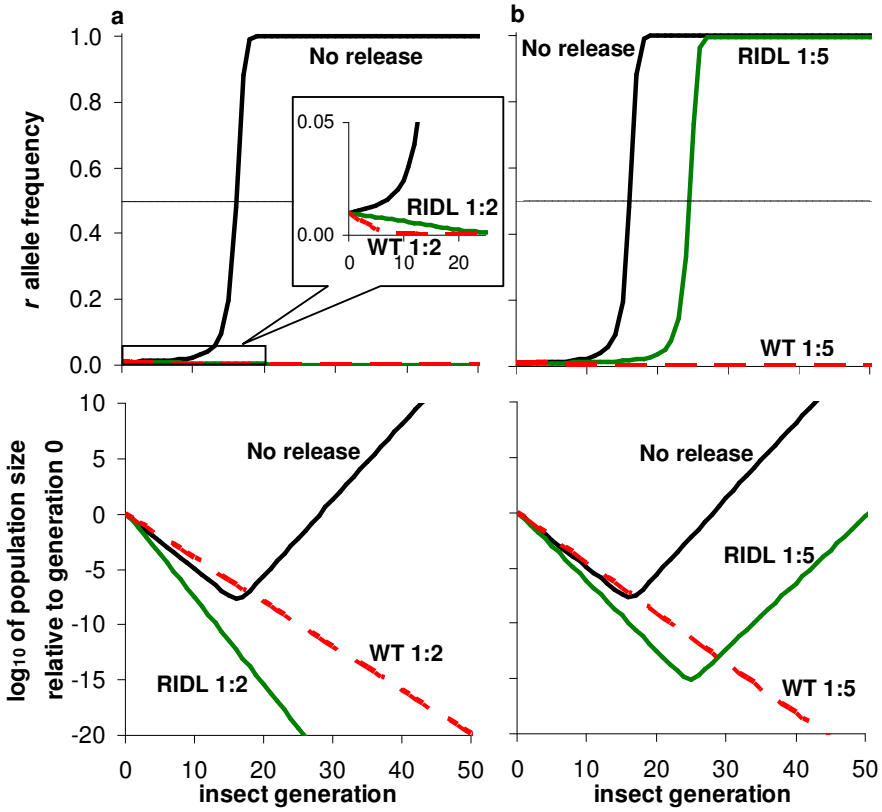

Figure 4.1 RIDL release acts in synergy with Bt crops, and wild type release is of variable relative merit.
The upper graphs show the frequency of the *r* allele in naturally emerging adults and the lower graphs show the relative population size, over time, for no release of insects (black lines), RIDL release (green lines), and wild type ('WT') release (dashed red lines) with release ratio (a) 1:2, i.e., one RIDL male or WT adult (male or female in equal numbers) per two males in the wild or (b) 1:5. Genotype relative fitness values represent a generic pest for which Bt plants are high-dose and resistance is recessive: in refuge $\upsilon_{sr} = 0.9$, $\upsilon_{rr} = 0.7$; on Bt plants $\omega_{ss} = \omega_{sr} = 0$, $\omega_{rr} = 0.6$. Initial *r* allele frequency p_0 is 0.01. Offspring per female $2R_0 = 16$. Refuge is 4%.

If the release ratio is sufficient (for the refuge and fitness values) that both RIDL and WT release strategies reverse the spread of resistance (Fig. 4.1a top), RIDL release is better at suppressing the population than WT release and no release (Fig. 4.1a bottom). (For

parameters as in Fig. 4.1 the minimum sufficient release ratio is 0.2916, i.e., a little over 1:3.5). The direction of change in population size depends on the population growth rate. With relatively low growth both release regimes can reduce the population size (Fig. 4.1a). For some higher values of population growth rate, population growth is only slowed not reversed with WT release but the population decreases with RIDL release (for example, with parameter values as in Fig.4.1a this happens where $2R_0$ >40 instead of 16, but with p_0 0.05 and 20% refuge it occurs with $2R_0$ =16). With very high growth rates the pest densities can increase under all three regimes.

WT releases are more effective than RIDL releases at managing resistance because *s* alleles are inherited by both male and female progeny of released insects, not just through the male line as with RIDL insects. Release of wild type insects, including fertile females, contributes to pest population growth. However, this effect can be mitigated by the resistance management aspect; by reversing the spread of resistance, releases help the Bt crops to be more effective at population suppression. If WT releases control resistance, they initially cause the population to decline more slowly than with no release, but after resistance becomes substantial (> 0.5 frequency) with no release the population quickly starts to grow and no release becomes the worst strategy in the long term (Fig. 4.1a and b).

If the release ratio is too low, neither strategy controls resistance (for parameters as in Fig.4.1, this occurs for release ratios below 0.0750, i.e., 1:13.3). Population sizes are higher with inadequate WT release than with insufficient RIDL release in the shorter term (while the Bt crops are still reducing the pest population). After resistance becomes substantial under RIDL release, populations may become larger than they would with WT release. If there is only a short period before resistance becomes widespread under WT

release too, this effect might only be temporary and in the longer term (after resistance is widespread) population sizes are again lower with RIDL releases than with WT releases. (For example, we observed such effects with higher p_0 and larger refuge than in Fig. 4.1). However, if resistance spreads significantly later with WT release than with RIDL release, the further population decline during that period may be too great for the female-lethal effect of the RIDL strategy to catch up with over a reasonable timeframe. (We observed this with parameters as in Fig.4.1 and smaller release ratios). Again, the population growth rate can affect whether the population ultimately grows or declines. In situations where neither strategy controls resistance, it is possible after resistance has spread for the population to decline with RIDL releases but increase with WT releases (for example, with parameter values as in Fig. 4.1b except with p_0 0.05, 20% refuge and low growth rate $2R_0$ =4).

For a small range of release ratios, WT release reverses the spread of resistance whereas RIDL release only slows its spread and prevents it reaching fixation (Fig. 4.1b). By controlling resistance, the combination of WT release and Bt crops reduces the pest population effectively. With the other strategies, the population starts to grow again after resistance becomes substantial. The value and spread of release ratios for which this situation arises depends on the *r* allele frequency, fitness parameters and refuge size. With parameters as in Fig.4.1 it occurs with release ratios from 0.075 (≈1:13.3) to 0.2916 (≈1:3.5) (with p_0 0.001 and 0.5% refuge the range is roughly 1:29 to 1:8; with p_0 0.1 and 15% refuge it is approximately 1:3 to nearly 2.5:1).

We have not modelled in detail the situation where RIDL genetic construct is not fully lethal ($\varepsilon_{\text{female}} < 1$). In that case, the performance of the RIDL insects would be

intermediate between the fully lethal RIDL ($\varepsilon_{female} = 1$) and WT ($\varepsilon_{female} = 0$) releases as modelled above, with a similar trade-off between population suppression and resistance management.

We stress that the proportional release policy inherent in our model is not the only possibility. For example, an alternative would be to release a constant number of RIDL males or wild type insects at every generation, rather than a number proportional to the number of males in the current population. If the number released in the first generation were the same, the constant policy would have a greater impact than the proportional policy, but success would be more expensive, as the release numbers do not decline with the population size. However, the constant policy would have an advantage in terms of monitoring; a limitation of the proportional policy is that the adult male population at each generation should be known or predictable (but apart from pink bollworm *Pectinophora gossypiella* (Saunders) in recent years, populations of Bt crop pests are generally highly variable in space and time), which would complicate implementation in practice.

RIDL release could be used for pest control in its own right, being a modified version of the SIT, and higher release ratios improve its effectiveness for resistance management. Together these features show that RIDL is a potential tool for remedial action where a resistance event has occurred. Simulations confirmed that RIDL release can reverse the spread of resistance even from very high frequencies (for parameter values in Fig. 4.2a, RIDL release at 10:1 can reverse the spread of resistance, although with a refuge of only 15% it takes over 30 generations to return to a low value). Where p_0 exceeds 0.5, RIDL release might cause the *r* allele to decline to an internal equilibrium value rather than to extinction. If the combination of release ratio and refuge size are not sufficiently high, this

equilibrium value can be above 0.5 and so the resistance problem would not have been dealt with (Fig. 4.2b), or the rate of decline to extinction can be too slow to be of practical use. For the extensive resistance modelled in Fig. 4.2b, increasing the release ratio from 5:1 to 10:1 would result in *r* allele frequency 0.37; also increasing the refuge from 10% to 20% refuge would reduce the frequency below 0.01 in 27 generations.

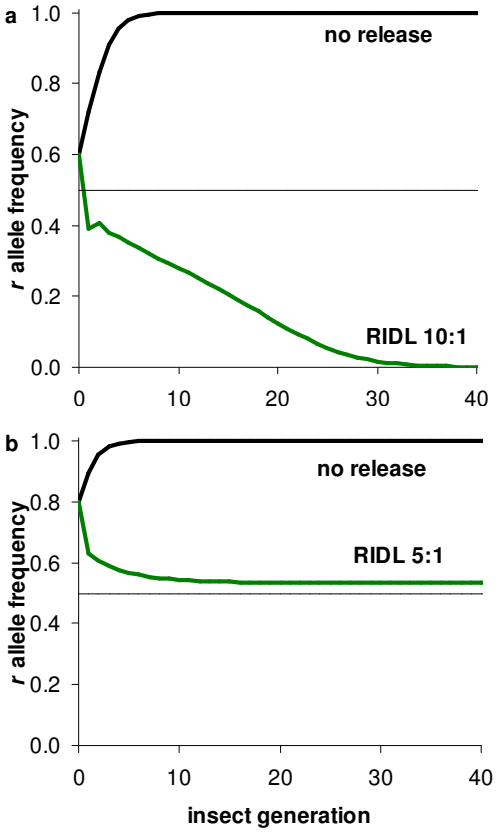

Figure 4.2 RIDL release can "clean up" extensive resistance, provided release ratio and refuge are sufficiently high.
The graphs show the frequency over time of the *r* allele for no release (black lines) and for RIDL release (green). (a) Relative fitnesses are: in refuge $\upsilon_{sr} = 0.9, \upsilon_{rr} = 0.4$; on Bt plants $\omega_{ss} = 0$, $\omega_{sr} = 0$ and $\omega_{rr} = 0.3$. Initial *r* allele frequency $p_0 = 0.6$. Refuge 15%. (b) Relative fitness values, representing non-seasonal pink bollworm data: in refuge $\upsilon_{sr} = 1$, $\upsilon_{rr} = 0.485$; on Bt plants $\omega_{ss} = \omega_{sr} = 0$, $\omega_{rr} = 0.223$. $p_0 = 0.8$. Refuge 10%.

4.3.2 Seasonality

Although Bt crops control pest densities initially, the population would increase rapidly after resistance is established, especially in an environment with no seasonal effects, whereas RIDL release could suppress the population effectively and local elimination might result (Figs. 4.1 and 4.3). Where larvae in each relevant generation incur costs of overwintering, the population suffers a step reduction once in every year (Fig. 4.3). These

steps can be seen in the population curves for both no release and RIDL release, but an overall trend in pest numbers is clear and dominates the results more than the in-year variation.

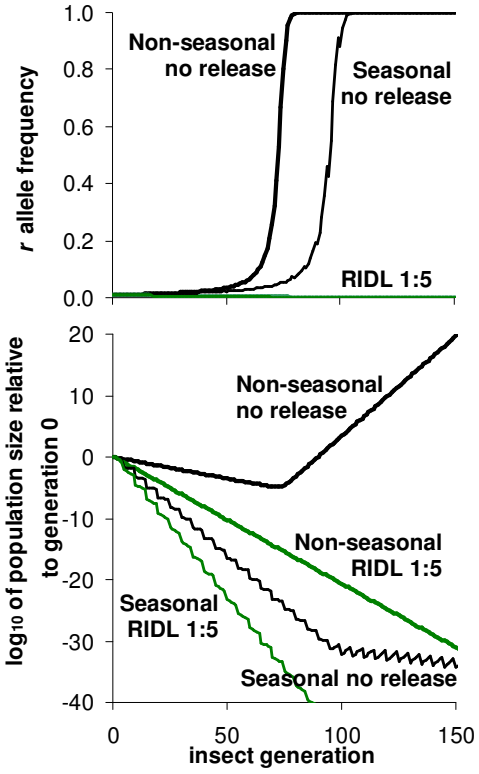

Figure 4.3 Winter slows resistance evolution and population growth but does not significantly alter the relative benefits of RIDL release.
The upper graph shows the frequency of the *r* allele and the lower graph shows the relative population size, over time, for no release of insects (black lines) and for a RIDL release ratio of 1:5 (green). Parameter values represent pink bollworm: relative fitness in refuge $\upsilon_{sr} = 1,\ \upsilon_{rr} = 0.485$; on Bt plants $\omega_{ss} = \omega_{sr} = 0,\ \omega_{rr} = 0.223$; winter occurs every 5th generation, overwinter survival 0.05, *rr* relative overwinter survival 0.29; offspring per female $2R_0 = 17$. Refuge is 10%. Initial *r* allele frequency $p_0 = 0.01$. Where conditions are maintained year-round (thick lines), population reduction is slower and resistance management is weaker than where winter imposes survival penalties for all genotypes and extra fitness costs for resistant homozygotes (thinner lines).

Winter mortality helps to suppress the population. For example, with seasonal parameter values for pink bollworm (Table 4.1), although the efficacy of Bt crops is significantly reduced after resistance arises, it appears to remain just good enough to keep pest densities in check (Fig. 4.3). If individuals harbouring one or more *r* alleles die disproportionately in winter, then the allele progresses more slowly towards fixation, or more rapidly towards extinction, than in a non-seasonal scenario, especially bearing in mind that without any winter the insects could have more generations per year (Fig. 4.3; with no release and 10% refuge, from $p_0 = 0.01$ the *r* allele exceeds 0.5 in 73 generations with no seasons but 96

generations with winter). Comparing the two scenarios, the programme of RIDL release has a greater effect on the evolution of allele frequency than winter has.

Winter appears to have little effect on the relative benefit of the RIDL strategy. For example, the non-seasonal model with no release, using pink bollworm data, has a critical proportion of Bt crops (eq. 4) at 0.698, i.e., a critical refuge size of about 30%; with a smaller refuge the *r* allele frequency will increase towards fixation. Simulated RIDL release at 1:5 reduces that minimum refuge to 6.4%, only about a fifth (21%) of the refuge required with no release. When overwintering costs are taken into account, the minimum refuge sizes are approximately 25% for no release and 5.5% for RIDL release, which is also about a fifth (22%) of the refuge needed with no release.

4.3.3 Dominance of resistance

With no released insects, we can deduce what effects varying dominance of resistance can have on the equilibrium *r* allele frequency. Increasing dominance of resistance moves the relative fitness of heterozygotes on Bt plants ω_{sr} further from ω_{ss} and closer to ω_{rr}. Altering ω_{sr} in that manner, while keeping all other fitness values constant, decreases the Φ_1 critical threshold for Bt crops (eq. 3) (except where costs of resistance are recessive, in which case $\Phi_1 = 0$) and increases Φ_2 (eq. 4) (except where costs are dominant, which forces $\Phi_2 = 0$). If the relative fitness values are such that $\Phi_1 < \Phi_2$, greater dominance widens the range of Φ for which $\Phi_1 < \Phi < \Phi_2$ (where heterozygotes have the overall advantage, i.e. 'marginal over-dominance') and the *r* allele will persist and settle at an internal equilibrium value, in some cases instead of going to extinction (where $\Phi < \Phi_1$ with the lower dominance) or to fixation (where previously $\Phi_2 < \Phi$). If $\Phi_1 > \Phi_2$, increasing dominance could change Φ_1 and Φ_2 sufficiently to reverse that relationship.

This would add to the range of potential outcomes by making an internal equilibrium allele frequency possible (which only occurs when $\Phi_1 < \Phi < \Phi_2$). In summary, increasing dominance of resistance can change outcomes by altering thresholds and creates more possibilities for the *r* allele to persist.

We investigated the relationship between the minimum refuge size required to prevent the spread of resistance and the RIDL release ratio. We illustrate this using parameter values estimated for *H. zea* (Table 4.1) and varying values of *h*. Here, the Bt toxins are not high dose (some *ss* and *sr* insects survive). There are no fitness costs of resistance (all genotypes survive equally well in the refuge), so the *r* allele has no disadvantage and with no releases the favourable selection pressure on Bt crops will drive it to fixation, whatever the dominance of resistance and whatever the refuge size. First, we consider starting a release programme from $p_0 = 0.01$. For all values examined, RIDL male release can reduce the minimum refuge size required (Fig. 4.4a). Even for very low RIDL release (1:1000), some Bt crops can be grown (refuge < 100%), and with higher release ratios the reduction in minimum refuge size is considerable. Compared with fully recessive *r* alleles, where low RIDL release ratios can have a significant effect on minimum refuge size, for partially dominant or dominant resistance ($h > 0$) the refuge reduction with low release ratios is less substantial.

For any given release ratio up to roughly 1:10, a higher refuge is needed to control a more dominant *r* allele. However, for release ratios rising from about 1:1 that effect is reversed (Fig. 4.4a). For example, if 6.5 RIDL males are released for each male in the wild, the minimum refuge required is 6.1% for $h = 0.1$, and this decreases through intermediate levels of dominance, down to 0% (no refuge needed at all) for $h = 0.826$ (the actual

estimate from *H. zea* data) and $h = 1$. This phenomenon could be explained by the genetics of this resistance. There are no fitness costs of resistance, so neither allele has any advantage in the refuge. At low refuge sizes, almost the whole area is planted with Bt crops so very few susceptible *ss* insects will survive and the evolution of the *s* allele frequency depends mainly on the heterozygotes, more of which will survive to reproductive maturity if the resistance is more dominant.

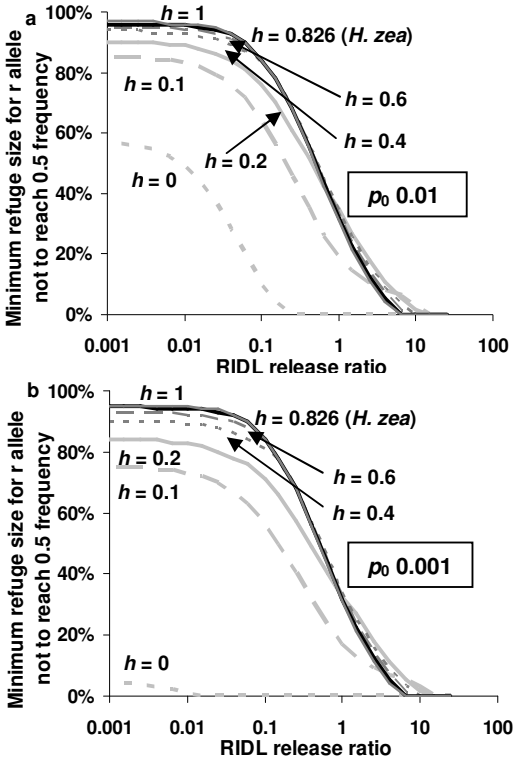

Figure 4.4 At low RIDL release ratios increasing dominance of resistance requires a larger refuge but at higher RIDL release ratios a partially reversed effect can be observed. Relative fitnesses, representing *H. zea* data are: in refuge $\upsilon_{ss} = \upsilon_{sr} = \upsilon_{rr} = 1$; on Bt plants $\omega_{ss} = 0.1$, $\omega_{sr} = 0.1$ to 0.404 (by varying h), $\omega_{rr} = 0.404$. Initial *r* allele frequency p_0 is (a) 0.01 or (b) 0.001. Critical refuge sizes for controlling the *r* allele, were approximated for a range of RIDL release ratios from 1:1000 to 25:1 by determining the minimum refuge size for which the allele frequency does not reach 0.5 in 500 generations. This was repeated for resistance of varying dominance: $h = 0$ recessive (light grey dotted line), 0.1 (light grey dashed line), 0.2 (light grey solid line), 0.4 (dark grey dotted line), 0.6 (dark grey dashed line), 0.826 (as reported for *H. zea*, black line) or 1 dominant (dark grey solid line).

The same patterns are observed if releases are started when p_0 is 0.001 rather than 0.01 (Fig 4.4b). Starting the programme earlier (Fig. 4.4 compare panels a and b) reduces the minimum refuge size considerably for recessive resistance ($h = 0$, light grey dotted lines) and there is a lesser but still noticeable effect for nearly recessive resistance ($h = 0.1$, light grey dashed lines). For more dominant resistance (higher h), the improvement is slight.

We investigated the effect of dominance of resistance on the ultimate fate of the *r* allele for a range of RIDL release ratios, for several refuge sizes (Fig. 4.5). First, we assumed 39% refuge (Fig. 4.5a). With the fitness values adapted from *H. zea* data, the recessive version of the *r* allele becomes extinct and so do versions with low dominance *h*. There are threshold *h* values, which rise with increasing release ratio, where the *r* allele equilibrium jumps from extinction to persistence. The threshold effect is not discontinuous for the relatively higher ratio (5:1). The *r* allele persists above 0.5 frequency for lower RIDL release ratios (1:10 and 1:5) and below 0.5 for moderate release ratios (1:1 and 5:1). The equilibrium allele frequency decreases with increasing release ratio, which is consistent with our results for recessive resistance with non-recessive costs (Table 4.2). Once over the threshold dominance level, increasing *h* decreases the equilibrium frequency if it is over 0.5 and increases it if it is under 0.5.

At the value estimated for *H. zea* ($h = 0.826$), the *r* allele goes to fixation with no releases (reaching 0.5 in 19 generations starting from 0.01 frequency), to 0.837 frequency with 1:10 RIDL release ratio (reaching 0.5 in 20 generations), and to 0.745 with 1:5 releases (0.5 in 23 generations). RIDL release at 5:1 only achieves a potentially acceptable equilibrium frequency 0.215. With those fitness values, 39% refuge and initial allele frequency 0.01, low levels of RIDL release are an improvement on no release, but the *r* allele can still become very common fairly rapidly.

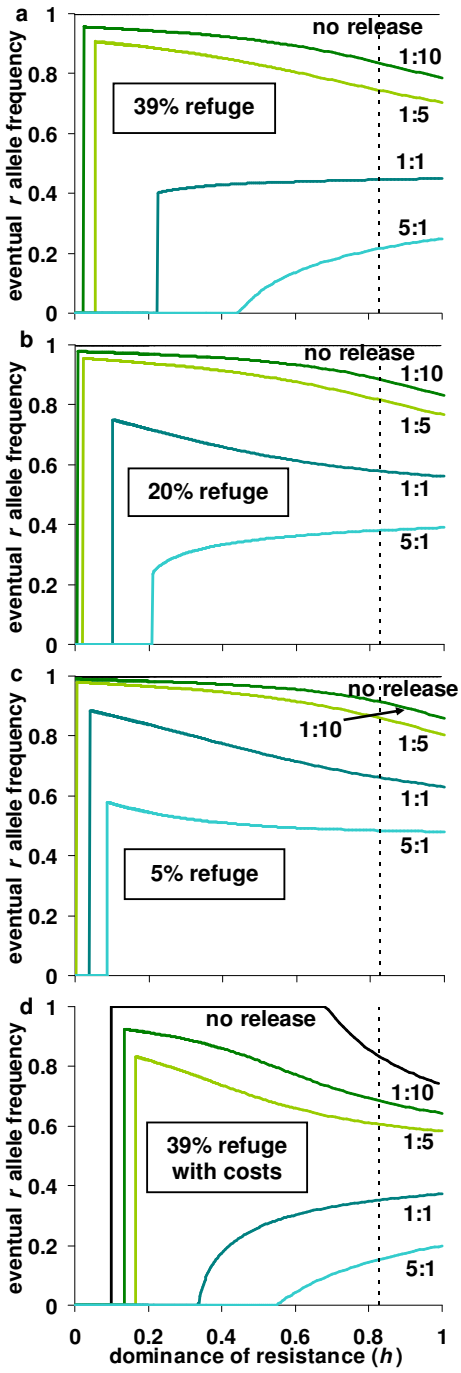

Figure 4.5 There are threshold values of dominance of resistance at which the *r* allele switches from extinction to persistence. The graphs show the equilibrium allele frequency towards which the *r* allele converges for resistance varying from recessive ($h = 0$) to dominant ($h = 1$). Relative fitness values and p_0 are as in Fig. 4.4a, refuge is (a) 39%, (b) 20% or (c) 5%, and release ratios are as marked. The dotted line indicates $h = 0.826$ as estimated from *H. zea* data. Panel (d) is as for panel (a), except there are fitness costs of resistance: in refuge $\upsilon_{sr} = 0.95$ and $\upsilon_{rr} = 0.8$ (instead of 1).

Simulations with p_0 at 0.001 instead of 0.01 produce virtually identical results (indistinguishable by eye from Fig. 4.5a). The threshold values of h are very slightly higher with lower p_0, but outside of that small range of h values the eventual allele frequencies are identical. This is consistent with our results for recessive resistance with

non-recessive costs (Table 4.3); the initial allele frequency acts as a switch between extinction or persistence, but does not alter the value of that equilibrium frequency of the persistent allele.

We obtain broadly similar results with a 20% refuge (Fig. 4.5b) instead of 39%. The thresholds are at lower values of *h* and the persistent allele frequencies are higher. Also, there is a discontinuity in the threshold effect even at 5:1 RIDL release. With 5% refuge (Fig. 4.5c), the *h* threshold values are lower still, and above that threshold with 5:1 release ratio the persistent allele frequency is above 0.5 (this consistent with Fig. 4.4a, which shows that 5:1 ratio is sufficient to prevent the *r* allele spreading if $h = 0$, but not if $h = 0.1$).

For these simulations with varying dominance of resistance, we have so far assumed no fitness costs of resistance. When we include such costs we obtain qualitatively similar outcomes (Fig. 4.5d). The presence of fitness costs creates selection pressure against resistance in the refuge, making it easier to control. With fitness costs (compare Fig. 4.5 panels a and d) thresholds are at higher values of *h* and eventual allele frequencies are lower. As illustrated, with no release the *r* allele can go extinct at low *h* (because of the extra selection pressure) and tends to an equilibrium below 1 at high values of *h* (where having a second copy of the *r* allele confers little or no advantage but has some extra cost, so the heterozygote has an advantage over the resistant homozygote).

Although the equilibrium value conveys information about the evolution of *r* allele frequency, the pattern during the early years is of more practical interest. These transient dynamics follow the classic sigmoid curves evident in Figs. 4.1-4.3. If the *r* allele exceeds

0.5 frequency, the population growth rate then increases significantly, as illustrated in Figs. 4.1 & 4.3. We investigated the effect of RIDL release ratio on the time taken to reach 0.5 frequency for a range of values of dominance of resistance. With fitness parameters adapted from *H. zea* data and 5% refuge (Fig. 4.6), a recessive *r* allele ($h = 0$) would not reach 0.5 frequency with no (or any) releases, but with dominance $h = 0.1$, the frequency passes 0.5 in 29 generations (from p_0 0.001) with no release, and that period is reduced as dominance increases. Very low RIDL releases have little effect on the timing, but at moderate levels they can slow the spread of resistance. This slowing effect is less marked as dominance of resistance increases, although greater dominance *h* reduces the critical release ratio with which the *r* allele will not reach 0.5 frequency.

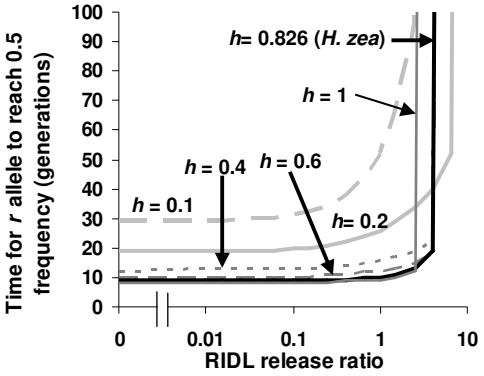

Figure 4.6 RIDL releases slow the spread of the *r* allele, with higher dominance of resistance reducing this slowing effect but also reducing the critical release ratio that is sufficient to stop resistance spreading.
The graph shows the number of generations after which the *r* allele exceeds 0.5 frequency, starting from $p_0 = 0.001$, for resistance of varying dominance as marked: $h = 0.1$ (light grey dashed line), 0.2 (light grey solid line), 0.4 (dark grey dotted line), 0.6 (dark grey dashed line), 0.826 (as reported for *H. zea*, black line) or 1 dominant (dark grey solid line). Relative fitness values are as in Figs. 4.4 & 4.5. Refuge is 5%. Recessive resistance ($h = 0$) is not shown, because the *r* allele does not reach 0.5 frequency regardless of releases. The RIDL release ratio axis is on a log scale except that the values for 0 (no release) are plotted and joined to those for 0.01 (1:100) by straight lines.

We conclude that for highly dominant resistance the advantages of the RIDL strategy are not as substantial as they are for recessive or nearly recessive resistance. As with other resistance management strategies, more dominant resistance is harder to defeat.

4.3.4 Dominance of fitness costs of resistance

Greater dominance of fitness costs of resistance reduces the relative fitness of heterozygotes in the refuge (υ_{sr}) while keeping all other fitness values constant. We can analyze the situation with no releases. More dominant costs raise the critical proportion of Bt crops Φ_1 (eq. 3) (unless resistance is recessive, which forces $\Phi_1 = 1$) and lower Φ_2 (eq. 4) (unless resistance is dominant, which makes $\Phi_2 = 1$). These are the opposite of the effects seen for increasing *h*. Generally, increasing dominance of fitness costs creates fewer possibilities for the *r* allele to tend to an internal equilibrium frequency and more opportunities for extinction.

To illustrate this, consider an example (data for *Helicoverpa armigera*, Table 4.1) where the Bt toxins are not high-dose, fitness costs are not very dominant ($g = 0.155$) and the no-release critical proportions of Bt crops are $\Phi_1 = 0.504$ and $\Phi_2 = 0.704$ (to 3 d.p.) so $\Phi_1 < \Phi_2$. With 70% refuge ($\Phi = 0.3$, so $\Phi < \Phi_1$) the *r* allele will decline to extinction. With 39% refuge ($\Phi = 0.61$ and $\Phi_1 < \Phi < \Phi_2$) the *r* allele will tend toward an intermediate equilibrium frequency, in this case 0.224. With a 20% refuge ($\Phi = 0.8, \Phi_2 < \Phi$), the *r* allele will go to fixation. If we now make the costs of resistance more dominant by lowering heterozygote fitness in the refuge, the critical values would change as described above. With 39% refuge the *r* allele equilibrium frequency starts at 0.349 with recessive costs of resistance ($g = 0$), decreases in value with increasing *g* and reaches 0 when *g* is 0.239. (This happens because if $g < 0.239$ then $\Phi_1 < 0.61 < \Phi_2$, if $0.239 < g < 0.301$ then $0.61 < \Phi_1 < \Phi_2$, and if $g > 0.301$, $\Phi_2 < \Phi_1$ and with $p_0 = 0.01$ Φ lies below Φ_3). With 20% refuge and p_0 0.01, the *r* allele switches from fixation to extinction when *g* increases above 0.621 (because Φ_3 changes from below 0.8 to above

0.8). These outcomes for no releases are confirmed by simulation (black lines in Fig. 4.8), see below.

According to this analysis, with no releases the lowest refuge size for the *r* allele represented in this example data (*H. armigera*) to tend to extinction is about 49.7% (so that $\Phi < \Phi_1 = 0.504$). Decreasing the refuge below 49.7% at first results in the *r* allele tending towards an internal equilibrium and while that remains below 0.5 frequency, the lower refuge size would still satisfy our criterion that the *r* allele frequency does not reach 0.5. Simulations show that the minimum refuge size defined this way is about 33.7%. If the fitness costs were instead dominant (make $g = 1$ by setting υ_{sr} equal to υ_{rr}), that minimum refuge size is 13.6%.

We investigated the relationship between the minimum refuge size required to prevent the spread of resistance (starting from $p_0 = 0.01$) and the RIDL release ratio, for various values of *g* (Fig. 4.7). RIDL release over about 1:10 reduces noticeably the minimum refuge required (at lower ratios RIDL release gives only a marginal improvement on no release). The reduction in minimum refuge size can be considerable and there is more benefit where the costs of resistance are less dominant. When costs of the *r* allele are less dominant, more heterozygous insects survive in the refuge and the extra *s* alleles they contribute reduce the effect of introgression of *s* alleles from released insects. For a given release ratio, a higher minimum refuge is needed to deal with an *r* allele that has less dominant fitness costs. However, the minimum refuge size varies little for *g* in the range $0 \le g \le 0.3$ across the whole range of release ratios; it is only at higher values of *g* that the influence of dominance on minimum refuge size becomes obvious. At higher release ratios the dominance of fitness costs has negligible effect on the minimum refuge size.

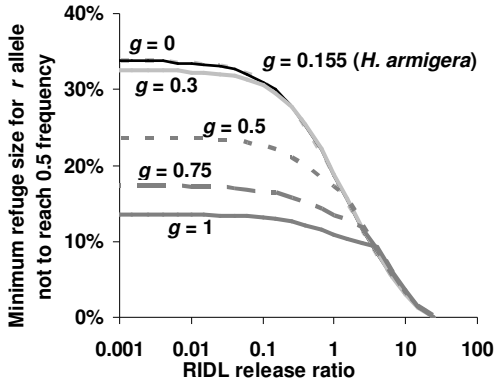

Figure 4.7 More dominant fitness costs of resistance reduce the minimum refuge size needed to contain the *r* allele, although the differences are slight at higher release ratios. Relative fitnesses, representing *H. armigera* data, are: in refuge $\upsilon_{sr} = 0.515$ to 1 (by varying *g*), $\upsilon_{rr} = 0.515$; on Bt plants $\omega_{ss} = 0.047$, $\omega_{sr} = 0.121$, $\omega_{rr} = 0.293$. Initial *r* allele frequency p_0 is 0.01. Minimum refuge sizes for which the *r* allele frequency does not reach 0.5 in 500 generations were determined for a range of RIDL release ratios from 1:1000 to 25:1. This was repeated for resistance costs of varying dominance: $g = 0$ recessive (light grey dotted line, almost entirely obscured beneath black line), 0.155 (as reported for *H. armigera*, black line), 0.3 (light grey solid line), 0.5 (dark grey dotted line), 0.75 (dark grey dashed line), or 1 dominant (dark grey solid line). Note that the vertical axis only extends to 0.4, not to 1.

RIDL releases lower the equilibrium frequency and lower the critical value of *g* above which the *r* allele goes extinct; higher release ratios increase those effects (Fig. 4.8). With 39% refuge the equilibrium allele frequency decreases continuously with increasing *g* until it reaches zero (Fig. 4.8a). However, with 20% refuge, except for RIDL release at 5:1, the outcome for the *r* allele switches discontinuously from persistence to extinction as dominance of resistance costs increases (Fig 4.8b). The same kinds of changing outcomes are seen with RIDL releases as we described analytically for no release (above): the nature of the *g* threshold (continuous or discontinuous effect) can be altered by having different refuge sizes (Fig. 4.8 compare a and b), or by varying the RIDL release ratio (compare 5:1 to other lines in Fig. 4.8b). A 5% refuge is insufficient to prevent the *r* allele spreading with any release ratio below nearly 10:1 (see Fig. 4.7), so there is no threshold effect and the dominance of fitness costs has only a slight effect on the eventual frequency of the *r* allele (Fig. 4.8c).

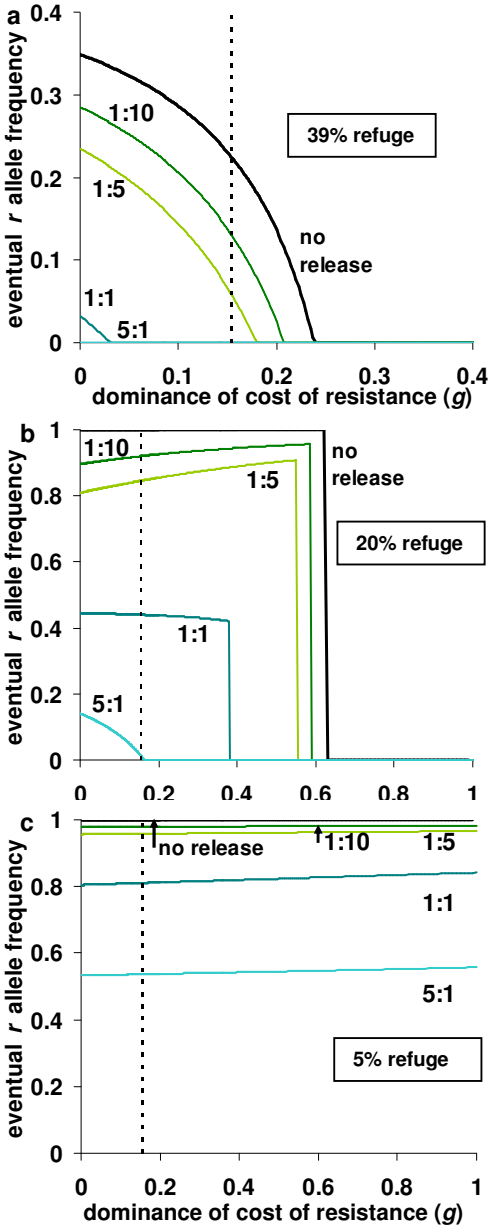

Figure 4.8 The equilibrium *r* allele frequency decreases with increasing dominance of fitness costs of resistance, from persistence to extinction.
The graph shows the equilibrium frequency towards which the *r* allele converges for fitness costs of resistance varying from recessive (g = 0) to dominant (g = 1). Relative fitness values and p_0 are as in Fig. 4.7 and release ratios are as marked. The dotted line indicates g = 0.155 as estimated from *H. armigera* data. Note that panel (a) is on a different scale; axes extend only to 0.4. With 39% refuge (a) there is no discontinuous threshold effect, but with 20% refuge (b) there is, and with 5% refuge (c) there are no thresholds.

4.4 Discussion

We have shown that a RIDL approach could combine synergistically with Bt crops to form an effective component of a pest management strategy. The effectiveness for managing resistance can be improved by any combination of increasing the RIDL release ratio, commencing releases earlier (when the resistant allele is at a lower frequency) and

increasing the refuge size. These measures slow the onset of resistance and may reverse it. Increasing the ratio of released RIDL males to males in the wild also has a beneficial impact on the size of the pest population.

Simulated RIDL release is consistently better than Bt crops and refuge alone for both resistance management and population control. Wild type release is predicted to be more effective for resistance management than the release of RIDL males. However, release of wild type males and females has a marginally detrimental effect on population size (and therefore on crop damage) compared with no release, until such time as resistance would have become substantial with no releases (likely to be several years for most key pests targeted by Bt crops).

RIDL release could potentially achieve a considerable reduction in the minimum refuge size required to prevent the spread of resistance. The relative benefit seems unaffected by environmental seasonality. The degree of advantage for resistance management depends on the fitness parameters associated with the resistant allele and the allele frequency. Generally, RIDL is of greater benefit in situations that are already more favourable for resistance management – where resistance is rarer, is less dominant or has more dominant fitness costs. Like the high-dose refuge strategy itself, RIDL release is less effective when applied to dominant resistant alleles in pest populations where Bt plants do not kill all susceptible individuals.

The existence of significant threshold effects arising from multiple parameters highlights the need for good information about the target pest population, when planning a resistance management strategy. The genetic fitness parameters and frequency of rare (possibly non-

existent) resistant alleles are difficult to measure or estimate in practice. It would be advisable to select a release ratio comfortably above the estimated critical value to avoid inaccuracy or fluctuations switching the situation to one where a large frequency could result instead of extinction.

The economic attractiveness of the RIDL strategy depends on an assessment of the costs of a release programme compared to the net benefits of refuge costs foregone. For growers, refuges are an inter-temporal economic trade-off; farmers lose the short-term benefits of Bt technology in the areas planted as refuge in return for extending the period over which Bt plants retain their efficacy (Frisvold and Reeves 2008). Estimates of the costs of refuges vary widely and, although generally agreeing that some refuge is preferable to none, various economic studies have come to inconsistent conclusions about what refuge policies would be optimal for grower returns. Entomological and economic information, both subject to uncertainty, are needed to assess where any particular region's agricultural producers are on the trade-off surfaces charting the longer-term benefits and costs of refuge policies. RIDL release would add an extra dimension to such analyses. The costs of a RIDL release programme depend on factors such as pest densities (the number of insects released vary with population size and change over time) and the availability of modest spare capacity in existing SIT facilities. RIDL release is more likely to be implemented as an area-wide programme and so would be influenced more by regional adoption choices than by individual growers' decisions.

There is evidence that many growers have planted less Bt crops than permitted (Carrière et al. 2005a, Frisvold and Reeves 2008). This suggests that non-economic factors are also influencing decisions, such as cautious adoption of new technology and a risk-averse

attitude if the variance of profits is higher for Bt plants (Frisvold and Reeves 2008). Another possible explanation is a halo effect, where long-term regional pest suppression in areas of high adoption can reduce pest pressure in the region generally, thereby generating benefits for non-Bt plants too and reducing the marginal advantage of planting more Bt crops (Carrière et al. 2003, Frisvold and Reeves 2008). In such circumstances, where mandatory refuge sizes might not actually be binding constraints, a RIDL option with lower permitted refuges may not have an apparent economic benefit.

An economic case for a RIDL approach might be more persuasive in situations where the strategies in current use have failed. Potential short-term responses to a resistance event include additional chemical control, early harvest, SIT releases and biological control (such as parasitic nematode treatments), and remedial action the following year could include temporary cessation of cultivating the relevant Bt crop and upward revision of mandatory refuge sizes (Frisvold and Reeves 2008). RIDL release could be added to this toolkit and, unlike the other options available, it would contribute to both reduction of pest densities and decrease of resistant allele frequencies. For a successful outcome using only RIDL release and refuge manipulation, it would be important to use a sufficiently high combination of refuge and release ratio to bring the resistant allele rapidly down to a low level.

Dynamic selection pressures and rapidly updated and expanding technology make it impossible to formulate a universally optimal resistance management strategy. Instead, it is sensible to adopt a framework within which an appropriate mix of measures can be selected, tailored to the particular genetically modified crop and incorporated toxin(s), and the local pest species, economic factors, environmental conditions and agricultural

practices. For example, in the USA, for each approved crop type a few alternative refuge requirements are specified, with some choice as to the position of refuges and whether they may be sprayed, and special cases for particular geographic regions. Within this context, the addition of appropriate RIDL strategies to the menu of options could be a sensible extension to existing crop pest management regimes.

5 Managing resistance to genetic control of insects

Summary Genetic RIDL® technology (Release of Insects carrying a Dominant Lethal), is a proposed modification of the Sterile Insect Technique that involves releasing insects that are homozygous for a repressible dominant lethal genetic construct rather than being sterilized by irradiation. Hypothetical resistance to the lethal mechanism is a potential threat to RIDL strategies' effectiveness. Using population genetics and population dynamics models, I assess the circumstances under which monogenic biochemically-based resistance could have a significant impact on the effectiveness of releases for population control. I assume that released insects would be homozygous susceptible to the lethal genetic construct and therefore releases would have a built-in element of resistance dilution. I find that this effect could prevent or limit the spread of resistance to RIDL constructs; the outcomes are subject to competing selective forces deriving from the fitness properties of resistance and the release ratio.

5.1 Introduction

Pest insects cause enormous harm to public health and economic damage to agriculture. Arthropods act as vectors for many human and veterinary diseases and destroy around 18% of the world annual crop production (Nicholson 2007). The Sterile Insect Technique (SIT) is a pest population control method in which insects are mass-reared, sterilized by irradiation and released (Knipling 1955, Dyck et al. 2005). Some wild insects mate with the released sterile insects and produce fewer progeny as a result. If sufficient insects are released for a sufficient period, populations of pest insects can be suppressed or perhaps eliminated locally. The SIT has been successful against a range of target species, mainly agricultural pests (Dyck et al. 2005).

Genetic technology, such as RIDL (Release of Insects carrying a Dominant Lethal), could modify the SIT by releasing insects that are not irradiated, but instead are homozygous for one or more dominant lethal genetic constructs that are repressible during mass-rearing (Thomas et al. 2000, Alphey et al. 2007a, Alphey 2007). This approach is also known as autocidal biological control (Fryxell and Miller 1995). Among the potential advantages claimed for genetic control over radiation-based SIT is the ability to engineer the timing or nature of the lethality. The preferred time of death varies between pests and contexts. Very early acting lethality would be preferable for pests where the larval stage causes the most damage; an embryonic lethal system has been developed in a model organism and work in a pest fruit fly is ongoing (Horn and Wimmer 2003, Schetelig et al. 2007). Late-acting lethality that delays the time of death until after a density-dependent mortality phase, such as has been developed for RIDL technology in mosquitoes, can greatly enhance the SIT's effectiveness against a population that is limited by density-dependent processes (Atkinson et al. 2007, Phuc et al. 2007). Female-specific lethality would be advantageous for several reasons connected with facilitating separation of the sexes (Dyck et al. 2005). Release of only males, rather than both males and females, has been shown to make the SIT much more effective (Rendón et al. 2004). The efficiency of releases is improved because the released males have to seek out wild females; if both sexes were released some of the released sterile males and females would mate with each other and have no effect on the natural population. For insect vectors where females transmit disease, such as mosquitoes, release of females would be highly undesirable. However, in the absence of genetic sexing mechanisms, the biology of most species does not permit sex separation on the large scale required for the SIT and male-only releases are currently the exception rather than the norm.

A female-lethal version of RIDL, with insects homozygous for one or more female-specific dominant lethals, has been constructed in several species (Fu et al. 2007, Stainton et al. unpublished data). F_1 progeny of RIDL males and wild females inherit a dominant female-specific lethal; the F_1 females die, thereby reducing the reproductive potential of the wild population, but the F_1 males are viable and fertile. This provides a genetic sexing mechanism facilitating male only release, either by employing the female-lethal version of RIDL and withdrawing the repressor from the generation prior to release, or by combining a bisex-lethal system with female-lethality (with an independent means of repressing or inducing lethality) to permit male only release of bisex-lethal strains designed to kill progeny of both sexes in the field.

Insects are well-known for developing resistance to insecticides. Insecticide resistance has been reported in most major insect disease vectors and against every class of chemical insecticide and insecticidal crops (Nicholson 2007, Reynolds 2007). "Behavioural resistance" to the SIT has been observed in isolated cases, where selection favours females that are able to identify and avoid mating with released sterile males (Dyck et al. 2005). As with any other new control method, resistance to the lethal mechanisms of the RIDL technology or other prospective methods of autocidal biological control is therefore a potential problem. None has so far been detected in any species at various stages of developing the technology (e.g. explicitly tested in Gong et al. 2005), but that does not preclude the possibility of resistance arising after introduction of released insects, whether through new mutations or existing mutations in some wild populations that are capable of conferring resistance.

In practice, prior to commencing any release programme there is no RIDL genetic construct in the wild, so there is no positive selection pressure and any resistance-associated allele would be expected to be at very low levels (especially if it impairs fitness and therefore carries a disadvantage that would tend to reduce its frequency) unless it also conferred some unrelated pleiotropic advantage. Evidence about resistance to other pest control methods generally supports this expectation. For example, from field data for several pests, if any resistance to insecticidal Bt crops could be detected at all, allele frequencies were of the order of 10^{-3} (Gould et al. 1997, Tabashnik et al. 2005, Stodola et al. 2006, Huang et al. 2007, Mahon et al. 2007a). This was not surprising because the toxins were derived from a soil dwelling organism *Bacillus thuringiensis* and so there was some prior natural exposure. However, mutant alleles associated with resistance to malathion have been found in pinned specimens of Australian sheep blow fly *Lucilia cuprina* collected before the release of organophosphate insecticides (Hartley et al. 2006). This finding, in a small sample, suggests that the frequency of alleles that could confer resistance to a new product might be much higher than expected. If such polymorphisms are present at a high frequency before insecticide use, they are probably balanced polymorphisms (maintained by balancing selection such as heterozygote advantage) and therefore do not carry a clear net fitness cost in the absence of the new insecticide as would be expected for a new mutation (ffrench-Constant 2007).

One possible measure to prevent or reverse the spread of resistance is to dilute the gene pool in the wild population with susceptible alleles. In radiation-based SIT, no viable hybrids are formed so there is no introgression of alleles from released insects into the wild population. With female-lethal RIDL, the F_1 males are viable and fertile so alleles from the mass-reared strain could be spread into the wild population, with the female-lethal

aspect still contributing to population suppression. Alleles from bisex-lethal RIDL strains might be pushed into the wild population if there were resistance to the RIDL genetic construct so that some progeny survive and reproduce.

In previous work, we explored insect resistance to crop plants engineered to produce insecticidal toxins (Bt crops), under arrangements where refuges of non-Bt plants are provided near insecticidal crops to provide a source of alleles conferring susceptibility to the toxins (Alphey et al. 2007b, Alphey et al. 2009). We proposed that control programmes could combine Bt plants with the release of Bt-susceptible homozygote insects, either natural or engineered insects, to manage economically important agricultural pests. By providing an alternative source of susceptible alleles, release of homozygous susceptible female-lethal RIDL males (or wild type insects) is predicted to slow the spread of resistance to Bt and sufficient releases can prevent or reverse the spread of the resistant allele. Here I investigate the potential for introgression of alleles from released insects to act in a similar manner, to help manage biochemically-based genetic resistance to the autocidal genetic construct itself. This could apply to any form of biochemical resistance to the lethal effector molecule expressed by the transgenic construct, for example, through alteration of the target site or increased metabolism to clear the toxin.

The evolution of allele frequencies depends on selection pressure due to relative fitnesses of the different genotypes and properties of the resistance trait. Resistance to RIDL constructs has not yet been observed, so there is no data about its potential properties, and consequently I explore a wide range of theoretical values. There is limited data about the fitness penalties of the RIDL construct in the absence of resistance. Lethality achieved by a single copy of the construct in bisex-lethal or female-lethal strains can be 97-100%

(Gong et al. 2005, Fu et al. 2007, Phuc et al. 2007). Some female-lethal strains have only a modest fitness penalty, if any, in males at 0-2% lethality (Fu et al. 2007). Generally, from any panel of transgenic insertion lines created, only those with sufficiently close to 100% lethality in heterozygous form, and female-lethal strains with little or no fitness cost to heterozygous males, are chosen for further development. In such lines, lethality in homozygotes is 100% or very close (Fu et al. 2007, Stainton et al. unpublished data).

To some extent I can investigate the evolution of resistant allele frequency in isolation. However, to assess its impact on the main objective, which is to suppress or eliminate a population, I must explore the interaction between allele frequencies and population size, and to do this I must combine a population genetics framework with a population dynamics approach. For pests or disease vectors where both sexes are responsible for damage, the number of adults emerging at each generation is a proxy measure for the level of damage. For example, it is indicative of the level of crop damage by phytophagous agricultural pests, because every individual that survives to reproductive maturity has completed larval development on plants. For those disease vectors, such as mosquitoes, where only adult females transmit disease (when they take a blood meal), the number of adult females emerging at each generation is the relevant measure of potential harm. This might also be a good measure for some agricultural pests, such as tephritid fruit flies where oviposition "sting" damage is economically harmful.

Here, through mathematical modelling, I explore the potential for proposed release strategies to contribute to managing a hypothetical heritable biochemical resistance to RIDL genetic constructs. I examine the evolution of RIDL-resistant allele frequency and of the frequency of the RIDL construct, considering both dynamics over time and

equilibria. I investigate the impact of the release ratio, initial resistant allele frequency and genetic properties of the resistant allele. First I consider dominant or recessive, completely effective resistance with no fitness costs and then gradually relax assumptions to allow the strength and fitness costs of resistance to vary across the whole range of possible values. I then explore the interaction between pest control and resistance management by modelling relative population size over time.

5.2 The model

Parameter definitions and symbols are set out in Table 5.1.

5.2.1 Population genetics model

I use a deterministic, non-spatial, discrete-generation population genetic model and assume a closed homogeneous population, with random mating, and no immigration, emigration or mutation. I assume a 1:1 sex ratio.

I assume the number of individuals surviving to reproductive maturity depends on the relative fitness values of the immature life stages. Embryonic, larval or pupal susceptibility to the lethal effect of a RIDL genetic construct is assumed to be controlled by a single autosomal locus with two alternative alleles: resistant *R* (frequency p in adults emerging in the wild) and susceptible *S* (frequency q, $p + q = 1$). There are three possible genotypes at this locus: *SS*, *SR* and *RR*. For convenience, I use the terms "larvae" and "larval stage" to refer to all immature stages, although engineered lethality might in principle be designed to prevent egg hatch, pupation or adult eclosion instead of being fatal during larval development.

Table 5.1 Parameters and symbols

Symbol	Parameter	Constraints
p	frequency of resistant *R* allele in current adult generation	$0 \le p \le 1$
q	frequency of susceptible *S* allele	$0 \le q \le 1,\ p + q = 1$
p_0	initial *R* allele frequency	$0 < p_0 < 1$
p^*	equilibrium *R* allele frequency	
l	frequency of the RIDL genetic construct *L*	$0 \le l \le 1$
l_0	initial *L* allele frequency	$0 \le l_0 < 1$
l^*	equilibrium *L* allele frequency	
i	genotype at *S*/*R* locus	*SS*, *SR* or *RR*
j	genotype at locus of construct insertion (*L* allele), or wild type absence of the construct (*w* allele)	*ww*, *Lw* or *LL*
k	genotype for effect of RIDL construct: target (both sexes for BL, females only for FL) non-target (males only for FL)	target or non-target
ψ_i	relative fitness of larvae of genotype *i*	$0 \le \psi_{RR} \le \psi_{SR} \le \psi_{SS} = 1$
ε_k	fitness penalty of RIDL construct	$0 \le \varepsilon_k \le 1$
γ_i	susceptibility to the RIDL construct (scaling factor applied to fitness penalty ε)	$0 \le \gamma_{RR} \le \gamma_{SR} \le \gamma_{SS} = 1,$ $\gamma_{RR} \ne \gamma_{SS}$
η_j	number of copies of the RIDL construct	0 for *ww*, 1 for *Lw* or 2 for *LL*
Ω_{ijk}	relative fitness of larvae of genotype *i*, *j*, *k*	(see Eq. 2)
d	release ratio of released RIDL males to naturally emerging adult males in the wild (of any genotype)	$d > 0$
R_0	average number of female progeny produced per adult female that survive to adulthood in a density-independent population	$R_0 > 0$
N_t	population size of mature adults at generation *t* relative to the initial pest density	$N_0 = 1$ (see Eqs. 4 or 8)
F_t	relative population size of mature females at generation *t*	(see Eqs. 5 or 9)
σ_t	simulated proportion of all offspring that survive to maturity	
$\sigma_t^{(F)}$	simulated proportion of female offspring that survive to maturity	

I assume that the resistant allele may have fitness costs, which also take effect during the larval stage. The relative fitness of immature individuals of genotype *i* (*i*: *SS, SR* or *RR*) is ψ_i, where fitness is measured relative to *SS* homozygote wild type insects (so $\psi_{SS} = 1$). I assume that fitness costs are not over- or under-dominant ($\psi_{RR} \le \psi_{SR} \le \psi_{SS} = 1$).

Engineered males for release ("RIDL males") carry a repressible dominant lethal genetic construct, for which they are homozygous. The RIDL construct is either bisex-lethal "BL" (where individuals of either sex inheriting one or more copies of the construct are non-viable in the absence of the repressor), or female-lethal "FL" (which similarly renders females non-viable but allows males to survive). There are two possible alleles at the insertion site of the RIDL construct: *L*, representing insertion of the construct (frequency l in adults emerging in the wild), and the wild type allele *w* (frequency $1-l$), representing absence of the construct, giving three possible genotypes at this locus: *ww*, *Lw* and *LL*. I assume there is no linkage between the RIDL insertion and the locus controlling resistance and neither of these is sex-linked.

I assume the RIDL construct imposes a fitness cost ε during the larval stage. For a bisex-lethal (BL) construct this fitness penalty takes a single value, and is equal to 1 if the construct is fully lethal. The relative fitness cost imposed by a female-lethal (FL) RIDL construct is sex-specific. It takes a value of (or near) 1 for the target sex (females). In the non-target sex (males) it either takes a lower value, to allow for a modest associated fitness penalty (for example, due to insertional mutagenesis and/or expression of a marker gene incorporated into the construct (Marrelli et al. 2006)), or is zero (having no effect). I denote this fitness cost ε_k where *k:* target (both sexes for BL, females only for FL) or non-target (males only for FL). I assume that the effect of the construct is multiplicative, so relative fitness of genotype *j* (*j: ww, Lw* or *LL*) in the absence of resistance is

$$\Omega_j = (1-\varepsilon_k)^{\eta_j} \qquad (1)$$

where η_j is the number of copies of the RIDL construct that the individual possesses (0 for *ww*, 1 for *Lw* or 2 for *LL*).

I represent the resistance trait as reduced susceptibility to the RIDL construct, by scaling the fitness penalty ε by a factor γ_i (*i*: *SS, SR* or *RR*). By definition of resistance, susceptible homozygotes are fully susceptible to the RIDL construct ($\gamma_{SS} = 1$) and *RR* individuals are less susceptible than *SS* ($\gamma_{RR} < \gamma_{SS}$). Resistance may be incomplete ($\gamma_{RR} \geq 0$), meaning that resistance might only reduce the fitness penalty associated with the *L* construct ($\gamma_{RR} > 0$), rather than negate its effect altogether (complete resistance, $\gamma_{RR} = 0$). I assume that resistance is not over- or under- dominant ($\gamma_{RR} \leq \gamma_{SR} \leq \gamma_{SS} = 1$).

Combining all these features, the relative fitness of larvae of genotype *i, j, k* (*i*: *SS, SR* or *RR*, *j: ww, Lw* or *LL*, *k:* target or non-target) is then

$$\Omega_{ijk} = \psi_i \left(1 - \varepsilon_k \gamma_i\right)^{\eta_j} \qquad (2)$$

There are potentially 18 genotypes in this model: combinations of 3 variants at the *S/R* locus, 3 variants at the *L/w* locus, and (for FL only) 2 variants in target or non-target sex.

5.2.1.1 No release

My baseline case is where the *R* allele is present but not at fixation ($0 < p_0 < 1$) in a naïve population (i.e., one with no *L* alleles in the wild, $l_0 = 0$, which would be the case before any RIDL release programme commenced) and no release strategy is being employed ("NR"). In this scenario, only the *S/R* genotype is relevant (no *L* allele is ever present as none are introduced) and relative fitness is $\Omega_{ijk} = \psi_i$ for all genotypes that can arise.

Standard population genetic theory (Gillespie 1998) tells us that the change in resistance allele frequency in a generation, Δp, is

$$\Delta p = \frac{p^2\Omega_{RR} + pq\Omega_{SR}}{q^2\Omega_{SS} + 2pq\Omega_{SR} + p^2\Omega_{RR}} - p$$
$$= \frac{pq\big(p(\psi_{RR} - \psi_{SR}) + q(\psi_{SR} - \psi_{SS})\big)}{q^2\psi_{SS} + 2pq\psi_{SR} + p^2\psi_{RR}} \qquad (3)$$

Where $p, q \neq 0,1$ (resistance allele present but not at fixation), the sign of Δp is determined by the sign of $p(\psi_{RR} - \psi_{SR}) + q(\psi_{SR} - \psi_{SS})$, which is never positive in my model. In the NR scenario, if there is no cost associated with the *R* allele it experiences no selection pressure, otherwise it would decline over time due to the effect of fitness costs.

In practice, rather than determining the *R* allele frequency by calculating Δp from Eq. (3), I use a simulation approach that involves calculating the frequencies of every genotype and applying the genotype-specific relative fitness values (Eq. 2). This approach allows me to analyze the model in the same way for no release (NR) and the more complicated cases of release of engineered insects (BL or FL); the latter cases involve a complex system of up to 18 difference equations that cannot be readily reduced to expressions in terms of the allele frequencies.

5.2.1.2 Release of engineered insects

To model the effects of the release of RIDL males carrying either a bisex-lethal or female-lethal construct ("BL" or "FL" RIDL release strategies), I assume that at each generation, as adults emerge prior to mating, mass-reared adult RIDL males are released at a fixed ratio *d* (the "RIDL release ratio") to the number of males in the wild population (of any genotype) that survived to reproductive maturity. All engineered released males are assumed to be homozygous susceptible to the RIDL construct (*SS*), and homozygous for

the RIDL construct (*LL*). Wild and released adults mix homogeneously and females mate with wild or released males at random.

Inherent in my model is a proportional release policy, where the number of insects released is proportional to the number of adult males in the current population. An obvious alternative would be a constant release policy, where the same fixed number of RIDL males is released at every generation (which could be described by the "release ratio" of that number to the number of adult males at the start of the programme). Implementing the proportional policy would require more sophisticated monitoring to provide data for reasonably accurate prediction of the adult male population at each generation so that the numbers for release can be estimated. I model the proportional policy because working in terms of proportions permits me to study the evolution of allele frequencies independently of population size (so those results are independent of any assumptions about population dynamics).

5.2.2 Population dynamics

5.2.2.1 Density-independent populations

R_0 is the average number of progeny produced per adult pest insect that survive to adulthood in a density-independent population, commonly expressed as female offspring per adult female. For agricultural pests under field conditions, after taking into account factors such as egg hatch rates, predation and pathogens that are common to all genotypes throughout the habitat, R_0 might typically range from 3 to 11 but could be much higher in some populations (Dye 1984, Dyck et al. 2005). For NR, BL and FL strategies the (dimensionless) relative population size is determined by

$$N_{t+1} = 2R_0 F_t \sigma_t \qquad (4)$$

$$F_{t+1} = R_0 F_t \sigma_t^{(F)} \qquad (5)$$

where N_t is the population size of mature adults at generation t relative to the initial pest density (set $N_0 = 1$), of which F_t are female. The population growth rate is $2R_0$ (each adult female contributes R_0 male and R_0 female larvae to the next generation) and σ_t and $\sigma_t^{(F)}$ are the proportions of all offspring or female offspring, respectively, that survive to maturity (simulated by applying the fitness costs of *R* alleles and the RIDL construct, and the lethal effect of the RIDL construct tempered by the resistance trait, as appropriate to each genotype).

5.2.2.2 Populations subject to density-dependent processes

Density-dependent processes play an important role in the dynamical behaviour of biological populations, and a number of difference equation models to portray them have been described (Bellows 1981). I adapt a general model of the form

$$\tilde{N}_{t+1} = R_0 \tilde{N}_t e^{-\alpha \tilde{N}_t} \qquad (6)$$

where $\tilde{N}$ denotes absolute (rather than relative) population size, and α is a measure of the strength of density dependence. Here $1/\alpha$ can be thought of as related to the carrying capacity of the habitat, such that if $\alpha = 0$ we recover the density-independent model, and in the limit as $\alpha \to \infty$, $\tilde{N}_{t+1} \to 0$ (the carrying capacity approaches zero and nothing survives). This system has equilibria at $\tilde{N}^* = 0$ and

$$\tilde{N}^* = \frac{\log(R_0)}{\alpha} \qquad (7)$$

To make this model dimensionless, I assume that the population starts at the equilibrium in Eq. (7) ($\tilde{N}_0 = \tilde{N}^*$) and measure the population size relative to that value $\left(N_t = \frac{\tilde{N}_t}{\tilde{N}^*} \right)$, so that $N_0 = N^* = 1$. It is important for my purpose to distinguish between males and females, so I express population growth in terms of progeny per female as I did in the

density-independent model. Finally, I multiply by the survival proportions (σ), which implies that the genetically-induced lethality occurs after the density-dependent processes (representing a late-acting rather than early-acting genetic construct).

With parameters and symbols as in Table 5.1, the relative population size in my density-dependent model is determined by

$$N_{t+1} = 2R_0 F_t e^{-(\log(R_0)N_t)}\sigma_t \qquad (8)$$

$$F_{t+1} = R_0 F_t e^{-(\log(R_0)N_t)}\sigma_t^{(F)} \qquad (9)$$

5.2.3 Simulations

I assume a naïve population ($l_0 = 0$) with genotypes initially in Hardy-Weinberg equilibrium (frequencies *SSww*: q_0^2, *SRww*: $2p_0q_0$, *RRww*: p_0^2). In the case of RIDL releases, other genotypes (*Lw* and *LL* types) arise from matings in the field.

For simplicity, I assume the RIDL construct is fully dominant and completely lethal ($\varepsilon_{\text{target}} = 1$) and the FL version is lethal to females but not to males; in these simulations, $\varepsilon_{\text{non-target}}$ was set to 0. As no resistance has been detected, there are no data about its potential properties, so I consider values across the permissible range (the constraints imposed in my model are summarised in Table 5.1).

I previously showed that lower release ratios than typically employed for the purpose of population suppression could be useful in some cases where the main objective is to manage resistance to an independent control measure such as Bt crops (Alphey et al. 2007b, Alphey et al. 2009). However, in the context of resistance to a RIDL construct, a higher release ratio of RIDL insects would provide both more *S* alleles to help dilute

resistance and greater selection pressure in favour of resistance, so it is not clear what the effect of a higher or lower RIDL release ratio might be.

I explore the effects of release of RIDL males by simulating different combinations of γ and ψ representing varying strength and fitness costs of resistance, over a range of release ratios from 1:10 (one RIDL male per ten males in the wild, 0.1) to 1000:1, or a subset thereof. Generally I model a release ratio of 10:1, which could be a realistic ratio for a control programme; overflooding ratios quoted in practice (typically 10:1 to 40:1 (Dyck et al. 2005)) may be higher to allow for poor dispersal and other losses during distribution, whereas my release ratio is net of any such effects and all the released insects are assumed to be available for mating. I simulate the frequencies of all genotypes over at least 30 generations to explore transient dynamics and more as necessary (30 up to several thousands) to estimate the value of an equilibrium allele frequency. Those higher numbers are far beyond the realistic practical duration of any release programme, but I use them for accurate estimation of equilibria by generating results past any transient behaviour to a time when the allele frequencies should have converged.

I begin by modelling initial *R* allele frequency p_0 of 0.01 or 0.1, to investigate resistance that is present at perceptible levels in the wild population (which would be of greatest practical concern). I also model lower values (0.001) to represent a rarer allele, and high values (for example, 0.7 or 0.99) to explore the potential reversal of very common resistance. A recent mutation might only have frequency of the order of 10^{-6}; I do not present results for such very rare alleles as I expect that any trend indicated by results for p_0 of 0.01 or 0.1 (or 0.001 where simulated) would extend to lower values, and because I

anticipate that it would take relatively many generations for the impact of such a very rare resistant allele to manifest.

5.3 Results

5.3.1 Managing resistance to a fully-lethal genetic construct in an initially naïve population

I begin by considering the baseline case where there is no resistance and then investigate the simple cases where resistance is either dominant or recessive, is complete (entirely removes susceptibility to the RIDL construct) and has no fitness costs. Later I progressively relax these assumptions to consider the full range of resistance properties and fitness costs.

5.3.1.1 Resistance is absent or lethal

There are three situations in terms of model parameters that are equivalent in that they represent the case where there is no resistance in the population: no *R* allele ($p_0 = 0$), a candidate "*R* allele" that does not actually confer any resistance property (susceptibility $\gamma_{SS} = \gamma_{SR} = \gamma_{RR} = 1$) and so is not a resistant *R* allele, or an *R* allele that is dominant fully lethal (relative fitness $\psi_{SR} = \psi_{RR} = 0$) and so never confers any resistance trait. In this baseline case I consider the implications for *R* and *L* allele frequencies.

In these circumstances, a resistant *R* allele tends toward (or remains at) zero frequency, i.e. the only stable equilibrium is extinction:

$$p^* = 0 \qquad (10)$$

As I have no mutation or immigration in my model, no resistant allele ever arises if it is initially absent. A dominant fully lethal allele will not survive into subsequent generations. A candidate allele that turns out to be non-resistant is not of interest. For completeness, note that such an allele has no fitness advantage, and will either remain

stable (under NR or BL scenarios if it has no fitness costs) or decline to extinction due to dilution and/or fitness costs (in all other situations).

The evolution of *L* allele frequency differs between BL and FL strategies. With no resistance and BL releases carrying a fully lethal *L* construct, all progeny of released males are non-viable, so no *Lw* or *LL* genotypes survive and the *L* allele does not spread. (There is no introgression of other alleles either, including any *S* alleles). Frequency *l* remains zero in all generations (frequencies are measured in adults emerging in the wild, and so exclude the released males).

$$\text{BL:} \quad l^* = 0 \qquad (11)$$

Under the FL strategy, in the absence of resistance all *Lw* or *LL* females die (and so all adult females are *ww*), but males survive and so the *L* allele will introgress into the population. The equilibrium *L* allele frequency can be shown to be

$$\text{FL:} \quad l^* = \frac{½d}{1+d} \qquad (12),$$

which is a stable equilibrium that increases with increasing release ratio (see Appendix to this chapter).

5.3.1.2 Resistance is present

I now consider the situation where a viable resistance trait is present in the wild population ($p_0 > 0$, $\gamma_{RR} < 1$ and $\psi_{SR} > 0$). The equilibria set out above for no resistance (Eqs. 10-12) are minimum values for allele equilibria in the presence of resistance; they will still apply unless the *R* allele's properties are such that that the fitness advantages outweigh the fitness penalty of the *L* allele and the effects of dilution by *S* alleles (which depend on the release ratio *d*). This can occur if the *R* allele is not lethal (fitness ψ_{RR} and perhaps ψ_{SR} not zero) and is effective against the RIDL construct (susceptibility γ_{RR} and perhaps γ_{SR} below

1). If so, the *R* allele can persist ($p^* > 0$), and so some target *LL* or *Lw* genotypes can survive (both sexes are targeted by BL and females are the target for FL), which allows the *L* allele to persist at higher frequency ($l^* > 0$ (NR, BL) or $l^* > \frac{½d}{1+d}$ (FL)). In a region of parameter space near $\psi_{SR} = \psi_{RR} = 0$ and $\gamma_{SS} = \gamma_{SR} = \gamma_{RR} = 1$, the *R* allele's advantages will not be sufficient to achieve this and the *R* and *L* allele frequencies will settle at those minimum equilibrium values (Eqs. 10-12). For convenience, I will refer to these minimum equilibrium values (Eqs. 10-12) as "floor" values and to the combinations of susceptibility parameter values where they are the outcome as "floor regions".

With RIDL releases (BL or FL), a proportion $\frac{d}{1+d}$ of progeny embryos (prior to the effects of *R* and *L*) will be sired by released *SSLL* males, and so will have genotype of the form *S*▫*L*▫ (meaning at least one S allele, i.e. the *S*/*R* type is *SS* or *SR*, and at least one *L* allele, i.e. the *L*/*w* type is *Lw* or *LL*). As release ratios would usually be 1:1 or higher ($d \geq 1$, with realistic $d >> 1$ for a suppression or elimination programme), the majority of embryos will have genotype of that form. I therefore expect that the fitness costs for *SR* heterozygotes (fitness ψ_{SR}) and the effectiveness of heterozygote resistance (susceptibility γ_{SR}) will have more influence on the evolution of *R* allele frequency, and hence on *L* allele frequency, than those of *RR* homozygotes do. This effect is more pronounced as the release ratio, *d*, increases.

Below, I confirm and expand on these general deductions by progressively relaxing assumptions about the genetic properties of resistance.

5.3.1.3 *Complete dominant no-cost resistance*

The strongest combination of resistance properties is an *R* allele with no fitness costs (fitness $\psi_{SR} = \psi_{RR} = 1$), conferring resistance that is complete (i.e. negates any effects of the *L* allele) and dominant (susceptibility $\gamma_{SR} = \gamma_{RR} = 0$). Such an allele has the greatest potential to spread among the population in the presence of the RIDL construct, and simulations show that it spreads rapidly when RIDL releases are in progress (Fig. 5.1a, BL and FL). The *R* allele equilibrium values are above ½ for BL and above ⅓ for FL releases, which is abundant enough to allow the *L* allele to spread very quickly to fixation (Fig. 5.1b). FL is better than BL at controlling resistance (Fig. 5.1a) and the *L* allele spreads marginally faster with FL than BL (Fig. 5.1b), because the female-specific *L* allele is inherited through all males not just resistant ones. In the absence of the RIDL construct, the *R* allele encounters no selection pressure and will remain constant (Fig. 5.1a, NR).

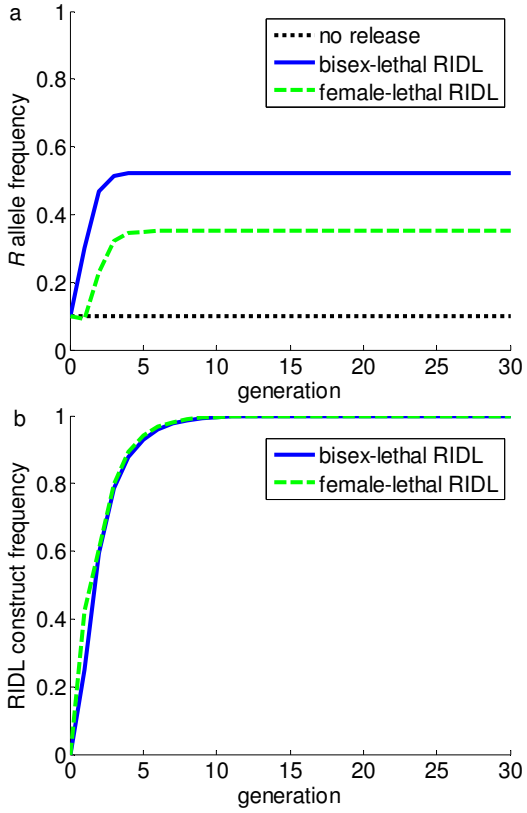

Figure 5.1 Allele frequency evolution with complete, dominant, no-cost resistance
The graphs show the frequency over time in naturally emerging adults of the *R* allele (a) and the *L* allele (b), for no release of insects (dotted black lines), bisex-lethal RIDL release (solid blue lines) and female-lethal RIDL release (dashed green lines). The release ratio is 10:1 ($d = 10$), i.e. ten RIDL males per male in the wild. The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. Resistance is dominant and complete: susceptibility $\gamma_{SR} = \gamma_{RR} = 0$. Initial *R* allele frequency p_0 is 0.1. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0.

Similar patterns are observed if the initial *R* allele frequency is lower. Starting with p_0 at 0.01 or 0.001 instead of 0.1, both yield the same broad pattern and identical equilibrium frequencies. Even from near fixation (p_0 of 0.99), the same equilibria result, with the *R* allele declining rapidly to that value and the *L* allele spreading very rapidly from 0 to fixation.

The shapes of these allele frequency curves against time are examples of patterns that can be seen more generally, not only for complete, dominant, no-cost resistance. The first two generations of RIDL releases (BL or FL) into a naïve population may exhibit distinctive changes, because those are when *Lw* (first generation) and *LL* (second generation) individuals first appear among the population emerging in the wild. Apart from this, the frequency of a spreading *R* allele is consistent with the sigmoid pattern often associated with resistance generally: a potentially long period (for a rare allele, not illustrated) with little change, followed by a period of rapid increase, then levelling off approaching the equilibrium frequency (Fig. 5.1a, BL, FL). The *R* allele never reaches fixation with RIDL releases because of the dilution effect of adding new *S* alleles at each generation, but the equilibrium frequency can potentially be close to 1 (see later examples). The same general patterns can be observed in the frequency of a RIDL genetic construct (*L* allele), except that the *L* allele can reach fixation with sufficiently fit and effective resistance (Fig. 5.1b), and if the *R* allele starts at high frequency the *L* allele can at first increase to a higher level and then decline to its equilibrium value.

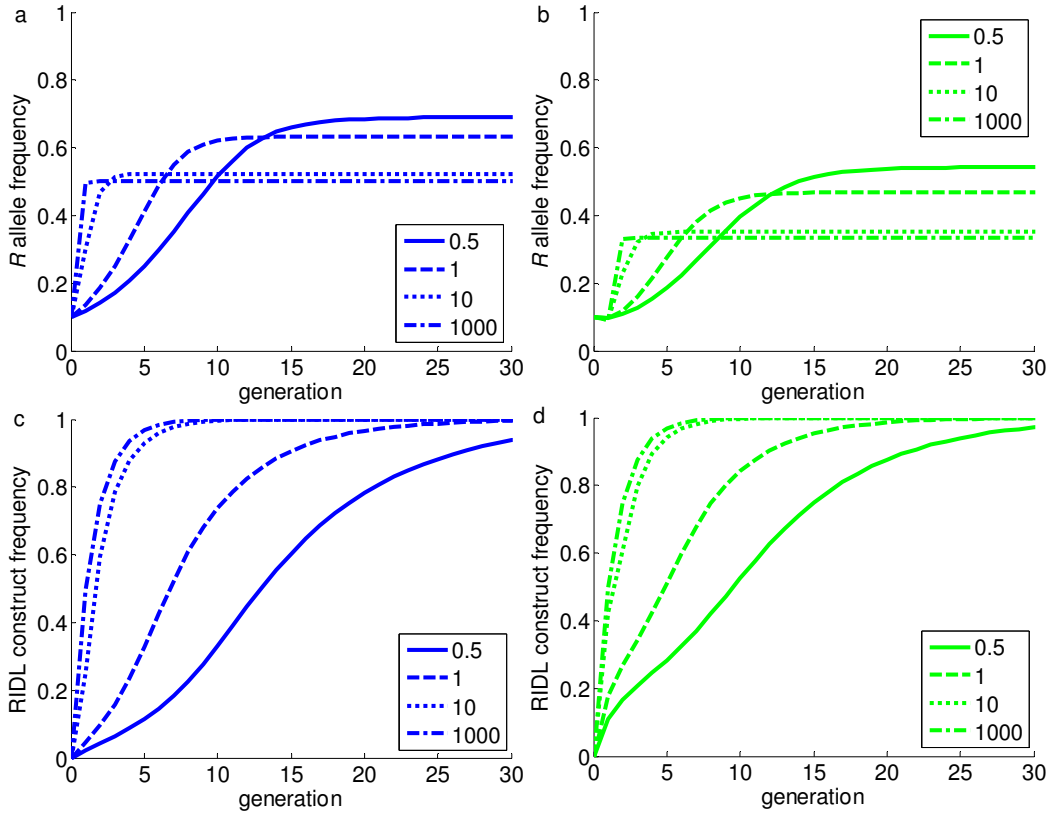

Figure 5.2 The effect of release ratio on allele frequency evolution
The graphs show the frequency over time of the *R* allele (a & b) and the *L* allele (c & d), for bisex-lethal RIDL release (a & c) or female-lethal RIDL release (b & d). The release ratios are 1:2 (d = 0.5, solid lines), 1:1 (d = 1, dashed lines), 10:1 (as Fig. 5.1, d = 10, dotted lines) and 1000:1 (d = 1000, dash-dot lines). Genetic parameters of *R* and *L* alleles are as in Fig. 5.1 (complete, dominant, no-cost resistance). Initial *R* allele frequency p_0 is 0.1.

The values of *R* allele equilibria under BL and FL strategies depend on the release ratio. The dominant *R* allele has a significant advantage in the presence of the *L* allele and spreads as far as it can subject to dilution (which effect is stronger at higher release ratios). As release ratio d increases, p^* decreases toward ½ for BL or ⅓ for FL, and that equilibrium is approached more quickly (Fig. 5.2a,b). A higher release ratio also speeds the spread of the *L* allele (Fig. 5.2c,d). These limits for p^* can be deduced from the genetics. With a very high release ratio virtually all females will mate with a released *SSLL* male, so virtually all offspring have genotype of the form *S*▫*L*▫. Under the BL

strategy, all progeny inheriting an *S* allele from their mothers are *SSL*▫ (i.e. *SSLw* or *SSLL*) and will die, so as $d \to \infty$ all viable offspring are *SRL*▫ (i.e. *SRLw* or *SRLL*) and the *R* and *S* alleles both occur at frequency 0.5. Under the FL strategy, all female progeny inheriting an *S* allele from their mothers will die, so as $d \to \infty$ the only viable females are *SRL*▫. In the next generation these females mate with released *SSLL* males, producing offspring that are half *SSL*▫, of which half are female and die, and half *SRL*▫. Among the survivors (one third *SSL*▫ and two thirds *SRL*▫), the *R* allele frequency is ⅓. With less than infinite release ratio, there are also some *R* alleles among the males that emerged in the wild, so more *R* alleles survive and the equilibria are higher than these limits.

5.3.1.4 Complete recessive no-cost resistance

If the *R* allele has no fitness costs (fitness $\psi_{SR} = \psi_{RR} = 1$) and gives complete resistance ($\gamma_{RR} = 0$) but is recessive (susceptibility $\gamma_{SR} = \gamma_{SS} = 1$) rather than dominant, this is weaker resistance than the dominant equivalent. The *R* allele only has an effect in homozygous form.

As before, in the absence of the RIDL construct (no release), the *R* allele encounters no selection pressure and will remain constant (Fig. 5.3a, NR).

With BL release, all progeny of released males are *SSL*▫ or *SRL*▫ and so are non-viable, and there can be no introgression of *S* or *L* alleles into the wild population. The *R* allele remains at its original frequency because *RRL*▫ genotypes (i.e. *RRLw* or *RRLL*) never arise to create selection pressure, and the *L* allele does not enter the population (Fig. 5.3, BL). This holds true for all recessive resistance, whether or not it is complete ($\gamma_{RR} \geq 0$).

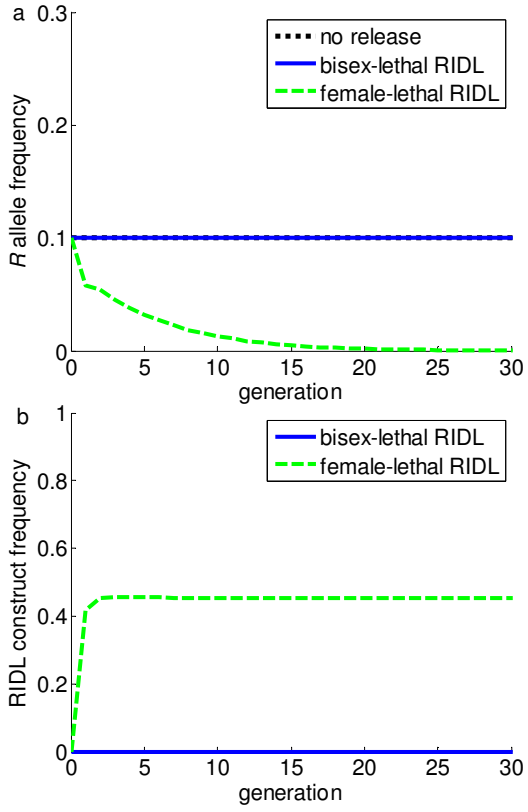

Figure 5.3 Allele frequency evolution with complete, recessive, no-cost resistance
The graphs show the frequency over time of the *R* allele (a) and the *L* allele (b), for no release of insects (dotted black lines), bisex-lethal RIDL release (solid blue lines) and female-lethal RIDL release (dashed green lines). The release ratio is 10:1 ($d = 10$). The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. Resistance is recessive $\gamma_{SR} = \gamma_{SS} = 1$ and complete: susceptibility $\gamma_{RR} = 0$. Initial *R* allele frequency p_0 is 0.1. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0. The *L* allele equilibrium frequency is $l^* = \frac{½ \times 10}{1+10} = 0.4545$ (Eq. 17). Note the y-axis in (a) is scaled 0 to 0.3 (not 0 to 1).

With FL release, all female progeny of released males are *SSL*▫ or *SRL*▫ and so are non-viable, but there is introgression of *S* or *L* alleles through the male line. With release ratio 10:1, the dilution effect of *S* alleles from released RIDL males is strong enough that the *R* allele declines to extinction (Fig. 5.3a), which means that the *L* allele settles at its floor equilibrium value (Eq. 12) and does so rapidly at this release ratio (Fig. 5.3b). Increasing the release ratio increases l^*, with a limit of 0.5 as $d \to \infty$.

The equilibria, and the finding that there is no change in *R* allele frequency with BL, are independent of initial *R* allele frequency. With low p_0 of 0.001 broadly similar patterns over time are observed. With high p_0 0.99 the equilibria (or no change) are the same, but the transient dynamics differ. With FL releases, *R* frequencies decline monotonically from

that high level and the *L* allele frequency increases at first (reaching a maximum exceeding 0.62 in the 15th generation) before declining to the same equilibrium value.

5.3.1.5 Incomplete no-cost resistance

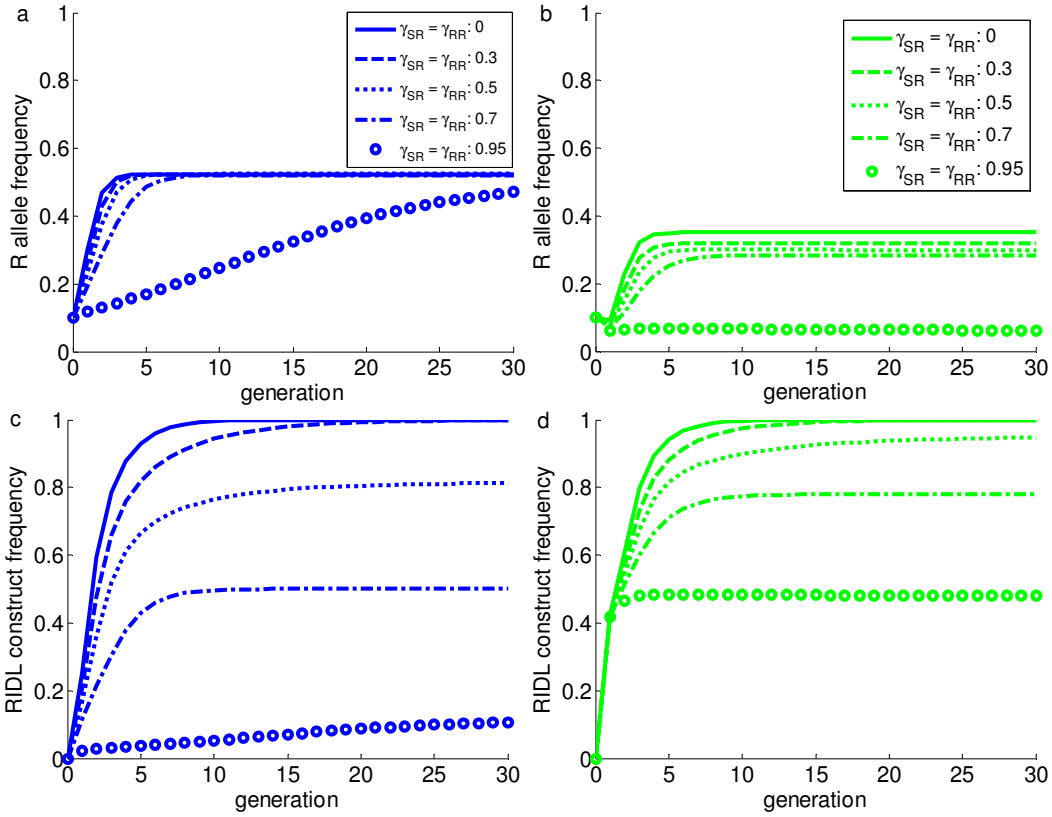

Figure 5.4 The effect of varying susceptibility on allele frequency evolution
The graphs show the frequency over time of the *R* allele (a & b) and the *L* allele (c & d), for bisex-lethal RIDL release (a & c) or female-lethal RIDL release (b & d). The release ratio is 10:1 ($d = 10$). Initial *R* allele frequency p_0 is 0.1. The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. Resistance is dominant: susceptibility $\gamma_{SR} = \gamma_{RR}$ taking values 0 (complete resistance, solid lines), 0.3 (dashed lines), 0.5 (dotted lines), 0.7 (dash-dot lines) or 0.95 (circles). Initial *R* allele frequency p_0 is 0.1. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0.

Resistance might reduce the fitness penalty of the RIDL construct rather than cancel it, i.e. resistance might be incomplete. The effectiveness of a dominant *R* allele with no fitness costs (fitness $\psi_{SR} = \psi_{RR} = 1$), varies with the susceptibility of resistant insects ($0 \le \gamma_{RR} = \gamma_{SR} \le 1$). As susceptibility to the RIDL construct decreases from 1 to 0 (i.e. as resistance becomes more effective), both *R* and *L* alleles spread more quickly (Fig. 5.4).

With FL releases, the *R* allele equilibrium decreases with less effective resistance, but with only small variation unless the resistance is weak (high susceptibility) (Fig. 5.4b). Under the BL strategy there is little variation in the *R* allele equilibrium (with releases at 10:1 p^* remains quite close to 0.52, Fig. 5.4a). For both strategies, the additional survival of *L* alleles with stronger resistance can cause a significant increase in their frequency (Fig. 5.4c,d).

When I allow susceptibility to the RIDL construct to range over all permitted values ($0 \le \gamma_{RR} \le 0 \gamma_{SR} \le 1$) with no assumptions about dominance, we see that this variation generally has more effect on l^* than on p^* (Fig. 5.5). As predicted (see §5.3.1.2) varying *SR* susceptibility has a greater effect on equilibria than varying *RR* susceptibility; the slopes of the p^* and l^* surfaces are generally steeper for fixed γ_{RR} than for fixed γ_{SR} (this is more visible for l^*). As explained above (see §5.3.1.4), with BL releases where resistance is recessive ($\gamma_{SR} = 1$) such an *R* allele remains at its initial frequency ($p^* = p_0$, which is 0.01 in Fig. 5.5a). Except for this special case, at realistic release ratios equilibria are independent of initial *R* allele frequency (I modelled p_0 of 0.01 (Fig. 5.5), 0.1, 0.7 and 0.99 with d = 10), and where resistance is recessive ($\gamma_{SR} = 1$) the *R* and *L* alleles tend to their floor values (Eqs. 10-12) (Fig. 5.5b-d). At the opposite corner of parameter space representing complete dominant resistance ($\gamma_{SR} = \gamma_{RR} = 0$), the *R* allele spreads as far as it can subject to the effects of dilution (Fig. 5.5a,b) and the *L* allele goes to fixation (Fig. 5.5c,d). The *L* allele also goes to fixation for parameter values in a region around that corner (γ_{SR} and γ_{RR} near zero), where the lethality of the RIDL construct is still overcome by the very effective resistance compared with the effects of dilution. What happens in between is slightly different for BL and FL strategies.

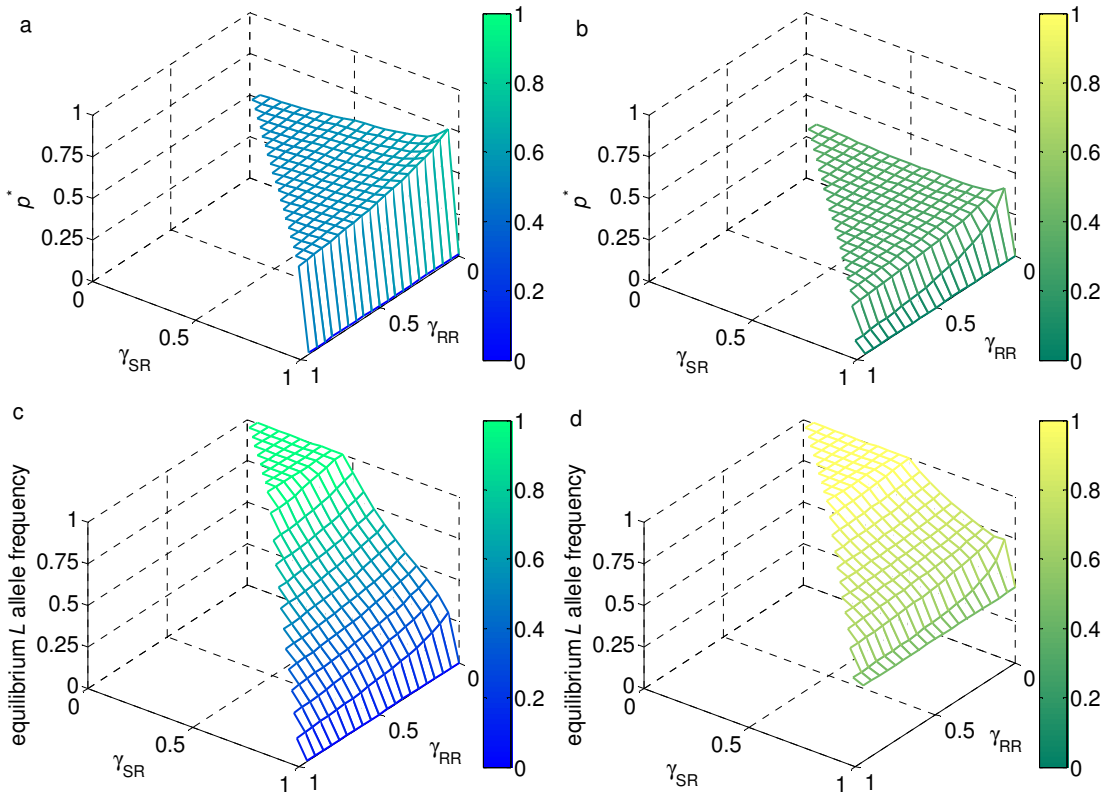

Figure 5.5 The effect of susceptibility to the RIDL construct on equilibrium allele frequencies
The graphs show the equilibrium frequencies of the *R* allele (a & b) and the *L* allele (c & d), for bisex-lethal RIDL release (a & c) or female-lethal RIDL release (b & d) against susceptibility to the RIDL construct ranging over all permitted values ($0 \le \gamma_{RR} \le 0\gamma_{SR} \le 1$). The release ratio is 10:1 ($d = 10$). Initial *R* allele frequency p_0 is 0.01. The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0.

In the BL scenario, for any given γ_{RR}, as heterozygote susceptibility γ_{SR} increases toward higher values (weaker heterozygote resistance), p^* increases (Fig. 5.5a) and l^* decreases (Fig. 5.5c) toward extinction (its floor, Eq. 11). This is the result of fewer *SRL*▫ individuals surviving; this reduces the effect of dilution by *S* alleles and introgression of *L* alleles from released RIDL males because that is the genotype of their potentially resistant offspring. For given γ_{SR}, as homozygote susceptibility γ_{RR} increases, p^* (Fig. 5.5a) and l^* (Fig. 5.5c) both decrease (although not by much) because reduced survival of *RRL*▫ types

reduces the frequency of *R* alleles a little. With lower release ratio ($d = 5$), the equilibrium surfaces look much the same, but with higher p^* values due to lesser effects of dilution, and lower l^* values, with *L* allele reaching fixation in a slightly smaller region of parameter space near the corner $\gamma_{SR} = \gamma_{RR} = 0$ than it did with $d = 10$. At an even lower release ratio ($d = 1$) those comments remain true, although the p^* surface appears a little less flat near one edge ($\gamma_{RR} = 0$), and again, the equilibrium results are the same with p_0 0.01 as with 0.99.

In the FL case, the outcomes are more sensitive to some parameter values. As in the BL case, for any given γ_{SR}, as homozygote susceptibility γ_{RR} increases, the equilibria p^* and l^* decrease a little (Fig. 5.5b,d), and over most of the range of values, holding γ_{RR} fixed and increasing γ_{SR} (less effective heterozygote resistance) reduces l^* towards its floor (Eq. 12) (Fig. 5.5d). This is consistent with fewer target *SRL▫* females surviving and so reducing the introgression of *L* alleles from released RIDL males through their female progeny. But there is interesting distinct behaviour near one corner, where γ_{SR} is near 1 (resistance is nearly recessive) and $\gamma_{RR} = 0$ (homozygote resistance is complete). With release ratio 10:1, there is little change in the *R* allele equilibrium frequency p^* over most of the surface (Fig. 5.5b). Holding γ_{RR} constant, increasing γ_{SR} can slightly increase or decrease p^* at different values of γ_{SR}, and again there is atypical behaviour near the same corner. With a release ratio 10:1, the p^* and l^* equilibria surfaces are the same regardless of initial *R* allele frequency (identical equilibrium results for p_0 0.01 and 0.99). With a lower release ratio (d = 5 or 1, Fig. 5.6), the surfaces appear similar to Fig. 5.5b & d except that the l^* floor is lower (Eq. 12) and the p^* and l^* floor regions extend further from the edge where $\gamma_{SR} = 1$; the lower release ratio the greater those effects are. The outcome in the atypical corner is affected by initial *R* allele frequency; when p_0 is higher the unusually higher equilibrium

values extend nearer to the corner and the floor does not extend right to that edge. For example, with $d = 5$, $\gamma_{SR} = 0.95$ and $\gamma_{RR} = 0$ both p^* and l^* go to their floor values starting from p_0 of 0.01, but starting from p_0 at 0.99 the equilibria are instead $p^* = 0.5761$ and $l^* = 1$, giving more pronounced peaks near the corner of interest (Fig. 5.6a,c).

With the FL strategy, this interesting behaviour occurs at the edge of parameter space where $\gamma_{RR} = 0$ (homozygotes have complete resistance), at higher values of γ_{SR} (resistance is closer to recessive than dominant). In these circumstances, *SSLw* and *SSLL* females are non-viable, and because there are no fitness costs associated with the *R* allele the only other genotypes with a fitness disadvantage are *SRLw* females (relative fitness $1-\gamma_{SR}$) and *SRLL* females (relative fitness $(1-\gamma_{SR})^2$). As susceptibility γ_{SR} moves away from 1 (recessive resistance), the relative fitness of *SRLw* and *SRLL* females improves. What effect those *SR* females have on allele frequencies is also dependent on the release ratio. With a high release ratio most *SS* females mate with released *SSLL* males and produce *SSL▫* progeny, the females among which are non-viable. Any *RR* females produce *SRL▫* progeny, of which females suffer a fairly high fitness penalty. The *SR* females that have survived to maturity, will produce a mixture of *S▫L▫* progeny, with females being non-viable or at low fitness. This means that many of the relatively few females remaining in the population will have been sired by non-released males (including genotypes other than *SSLL*). At lower release ratios, more non-released males are available for mating and more different mating crosses are possible, amplifying the effects that I observe in the simulations.

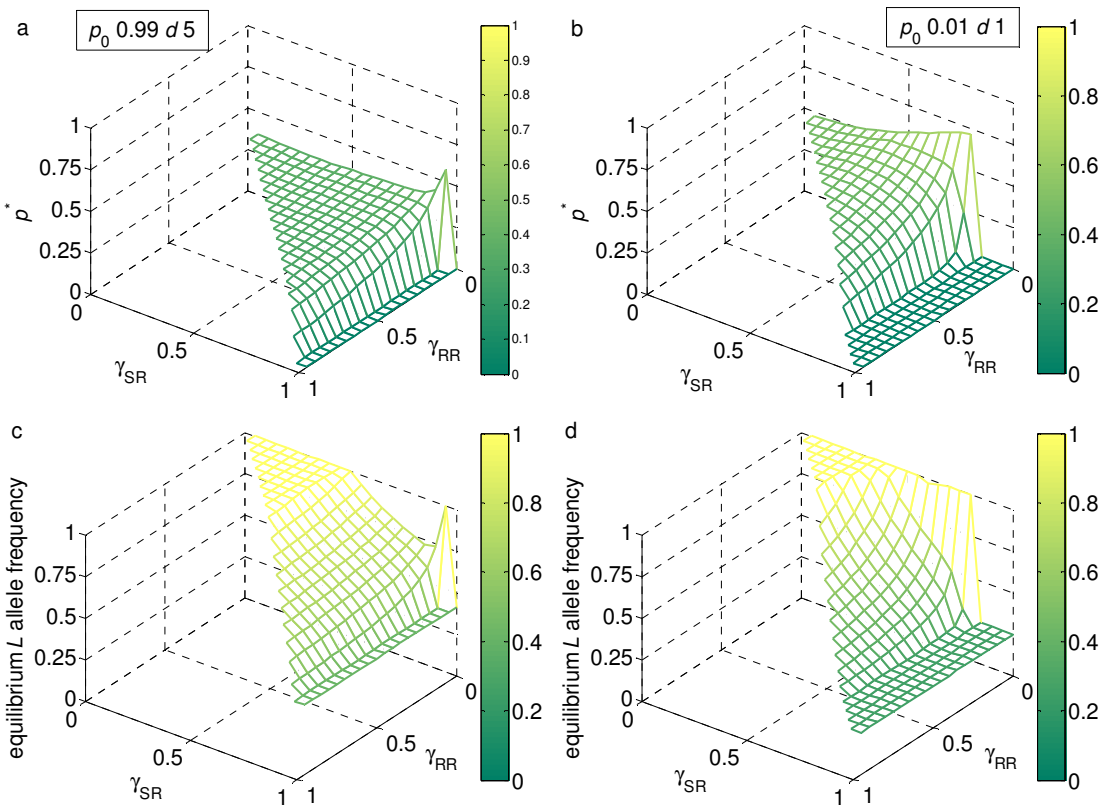

Figure 5.6 The effect on female-lethal equilibria of varying release ratio and initial *R* allele frequency
The graphs are as for Fig. 5.5 b & d, except for release ratio and initial *R* allele frequency. They show the equilibrium frequencies of the *R* allele (a & b) and the *L* allele (c & d), for female-lethal RIDL release against susceptibility to the RIDL construct ranging over all permitted values ($0 \le \gamma_{RR} \le 0 \gamma_{SR} \le 1$). The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0. The release ratio is (a & c) 5:1 (d = 5) or (b & d) 1:1 (d = 1). Initial *R* allele frequency p_0 is (a & c) 0.99 or (b & d) 0.01.

At high heterozygote susceptibility γ_{SR} (for example, with d = 5, the region marked A in Fig. 5.7a,b), both alleles to tend to their floor values (no *R* alleles and *L* alleles only in males). The γ_{SR} threshold above which this occurs, is dependent on the release ratio d (Fig. 5.7a,b) and on p_0. The dilution effect on *S* alleles is the most influential force (which is why the threshold changes with release ratio), and as *R* alleles are driven out of the population the *L* allele comes to kill all females possessing it. Eventually all females are *SSww* and all males emerging in the wild are *ww* (from low p_0 this happens rapidly, Fig.

5.7 c,d). Those males are mostly *SSLw*, with the remainder being *SSww*. Progeny of *SSww* females and released males are non-viable, and the population of wild type females is maintained by the *SSww* or *SSLw* males. The *SSLw* males are maintained by the released males.

Immediately below that γ_{SR} threshold is a region (Fig. 5.7a,b, marked B) where the *L* allele reaches fixation and the *R* allele spreads to be very common (e.g. at $\gamma_{SR} = 0.92$ with $d = 5$, p^* is 0.5652). Lowering the release ratio raises the value of p^* at that threshold edge (Fig. 5.7a) and widens the plateau (i.e. range of γ_{SR} values for which these outcomes occur) (Fig. 5.7a,b). On crossing that threshold (from the floor side), the slight improvement in fitness of *SRLw* females is sufficient to allow the *R* allele to spread widely, and that allows the *L* allele to go to fixation; the selection pressure in favour of the *R* allele appears to outweigh the effect of dilution by *S* alleles in this region. The transient dynamics (Fig. 5.7c-f) show that *RRLw* and *SRLw* females increase for some time while the RIDL construct first spreads among *Lw* heterozygotes and then they decrease while the *L* allele spreads into *LL* homozygotes. In this case the spread of *R* and *L* alleles happens very slowly, with only small changes during the first 300 generations or so, so the practical implications for the short or medium term are quite different from the long-term behaviour. At equilibrium, all insects are *LL*; females are mostly *RRLL* with some *SRLL*, and males are mostly *SRLL* but with both *SS* and *RR* homozygotes present. In this region, decreasing heterozygote susceptibility γ_{SR} allows more *SRLL* females to survive, so there are more *S* alleles among the female population at equilibrium and the *R* allele equilibrium is lower (i.e. the top of the plateau on the p^* graph (Fig. 5.7a) slopes downward to the left).

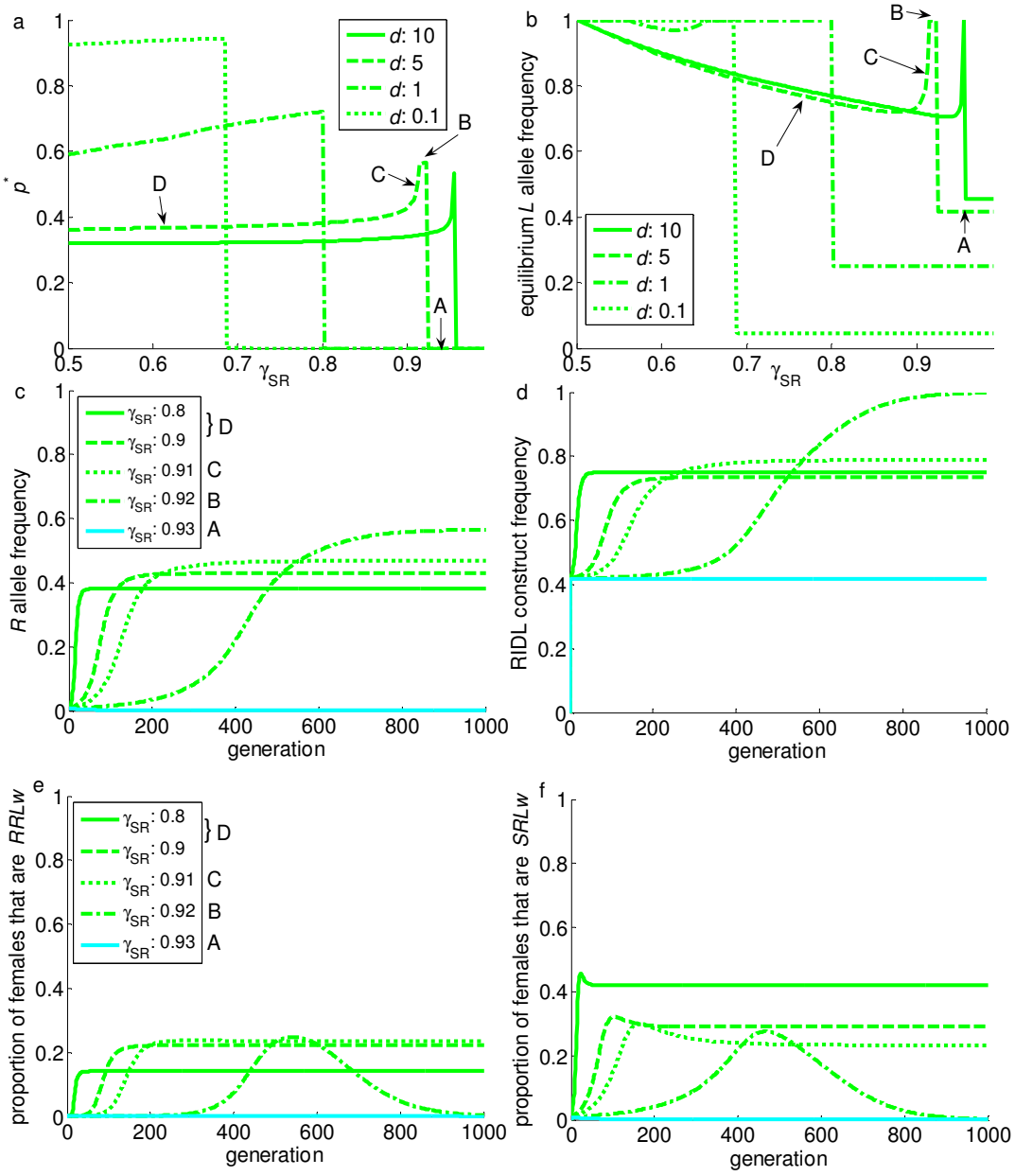

Figure 5.7 Complete homozygote resistance (FL)
The upper graphs show, for female-lethal RIDL release, the equilibrium frequencies of the *R* allele (a) and the *L* allele (b) against heterozygote susceptibility to the RIDL construct over the range $0.5 \le \gamma_{SR} \le 0.99$ with release ratios as shown: 10:1 ($d = 10$, solid line), 5:1 ($d = 5$, dashed line), 1:1 ($d = 1$, dash-dot line) or 1:10 ($d = 0.1$, dotted line). The remaining graphs show the frequency over time of (c) the *R* allele, (d) the RIDL construct *L* allele, (e) genotype *RRLw* among females and (f) genotype *SRLw* among females, with release ratio 5:1, for the following values of heterozygote susceptibility: 0.8 (solid green line), 0.9 (dashed green line), 0.91 (dotted green line), 0.92 (dash-dot green line) or 0.93 (solid cyan line). In all panels resistance is complete in heterozygotes ($\gamma_{RR} = 0$). Initial *R* allele frequency p_0 is 0.01. The *R* allele has no fitness costs: relative fitness $\psi_{SR} = \psi_{RR} = 1$. The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0. (A) – (D) indicate regions of outcomes described in the text.

The transition at the next threshold is not discontinuous. At the lower end of the plateau is another region (marked C in Fig. 5.7a,b), where both p^* and l^* decrease steeply as γ_{SR} decreases. Here, the additional *S* alleles surviving in *SRLw* and *SRLL* females reduce the proportion of *R* alleles and that reduces the *L* allele survival over all. All genotypes exist at equilibrium (except the two nonviable female types *SSL*▫), with the bulk of the males in *SR* form particularly *SRLL*, and *Lw* females fairly abundant. This suggests that in this region there is a delicate balance between the various forces acting – direct and indirect selection pressure and allele dilution (*S*) or injection (*L*) - that is easily affected by small changes in fitness. This changes smoothly to a region (marked D in Fig. 5.7a,b) where p^* remains fairly flat and l^* increases as γ_{SR} decreases, eventually reaching fixation at lower values of γ_{SR}, where resistance is nearly dominant. The behaviour in that region is consistent with that for other non-zero values of homozygote resistance γ_{RR} as described above; as γ_{SR} decreases more target *SRL*▫ females survive and that increases the introgression of *L* alleles from released RIDL males through their female progeny.

5.3.1.6 The impact of fitness costs of resistance

Where the *R* allele has fitness costs, recessive resistance ($\gamma_{SR} = 1$) is no longer a special case for the BL strategy; the costs cause the *R* allele to decline to extinction rather than remain unchanged at its initial frequency. The existence of fitness costs for resistance to a FL RIDL construct removes the interesting behaviour discussed above from the corner of parameter space where γ_{SR} is near 1 (resistance is nearly recessive) and $\gamma_{RR} = 0$ (homozygote resistance is complete). (I modelled p_0 of 0.01, 0.1 or 0.8 with d = 10, and p_0 of 0.01 with d = 1, to confirm both of these findings). As a result, the equilibrium surfaces for BL and FL cases against susceptibility to RIDL are similar in shape (but not scale) and behaviour, so I illustrate only FL.

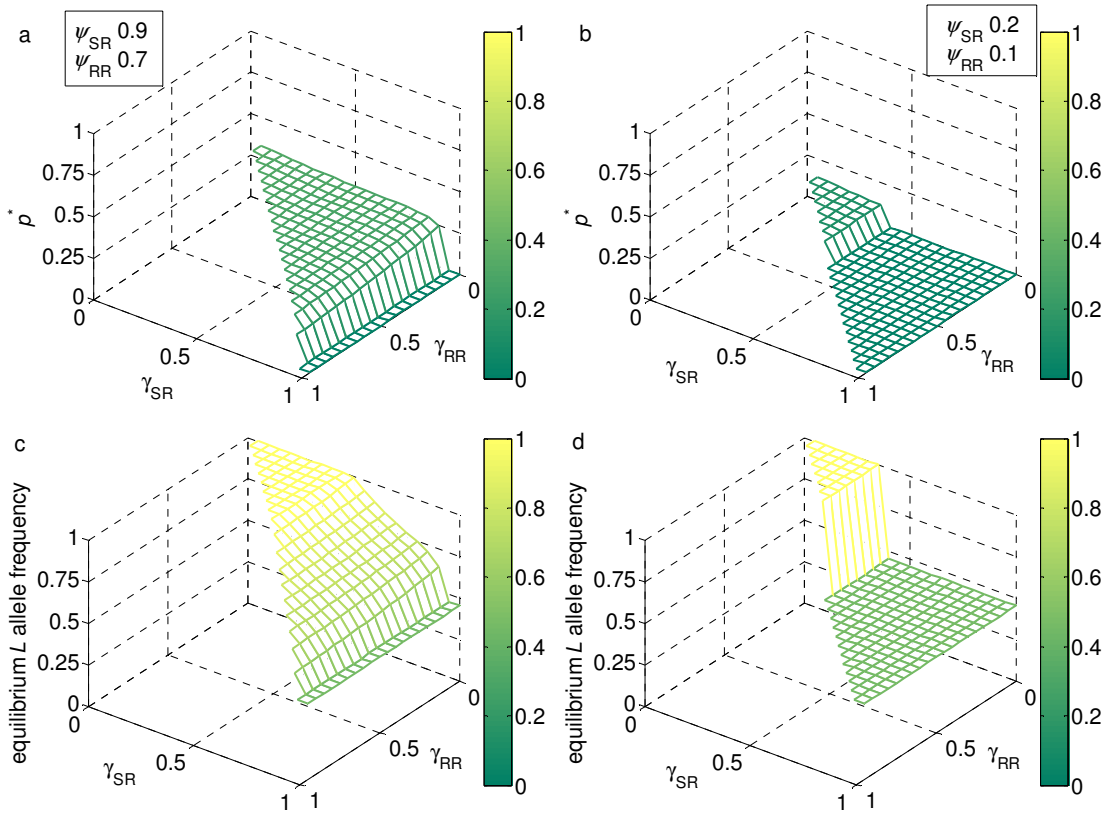

Figure 5.8 The effect of fitness costs of resistance on equilibrium allele frequencies The graphs are as for Fig. 5.5 b & d, except for fitness costs of resistance. They show the equilibrium frequencies of the *R* allele (a & b) and the *L* allele (c & d), for female-lethal RIDL release against susceptibility to the RIDL construct ranging over all permitted values ($0 \le \gamma_{RR} \le 0 \gamma_{SR} \le 1$). The *L* construct is fully-lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0. The release ratio is 10:1 (d = 10). Initial *R* allele frequency p_0 is 0.01. The *R* allele has fitness costs: relative fitness (a & c) $\psi_{SR} = 0.9, \psi_{RR} = 0.7$, "fit resistance" or (b & d) $\psi_{SR} = 0.2, \psi_{RR} = 0.1$, "costly resistance".

With both BL and FL strategies, as fitness costs associated with the *R* allele increase, especially those for *SR* homozygotes, the *R* allele goes extinct for a greater range of heterozygote susceptibility values, and where it persists it tends toward a lower equilibrium (compare Fig. 5.5b,d no costs, Fig. 5.8a,c low costs and Fig. 5.8b,d high costs). This reduction in p^* is smaller for more effective *R* alleles (low values of susceptibility γ_{SR} and γ_{RR}). Resistance with high heterozygote susceptibility γ_{SR} is not

sufficiently effective, in the presence of fitness costs, for the *R* and *L* alleles to persist above their floors (Eqs. 10-12) and those floors extend over a larger region (mostly defined by a γ_{SR} threshold).

A resistant allele that is very costly (high fitness penalty in heterozygotes and homozygotes) is harder to control when it is very abundant than when it is rare. (However, such a high-cost allele would not be expected to be abundant, because of the selection pressure, so this is unlikely to arise in practice). With 10:1 release ratio, the initial *R* allele frequency p_0 only makes a difference to p^* and l^* for the most costly *R* alleles (ψ_{SR} and ψ_{RR} near zero). It affects the position of the threshold (which occurs at higher γ_{SR} for higher p_0, giving a smaller floor region) but for effective resistance (on the low susceptibility γ_{SR} side of that threshold) the equilibrium values are the same regardless of p_0. That is, for costly resistance with given susceptibility, p_0 determines whether the *R* and *L* alleles will persist above their floors (Eqs. 10-12) but, if they do, it does not change the equilibrium values at which they will persist. These results suggest some kind of bifurcation of surfaces (floor versus persistent equilibria) for each of the *R* and *L* alleles for a given release ratio, with p_0 determining on which surface the result lies. The critical p_0 appears to be moderated by the release ratio and fitness of resistance, and if it does not fall between 0 and 1, floor values will result either at all values of susceptibility or only for recessive resistance (the edge where $\gamma_{SR} = 1$). At the edge of the floor, the transition can be continuous (Fig. 5.8 a,c) or discontinuous (Fig. 5.8b,d).

A lower release ratio (1:1 instead of the 10:1 shown in Fig. 5.8) reduces the γ_{SR} threshold thereby enlarging the floor regions (for example, with $d = 1$ and costly resistance, floors for both *R* and *L* alleles extend across the whole range of susceptibility values for BL and

FL). This is because there is less selection pressure in favour of the *R* allele at lower release ratios. With effective resistance (on the low susceptibility γ_{SR} side of that threshold) the *L* allele goes to fixation over a smaller range of susceptibility values and p^* is higher. The *R* allele persists at higher equilibrium value because the lower release ratio lessens the effect of dilution by *S* alleles from released males.

These effects of reducing the release ratio suggest that for any given set of parameter values (γ, ψ) and initial *R* allele frequency there is a critical release ratio, below which the *R* allele tends to extinction and the *L* allele tends to extinction (BL) or its non-zero floor (FL). This is borne out by simulations. This critical release ratio depends on the genotypes' relative fitness values, with regard to both costs and efficacy of resistance and, for some parameter combinations, the initial *R* allele frequency. For example, with $\psi_{SR} = 0.4$, $\psi_{RR} = 0.3$, $\gamma_{SR} = 0.9$ and $\gamma_{RR} = 0.4$ (nearly recessive incomplete resistance with nearly dominant, moderately high fitness costs), the critical release ratio for FL is roughly 37:1 with p_0 of 0.001 or 0.9, but the critical release ratio for BL is 58:1 with p_0 of 0.001 or 47:1 with p_0 of 0.9. More effective resistance and/or lower fitness costs gives a lower critical release ratio, which could be significantly below the release ratios expected for a suppression or elimination programme. Homozygote costs and susceptibility have little effect compared with heterozygote costs and susceptibility. For example, having moderate or high homozygote fitness costs $\psi_{RR} = 0.4$ or 0.1, does not change the critical release ratios of roughly 0.37:1 for FL and 0.41:1 for BL, with $\psi_{SR} = 0.9$, $\gamma_{SR} = 0.3$ and $\gamma_{RR} = 0.1$ (partially dominant, very effective resistance with near recessive fitness costs) and p_0 of 0.001 or 0.9. There can be a large discontinuity at the critical release ratio, or not, regardless of p_0. For example, starting BL releases from p_0 of 0.9, with $\psi_{SR} = 0.9$,

$\psi_{RR} = 0.1$, $\gamma_{SR} = 0.9$ and $\gamma_{RR} = 0.4$ the critical release ratio (below which $p^* = l^* = 0$) is roughly 4.2:1, and the slightly higher release ratio 4.5:1 results in very low equilibria $p^* = 0.0087$ and $l^* = 0.0017$. With alleles that rare (and from high p_0 becoming so fairly quickly - the *R* allele reduces from 0.9 to just above 0.05 in 30 generations) a release ratio a little over the critical value need not be cause for practical concern. However, with $\psi_{SR} = 0.4$, $\psi_{RR} = 0.3$, $\gamma_{SR} = 0.3$ and $\gamma_{RR} = 0.1$ the critical release ratio is 2.1:1, but releases at 2.15:1 result in very much higher equilibria $p^* = 0.6009$ and $l^* = 1$.

5.3.2 Population control and resistance management

For practical purposes, it is not the frequency of a resistant allele that is directly of interest, but the size of the pest population. I explored the interaction between the evolution of allele frequencies and the impact on the relative size of the pest or vector population. I also examined the relationship with the insect's net reproductive rate R_0 (the per capita rate of population growth in a generation), employing a higher release ratio for a pest with higher growth potential. I limited these investigations to situations where the *R* allele will not decline to extinction, because that is where concern would focus, and compared the outcomes with the equivalent release regimes in the absence of any resistance. I selected release ratios that would lead to a successful programme (local elimination of the pest) in the absence of resistance.

With no release and bisex-lethal RIDL release strategies, the females are always half of the population. Under the female-lethal RIDL release strategy this is not true; the relative number of females is less than half the relative total population size because females are targeted and are killed off disproportionately.

Under my simulated proportional release policy, female-lethal releases perform better in terms of relative population size than bisex-lethal releases at the same release ratio (Figs. 5.9 & 5.10). This is because the number of insects released is proportional to the number of males emerging in the wild; the BL strategy kills males and females in equal numbers but FL strategy kills only females. The same release ratio for both strategies leads to more insects being released under the FL strategy than the BL strategy, so the two are not directly comparable in terms of costs.

5.3.2.1 Density-independent populations

The presence of a resistant allele that spreads to non-zero equilibrium has greater effect on the population size if it is a stronger *R* allele (with low fitness costs and low susceptibility to the lethal genetic construct) and/or if the insect has a high per capita population growth rate. A weaker *R* allele (with a smaller reduction in susceptibility and higher fitness costs) can have a fairly small impact on population control, by marginally slowing the rate at which releases drive the pest to local elimination (Fig. 5.9a). If the pest has a low population growth rate, such resistance can have negligible impact on population management (with lower R_0 and *d* values, as used in Fig. 5.9b, the results with and without resistance are indistinguishable on the equivalent of Fig. 5.9a until the relative population size is below 10^{-20}). A stronger *R* allele has a greater effect on population size, although with lower R_0 the population may remain suppressed below its original size for many generations (Fig. 5.9b). At worst, at a release ratio that would be very effective in the absence of resistance, strong resistance in a pest with higher R_0 can mean that instead the releases only slow the rate of explosion of the population (Fig. 5.9c). I did not find any instances where releases did no better than no release: even complete dominant non-cost resistance (fitness $\psi_{SR} = \psi_{RR} = 1$, susceptibility $\gamma_{SR} = \gamma_{RR} = 0$) resulted in significantly slower population growth than for the uncontrolled population.

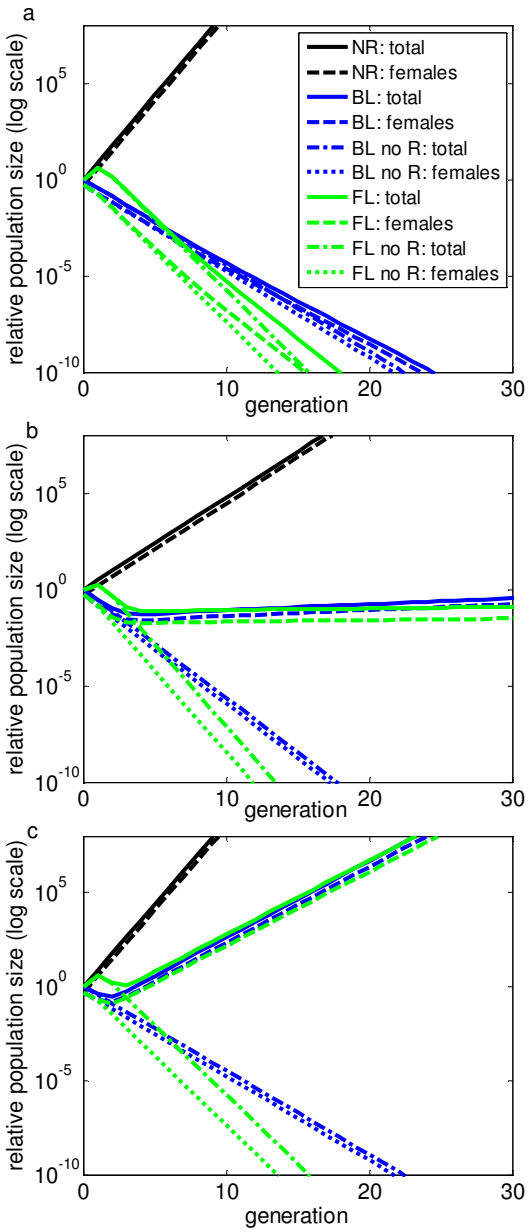

Figure 5.9 Population dynamics with no density-dependent mortality
The graphs show the relative population size over time, for no release of insects (black lines), bisex-lethal RIDL release (blue lines) and female-lethal RIDL release (green lines). The relative numbers are totals for both sexes (solid) or females only (dashed). For comparison, totals (dash-dot lines) and females (dotted) with no resistance present are shown for bisex-lethal and female-lethal RIDL release. Initial R allele frequency p_0 is 0.01. The L construct is fully lethal to targets, with no other fitness costs: $\varepsilon_{\text{target}}$ is 1 and $\varepsilon_{\text{non-target}}$ is 0. Progeny per female $2R_0$ = (a & c) 15 or (b) 6, with release ratio (a & c) 20:1 (d = 20) or (b) 10:1 (d = 10). The R allele has (a) minor fitness costs, with relative fitness $\psi_{SR} = 0.95, \psi_{RR} = 0.85$, or (b & c) no costs $\psi_{SR} = \psi_{RR} = 1$. Resistance is (a) modest, with susceptibility $\gamma_{SR} = 0.9$, $\gamma_{RR} = 0.7$, or (b & c) strong, susceptibility $\gamma_{SR} = 0.2$, $\gamma_{RR} = 0.1$. The corresponding equilibrium allele frequencies are (a) BL: p^* = 0.5054, l^* = 0.3312, FL: p^* = 0.2412, l^* = 0.6906, (b) BL: p^* = 0.5288, l^* = 1, FL: p^* = 0.3320, l^* = 1, (c) BL: p^* = 0.5151, l^* = 1, FL: p^* = 0.3215, l^* = 1.

As described above (§5.3.1), the initial R allele frequency does not make a difference to the equilibrium R or L allele frequencies for most combinations of fitness costs and susceptibility. If the allele starts at a much higher frequency its impact on the population size in the early generations could potentially affect the relative population size after the equilibria have been reached. However, I generally observed only a slight slowing of population decline or increase of population explosion (by a small number of generations).

This might or might not be large enough to have a significant economic or public health effect. For example, with parameters as in Fig. 5.9 but p_0 of 0.9 instead of 0.01, the largest difference was with the stronger resistance with the higher growth rate (Fig. 5.9c), where the population became more than a million times its original size after 14 generations, compared with 19. The impact was much smaller for the other panels.

5.3.2.2 Populations subject to density-dependent processes

Broadly, I draw the same general conclusions about the impact of resistance to the RIDL construct when there are density dependent processes operating as for density independent populations. The main difference is that the population is assumed to start at equilibrium, so if the resistant allele has no fitness costs, the relative population size will remain at 1 with no releases, of which half are female (Fig. 5.10). The RIDL construct fitness penalty takes effect after density dependent mortality, because of its late-acting lethality, so successful RIDL releases can drive the population to local elimination. Where resistance disables the efficacy of the RIDL construct, the population will return to (or remain at) equilibrium densities.

Density dependent processes do not alter the prediction that a spreading resistant allele has greater effect on the population size if it is a stronger (low susceptibility and low cost) *R* allele and/or if the insect has a high per capita population growth rate. The presence of a weak *R* allele can mean releases take slightly longer to drive the pest to local elimination (Fig. 5.10a); with low population growth rate the impact on population control might be negligible (e.g. as Fig. 5.10a except with the lower R_0 and *d* values). A strong *R* allele is more detrimental for the effectiveness of releases, although with lower R_0 the initial suppression while resistance is still uncommon is enough to keep the population below its original size for many generations (Fig. 5.10b). At high release ratios, strong resistance

spreading in a pest with higher R_0 might only suppress the pest for a few generations before the population recovers to near-equilibrium densities (Fig. 5.10c). If that *R* allele is already widespread (e.g. as Fig. 5.10c but with p_0 of 0.9 instead of 0.01) that initial suppression phase does not occur and pest densities move quickly to that new level just below equilibrium.

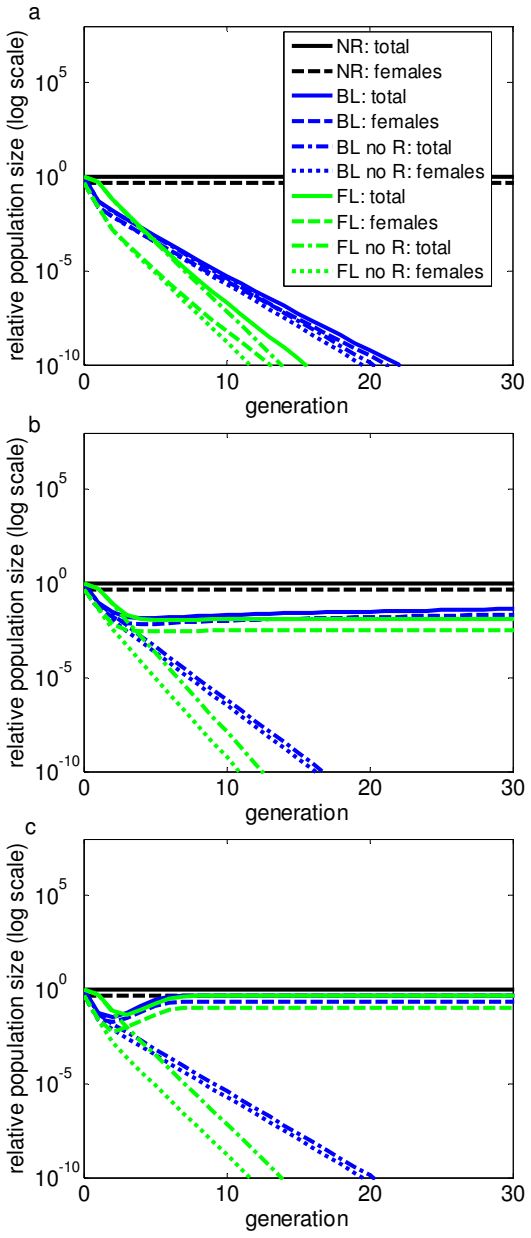

Figure 5.10 Population dynamics with density-dependent mortality
Line styles, parameter value combinations and corresponding *R* and *L* allele frequencies are the same as in Fig. 5.9 and panels therein.

I did not find any instances where releases did not reduce the population below equilibrium. Even complete dominant no-cost resistance (fitness $\psi_{SR} = \psi_{RR} = 1$, susceptibility $\gamma_{SR} = \gamma_{RR} = 0$) resulted in population densities slightly below equilibrium and could exhibit suppression temporarily while resistance was uncommon. (For example, with other parameters as in Fig. 5.10b, population size declined to 0.03 relative to equilibrium in 3 (BL) or 4 (FL) generations, then recovered to 0.45 (BL) or 0.41 (FL) by the 17th generation.)

5.4 Discussion

I have shown that a RIDL approach could potentially limit the effects of biochemically based resistance against the RIDL genetic construct (*L* allele) engineered into the released strains, due to a built-in dilution mechanism. Resistant alleles are unable to spread to fixation, although they can become more common than the alternative susceptible allele. However, the evolution of resistant allele frequency is subject to a complicated mix of competing forces.

A resistant allele with no fitness costs has a net advantage in a population containing the RIDL genetic construct, the magnitude of which depends on the effectiveness and dominance of resistance (which I express in terms of genotype susceptibility to the RIDL construct). That advantage depends on the frequency of the RIDL allele, which in turn depends on the release ratio. However, whilst a higher release ratio increases the frequency of the *L* allele and thereby acts in favour of resistance, it also increases the dilution of the gene pool by susceptible alleles to reduce resistance. The existence of fitness costs associated with resistance adds another complication by affecting the direction and magnitude of the resistant allele's net advantage or disadvantage. This is a multi-dimensional problem for which several parameter values cannot reasonably be

predicted because resistant alleles are likely to be rare or non-existent at the commencement of a release programme, and may be difficult to measure or estimate accurately even if resistance has been identified, especially if resistance is recessive or nearly recessive so that it is only observed in homozygous form.

In the absence of resistance, increasing the ratio of released RIDL males to males in the wild has a beneficial impact on the size of the pest population. As with classical SIT, there is a critical release ratio above which the size of the population will be reduced (Thomas et al. 2000, Dyck et al. 2005, Phuc et al. 2007). Here I have identified that there is a critical release ratio below which resistance will be prevented from spreading through the population and the RIDL construct will not spread among individuals of the target sex(es). Clearly, there may be situations where those two requirements are incompatible, which arise when resistance is more effective and/or has lower fitness costs. However, the population might still be effectively controlled even if resistance becomes common, and with weak resistance that is initially rare there may even be little practical impact for population control. Stronger, fitter resistance has greater potential to be detrimental for the aim of reducing population size.

Stronger resistance (with low fitness costs and low susceptibility to the lethal genetic construct) is the main threat to the success of a genetic control release programme. Such resistant alleles have greater selection pressure in their favour and tend to spread very rapidly, even from low levels. At worst, against target insects that have little or no regulation by density dependent processes, the presence of such resistance should therefore become apparent quickly, as monitoring data would reveal a clear detrimental change in the rate of population growth after a promising start. Similarly, population monitoring

should identify such resistance among populations that are subject to density dependent mortality, as a new equilibrium density would be reached fairly rapidly. In practice, though, pre-implementation preparatory trials should be expected to detect such fit and effective resistant alleles, unless they are very rare. All RIDL strains incorporate a heritable genetic marker that allows easy identification, so survival of RIDL heterozygotes (*Lw* individuals) should be readily detected from trapping data during release trials. Laboratory trials involving crosses with the wild population might potentially identify resistant alleles present in the field at a much earlier stage. The detection threshold could be of the order of 0.01 allele frequency or lower, depending how recessive the resistance trait is (a resistance effect in *SR* heterozygotes is easier to detect).

Some of the low release ratios that I have modelled would not intentionally be used in an actual population control programme. However, these values but might be relevant outside the programme's target area if there are regions to which engineered insects could emigrate. It is known that with density-dependent populations SIT releases can in theory have unintentional consequences – sometimes detrimental - in neighbouring areas, although a related modelling study suggest this would not occur with (bisex-lethal) late-acting RIDL strategies (Yakob et al. 2008). My findings here suggest that any resistance to the genetic construct in areas of low effective release ratio would be less likely to spread, because of the lower favourable selection pressure; it might therefore be expected to interfere less with population reduction outside the target region, than inside it. This is unlike resistance to chemical insecticides, where lower doses (such as spray drift, or early prototype "medium-dose" GM crops) tend to encourage resistance by allowing survival of partially resistant individuals. A model incorporating spatial aspects would be required to fully investigate this matter.

I have not simulated the situation where RIDL genetic construct is not fully lethal ($\varepsilon_{\text{target}} < 1$). I might reasonably expect a trade-off between population suppression and resistance management, but the competing selective forces acting on the evolution of resistance might lead to counterintuitive results, so this could be worthy of exploration. I did not simulate scenarios where the female-lethal RIDL construct has a modest fitness cost in males ($\varepsilon_{\text{non-target}} > 0$). This also could be potentially interesting in conjunction with the population dynamics.

I assumed that the insects released are homozygous susceptible. The dynamics could be different if quality control processes are not able to ensure this. In particular, if resistance is of low dominance, it might be that the release population includes a small number of heterozygous susceptible RIDL males (*SRLL* types as well as *SSLL*). In principle my model could be adapted to explore this issue.

Compared with the proportional release policy of my model, a constant release policy does not enable population genetics to be studied separately from the population dynamics, so some population growth assumptions have to be made, however simple. If the release ratio of released males to initial population of males, say, is high enough to reduce the population initially, in subsequent generations the ratio of released males to actual males in the wild increases. This could mean that the resistance becomes more able to spread as the effective release ratio increases, which in turn could impact the population size. This adds a further layer of competing influences above those encountered in my proportional policy model. In practice, what is implemented might be somewhere between those two, with a larger release at first, reduced in one or more steps as the population declines.

Releasing fertile susceptible wild type insects would allow introgression of susceptible alleles into the population in a similar manner to release of RIDL insects. However, given the difficulty of separating wild males from females on an industrial scale, both sexes would probably have to be released and any released fertile females would contribute to population growth. Susceptible alleles would be inherited through both sexes and none of the progeny would suffer reduced survival (unlike progeny of RIDL males), so this should be more effective at reducing the frequency of resistant alleles than equivalent RIDL releases. I could incorporate wild type releases into my models, to explore combinations of release strategies to manage resistance and maintain the efficacy of an established RIDL release programme. In theory, I could also consider the sequential or alternating use of bisex-lethal RIDL releases and female-lethal RIDL releases, with one being used to combat unrelated resistance to the other while still managing the population size. This would only be appropriate if the two genetic constructs involved had independent modes of action and there was no cross-resistance. Given the molecular basis of the RIDL strains currently in existence or under development (Thomas et al. 2000, Gong et al. 2005, Fu et al. 2007, Phuc et al. 2007, Stainton et al. unpublished data), these may or may not be reasonable assumptions.

A potential concern about releasing GM insects into the wild is that the inserted DNA may have unforeseen consequences. This has particularly been raised regarding strategies that aim to replace a wild population, for example with a version engineered to be unable to transmit a pathogen. One advantage claimed for genetic SIT strategies over population replacement strategies is that autocidal engineered insects are programmed to die and therefore the lethal genetic construct should die out if releases cease. This relies on the

construct having some fitness costs even if it does not retain its efficacy. I have shown that resistance to the construct might significantly increase its frequency. In theory, there are combinations of genetic properties of resistance for which the construct could eventually be driven to fixation; that scenario is highly unlikely to be played out in real life because the release programme could be stopped if substantial resistance were detected, long before the RIDL construct became common, and the wild type would reinvade through immigration.

It is remarkable that a fairly simple two-locus genetic system with forcing (from releases) can produce such a complicated array of potential behaviours. Some combinations of genetic properties of resistance can generate unexpected results, especially with the female-lethal strategy, and small changes in a single parameter can make the difference between resistance going extinct and resistance spreading so widely as eventually to render the lethal construct largely ineffective. However, the theoretical equilibrium results are not always relevant to planning suppression or elimination programmes, where it is short to medium term behaviour (the transient dynamics) that is important. Ultimately, it is the effect of the resistance on the population size that matters and that can vary from negligible to substantial impact on the results of a release programme, with strong (effective low-cost) resistance and high growth-rate insects being harder to control. Fortunately, those stronger resistance alleles are the ones that are easier to detect in the population either during preparatory trials or during early stages of a release programme.

Appendix: Female-lethal L *allele equilibrium frequency*

Where resistance is not present or the resistant allele is dominant fully-lethal (so only *SS* genotypes exist or are viable, and p is always zero), the *S*/*R* genotype is irrelevant and I consider only the *L*/*w* locus. The *L* allele is assumed to be fully lethal in females ($\varepsilon_{\text{target}} = 1$), with no fitness costs in males ($\varepsilon_{\text{non-target}} = 0$).

The only viable female genotype is *ww*, so *LL* males cannot arise from matings in the field. Eggs are male (♂) or female (♀) in equal numbers and *ww* males and females have equal fitness, so they will occur in equal numbers as adults. Proportions of genotypes in adults emerging naturally in the wild will be of the following form:

ww♂: x, $\quad Lw$♂: $1-x$, $\quad (0 < x \leq 1) \quad ww$♀: x (numbers equal ww♂).

Immediately before releases, the frequency of the *L* allele in the population is therefore:

$$l_t = \frac{½(1-x)}{1+x} \qquad \text{(A1)},$$

which can be rearranged to show that

$$x = \frac{½ - l_t}{½ + l_t} \qquad \text{(A2)}.$$

The proportions of males immediately after release of d RIDL males for every male in the wild will be: $\quad ww$♂: $\frac{x}{1+d}$, $\qquad Lw$♂: $\frac{1-x}{1+d}$, $\qquad LL$♂: $\frac{d}{1+d}$.

Mating crosses of wild females with these males produce offspring in the following proportions:

ww: $\frac{x}{1+d} + ½\frac{1-x}{1+d}$ (of which half are ♂, half are ♀), and

Lw: $\frac{1}{2}\frac{1-x}{1+d}+\frac{d}{1+d}$ (of which half are ♂, and half are ♀ which do not survive).

Among the offspring surviving to adulthood, the *L* allele frequency is:

$$l_{t+1}=\frac{\frac{1}{2}\left[\frac{1}{2}\left(\frac{1}{2}\frac{1-x}{1+d}+\frac{d}{1+d}\right)\right]}{\left(\frac{x}{1+d}+\frac{1}{2}\frac{1-x}{1+d}\right)+\left[\frac{1}{2}\left(\frac{1}{2}\frac{1-x}{1+d}+\frac{d}{1+d}\right)\right]}$$

$$=\frac{1-x+2d}{6+2x+4d} \qquad \text{(A3)}$$

By calculating the change in allele frequency over one generation ($\Delta l = l_{t+1} - l_t$) from Eqs. (A1) and (A3), and substituting for *x* (Eq. A2) it can be shown that the non-trivial equilibrium allele frequency (where $\Delta l = 0$) is

$$l^* = \frac{\frac{1}{2}d}{1+d} \qquad \text{(A4)}$$

and that this is a stable equilibrium ($\Delta l > 0$ where $l_t < l^*$ and $\Delta l < 0$ where $l_t > l^*$). The equilibrium increases with release ratio ($d > 0$), with a minimum of zero (as $d \to 0$) and an upper limit of 0.5 (as $d \to \infty$). This result (Eq. A4) was confirmed by simulations.

6 Genetic vector control strategies to reduce the burden of mosquito-borne diseases

Summary Vector-borne diseases impose an enormous health and economic burden around the world and new methods to control vector populations are needed. The Sterile Insect Technique (SIT) has been successful against agricultural pests, but is not in large-scale use for suppressing or eliminating populations of mosquito disease vectors. This is due in part to technical difficulties with the current technology. Genetic RIDL® technology (Release of Insects carrying a Dominant Lethal), is a proposed modification that involves releasing insects that are homozygous for a repressible dominant lethal genetic construct rather than being sterilized by irradiation, and could potentially overcome some of those problems. Using the arboviral disease dengue as an example, I combine a vector population dynamics model with an epidemiological model to explore the effect of a programme of RIDL releases on disease transmission, and investigate the potential cost-effectiveness by applying estimates of the costs of RIDL-based SIT. Through mathematical modelling I find that this genetic control strategy could eliminate dengue from a human community within one year, and at lower cost than the direct and indirect costs of disease. I also consider the wider economic benefits.

6.1 Introduction

Around the world, the medical and economic burden caused by vector-borne diseases continues to grow as the current control measures fail to cope. There is an urgent need to identify new strategies that will remain effective, even in the face of growing insecticide and drug resistance (Sachs and Malaney 2002). Genetic techniques targeting insect vectors may provide new approaches to disease control. I focus on dengue to illustrate the potential benefit of genetic vector control strategies for vector-borne diseases.

6.1.1 Dengue: a candidate disease for genetic vector control

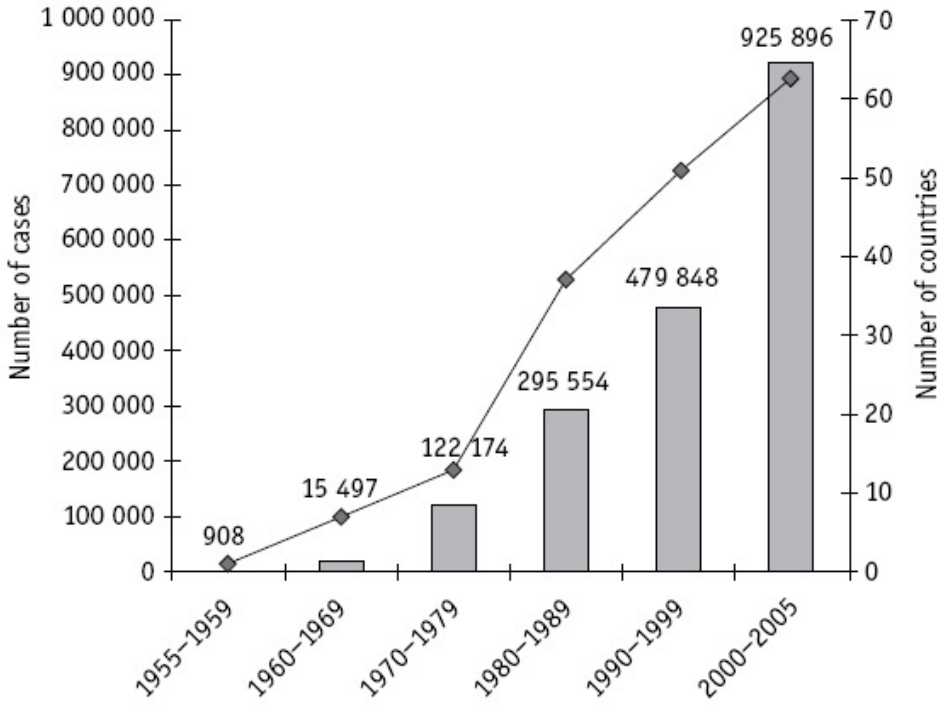

Figure 6.1 Average annual number of cases of dengue or severe dengue reported to WHO (bars), and average annual number of countries reporting dengue (lines) (reproduced from WHO-TDR 2007).

Arguably the most important arboviral disease of humans today is dengue, including its most severe forms dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler 2002). It is the most rapidly spreading vector-borne disease in tropical and subtropical countries (see Figure 6.1) and it is estimated that over 40%, perhaps now almost half, of the world's population live in urban areas at risk of dengue (WHO-TDR 2007, WHO 2009). Dengue is endemic in over 100 countries, of which more than 60 have DHF, and over 2.5 billion people are at risk of infection (Gubler 2002, WHO-TDR 2007, WHO 2009). An estimated 50-100 million dengue infections occur annually, particularly in South-East Asia, the Americas, and the Western Pacific islands; in the first half of the present decade an annual average of over 0.9 million severe dengue cases were reported to the World Health Organization (WHO) and roughly 18-19,000 dengue-related deaths are

registered each year (Gubler 2002, WHO-TDR 2007, WHO 2008, WHO 2009). Dengue affects all ages, with most deaths and cases reported to WHO occurring among children (age 0-14 years) (WHO 2008, WHO 2009). The disease mainly affects low and middle income countries (World Bank estimate of 2004 gross national income per capita below US$10,066) (WHO 2008).

Dengue is a flu-like illness. It is potentially severe, although it can present as a spectrum of symptoms from near-asymptomatic or mild febrile illness, through the classic incapacitating disease with abrupt onset, severe headaches and pain behind the eyes, in muscles and joints, with a rash, to the complications DHF and DSS. Classic dengue fever is seldom fatal, but case fatality rates (CFR) for DHF range from below 1% with modern intensive supportive therapy to over 20% without proper treatment, with an average of 5% (Gubler and Kuno 1997).

Dengue is caused by any of four related dengue viruses. Infection with any one serotype confers lifelong immunity to that type, and temporary cross-immunity to other serotypes (Sabin 1952, Gubler and Kuno 1997). On subsequent heterotypic infection after the period of cross-immunity (a few months), antibody-dependent enhancement increases the risk of more serious disease (and DHF) (Sangkawibha et al. 1984, Gubler and Kuno 1997). All four dengue virus serotypes are circulating in Asia, Africa and the Americas (WHO-TDR 2007).

Dengue is transmitted to humans by the bite of an infective female mosquito; the main vector is the yellow fever mosquito *Aedes* (*Stegomyia*) *aegypti* (L.), with the Asian tiger mosquito *Aedes albopictus* (Skuse) (*Stegomyia albopicta*) implicated as a secondary vector

in Asia. *Ae. aegypti* is a predominantly urban species (WHO 2009). In Asia and the Americas it breeds mainly in man-made containers (such as storage jars and tanks) and items that collect rainwater (including discarded plastic food containers and used tyres). In Africa, where this species originated, it also breeds extensively in natural habitats (for example, tree roots).

There are currently no specific clinically useful diagnostic test and no drugs (prophylactic or therapeutic) for dengue (Farrar et al. 2007). The prospect of an effective and safe vaccine against dengue available for use in endemic countries to prevent epidemics remains fairly remote; although work is in progress on several candidate vaccines, the challenges facing development and delivery are significant (Farrar et al. 2007, Whitehead et al. 2007). Bednets are of little use, as the *Aedes* vectors bite in the day time (Gubler and Kuno 1997). The only practical prevention method presently available is to control the principal vector, *Ae. aegypti*. Prevention efforts focus on vector control and larval source reduction (Clark et al. 2005, Torres and Castro 2007, WHO 2009); in practice the effectiveness of vector control has been compromised due to issues of delivery, coverage and acceptability (WHO-TDR 2007). Dengue appears to be a particularly suitable target for genetic control strategies because it is specific to humans across most of its range (it has no significant animal reservoirs), it has a single dominant vector (unlike malaria), and area-wide vector control programmes have in the past proven to be effective in controlling this disease (Slosek 1986, Ooi et al. 2006).

6.1.2 Vector control to prevent the spread of dengue

Existing vector control and clinical management measures that are known to help to reduce the vector population and reduce case fatality rates have failed to be implemented widely or effectively (WHO-TDR 2007). Complex urban settings, such as some highly urbanised

areas in Brazil where a substantial proportion of the population is living in crowded, impoverished areas with poor sanitation, present a major challenge for vector control activities (Siqueira et al. 2005). During epidemics, politicians and health officials are under pressure to take emergency measures that are highly visible demonstrations of action. Ultra low volume (ULV) insecticide sprays are often used in such situations; these are ineffective at controlling *Ae. aegypti*, but may also give a false sense of security to the public, who then continue to do little to control mosquitoes in and around their homes, and so epidemics are perpetuated (Gubler 2002).

The Sterile Insect Technique (SIT) is one method potentially available for suppressing or eliminating vector populations. It has been used successfully against a number of agricultural pests (Dyck et al. 2005), but has had limited application to disease vectors, with a rare example being the elimination of the tsetse fly *Glossina austeni* from Unguja Island, Zanzibar, in 1994-1997 (Vreysen et al. 2000). There are no large-scale SIT programmes in operation against any mosquito species. However, a number of trials were conducted against mosquitoes mostly in the 1960s and 1970s (reviewed in Benedict and Robinson 2003), mainly to answer specific research questions, but with some success in population suppression or elimination. In recent years extensive research and development has been underway in preparation for commercial scale SIT (Asman et al. 1981, Benedict and Robinson 2003, IAEA 2008). It is important to be able to separate the sexes and release only males. As for any SIT programme this would aid efficiency - killing females early in the last generation (for example, using inducible female-specific genetic lethality) reduces rearing and distribution costs, and increases the control efficiency of released males which will all seek wild females to mate with as there are no released females – but it is also essential for public health because even sterile female mosquitoes bite and could

contribute to disease transmission. Some technical difficulties with SIT using radiation-sterilisation, particularly loss of male fitness and absence of sexing mechanisms applicable on a large scale, can potentially be overcome using genetically engineered strains (Benedict and Robinson 2003, Alphey et al. 2007a). Recently, *Ae. aegypti* mosquitoes have been genetically modified using the RIDL (Release of Insects Carrying a Dominant Lethal) system incorporating dominant, late-acting, bisex-lethal genetic constructs (Phuc et al. 2007), and such strains are the subject of this chapter.

6.1.3 Modelling dengue dynamics

Dengue is an inherently complex disease to model due to the interactions between its four serotypes, temporary cross-immunity and antibody-dependent enhancement (ADE). There are wide discrepancies between published epidemiological models of dengue in terms of their assumptions, parameter values and predictions. Most epidemiological models in the literature only incorporate one of cross-immunity or ADE, and focus only on one or two serotypes. Many do not explicitly include the mosquito vector.

Preliminary collaborative work (Atkinson et al. 2007`, appended to this thesis) formulated and analysed a mathematical model to enable an initial assessment of the potential impact of RIDL strategies on the spread of a mosquito borne disease (see Figure 6.2). This modelled releases of adult male insects homozygous for a dominant, repressible, bisex-lethal genetic trait, using a simple dynamic model for the female adult mosquito population, incorporating density-dependent competition during the larval stage using delayed logistic growth, embedded into a susceptible–exposed–infectious–susceptible human-vector epidemic model for the spread of the disease. Analytical and numerical results suggested that eradication in approximately one year is feasible for human populations of the order of 10^5 to 10^6 and confirmed earlier results (Phuc et al. 2007) that

late-acting lethality (causing death after the effects of density-dependent mortality) was expected to be more effective than early-acting lethality.

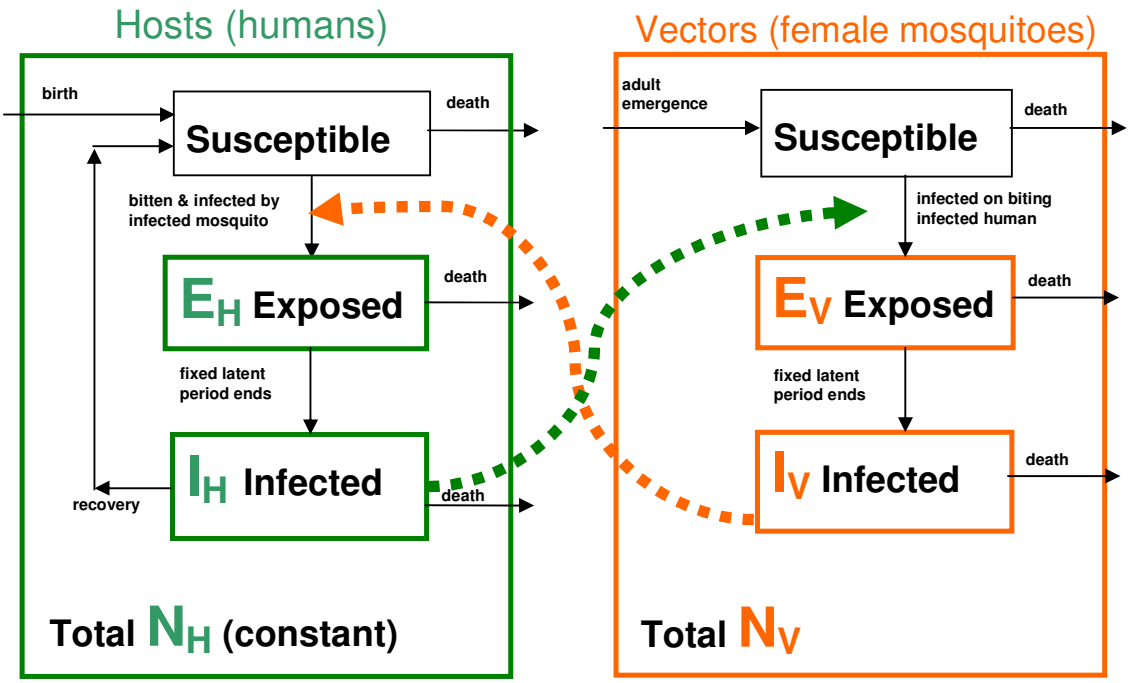

Figure 6.2 Compartment diagrams for the SEIS/SEI model of dengue Subscripts denote human (H) or adult female mosquito vector (V). Individuals that are susceptible (S) may acquire the virus through biting and are then classed as "exposed" (E) for a fixed latent period before becoming infectious (I). Humans, but not vectors, recover and are then susceptible to another infection. The total number (N) of humans is constant, and the number of vectors is governed by a temporal formula involving population growth, larval density dependence, control by RIDL releases and adult mortality.

In that study, we illustrated the results using parameter values for dengue fever. However, it was effectively a single serotype model, with the consequent omission of lifelong homologous immunity and temporary cross-immunity being dealt with by treating all recovered individual hosts as susceptible to another infection (this would tend to overestimate the number of infections). It did not reflect any additional disease-related mortality. The simplified model enabled some analytical results for a proportional release policy, the main one being the condition for "eradication" of the disease (i.e. reducing the number of infectious hosts and vectors to zero), and further approximations were possible

in cases where the total vector population is also eliminated. The number of RIDL mosquitoes necessary for eradication, and the time to eradication were derived or approximated, as were formulae for release parameters that minimise the overall number of released mosquitoes needed. There is a trade-off between minimising the time to virus eradication and minimising the total number of mosquitoes released for virus eradication.

Here I develop a new and extended model framework by combining vector population dynamics and epidemiology to assess economic cost-effectiveness of genetic vector control. For the purposes of this study I want a combined model that is sufficiently sophisticated to capture key features of both of these aspects, including the density-dependent competition among *Ae. aegypti* larvae and the multi-annual cycles that are characteristic of dengue. However, in order to make the overall problem tractable, I make appropriate simplifying assumptions in each component, rather than attempting to incorporate all that is known or theorised about vector populations and dengue dynamics.

6.1.4 Costs of dengue

It is difficult to place a monetary value on dengue, particularly because it causes social disruption, which is hard to measure, and has a relatively low case fatality rate (CFR) (Gubler 2002). Several studies have estimated costs of dengue, reflecting a range of types of cost and using different measures. Most estimates relate only to epidemics; there is little information readily available on the economic impact of endemic dengue (Meltzer et al. 1998). Often these are gross underestimates of the true economic impact because they do not measure the total cost of a large epidemic of flu-like illness, omitting effects such as lost productivity, time off work or school, social disruption and lost tourism, much of which goes unreported yet contributes to a considerable actual disease burden even during periods between epidemics (Gubler 2002).

Several studies report estimated costs of dengue in various countries, but these vary in details, methods, timing and the nature of costs included.

- Puerto Rico (Von Allmen et al. 1979, Meltzer et al. 1998): The 1977 epidemic was estimated to have cost between US$6.1 million and $15.6 million in direct (including medical costs and control measures) and indirect costs. This represents about $26-31 per clinically ill case.
- Cuba (Kouri et al. 1989): The 1981 epidemic of 344,203 reported cases had an estimated economic cost of US$103 million, including medical care $41 million, lost productivity $14 million, social security salaries for adult patients $5 million and vector control measures $43 million.
- Nicaragua (Torres and Castro 2007): The overall economic impact of the 1994 epidemic was estimated at US$2.7 million, with 60,916 cases of dengue and DHF. At about US$44 per case, this was influenced by the high cost of hospitalisation ($130 per day for a bed).
- Venezuela (Añez et al. 2006): In the study period 1997-2003 there were 33,857 cases of dengue and DHF in the State of Zulia, Venezuela, incurring direct (medical) and indirect (work absence by patients/mothers) costs totalling US$1.3 million, giving an average of US$40 per case.
- Thailand (Clark et al. 2005, Anderson et al. 2007): In a 2001 study of family-level costs (rather than societal costs), direct hospital costs per individual patient hospitalised averaged US$24; children under 12 years old receive free treatment in public hospitals but in practice higher values were incurred where patients first attended a clinic (most did, as a first step, and private clinics are not free) and by uninsured adults. Other studies put net hospital costs in 1997 at $39-55 per DHF

patient, or direct patient costs varying geographically, at $23-61 in rural Thailand but $67 in Bangkok. The 2001 opportunity costs of missing an average 4.2 days of work per hospitalisation was estimated at $44. In 1994 DHF caused direct costs of $13 million (51,688 reported DHF cases averaging $257 per case), including overall costs $5.7 million to patients and $6.9 million to providers. A prospective study of primary school children in 1998-2002, estimated patients' costs per illness, comprising clinic or hospital costs, medication and lost family income during clinic or hospital visits, at about US$17 on average, $10 for non-hospitalised case, $32 for hospitalised dengue fever and $39 for hospitalised DHF.

- India (Garg et al. 2008): The median cost per hospitalised patient in North India during the 2006 epidemic was US$432. The total economic burden in India was estimated to be US$27.4 million.
- Panama (Armien et al. 2008): Dengue mainly affects adults in Panama. The estimated cost of the severe dengue epidemic during 2005 was US$11.8 million (5489 reported cases, estimated 32900 actual cases). Cost per non-fatal ambulatory (outpatient) case was US$332 and per hospitalised (inpatient) case was $1065 (these results were obtained as part of a multi-country prospective study, see below).

A prospective study (Suaya et al. 2009) applying a common protocol in eight countries (five in the Americas and three in Asia), is the most comprehensive study of dengue costs published to date and the first to develop comparable data over two hemispheres. This study distinguished between ambulatory and hospitalised cases, and included direct medical costs, direct non-medical costs (such as transportation, food and lodging associated with seeking or obtaining medical care) and indirect costs (days of school or paid work lost by the patient or a household member providing care during the illness

episode). It reported the average cost per case at US$417 (standard deviation US$21) over all study countries, US$733 in the Americas, and US$330 in Asia.

Currently, the only practical method of reducing the occurrence of dengue is vector control in urban areas, which is costly (Torres and Castro 2007). For example, vector control measures cost US$43 million during the Cuban dengue epidemic of 1981 (Kouri et al. 1989) and Brazil's budget for vector control in 1997 was roughly US$0.6 billion (Suaya et al. 2009). Many of the most cost-effective health interventions across all diseases and conditions are preventative (rather than clinical, mostly curative), but not all preventative measures are cost-effective; the World Development Report "Investing in Health" cites spraying to control the mosquitoes that carry dengue as an example of poor value for money (World Bank 1993). As well as spraying adulticides, current methods include larviciding, source reduction and environment management (WHO-TDR 2007).

6.1.5 Wider economic impact of health improvements

As the World Development Report (World Bank 1993) points out, there are other potential benefits from interventions against disease in addition to the direct gains in terms of reduced morbidity and mortality. The most obvious sources of gain from improving health in developing countries are fewer work days lost due to illness or caring for sick relatives, increased productivity, greater opportunities to obtain better-paying jobs and longer working lives. Output and earnings lost by individuals struck by illness are often undetected and unreported within economic statistics because they are borne by the household. In many developing countries, unemployment insurance and disability insurance are rare and so healthier members of the household have to work longer and/or harder to make up for lost income, which effort is not measured. Healthier children not only miss less school, they are also able to learn more effectively while there.

Some wider effects of reducing the burden of disease may be less obvious. Health improves wealth by its effect on savings and investment; healthier people expect to live longer, so they have more incentive to save, and do actually live longer, so they have longer during which to save (Bloom et al. 2005). There is also a demographic benefit; as parents realize they need fewer children to achieve their intended family size, fertility falls, and resources are concentrated on fewer children, giving them a greater chance of receiving effective healthcare and a good education (Bloom et al. 2005). Some health investments can raise the productivity of land (World Bank 1993). Malaria was nearly eradicated in Sri Lanka in 1947-1977 and areas previously blighted by mosquitoes became attractive for settlement. Migrants moved in and output increased. Success against vector-borne disease in rural areas would be likely to cause gains among farmers, who are often risk-averse in order to hedge against sickness. For example, in Paraguay, farmers in malaria areas chose to grow lower value crops that can be worked outside of the malaria season (World Bank 1993).

A key additional indirect effect of diseases such as dengue is the impact on income from tourism; for example, media reports of resurgence of dengue in India in autumn 2006 and in Latin America in spring 2007 warned of the threat to tourism. It is difficult to assess and quantify this effect in countries where dengue is endemic, as no baseline statistics are available. However, the potential for significant reduction or suppression of tourism revenues is illustrated by serious outbreaks of other diseases.

An outbreak of foot and mouth disease (FMD) in the UK in 2001 resulted in restricted access to the countryside from March to August and, through its effects on both urban and

rural tourism by UK and overseas visitors, is estimated to have caused total losses of between £2.7 billion and £3.2 billion to businesses directly involved in tourism (Thompson et al. 2002). This amount is similar to the estimated £3.1 billion loss to agriculture and the food chain. This is an underestimate of the total economic impact for two reasons. Although the last counties were not declared disease-free until January 2002, these estimates do not include any effects from 11 September 2001 ("9/11") because they were overwhelmed by the impact of the international terrorism events in the USA that day. Indirect effects on industries supplying goods and services to the directly affected businesses were not quantified.

In 2002-3 severe acute respiratory syndrome (SARS) caused serious disruption to short-term economic growth in a number of Asian economies (Fan 2003, Siu and Wong 2004). Tourism and air travel were severely affected, especially in Hong Kong, with a WHO travel warning advising postponement of nonessential trips and intense media coverage drawing attention to the situation. Local consumption was also affected. Consumption spending and tourism would have been vulnerable to long-term effects, had successful identification and containment not stemmed the outbreak.

In §6.3.6 below, I discuss the impact on tourism of an outbreak of the mosquito-borne disease chikungunya on la Reunion, a French territory in the Indian Ocean, in 2006.

6.1.6 Outline of this study

Here I develop a combined model framework with which to analyse the cost-effectiveness of genetic vector control methods to control the transmission of dengue (Figure 6.3). The mosquito population dynamics model predicts the population size of the vector (adult female mosquitoes). This is coupled with the epidemiological model to predict the disease

dynamics, including the total number of dengue cases. I apply estimates of the costs of RIDL-based SIT for vector control to investigate the potential cost-effectiveness of this strategy. Finally, I draw conclusions about some of the direct and indirect effects of the intervention on the economy.

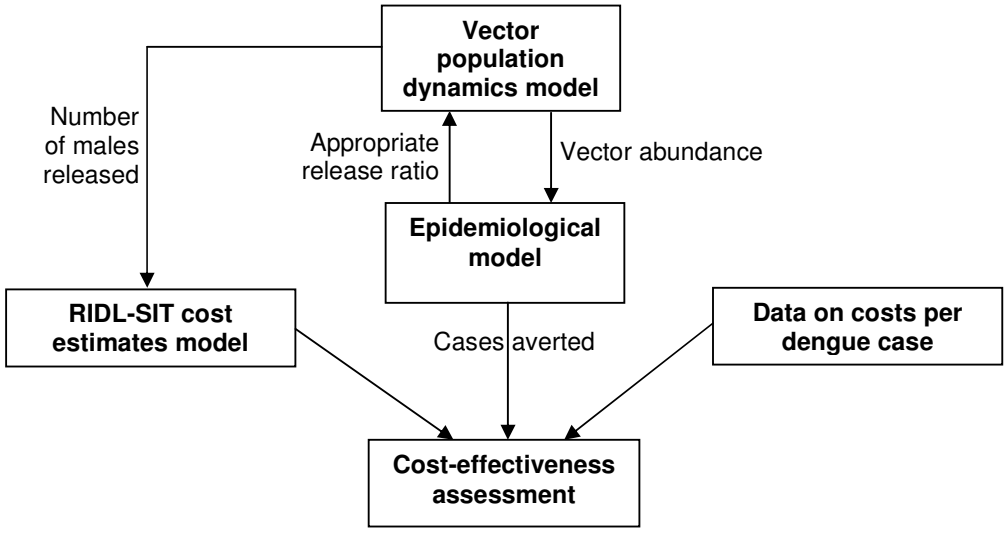

Figure 6.3 Overview of model components

6.2 Methods

For ease of reference, the state variables are listed in Table 6.1 and all parameters described in the text are shown in Table 6.2 (see §6.2.2 below).

6.2.1 Vector population dynamics model

Using data from field studies (Southwood et al. 1972), Dye (1984) developed a simulation model for the population dynamics of *Ae. aegypti* and assessed the likely range of several parameters (including growth rate and density dependence). I adapt Dye's deterministic, continuous time, time-delayed model of mosquito population dynamics, which assumes a closed homogeneous population, with random mating, overlapping generations, and constant life history parameters. I modify this model in three respects. First, to allow for different effective sizes of vector population, and relate this to the number of human hosts

in the population of interest, I define a new input parameter (k) the average number of vectors (adult female mosquitoes) per host prior to RIDL control, and use this to calculate one of Dye's density dependence parameters (α), related to the carrying capacity (number of breeding sites in the environment). Second, I must keep track of numbers of male and female adult mosquitoes separately (rather than using Dye's single variable $A(t)$ for adult mosquitoes). Third, I incorporate release of male mosquitoes that carry a dominant, late-acting, bisex-lethal genetic construct ("RIDL males").

Designating the number of vectors, i.e. female adult mosquitoes, at time t as $F(t)$, my version of Dye's formula with my first two modifications is:

$$\frac{dF}{dt} = PF(t-T)\exp\left\{-\alpha\left[2F(t-T)E\right]^{\beta}\right\} - \sigma F \qquad \text{(D1)}$$

Here, T is the generation time from egg to adult, $2E$ is the average daily egg production rate per female mosquito (Dye defined E as the average per adult), P is the per capita rate E reduced to reflect egg-to-adult mortality due to density-independent factors (and hence is the number of females a female would contribute to the next adult generation in the absence of density-dependent mortality), β is a measure of the strength of density-dependent competition between larvae, and σ is the adult mosquito per capita death rate per day (males and females are assumed to have the same average lifespan, $1/\sigma$). The first expression in equation D1 is the adult emergence of new females and is equal to the number of adult females (F) at the time the newly emerging vectors were laid as eggs (T days ago), multiplied by the maximum daily rate (P) at which each of those mothers could contribute adult females to the current generation, multiplied by the exponential term applying mortality due to density-dependent competition while developing as larvae. The final term is the density-independent death rate of vectors.

Equation D1 has a stable equilibrium value F^* for the number of vectors in the absence of, or prior to, RIDL release:

$$F^* = \frac{\left[\frac{1}{\alpha}\log\left(P/\sigma\right)\right]^{1/\beta}}{2E} \qquad \text{(D2)}$$

With weak density dependence (low β), the population tends monotonically to this equilibrium value. Moderate density dependence introduces damped oscillations, and if density-dependent effects are strong (particularly where $\beta \geq 1$), the population exhibits cycles. If the human host population is approximately constant ($\approx N_0$) over time, then by definition $F^* \approx kN_0$ (see Table 6.2 for calculation of α from k).

I modify equation D1 to incorporate the release of RIDL males starting at time $t = 0$. All engineered released males are assumed to be homozygous for the RIDL construct. I assume the RIDL insertion is not sex-linked and that the construct imposes no fitness cost. I assume a 1:1 sex ratio (as did Dye), so that the number of wild type males equals the number of wild type females. I assume a constant release policy, whereby mass-reared adult RIDL males are released in quantities that maintain their numbers at a fixed ratio (the "release ratio" C) to the equilibrium number of adult males (or females) in the wild population prior to initiation of the release programme (F^*, equation D2), i.e. the total number of adult RIDL males = $C\,F^*$ for $t > 0$. Females mate with wild or released males at random. The RIDL construct is assumed to be completely lethal, so the only insects that survive to adulthood are those that were sired by wild type males, i.e. the fraction of wild type within all adult males (wild type and released RIDL males) at the time these new adults were laid as eggs $\frac{F(t-T)}{F(t-T)+CF^*}$. All eggs laid ($2E \times F(t-T)$) contribute to the

density-dependence term, not just the progeny of wild type males, because the engineered lethality acts after the effects of larval competition.

$$\frac{dF}{dt} = PF(t-T)\frac{F(t-T)}{F(t-T)+\tilde{C}F^*}\exp\left\{-\alpha\left[2F(t-T)E\right]^{\beta}\right\} - \sigma F \qquad \text{(V1)}$$

$$\text{where } \tilde{C} = \begin{cases} 0 & \text{if } t-T \le 0 \\ C & \text{if } t-T > 0 \end{cases}$$

To maintain the number of RIDL males at the required constant value, CF^* of them must be released instantaneously at $t = 0$, and thereafter an average of σCF^* must be released daily to replace the losses from natural mortality. In practice, the initial release would likely be achieved by rapid build-up over a short period but I do not expect this simplification to have a significant effect on my conclusions. I ignore seasonality in vector dynamics; Wearing and Rohani (2006) found that seasonality in vector recruitment is necessary to explain intra-annual variation in dengue incidence but that it has a lesser impact on inter-annual dengue dynamics, and I am interested primarily in assessing a control programme running over several years.

6.2.2 Epidemiological model

In conjunction with the vector population dynamics model, I use a compartmental model to describe the status of vectors and hosts with respect to the disease. I develop two versions of this model, for reasons that will be explained below (§6.3.2). Vectors, the adult female mosquitoes, are classed as susceptible or infectious, and human hosts are classed broadly as susceptible, infectious, cross-immune or recovered. The intrinsic (in the host) and extrinsic (in the vector) incubation periods are represented using fixed duration time delays.

Table 6.1 State variables

Vectors (adult female mosquitoes, see Figure 6.4):	
F	total number of adult female vectors (susceptible, exposed and infectious)
X	susceptible
Y_j	infectious with serotype j
Hosts (humans, see Figure 6.5):	
N	total number of hosts (susceptible, exposed, infectious, cross-immune and recovered)
S	susceptible to all 4 serotypes
I_i	primary infection (infectious) with serotype i
C_i	recovered from primary infection with serotype i, temporarily cross-immune to all other types
R_i	recovered from primary infection with serotype i, susceptible to all other types
I_{ij}	secondary infection with serotype j, following primary infection with serotype i
R	recovered from secondary infection, immune to all serotypes

The choice and range of values for entomological and epidemiological parameters is drawn from published literature (Table 6.2).

6.2.2.1 Two-of-four serotype model

Full representation of all four dengue serotypes and the interactions between them requires a complicated mathematical model. To reduce the complexity I assume that a host can be infected by at most two of the four serotypes and is thereafter immune to further infection by the remaining types. I do not allow concurrent infections in either vectors or hosts. I assume no vertical transmission of dengue so all newly emerging vectors and all human new-borns are susceptible.

The vector compartments in the model are shown in Figure 6.4. As well as tracking the total number of vectors (equation V1) I now have equations for the numbers of susceptible vectors (X) and vectors infectious with each serotype i (Y_i). Vectors do not recover from infection (Siler et al. 1926). The last expression in each equation is natural deaths from that class (I assume dengue has no effect on vector mortality as there is no evidence of any significant effect).

Table 6.2 Parameters

Symbol	Description	Default value	Range	References
t	Time (days)	-250×365 to $+200\times365$	N/A	N/A
σ	Adult mosquito death rate (per day)	$1/14$	$1/15$ to $\frac{1}{3\,1/3}$ (=0.3)	0.12 (Dye 1984), 1/14 (Wearing and Rohani 2006), 0.15-0.3 *Ae. aegypti*, 0.079-0.15 *Ae. albopictus* (Gubler and Kuno 1997)
T	Mosquito generation time (days), i.e. development period from egg to emerging adult	18.5	18.5	18.5 (Southwood et al. 1972, Dye 1984), egg plus 9-12 (Gubler and Kuno 1997)
E	Daily egg production rate per adult mosquito (a female lays $2E$ per day on average, a male lays none)	8	8	inferred ≈ 7 – 9 (Dye 1984)
P	The number of offspring produced by each adult per day that will survive to adulthood in the absence of density-dependent mortality (i.e. E adjusted for density-independent egg-to-adult survival) – estimated value, calculated using field data, depends on value of σ	0.7	0.2 to 0.7	0.367-1.31 under- & over-estimates with $\sigma = 0.12$ (Dye 1984), which adjusts to 0.218-0.780 for $\sigma = 1/14$
k	Average number of vectors (adult female mosquitoes) per host (initial population N_0)	2	0.3 to 20	<1, 1.5, 2, "high" 5 (Gubler and Kuno 1997), 2, range 0.3 to >20 (Wearing and Rohani 2006)
α	$1/\alpha$ is related to the number of breeding sites Set $\alpha = \frac{\log(P/\sigma)}{(2kN_0E)^{\beta}}$ for given initial host population (N_0)	calculated $\approx 1.5\times10^{-8}$	calculated $\approx 4.5\times10^{-12}$ to $\approx 1.0\times10^{-2}$	(Dye 1984)
β	Strength of larval density dependence	1	0.302 to 1.5	0.302 – 1 (Dye 1984)
P/σ	rate of increase of the mosquito population (net reproductive rate)	calculated 9.8	calculated 2.33 to 10.5	3.1-11.2 under- & over-estimates (Dye 1984), 10.6 (Atkinson et al. 2007)

Symbol	Description	Default value	Range	References
C	Maintained ratio of RIDL males to pre-release equilibrium number of adult males (constant release policy)	10 or various	varies	N/A
v	Human per capita birth rate (per day)	$\frac{1}{60\times365}$	$\frac{1}{60\times365}$ to $\frac{1}{68\times365}$	Equal to human death rate (so population would be constant in the absence of DHF/DSS)
μ	Human per capita death rate (per day)	$\frac{1}{60\times365}$	$\frac{1}{60\times365}$ to $\frac{1}{68\times365}$	i.e. 60 year life span (Wearing and Rohani 2006), e.g. Thailand life span 59-68 years 1970 to 2000 (UN)
b	Biting rate (number of bites per mosquito per day)	0.5	0.33 to 1	0.5, range 0.33-1 (Wearing and Rohani 2006), 0.7 (Atkinson et al. 2007)
a	Proportion of bites that successfully infect a susceptible human	0.38	0.38 to 0.75	0.38 calculated from R_0, assuming $a = c$ (Wearing and Rohani 2006), 0.75 (Atkinson et al. 2007)
c	Proportion of bites that successfully infect a susceptible mosquito	0.38	0.37 to 0.75	0.38 calculated from the disease's basic reproduction number R_0, assuming $a = c$ (Wearing and Rohani 2006), 0.75 (Atkinson et al. 2007), <0.6 (Schneider et al. 2007)
τ	Virus latent period in humans (days) Intrinsic incubation period	5	3 to 12	5 (Wearing and Rohani 2006), 7 (Atkinson et al. 2007), 4-7, exceptionally up to 10 (Siler et al. 1926), 4-12 (Sabin 1952), 4-8, 4-5 for classic DF (Gubler and Kuno 1997)
ω	Virus latent period in vectors (days) Extrinsic incubation period	10	7 to 14	10 (Wearing and Rohani 2006), 9 (Atkinson et al. 2007), 10-14 (Gubler and Kuno 1997), 10 (Siler et al. 1926), 14 (Sabin 1952)
γ	Human recovery rate (per day)	$1/6$	$1/10$ to $1/2$	i.e. infectious period 6 days; in days: 6 (Wearing and Rohani 2006), 4 (Atkinson et al. 2007), 3-4 (Siler et al. 1926), 2-6 (Sabin 1952), 5-5.9 (Suaya et al. 2009), 4-5 viraemia, symptoms 2-7 DF or 7-10 DHF/DSS (Gubler and Kuno 1997)
ψ	Rate at which humans lose cross-immunity (per day)	$\frac{1}{365\times4/12}$	$\frac{1}{365\times5/12}$ to $\frac{1}{365\times2/12}$	i.e. cross-immunity lasts 4 months; in months: 4 (Wearing and Rohani 2006), 2-3 (Sabin 1952), 2-3 or "several" (Gubler and Kuno 1997)

Symbol	Description	Default value	Range	References
χ	Increased host susceptibility due to ADE	1.5	1 to 3	1-3 (Wearing and Rohani 2006), need ≥c.1.5 to get cycles by ADE alone (M. Recker, pers. comm.), >1 (Gubler and Kuno 1997); strictly, proportion of bites infecting a human $\chi a \le 1$, so maximum χ is $1/a \approx 2.63$ when $a = 0.38$
ζ	(Alternative to χ) Increased transmissibility due to ADE	1	1	Possibly >1 (Gubler and Kuno 1997); the proportion of bites infecting a mosquito $\zeta c \le 1$, so maximum ζ is $1/c \approx 2.63$ when $c = 0.38$
ρ	Proportion of hosts that recover from secondary infection (1-ρ die from DHF/DSS)	0.9999	$1-0.05(1-0.87)$ ≈ 0.9935 to 1	0.8% of 6% of 24% die ≈ 0.0001, i.e. $\rho = 0.9999$ (Shepard et al. 2004), 87% cases mild & 5% CFR for DHF (Gubler and Kuno 1997), 1-ρ is 0.00014 for five countries in the Americas, and 0.0027 for three countries in Asia, i.e. $\rho = 0.9973$ or 0.99986 (Suaya et al. 2009).

$$\frac{dX}{dt} = PF(t-T)\frac{F(t-T)}{F(t-T)+\tilde{C}F^{*}}\exp\left\{-\alpha\left[2F(t-T)E\right]^{\beta}\right\} - cb\frac{X}{N}\sum_{i=1}^{4}\left(I_i + \sum_{j=1,\,j\neq i}^{4}\zeta I_{ji}\right) - \sigma X \qquad \text{(V2)}$$

The first term on the right hand side is the emergence of wild type new adults, which is the same as in equation V1. The second term represents infections; mosquitoes bite at rate *b* and if susceptible (*X*), on biting an infectious host (I_i or I_{ji} of *N* hosts), a proportion (*c*) will become infected with serotype *i*. ADE may manifest as increased transmissibility from a host with a secondary infection (if $\zeta > 1$), but I do not invoke this alternative scenario in my simulations (I keep $\zeta = 1$). After a fixed time delay, the extrinsic incubation period (ω), those vectors become infectious (Y_i) with serotype *i* and remain infected for the rest of their lives.

$$\frac{dY_i}{dt} = \left(\exp(-\sigma\omega)\right)\left(\frac{cbX(t-\omega)}{N(t-\omega)}\left(I_i(t-\omega) + \sum_{j=1,\,j\neq i}^{4}\zeta I_{ji}(t-\omega)\right)\right) - \sigma Y_i \quad i = 1,2,3,4 \qquad \text{(V3)}$$

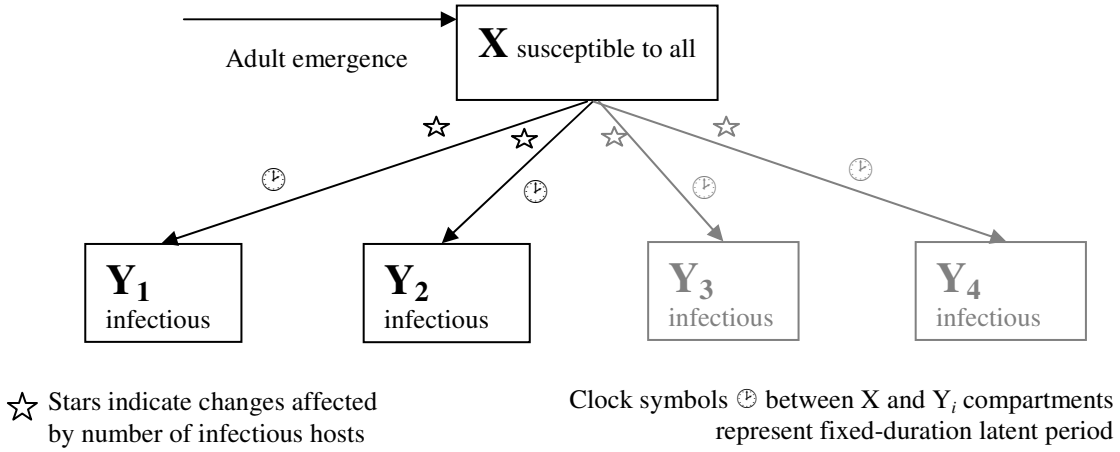

Figure 6.4 Vectors (adult female mosquitoes)
Deaths occur from all compartments (not shown). Paler symbols are elements of the two-of-four serotype model that are not included in the simpler two serotype model.

The disease dynamics in the hosts are more complicated and require more compartments (see Figure 6.5). The total number of hosts (*N*) will potentially grow or decline exponentially, although in my simulations I set the per capita birth rate (ν) equal to the per capita death rate

(μ) so that the population would remain constant if there were no deaths due to DHF, and will be approximately constant if the proportion of hosts surviving a secondary dengue infection (ρ) is close to 1 (see equation H7 below).

$$\frac{dS}{dt} = \nu N - \sum_{i=1}^{4}\left(ab\frac{F}{N}S\frac{Y_i}{F}\right) - \mu S = \nu N - \sum_{i=1}^{4}\left(ab\frac{SY_i}{N}\right) - \mu S \qquad \text{(H1)}$$

Each susceptible host (S) is bitten by mosquitoes at rate b, of which Y_i/N per host are infectious with serotype i, and a proportion a are successfully infected with the virus. After a fixed time delay, the intrinsic incubation period (τ), they become infectious (I_i), which lasts on average $1/\gamma$ days.

$$\frac{dI_i}{dt} = \left(\exp(-\mu\tau)\right)ab\frac{S(t-\tau)Y_i(t-\tau)}{N(t-\tau)} - (\gamma+\mu)I_i \qquad i = 1,2,3,4 \qquad \text{(H2)}$$

The host retains lifelong immunity to the serotype of infection and has temporary cross-immunity to all other serotypes (C_i, not to be confused with C, the release ratio) for an average period of $1/\psi$ days following the end of the infectious period. On challenge by a serotype not previously encountered cross-immunity is "classical", i.e. no infection arises and no immunity is gained (as opposed to "clinical" cross-immunity where no symptoms arise and the virus is not transmitted onward but immunity is gained to that serotype). When the temporary cross-immunity has waned, the host is susceptible to infection by serotypes other than that from which s/he has recovered (R_i).

$$\frac{dC_i}{dt} = \gamma I_i - (\psi+\mu)C_i \qquad i = 1,2,3,4 \qquad \text{(H3)}$$

$$\frac{dR_i}{dt} = \psi C_i - \sum_{j=1,\, j\neq i}^{4}\left(\chi ab\frac{R_iY_j}{N}\right) - \mu R_i \qquad i = 1,2,3,4 \qquad \text{(H4)}$$

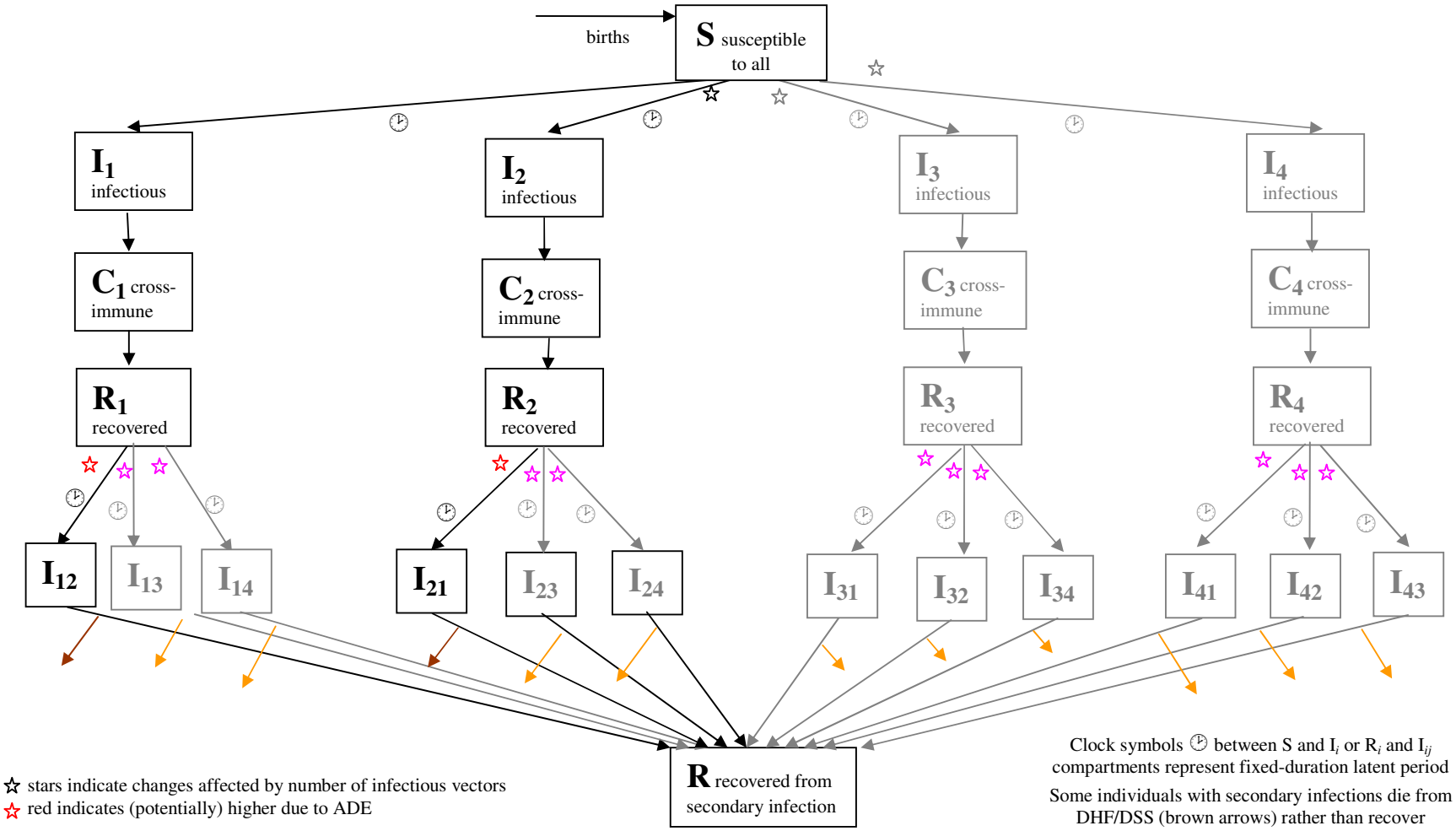

Figure 6.5 Hosts (humans)
Deaths occur from all compartments (not shown). Paler symbols are elements of the two-of-four serotype model that are not included in the simpler two serotype model.

ADE manifests as increased susceptibility to a secondary infection ($\chi > 1$),

$$\frac{dI_{ij}}{dt} = \left(\exp(-\mu\tau)\right)\chi ab\frac{R_i(t-\tau)Y_j(t-\tau)}{N(t-\tau)} - (\gamma+\mu)I_{ij} \qquad i, j = 1,2,3,4;\ j \neq i \qquad \text{(H5)}$$

and as a risk of DHF or DSS from secondary infection, with a proportion (1 - ρ) dying at the end of the infectious period. On recovery from secondary infection (R), I apply the simplifying assumption that the host is immune to infection from all serotypes.

$$\frac{dR}{dt} = \sum_{i=1}^{4}\sum_{j=1,\,j\neq i}^{4}\left(\rho\gamma I_{ij}\right) - \mu R \qquad \text{(H6)}$$

$$\frac{dN}{dt} = \nu N - \sum_{i=1}^{4}\sum_{j=1,\,j\neq i}^{4}\left((1-\rho)\gamma I_{ij}\right) - \mu N \qquad \text{(H7)}$$

Together, the equations V1 to V3 and H1 to H7 form a system of 33 delay-differential equations representing the vector-pathogen-host dynamics. I ran simulations in Matlab, incorporating the delay-differential equation solver dde23 (Shampine and Thompson 2001).

6.2.2.2 Two serotype model

Instead of assuming that hosts acquire immunity to all four serotypes after a secondary infection, an alternative simplification is to restrict the model to two serotypes in total. I label these 1 and 2, which could be any pair of the four known serotypes. In the above equations, the subscripts i and j therefore only take values 1,2 not 1,2,3,4, and some equations can be re-written for clarity:

$$\frac{dX}{dt} = PF(t-T)\frac{F(t-T)}{F(t-T)+\tilde{C}F^{*}}\exp\left\{-\alpha\left[2F(t-T)E\right]^{\beta}\right\} - cb\frac{X}{N}\left(I_1 + \zeta I_{21} + I_2 + \zeta I_{12}\right) - \sigma X \qquad \text{(V2)}$$

$$\frac{dY_i}{dt} = \left(\exp(-\sigma\omega)\right)\left(\frac{cbX(t-\omega)}{N(t-\omega)}\left(I_i(t-\omega) + \zeta I_{ji}(t-\omega)\right)\right) - \sigma Y_i \quad i, j = 1,2\,;\ j \neq i \tag{V3}$$

$$\frac{dS}{dt} = \nu N - \sum_{i=1}^{2}\left(ab\frac{F}{N}S\frac{Y_i}{F}\right) - \mu S = \nu N - \left(ab\frac{S(Y_1+Y_2)}{N}\right) - \mu S \tag{H1}$$

$$\frac{dR_i}{dt} = \psi C_i - \left(\chi ab\frac{R_i Y_j}{N}\right) - \mu R_i \qquad i, j = 1,2\,;\ j \neq i \tag{H4}$$

$$\frac{dR}{dt} = \left(\rho\gamma(I_{12} + I_{21})\right) - \mu R \tag{H6}$$

$$\frac{dN}{dt} = \nu N - \left((1-\rho)\gamma(I_{12} + I_{21})\right) - \mu N \tag{H7}$$

These equations V1 to V3 and H1 to H7 form a system of 15 delay-differential equations representing the vector-pathogen-host dynamics.

I tested the sensitivity of this model to parameter values in the ranges shown in Table 6.2, for release ratio (*C*) 10. I varied one or sometimes two or three related parameter values and assessed the qualitative effect on the behaviour of the host-vector-pathogen system, including the average number of infectious cases arising in the 50 years before ("baseline cases") and the 50 years after RIDL releases start.

My model is not restricted to integer values. For the purposes of my simulations I regard the vector population (*F*) or the virus (the sum of all *I* values) as eliminated (locally) if it drops below 0.1 and remains there, and I label the earliest time from when that is true as the time to elimination.

The two serotype model encapsulates the key properties of the disease dynamics. I use my two-serotype model to evaluate the RIDL genetic strategy for vector control by modelling the potential reduction in numbers of cases. Fewer cases will reduce the cost and burden of disease. The release ratio and duration of release of engineered male mosquitoes will drive the costs of the intervention.

6.2.3 Estimating the costs of implementing the Sterile Insect Technique using genetically engineered mosquitoes

A lot of the knowledge and expertise of designing, constructing and managing sterile insect production facilities has been transmitted by personal communication, internal memos, the involvement of the same governments and to some extent the same individual staff members in different programmes, bilateral cooperation and international facilitation by the United Nations Food and Agriculture Organization and International Atomic Energy Agency (FAO/IAEA) joint programme on nuclear techniques in food and agriculture, rather than through extensive literature, guides or published software (IAEA 2008). I draw information from the available literature and various public sources on the costs and cost structures of existing and proposed SIT programmes.

Most available information is already expressed in US dollars; where this is not so I convert using published exchange rates (US Federal Reserve 2009). I inflate values to current cost, 2008 US dollars, using GDP chained Price Index (US Office of Management and Budget 2008). This could be considered a compromise; even where information is presented in local currency, there is not usually any relevant official published index available, and the funder(s), the recipients of expenditure, the SIT facility and release programmes might operate in several different currency regions. I identify facility construction costs separately from

operational costs where possible and attempt to fit regression curves to the available data for each, using the least-squares method. This information is applied to generate a range of projected values for those two key broad elements of SIT cost.

6.2.4 Estimating the burden of disease

I use total cases, output from the simulation model, as my measure of the direct impact of the disease. The cost of dengue is estimated using a range of values for cost per case, based on the studies cited in §6.1.4 above. I also gather data on the per capita cost of conventional vector control methods. All amounts are restated in 2008 US$ (method as in §6.2.3 above).

6.2.5 Assessing cost-effectiveness of RIDL-based SIT to control dengue

The cost-effectiveness of any disease control strategy is the cost of achieving a given outcome, such as the cost per case averted by the intervention. This could be judged by comparing with a benchmark level, with the equivalent figure for an alternative strategy, or with that for the current policy. Using more sophisticated measures, a hypothetical vaccination programme against dengue has been claimed to be potentially very cost-effective (Shepard et al. 2004). It has been suggested that conventional mosquito spraying programmes are not cost-effective (World Bank 1993), although a recent study of larviciding campaigns in Cambodia claims those can be very cost-effective (Suaya et al. 2007). For my study, I compare the projected costs per case averted by genetic vector control with estimates of the average cost of an episode of illness.

In contrast to *cost-effectiveness analysis*, the aim of *cost-benefit analysis* is to assess a disease intervention by weighing an estimated value of all the benefits it achieves against the total cost of implementing the strategy. To identify and value the overall costs and benefits of genetic

vector control strategies to reduce dengue would require not only obtaining estimates of the costs of dengue, including direct costs (such as medical care) and indirect costs (such as lost income), and also attempting to assess the wider economic impact. It entails estimating the burden of disease, including the impact of mortality and morbidity, and putting some monetary value on that (WHO 2001). A full cost-benefit analysis is beyond the scope of this study, but I gather some information to illustrate the wider benefits of reducing or locally eliminating dengue and draw some broad conclusions (see §6.3.5 and §6.3.6).

6.3 Results

6.3.1 Vector population dynamics model

Equation V1 decouples from the others and can be investigated in isolation. This potentially has more than one equilibrium solution: $F = 0$ is always a solution (local elimination of the vector) and potentially there is a non-zero equilibrium vector abundance $F_R^* > 0$. If it exists, this satisfies the following equation, with F^* being the equilibrium in the absence of, or preceding commencement of, RIDL releases, as defined in equation D2:

$$C = \frac{F_R^*}{F^*}\left(\left(\frac{P}{\sigma}\right)^{\left[1-\left(\frac{F_R^*}{F^*}\right)^{\beta}\right]} - 1\right) \qquad \text{(D3)}$$

Note that this is scale-free; the post-release equilibrium F_R^* only appears as a fraction of the pre-release equilibrium F^*. It can be shown that $C > 0 \Rightarrow F_R^* < F^*$ (where it exists), i.e. releasing RIDL males reduces the equilibrium number of vectors, whether to a new lower equilibrium or to zero. For some values of the release ratio C, equation D3 has two positive solutions for F_R^* and for higher values of C it has no non-zero solutions (see Figure 6.6). The critical release ratio (C_R) above which the only possible equilibrium is zero satisfies:

$$C_R = \frac{F_R^*}{F^*}\left(\frac{1}{1-\beta\log\left(P/\sigma\right)\left(\frac{F_R^*}{F^*}\right)^{\beta}}-1\right) \qquad \text{(D4)}$$

With default parameter values (as in Table 6.2 and Figure 6.6 blue line) the critical release ratio is approximately 1.19 (i.e. maintain 1.19 RIDL males for every wild type male at pre-release equilibrium), at which the possible non-zero post-release equilibrium (F_R^*), is approximately 0.34 times the pre-release equilibrium (F^*).

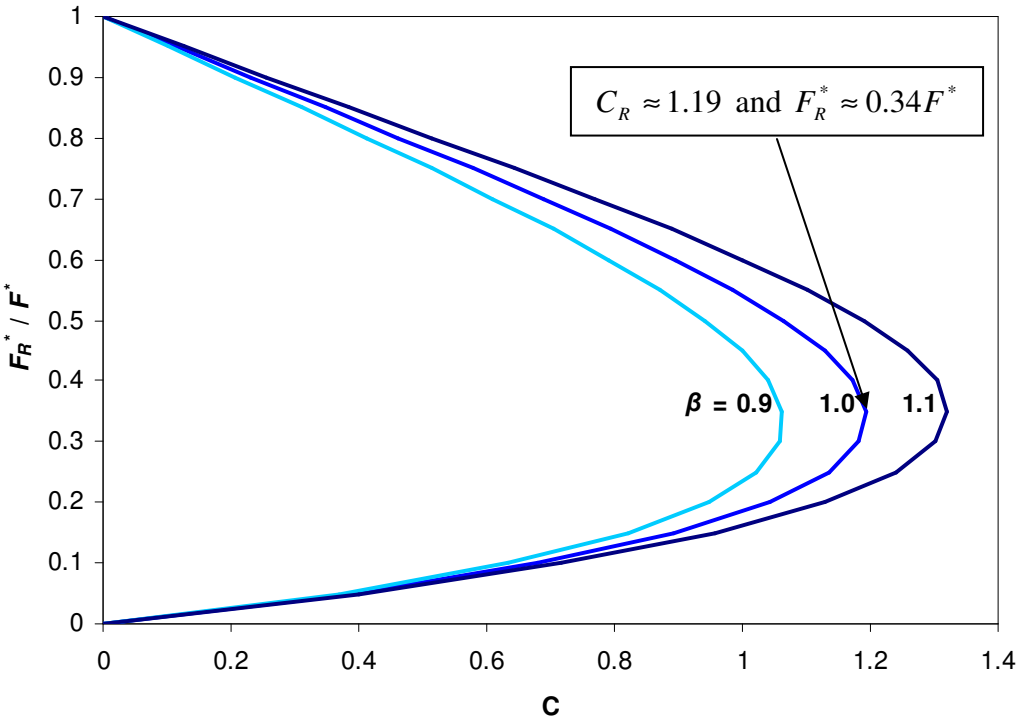

Figure 6.6 Solutions to equation D3, with default parameter values as in Table 6.2 and strength of density dependence (β) 0.9 (turquoise), 1 (blue) or 1.1 (indigo). If the release ratio (C) is in the range that has non-zero solutions, simulations show that the vector population is reduced from its pre-release equilibrium F^* to a new equilibrium represented by the upper edge of this curve.

Simulations confirm that a sufficiently high release ratio ($C > C_R$) will eliminate the vector population ($F = 0$), and reveal that with lower release ratios ($C \leq C_R$) the vector abundance can move to a new lower equilibrium value (Figure 6.7). That lower equilibrium is stable and is equal to the larger solution F_R^* of equation D4 (upper edge of curve in Figure 6.6) unless the vector population when releases begin is very small relative to its natural equilibrium F^* (for example, through intensive chemical control efforts prior to starting the RIDL release programme). If the population is sufficiently small when releases start, the vector population is eliminated even with sub-critical values of C, presumably because the initial over-flooding ratio of RIDL males to actual wild type males (which is not the same as C, their ratio to the equilibrium number) is so high that it outweighs the density-induced population rebound.

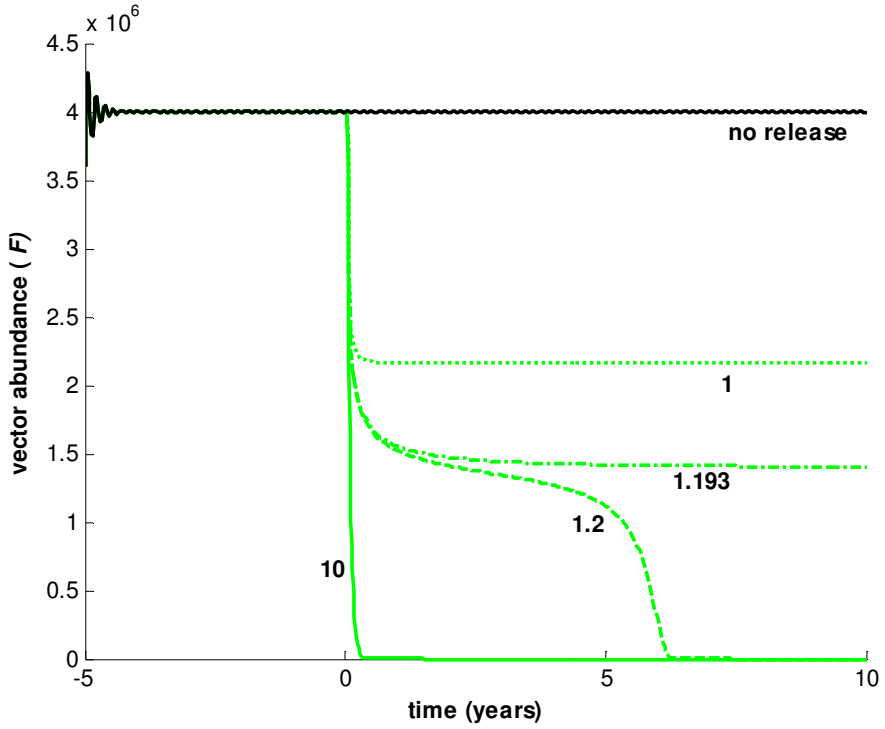

Figure 6.7 Vector dynamics
Vector population over time with no release (black), or RIDL release (green) at release ratio 1 (dotted), 1.193 (dash-dot), 1.2 (dashed) or 10 (solid). Default parameter values (Table 6.2) with F^* 4 million.

6.3.2 Epidemiological models (no RIDL release)

The simulations must be run for a period of years to allow the transient dynamics of the model (very large epidemics interspersed between deep troughs of negligible infection numbers) to pass and settle to the expected pattern of epidemic cycles. Typically, I run 50 to 250 years prior to beginning RIDL releases. Results here are presented after disregarding that initial period of atypical transient behaviour.

Before considering the effect of RIDL control measures, I must be satisfied with the epidemiological dynamics in their absence. The two-of-four serotype model (§6.2.2.1), where hosts become immune to all four serotypes after a second infection, does not produce the sustained cycles characteristic of dengue. It produces damped oscillations (Figure 6.8) and eventually the host and vector compartments settle to stable equilibrium states. The period of those temporary oscillations, at about 3.3-3.6 years average per epidemic, is in line with a 30-year four-serotype dataset from Thailand and a full four-serotype model (Wearing and Rohani 2006).

For this reason, the two-of-four model is not suitable for the current study, and so I adopted the simpler model incorporating only two serotypes of dengue (§6.2.2.2). The two serotype model does exhibit the characteristic dengue cycles. (This also represents the situation in some countries, such as Costa Rica, where only two serotypes are reported). Fewer serotypes lengthen the average period between epidemics to approximately 5.5 years. After the cyclical pattern is established, about 60% of the host population is recovered and immune to both serotypes. The two serotype model (and the single serotype version contained within it) can be analysed in part by applying ecological and population biology techniques.

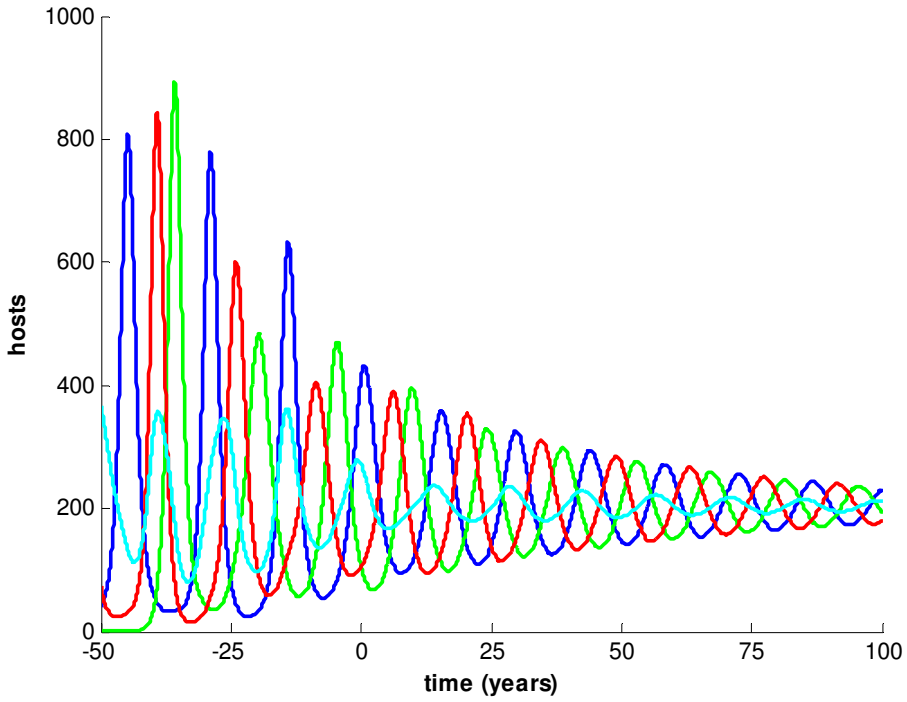

Figure 6.8 Disease dynamics using two-of-four serotype model
Total number of infectious hosts (primary or secondary infections) over time, by serotype (1: blue, 2: green, 3: red, 4: turquoise) using the two-of-four serotype model. Default parameter values (Table 6.2), with initial conditions host population (N_0) 2 million and primary infections I_1: 1, I_2: 2, I_3: 3, I_4: 4.

For simplicity, I may assume the vector population is at equilibrium (F^*), and the host birth (ν) and death (μ) rates are equal and dengue-associated deaths are negligible ($\rho \approx 1$) so that the host population (N) is constant. I can assess the conditions under which dengue (serotype 1, say) can invade naïve populations (where all hosts and vectors are susceptible to dengue, $S \approx N$ and $X \approx F^* \approx kN$) by expressing equations V1 to V3 and H1 to H4 in the form of an invasion matrix:

$$\frac{d}{dt}\begin{bmatrix} Y_1 \\ I_1 \end{bmatrix} = \begin{bmatrix} -\sigma & e^{-\sigma\omega}\frac{cb}{N}X(t-\omega) \\ e^{-\mu\tau}\frac{ab}{N}S(t-\tau) & -(\gamma+\mu) \end{bmatrix}\begin{bmatrix} Y_1 \\ I_1 \end{bmatrix} \approx \begin{bmatrix} -\sigma & e^{-\sigma\omega}cbk \\ e^{-\mu\tau}ab & -(\gamma+\mu) \end{bmatrix}\begin{bmatrix} Y_1 \\ I_1 \end{bmatrix} \qquad \text{(E1)}$$

Expression E1 is equal to the zero matrix on the zero-growth isocline, at which the numbers of infectious hosts and vectors in almost entirely susceptible populations do not grow (or decline). Invasion analysis ("can dengue invade?") is equivalent to linearizing the epidemiological model about the disease-free equilibrium ("is the disease-free state an unstable equilibrium?"). This threshold occurs when the determinant of the invasion matrix (E1) is equal to zero, i.e. where

$$\sigma(\gamma+\mu)-e^{\sigma\omega+\mu\tau}cab^2k=0 \qquad \text{(E2)}$$

Rearranging E2 gives the entomological threshold for disease transmission, i.e. the minimum number of vectors per host that are necessary to sustain the disease:

$$k_R=\frac{\sigma(\gamma+\mu)e^{\sigma\omega+\mu\tau}}{cab^2}\approx 0.67 \text{ with default parameter values (Table 6.2)} \qquad \text{(E3)}$$

Simulations with a reasonable estimate of actual mortality from DHF/DSS ($\rho = 0.9999$, Table 6.2), confirm the entomological threshold still falls approximately between 0.67 and 0.68.

The invasion threshold is where the initial reproduction number of dengue (R_0) is equal to one. With the assumption of constant host population, I can estimate the value of R_0 inherent in my model from the dengue-naïve scenario. Arguing from first principles, R_0 is the number of mosquito infections that result directly (via hosts) from a single infectious vector mosquito entering the population. If that female mosquito survives the latent period (a proportion $e^{-\sigma\omega}$ would), on average that vector will bite and successfully infect $\frac{ab}{\sigma}$ human hosts during her lifespan. Each of those hosts that survives the latent period ($e^{-\mu\tau}$) will be bitten by and successfully infect $\frac{cbF}{N(\gamma+\mu)}$ vectors on average while they remain infectious. Therefore

$$R_0 = \frac{e^{-(\sigma\omega+\mu\tau)}acb^2}{\sigma(\gamma+\mu)}\frac{F}{N} \approx \frac{e^{-(\sigma\omega+\mu\tau)}acb^2k}{\sigma(\gamma+\mu)} \qquad \text{(E4)}$$

More formally, I use Dobson's (2004) method for multi-species pathogens, which uses the result that R_0 can be computed as the dominant eigenvalue of a form of transmission matrix (Diekmann et al. 1990). In this case of a vector-borne (two-species) disease, I modify the "who acquires infection from whom" (WAIFW) matrix, which contains the rates of infection between all possible combinations of species, to form a "next-generation" matrix, which contains those transmission rates multiplied by the duration of time for which an individual of the transmitting species is infectious. The next-generation matrix for my model is

$$\begin{bmatrix} 0 & \dfrac{e^{-\sigma\omega}\dfrac{cbX}{N}}{\sigma} \\ \dfrac{e^{-\mu\tau}\dfrac{abS}{N}}{\gamma+\mu} & 0 \end{bmatrix} \approx \begin{bmatrix} 0 & \dfrac{e^{-\sigma\omega}cbk}{\sigma} \\ \dfrac{e^{-\mu\tau}ab}{\gamma+\mu} & 0 \end{bmatrix} \qquad \text{(E5)}$$

and solving for the dominant eigenvalue of E5 gives the same result as E4. Evaluating equation E4 with my default parameter values (Table 6.2) gives my estimated R_0 value of 2.97, for any of the dengue serotypes (I assign identical parameters to all of them).

These estimates for the entomological threshold k_R and the initial reproduction number R_0 are discussed below (§6.4) in comparison with other studies.

As well as an entomological threshold for disease transmission, there is also a demographic threshold. Using my criterion for local elimination (the sum of all *I* values falls below 0.1 and remains there, see §6.2.2), the disease is unable to persist in a human population of about 100 or fewer, because the peak number of infectious cases in the cycles is just below 0.1. If I

regard the infection as eliminated when the peak number of cases is below one, the threshold size for the human community is roughly 1000.

6.3.3 Control of dengue (two serotypes) by release of RIDL mosquitoes

There are potentially three possible scenarios:

- with sufficiently high release ratio ($C > C_R$), the vector and virus are eliminated (Figure 6.9);
- with intermediate release ratio ($C \leq C_R$), if the new equilibrium to which the vector population is reduced (F_R^* satisfying equation D3, see §6.3.1) is below the transmission threshold for vector abundance ($k_R N$, where k_R is the entomological threshold vectors per host defined in equation E3), the virus is eliminated;
- with too low release ratio ($C \leq C_R$), if the vector population is reduced but remains above the transmission threshold vector abundance ($k_R N$), the virus may be temporarily removed but the disease persists in the longer term (Figure 6.10).

The intermediate scenario cannot occur if the minimum possible non-zero equilibrium (F_R^* with release ratio C_R, satisfying equation D4, see §6.3.1) is more than $k_R N$. This is the case with my default parameter values, so with those values I could only eliminate the disease by eliminating the vector. For these values, a critical release ratio somewhere between 1.1 and 1.2 determines whether both or neither vector and virus are eliminated (this is comparable with the 1.19 calculated analytically for the situation where there is no DHF/DSS mortality, §6.3.1). However, with only 1 vector per host (k), and release ratio 1 (C), for example, the

vector population is only reduced to 54% of its pre-release equilibrium value but this is enough to disrupt transmission of the disease and eliminate the virus.

Without eliminating the virus, it is possible to reduce the disease below detectable levels and sustain this by continued releases for more than 50 years with my default parameter values and release ratio 1 (Figure 6.10c). In my model this occurs as the numbers of infectious hosts and vectors fall to very small values before resurgence. In practice, the virus might temporarily fade out but be reintroduced by an infectious person, or less likely by an infectious vector, moving into or returning to the area. (If this were of particular interest, it could be reflected in the model by including a small additional constant term in equation H2 to represent a low background level of reintroductions).

Both entomological and epidemiological parameter values influence the transmission threshold number of vectors per host (k_R). However, RIDL-SIT control would only be contemplated where the disease is able to persist, and given that situation, only vector-related parameters are involved in determining whether there is any intermediate level of control that only suppresses the vector and yet eliminates the disease. In practice, it is likely that a genetic control programme would aim to eliminate the vector if that were feasible. Uncertainty in measuring parameters in the field, and deviations from model assumptions, would also make it wiser to allow a good margin of error when setting the release ratio.

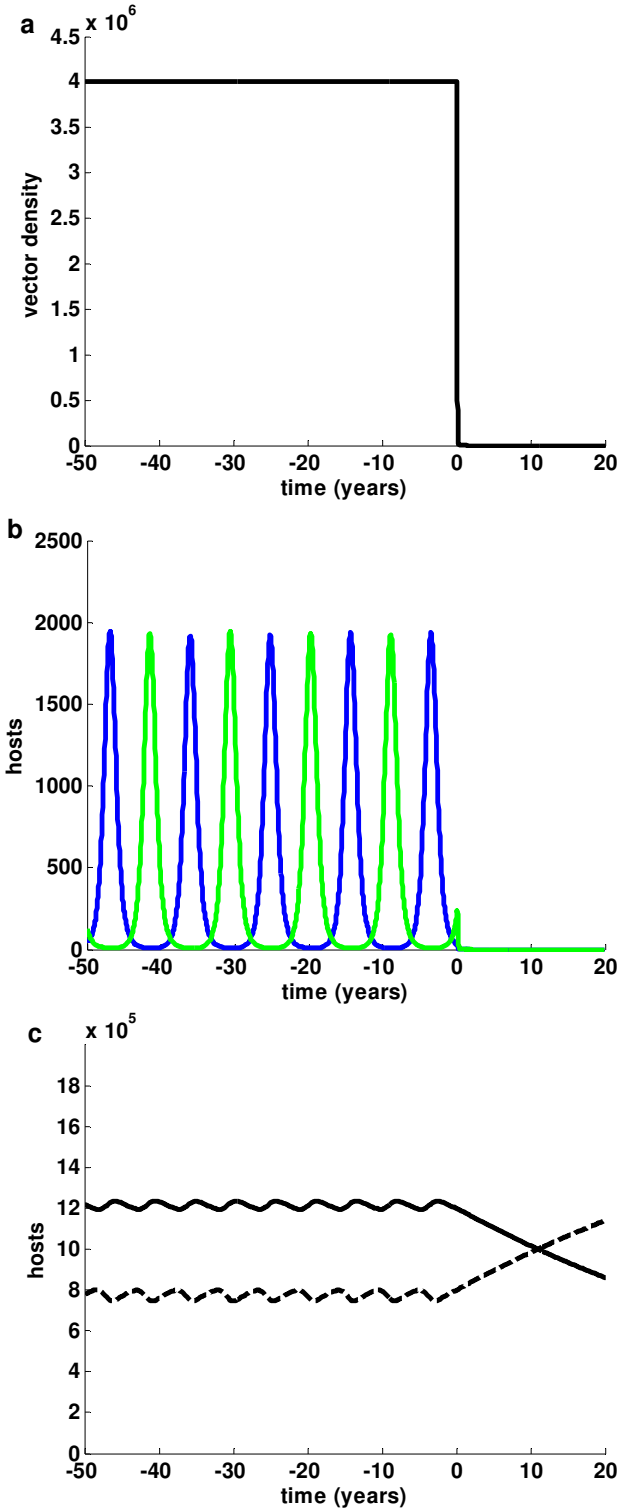

Figure 6.9 Epidemiological dynamics: release ratio C: 10.
Over time (a) total number of vectors, (b) total number of infectious hosts (primary or secondary infections), by serotype (1: blue, 2: green), (c) total number of hosts recovered from secondary (solid line) infection or susceptible to either or both serotypes (dashed). Default parameter values (Table 6.2), with initial conditions host population $N_{0:}$ 2 million and primary infections I_1: 1, I_2: 2.

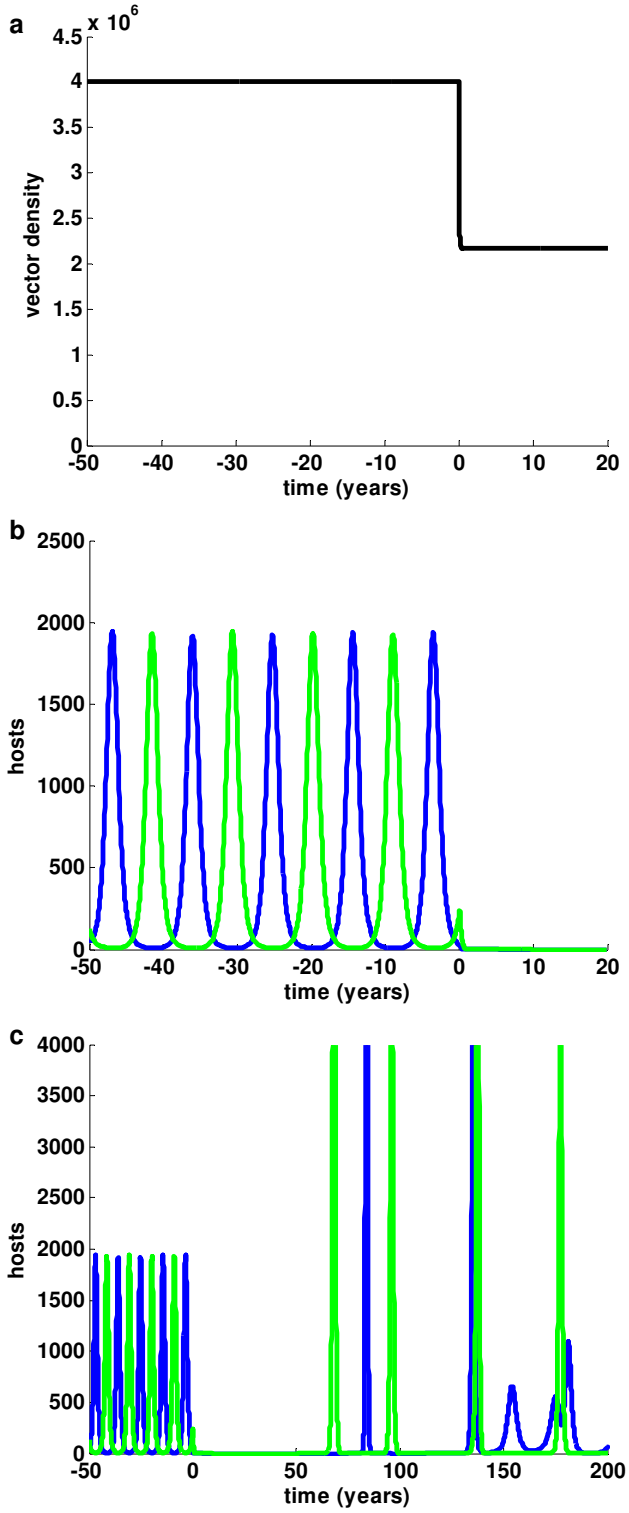

Figure 6.10 Epidemiological dynamics: release ratio C: 1.
Over time (a) total number of vectors, (b & c) total number of infectious hosts (primary or secondary infections), by serotype (1: blue, 2: green); (c) is on different scale and shows that the disease returns after initial suppression. Default parameter values (Table 6.2), with initial conditions host population N_0: 2 million and primary infections I_1: 1, I_2: 2.

I tested the sensitivity of my model to parameter values in the ranges shown in Table 6.2, for release ratio (C) 10, which is projected to achieve vector elimination, and initial human population 2 million. Changes to parameters other than vector life history traits do not alter the approximate time scale for elimination. Significantly shorter mosquito lifespan ($1/\sigma = 1/0.3$ days) prevented the disease from establishing at all. The disease could still become weakly endemic if the average vector lifespan ($1/\sigma = 8.5$) was shorter than the fixed extrinsic incubation period ($\omega = 10$ days), but at a lower prevalence; the benefit of genetic vector control would be less because there would be fewer baseline cases (i.e. average number of infectious cases in the 50 years before RIDL releases start) to avert, but this would be partly mitigated as the vector population could be eliminated more quickly (or with a lower release ratio) thus reducing the cost of doing so. A longer mosquito lifespan had the opposite effect, but my default value ($1/\sigma = 14$) was close to that end of the range ($1/\sigma = 15$) so the effect was not large. A significantly higher transmission success rate ($a = c = 0.75$) would reduce the entomological transmission threshold, so there could be fewer vectors per host ($k = 0.3$) causing fewer baseline cases, and needing fewer RIDL males for control. An extremely large number of vectors per host ($k = 20$) by itself significantly increases the baseline cases and increases the frequency of epidemic cycles, and would take a proportionately greater number of RIDL males to achieve the release ratio. A lower or higher biting rate ($b = 0.33$ or 1 per day), respectively decreases significantly (to 40%) or increases slightly the baseline cases and speeds or slows the epidemic cycles. A shorter or longer extrinsic incubation period ($\omega = 7$ or 14 days) increases or reduces the baseline cases (by about 10%). If hosts recover very quickly ($1/\gamma = 2$ days), very few mosquitoes acquire the infection, so the disease is barely

able to persist. If hosts recover slowly ($1/\gamma = 10$), more people are infected and stay infected for longer so the baseline cases increase dramatically (nearly double) and there is much greater potential benefit from the programme.

I simulated the effect of RIDL male release in a dengue-endemic setting with a host population size (before disease-related mortality) of either 2,000,000 (Figures 6.9 & 6.10) or 10,000, representing a city or small settlement, respectively. With default parameter values, the number of infectious hosts peaks at below 10% of the host population (e.g. under 2,000 in the city, Figure 6.9b), with the mean number of infectious hosts just below half that (800 in the city setting, corresponding to incidence of 48-49,000 cases per year out of a population of 2 million). The cyclic dengue epidemics occur with a period of about 5.4 years (Figures 6.9 & 6.10), so I assessed the outcomes for a programme running for 5 or 10 years (Table 6.3), ignoring any residual effects beyond that period.

As noted above, the high release ratio (C = 10) eliminates the vector and the virus, and although the low release ratio (C = 1) only suppresses the vector population and temporarily removes the disease, continuing releases at this low level gives protection for up to 50 years, which is far beyond the end of my assessment period (and likely beyond the practical duration of any genetic vector control programme). Assessed strictly over this 5 or 10 year time frame, the two release regimes have very similar effects and this is true in both settings. Where the vector is eliminated, this is projected to occur in well under a year, and the disease is eliminated in three to six months. (The disease can be eliminated earlier, because this only requires the vector to be below the transmission threshold, not at extinction.)

Table 6.3 Simulation results

Initial host population N_0	**2,000,000**		**10,000**	
Release ratio C	**1**	**10**	**1**	**10**
Time to vector elimination (days)	N/A	287	N/A	214
Time to disease elimination (days)	N/A	171	N/A	99
Mean numbers of infectious hosts:				
t: -5 to 0 years	803	803	4	4
t: 0 to +5 years	16	20	0	0
Cases averted in 5 years*	239,379	238,163	1,217	1,217
t: -10 to 0 years	795	795	4	4
t: 0 to +10 years	25	12	0	0
Cases averted in 10 years*	468,417	476,325	2,433	2,433
RIDL males released in the period (millions):				
Initially (CF^*)	4	40	0.02	0.2
Per year ($365\sigma CF^*$)	104.3	1042.9	0.5	5.2
Total released in 5 years	525.4	5254.3	2.6	26.3
Total released in 10 years	1046.8	10468.6	5.2	52.3
RIDL males released each week per person	1.0	10.0	1.0	10.0

*The average duration of infection is 6 days ($1/\gamma$), i.e. $\frac{6}{365}$ years, so an average of n infectious hosts at any time represents an average of $n\gamma = \frac{365n}{6}$ cases of infection per year. The cases averted are therefore calculated as ([mean no. before – mean no. after] / average duration) x no. years.

Production of RIDL males on this scale is potentially feasible, even applying release ratio 10 in a city of 2 million, which would require just over 1 billion RIDL males to be released annually over the duration of the programme. For comparison, the largest SIT facility in the world, at El Pino in Guatemala, produces over 2 billion sterile male Mediterranean fruit flies per week (Dyck et al. 2005), equivalent to over 100 billion annually, and even that is below its maximum production capacity (IAEA 2008).

6.3.4 Estimated cost-effectiveness of genetic vector control

Table 6.4 shows construction costs of SIT facilities. At smaller capacities, increasing size brings economies of scale, but large factories are built on a modular basis, so the cost

relationship becomes more linear. Figure 6.11 shows possible fits of a power law $y = ax^b$ and a rational function of the form $y = \frac{ax}{1+bx}$ to this data using non-linear least-squares regression. The proposed Australian facility to deal with a possible invasion of Old World screwworm was not approved, a decision that may have been influenced by the high cost – that data point significantly alters the regression curve (Figure 6.11, compare pink and aqua dotted lines) – so it seems reasonable to remove this outlier from the data. At its current size, the El Pino Medfly facility dwarfs all others in the data set; it does not significantly alter the fit of a power law curve, but it does alter the rational function (Figure 6.11, compare aqua and orange solid lines). For the smaller scale of facility commensurate with my results in §6.3.4 above, the rational function with both outliers removed (solid orange line) gives the best fit.

Table 6.5 shows budgeted or actual operational or production costs for SIT facilities, from a range of inconsistent sources. There is no discernable pattern to this data to relate the cost per million insects to the production quantity. Screwworm facilities have higher costs, because of the difficulty of rearing a myiasis pest (flesh-eating maggots) and partly also because there is no sex separation and so both sexes must be reared in the final generation and released. Codling moths are also unsexed, but even allowing for that they are very much more expensive than the other SIT programmes. I therefore exclude the codling moth data and use the mean cost (US$813 per million insects), and a range from the minimum (US$172 for mosquito SIT in India in 1971) to the mean plus standard error (US$813+819 = 1632).

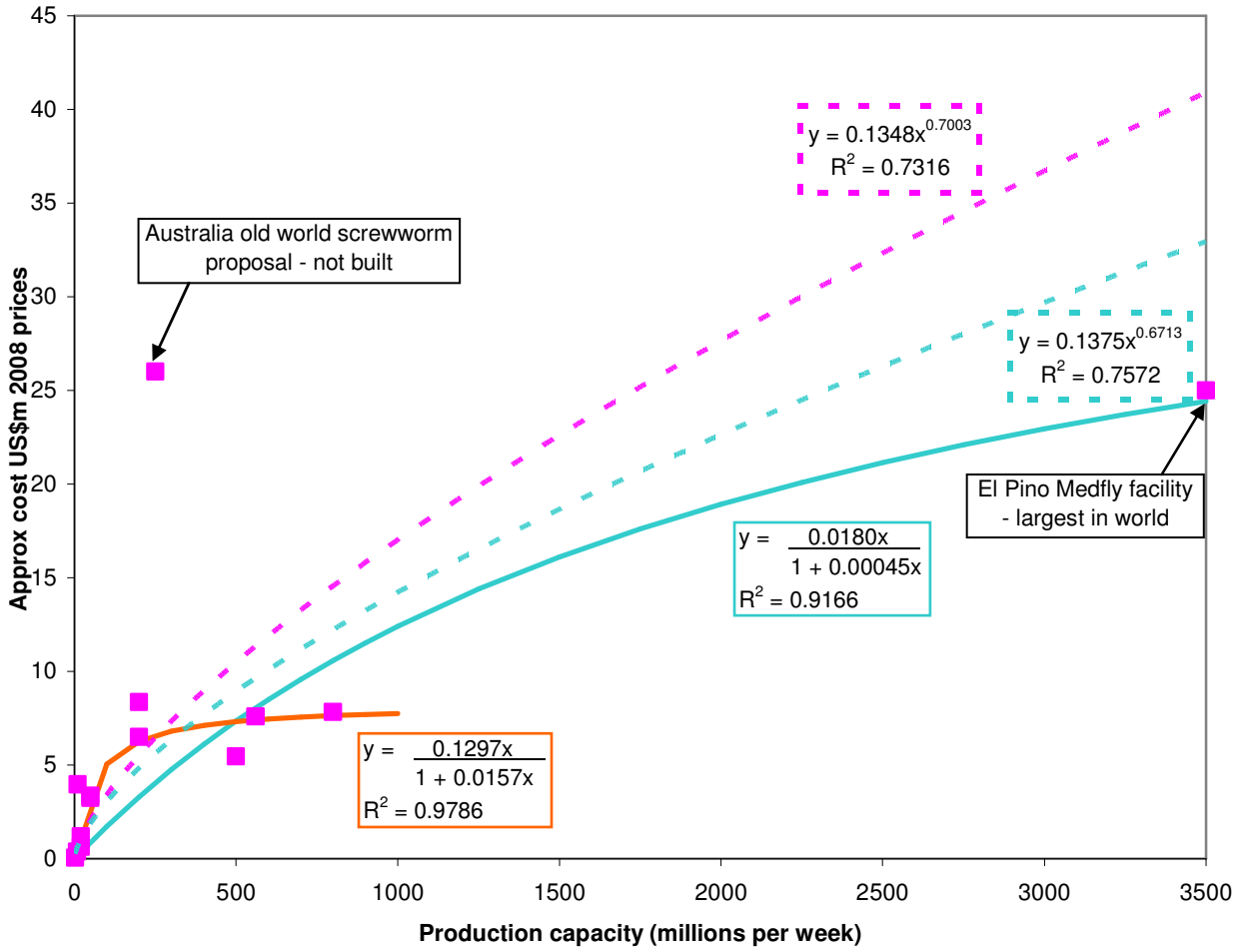

Figure 6.11 Construction costs for SIT facilities plotted against production capacity (pink squares); data from Table 6.4. Curves using rational functions (solid lines) or power law functions (dotted lines) are fitted to the data as follows: all data (pink dotted line), excluding the Australian screwworm facility proposal (aqua lines), or excluding both that Australian facility and the El Pino Medfly facility (orange solid line).

Table 6.4 SIT facility construction costs

Insect	Site	Construction from	Production capacity (millions sterile larvae/pupae per week)	Release	Approx. (cumulative) construction cost US$ million	Approx. cost US$ million at 2008 prices	Reference
Culex pipiens fatigans	India - development	1973	2.45	males	0.014	0.05	(Singh and Razdan 1975)
Medfly	Argentina	1991	200	males	4.5	6.50	(IAEA 2008)
Medfly	Chile	1992	50	males	2.3	3.24	(IAEA 2008)
Medfly	El Pino stage 1	1996	500	males	4.2	5.45	(IAEA 2008)
Medfly	Madeira	1996	50	males	2.6	3.37	(IAEA 2008)
Medfly	W Australia pilot	1997	20	males	0.5	0.64	(IAEA 2008)
Medfly	S Africa stage 1	1998	8	males	0.3	0.38	(IAEA 2008)
Medfly	El Pino stage 1+2	1999	800	males	6.3	7.83	(IAEA 2008)
Medfly	El Pino stage 1+2+3	2001	3500	males	21	25.00	(IAEA 2008)
Medfly	Valencia, Spain	2004	560	males	6.8	7.59	(IAEA 2008)
Medfly	Israel	2005	20	males	1.1	1.19	(IAEA 2008)
Medfly	Bahia, Brazil	2006	200	males	7.99	8.36	(IAEA 2008)
Old World Screwworm	Australia/Malaysia	1995	10	both sexes	3	3.97	(Institut Haiwan 2002)
Old World Screwworm	Australia - not built	(costed but not built) 1992	250	both sexes	18.44	26.00	(Anaman et al. 1994)

Table 6.5 SIT facility production or operational costs

Insect	Site	Year of cost estimate	Production (millions sterile larvae/pupae per week)	Release	Approx operational cost US$ per million released	Approx cost US$ per million at 2008 prices	Reference
Aedes aegypti	India - development	1973	2.8	males	58	228	(Ansari et al. 1975)
Culex pipiens fatigans	India - development	1973	2.45	males	50	196	(Singh and Razdan 1975)
Aedes aegypti / Culex quinquefasciatus	India	1971	?	males?	40	172	(Dame 1985)
Anopheles albimanus	El Salvador	1979	?	males	156	389	(Bailey et al. 1980, Dame 1985)
Old World Screwworm	Australia/Malaysia	1995	10	both sexes	1084	1,433	(Institut Haiwan 2002)
New World Screwworm	Jamaica	1998	20	both sexes	1700	2,141	(IAEA 2008)
NW Screwworm	Mexico	2001	?	both sexes	1900	2,262	(IAEA 2008)
Mexfly	Texas, USA	2004	55	both sexes	410	458	(USDA 2006)
Medfly	Hawaii, USA	2006	110	males	559	586	(USDA 2006)
Medfly	El Pino stage 1+2+3	2001	3500	males	220	262	(Parker 2005, IAEA 2008)
Codling moth	Canada	2001	14	both sexes	8500	10,119	(IAEA 2008)
All						**1,659** **2,912**	**Mean** **Standard error**
Excluding Codling moth						**813** **819**	**Mean** **Standard error**

Table 6.6 Estimated cost of simulated releases

Initial host population N_0	**2,000,000**		**10,000**	
Release ratio *C*	**1**	**10**	**1**	**10**
RIDL males released in the period (millions):				
Per year ($365\sigma CF^*$)	104.3	1042.9	0.5	5.2
Per week (7/365) =*capacity/production* (x)	2.000	20.000	0.010	0.100
Construction costs $\frac{0.1297x}{1+0.0157x}$ (US$ millions)	0.252	1.974	0.001	0.013
Five year programme:				
Total released in 5 years (z)	525.4	5254.3	2.6	26.3
Operational costs: mean 813z	0.427	4.272	0.002	0.021
range 172z to 1632z	0.090-0.857	0.904-8.575	0.000-0.004	0.005-0.013
Total costs: mean	0.679	6.246	0.003	0.034
range	0.342-1.109	2.878-10.549	0.002-0.006	0.018-0.056
mean cost per million insects	*0.001*	*0.001*	*0.001*	*0.001*
Cases averted in 5 years	239,379	238,163	1,217	1,217
Cost per case averted (mean)	US$2.83	US$26.22	US$2.47	US$27.93
Ten year programme:				
Total released in 10 years (z)	1046.8	10468.6	5.2	52.3
Operational costs: mean 813z	0.851	8.511	0.004	0.043
range 172z to 1632z	0.180-1.708	1.801-17.085	0.001-0.008	0.009-0.085
Total costs: mean	1.103	10.485	0.005	0.056
range	0.432-1.960	3.775-19.059	0.002-0.009	0.022-0.098
mean cost per million insects	*0.001*	*0.001*	*0.001*	*0.001*
Cases averted in 10 years	468,417	476,325	2,433	2,433
Cost per case averted (mean)	US$2.35	US$22.01	US$2.06	US$23.02

Numbers of insects released and cases averted are taken from Table 6.3. All costs are in 2008 US$ millions, except the mean costs per case averted.

Table 6.6 shows the projected costs applied to the simulated numbers of insects released and assessed against the simulated number of cases averted (Table 6.3). The construction costs are modelled using the best fit rational function (Figure 6.11 orange line) and the operational costs are modelled as the mean cost (or range from minimum to mean plus standard error) from Table 6.5. Cases averted beyond the end of the programme period are ignored. The unit cost is of the order of magnitude US$1000 per million insects in all scenarios simulated. The cost per case averted is roughly US$2-3 with release ratio 1 and $US20-30 with release ratio

10. The mean cost per person protected per year is US$0.05-0.07 or US$0.52-0.68 (release ratio 1 or 10, respectively). The risk of disease reintroduction with the lower release ratio is not priced into this model. An alternative strategy, which I do not evaluate here, would be to use the higher release ratio for a year, to be surer of eliminating the disease, and then revert to the lower release ratio for the remainder of the programme (or operate a reactive programme based on monitoring, to be activated in response to the detection of sentinel cases).

6.3.5 Estimated benefits of reducing the burden of disease

Table 6.7 shows the range of dengue costs reported in the studies described in §6.1.4 above, restated to 2008 US$. Where results are given separately, hospitalised cases typically cost roughly three times as much as non-hospitalised cases (Anderson et al. 2007, Armien et al. 2008, Suaya et al. 2009). There is inconsistency between studies about which cases are included; many people infected with dengue are not hospitalised, so figures that include non-hospitalised cases (i.e. patients that are ill at home, many of whom will make outpatient visits to a hospital or clinic) are more appropriate for my purpose. Estimates that focus on direct costs and narrow (if any) indirect costs, or only on costs to the patient and ignore higher level costs to society, will be underestimates of the cost per case of illness – and therefore of the potential value of the benefit of reducing the number of cases. As noted in §6.1.4 above the recent prospective study across eight countries is the most comprehensive and consistent research into the costs of dengue (Suaya et al. 2009), giving a range of costs per clinically ill case US$ 357-793. The lowest societal perspective cost in the table including direct and indirect costs is US$59 for the 1994 epidemic in Nicaragua.

Table 6.7 Estimated costs per dengue case

Country	Year	Nature of costs	Approx cost US$ million	Approx cost US$ million at 2008 prices	Approx cost per (ill / reported / clinically ill / hospitalised) case US$	Approx cost per case US$ at 2008 prices	Reference	Cases (if used)
Puerto Rico	1977 epidemic	direct costs (medical care & epidemic control measures), indirect costs (lost production workers/parents)	6.0-15.6	17.3-44.9	26-31	75-89	(Von Allmen et al. 1979)	-
Cuba	1981 epidemic	direct medical, patient social security pay, lost production, *Aedes* control	103	215.3	-	625	(Kouri et al. 1989)	344203 ill
		direct medical, patient social security pay, lost production (excludes vector control)	60	125.4	-	364		
Nicaragua	1994 epidemic	overall economic impact	2.7	3.6	44	59	(Torres and Castro 2007)	60916 DF&DHF
Venezuela	1997-2003 study	direct (medical), indirect (work absence patients/mothers)	1.3	1.5	-	44	(Añez et al. 2006)	33857 DF&DHF
Thailand	1997	direct patient costs	-	-	23-67	29-85	(Clark et al. 2005)	-
	2001 study	patient direct hospital costs, work days lost	-	-	68	81		
	1994	total direct costs of DHF	-	-	257	347		
	1998-2002 study	primary school patient costs clinic/hospital, medication, family lost income	-	-	17	19	(Anderson et al. 2007)	-
India	2006 epidemic	median cost of treatment per hospitalised patient	-	-	432	452	(Garg et al. 2008)	-
		economic burden (public & private healthcare, patient lost productivity)	27.4	28.7	-	-		
Panama	2005 epidemic	direct medical, non-direct medical, indirect – ambulatory / hospitalised	-	-	332 / 1065	359 / 1152	(Armien et al. 2008)	-
Eight countries Americas & Asia	2005 study	direct medical, non-direct medical, indirect - all 8 countries: mean ±S.D.	-	-	417±21	451±23	(Suaya et al. 2009)	-
		- Americas	-	-	733	793		
		- Asia	-	-	330	357		

The direct and indirect costs apply mainly to cases that are clinically ill, particularly those that are hospitalised, but also including those that are ambulatory. About 76% of dengue infections are thought to be asymptomatic or mild, and 24% of cases are clinically ill (Shepard et al. 2004), although reports of the ratio of apparent to inapparent cases range from 1:60 to 1.7:1 (Gubler and Kuno 1997). If only 24% of the infectious cases simulated in my model incur the costs of illness, the overall cost per case (of any severity) based on the above figures is US$86-190, or perhaps as little as US$14.16. These all substantially exceed the US$2-3 average cost of RIDL-based vector control per case averted assuming the lower release ratio ($C = 1$). The likely range of cost per case also significantly exceeds the US$20-30 cost per case averted of the high release ratio ($C = 10$) vector control programme.

A significant reduction of dengue prevalence, or local elimination, might be expected also to lead to a significant reduction in the costs associated with conventional mosquito control. The costs of vector control in several countries are shown in Table 6.8 (Shepard et al. 2004, Suaya et al. 2007, WHO-TDR 2007). More urbanised countries had higher spending (dengue is primarily an urban disease). The underlying studies were generally reporting on years when epidemics occurred. The reported actual or estimated expenditure varies by year and country, and they represent efforts that would have had differing levels of effectiveness; with few exceptions vector control has not achieved sustained control or prevention of dengue in recent decades. The data gathering protocols differ, but the amounts generally include both direct action such as larval source reduction or spraying adulticides, and related activities such as surveillance. For example, the figure reported for Malaysia was based on an estimated national cost in 2002 of US$5.8 million, split between the two major components of the programme, 74% inspection and 24% fumigation. The

figure for Cambodia is the weighted average of annual targeted larviciding campaigns in two regions reaching a population of 2.9 million, and comprises larvicide, operational costs, communications campaign and administrative costs.

Table 6.8 Estimated per capita spending on vector control
(sources: Shepard et al. 2004, Suaya et al. 2007, WHO-TDR 2007)

Country	**Year**	**Cost per capita US$**	**Cost per capita US$ at 2008 prices**
Thailand	1994	0.081	0.109
Thailand	1998	0.188	0.237
Indonesia	1998	0.015	0.019
Singapore	2000	2.400	2.925
Malaysia	2002	0.240	0.280
Cambodia	2001-2005	0.196	0.212
17 Caribbean Islands	1990	0.140-8.490 (median 1.340)	0.189-12.733 (median 2.010)
14 Latin American countries	1997	0.020-3.560 (median 0.260)	0.025-4.538 (median 0.331)
		Mean	0.765

This does not lead to a direct calculation of costs avoided because these costs of conventional vector control would not simply be entirely replaced by the costs of RIDL male releases. The SIT operational costs on which I base my cost estimates for the genetic vector control programme also include some monitoring and surveillance, which would already be incurred and included with these estimates for conventional mosquito control. The RIDL male releases would likely be conducted as part of an integrated vector control programme rather than as a replacement for the current practice. (My simulation model does not include any adjustments to the vector population dynamics to reflect cessation of background level vector control, but neither does it include positive effects of such measures other than any that might be implicit in the pre-release equilibrium number of vectors per host.) With a successful RIDL release programme the level of vector control activity would decrease, thus reducing costs. At first, fewer dengue cases occurring would

trigger fewer reactive measures in the affected premises and neighbourhood, and after local elimination of the vector has been confirmed, only surveillance would be needed for the purpose of watching for the return of the mosquito. Using the mean value from Table 6.8, vector control costs in my simulated city of 2 million people would be US$1.53 million and for 10,000 people would be US$7,650. A major proportion of this would likely be saved, in addition to the direct and indirect costs of illness shown above, but the amount cannot be quantified from the available data.

6.3.6 Tourism revenues

In principle, the potential wider economic impact of significant reduction of dengue virus transmission or local elimination of the vector could be estimated, but such an evaluation is beyond the scope of this study. Instead, I illustrate this by considering one particular benefit that has received little attention in the peer reviewed literature. In regions with significant tourism, a major outbreak of dengue could have a significant impact on visitor numbers. Likewise, sustained removal of dengue from a country or region where it is endemic could potentially have an economic benefit in the form of increased tourism.

Chikungunya, a febrile disease characterised by arthralgia or arthritis, is, like dengue, an arbovirus transmitted by *Ae. aegypti* or *Ae. albopictus*. In late 2005 and early 2006 a serious outbreak occurred on Reunion Island, a French territory, and neighbouring islands in the Indian Ocean. Until then, deaths associated with chikungunya had been reported rarely, but the outbreak was probably responsible for over 200 excess deaths in Reunion in the first four months of 2006 (Josseran et al. 2006). The outbreak received significant media attention. Tourism is an important source of income in this region. Travel medicine physicians strongly discouraged pregnant women, families with small children, people aged over 70 years, and those with significant comorbidity from travelling to the Indian

Ocean islands (Bodenmann and Genton 2006). Tourism and aviation businesses reported substantial declines in long-haul travel bookings from Europe, especially France, to Reunion and Mauritius and in short-haul travel in the region, and adverse effects on hotel occupancy levels (Air Mauritius 2006, Rogers and Company Limited 2007, Thomas Cook 2007, TUI Group 2007). Mauritius experienced 14.4% negative growth rate in tourist arrivals in March 2006 mainly explained by the chikungunya outbreak, and drops in tourist arrivals of 37.1% from the main market, France, and 30.6% from the second most important market, Reunion, compared with the corresponding period in 2005 (Mauritius National Assembly 2006).

Colleagues (Lim et al. 2009, Murtola et al. 2009, in press) have gone on to investigate such effects, using collected tourism data to make preliminary estimates of possible revenue losses due to a major epidemic of dengue or chikungunya, on the assumption that a proportion of tourists from countries where those diseases are not endemic are discouraged from visiting (or choose an alternative destination). A major outbreak causing a 4% decline in tourists from non-endemic countries (or a 2% decline in visits to the Gujurat region of India by Indians not resident in India), could reduce tourism revenues by US$8 million for Gujurat, US$65 million for Malaysia and US$363 million for Thailand (Murtola et al. 2009, in press). Under slightly different assumptions, a 5-15% reduction in tourism revenues in Malaysia could cause losses of US$57-171 million, which is of an order of magnitude comparable to the estimated US$88-215 million combined direct and indirect annual cost of dengue to that country (Lim et al. 2009). All these estimates are expressed in 2008 US$.

Thailand's population is roughly 67 million (National Statistical Office of Thailand 2009, United Nations Population Division 2009) and Malaysia's is about 27 million (Department of Statistics Malaysia 2009, United Nations Population Division 2009), and these estimates translate to approximately US$2-6 per capita at 2008 prices. On that basis, my simulated population of 2 million people might have lost annual tourism revenues of US$4-12 million because of dengue. With the likely overall average cost per case (of any severity) in the range US$86-190 (see §6.3.5) and total numbers of cases averted as in Table 6.3, the estimated costs of illness avoided by the simulated genetic vector control programmes are US$4.0-9.1 million per annum. The potential gains in tourism revenue as a result of eliminating dengue could be of similar magnitude, and possibly higher.

6.4 Discussion

I have shown by mathematical modelling that genetic control of the dengue vector, using RIDL male releases in a SIT programme, can eliminate the disease at a lower cost than the direct and indirect cost savings from the illnesses averted. The disease can effectively be removed from the population in a time scale of the order of six months or less. This can be achieved even where the vector is not suppressed to a level below the entomological threshold for disease transmission, provided there is sufficient disruption to transmission. Continuing the programme of releases can provide effective protection for another 50 years or more beyond the five or ten year assessment period, subject to reintroduction from outside the population. Further indirect benefits to the economy have not been quantified, and would be difficult to attribute and measure, but I have argued that they could comprise a significant extra gain for society.

One of my epidemiological models proved unsuitable for the purpose of this study. The assumption that hosts are immune to all four serotypes after two heterotypic infections is

made by other authors (for example, Ferguson et al. 1999, Billings et al. 2007), in models where the vector is represented implicitly, with only host compartments included in the model. My results show that when the vector is explicitly included, the characteristic persistent multi-annual cycles do not occur, rather oscillations are damped away. Wearing and Rohani (2006), who also modelled vectors explicitly, concluded that *temporary* cross-immunity was required to explain the multi-annual patterns exhibited in dengue data. My findings using the two-of-four serotype model are consistent with this; in effect, the key assumption that hosts can have at most two infections introduces *permanent* cross-immunity to the remaining two types once a secondary infection has occurred. This observation highlights the dangers of model assumptions such as using mass-action transmission to simplify the representation of transmission by vectors in epidemiological models.

I used a two serotype disease model to investigate the cost-effectiveness of genetic vector control, which is an appropriate simplification. Dengue-endemic countries might not have all four dengue serotypes circulating and two serotypes can cause a significant level of disease. For example, according to country reports to the Pan-American Health Organisation (PAHO) for 2008 (PAHO 2009), Honduras's nearly 19000 reported cases were infected with dengue serotypes 2 and 4, and the more than 7000 cases reported by Costa Rica were serotypes 1 and 2. In that year only Brazil (426) reported a higher incidence rate per thousand population than Honduras (288) and, in addition to those, only French Guiana (271) reported more than Costa Rica (221 in population at risk); those countries have three serotypes confirmed in circulation (Brazil: serotypes 1, 2 and 3; French Guiana: serotypes 1, 2 and 4) but many countries less affected had three or four serotypes. Where appropriate, my model could be extended to three or four serotypes, to

produce a more realistic pattern of cases prior to the simulated intervention, and so generate a more accurately projected reduction in the burden of disease.

My simulations identified an entomological threshold of about 0.67 to 0.68 adult females per person necessary to sustain dengue transmission in a susceptible population. This is consistent with another study on entomological transmission thresholds. Focks et al. (2000) used validated simulation models to estimate transmission thresholds in terms of the standing crop of pupae per person as a function of ambient air temperatures, initial immunity levels in the human population, and the size and frequency of viral introduction. My result is within the range determined by those authors, converted using their ratios of standing crops of adult females to pupae per person at a range of temperatures. It is consistent with their results at an average temperature of about 28-29°C, which is reasonable for Thailand, for example, and the data supporting my parameter values.

I estimated the value of R_0, the initial reproduction number of any dengue serotype, at 2.97. This result compares plausibly with other estimates of dengue R_0 obtained by different methods. Some older published estimates of 1.33 - 2, and a maximum of 2.5, are problematic (Gubler and Kuno 1997); for example one study used a formula developed for directly transmitted rather than vector-borne disease and another used a formula assuming a susceptible population applied to data from an area where dengue was endemic. For the 2005 outbreak in Singapore R_0 was estimated to be ~1.89-2.23 (95% confidence interval: 1.15-3.00) using a single-phase Richards (logistic-type) model (Hsieh and Ma 2009). Analysis of age-stratified serotype-specific data collected in Thailand, using various rigorous statistical methods, gave strain-specific estimates of 1.38 – 8.47 across all models,

and the best estimates put those R_0 values in the range 4 to 6 (data from Sangkawibha et al. 1984, analysed by Ferguson et al. 1999).

I have focussed on the projected number of dengue cases averted as a result of reducing the virus transmission by controlling the vector population, and assessed the estimated cost-effectiveness of that intervention for the improvement of public health. As I have not performed a full cost-benefit analysis, I have not (other than giving some preliminary estimates for potential lost tourism revenues) accounted for the wider economic benefits such as future productivity gains resulting from lives saved. In keeping the model relatively simple, I have also ignored other possible benefits within the epidemiological results. For example, I have assumed a constant case fatality rate, but this depends on the quality of care and the stage at which the patient is admitted to hospital. Hospitals can be overwhelmed during periods of intense epidemic dengue transmission (for example, in April 2008 military support was called on to expand care beyond beleaguered public hospitals in Rio de Janeiro, Brazil) and patient management may suffer at such times. By reducing the number of hospital admissions during peak dengue periods, the vector control strategy might also decrease case fatality rates and thus save further lives. The sheer scale of an epidemic can also have implications for other health costs and services; for example, the dengue outbreak in North Queensland, Australia, in 2008/9 has led to shortages of blood supplies at blood donor centres in the area (Wight 2009).

The simulated control programme costs set out in §6.3.4 are undiscounted costs at 2008 prices. I compared them with the simulated costs averted (§6.3.5) as though there were no time value of money. A formal cost-effectiveness or cost-benefit analysis would ideally determine the nature, amount and timing of the cash flows associated with those costs and

discount them to present values using an appropriate risk-adjusted discount rate. The timing of the dengue cases averted would also be modelled and the consequent cost savings discounted to present values. This model is not sophisticated enough to facilitate such analysis. The effect of discounting is to reduce the weight of cash flows further into the future; as capital costs are likely to be borne up front discounting would tend to reduce the costs saved more than the costs of the RIDL control programme and so I will tend to have somewhat overstated the benefit of genetic vector control. The costs of genetic vector control per case averted are very much smaller than the costs saved (§6.3.5). I conclude that discounting these amounts would have little impact on the relative values and would not affect the qualitative conclusions drawn.

The cost per case averted is a rather crude measure of potential cost-effectiveness of a health intervention. The World Development Report "Investing in Health" (World Bank 1993) makes extensive use of the Disability Adjusted Life Year (DALY) lost, a key measure of the burden of disease, together with cost-effectiveness, to judge which health improving interventions deserve the highest priority for public action. The DALY reflects the total amount of healthy life lost, to all causes, whether from premature mortality or from some degree of physical or mental disability over a period of time, taking into account how far into the future the loss occurs and weighting the relative importance of healthy life at different ages (Murray 1994, Homedes 1996). The effectiveness of a prevention or treatment measure can also be measured in DALYs, the reduction in disease burden that it produces. Eliminating the disease, which happens within about 6 months in my simulation, averts all of the DALYs. For an assessment quantifying the DALYS averted, the model would have to be expanded to include the age structure of host population.

This analysis could be customised for particular disease-endemic cities or countries. In principle, with relevant country- or region-specific epidemiological, entomological, healthcare and economic data, appropriate scenarios could be simulated for that country or region, detailed estimates of constructing and operating a sterile mosquito release programme could be prepared for a production facility of appropriate size and location, the projected DALYs saved could be valued at the per capita Gross National Income, and the effect on the wider impact of dengue on that economy could be assessed. In practice, such a wealth of specific data is unlikely to be available and an accurate fully inclusive cost-benefit analysis would be infeasible at least in the shorter term. However, preliminary modelling using whatever data and estimates are available (locally or from comparable regions), could be used to identify the parameter values and other estimates to which the conclusions are most sensitive and to direct experimental, ecological, economic and other studies towards filling those key knowledge gaps. The framework could also be extended to model interactions with a vaccine, which for dengue could conceivably be available within several years of genetically engineered insects.

The modelling and assessment process demonstrated here could be adapted to apply to other diseases and other scenarios. The genetically engineered lethality rather than radiation-induced sterilisation, and the robustness of *Ae. aegypti* eggs, particularly the ability to withstand desiccation, allow greater flexibility in the life stage at which RIDL males might be released. A programme involving the release of eggs into natural breeding sites would require some modifications to my vector population dynamics model because those released individuals would also contribute to density-dependent larval competition. To evaluate the prospects for genetic vector control of malaria, my approach could be

extended to include the costs and epidemiological interactions with insecticide impregnated bednets and possibly the release of more than one species of vector. The use of bednets also raises issues about the contribution of insecticide-susceptible RIDL males to resistance management, adapting the ideas in Chapters 2 to 4 of this thesis. Other alternative control strategies, such as replacement of the vector population with insects that are refractory to disease transmission, could also be modelled using the same underlying framework.

The epidemiology and assessment of cost-effectiveness could be significantly altered by modelling more than a single disease in isolation. Combined use of resources against multiple target infections could increase cost-effectiveness (for example`, see Hotez and Molyneux 2008). *Ae. aegypti* and *Ae. albopictus* each potentially transmit a number of diseases (yellow fever, for example), so control of those vectors would give a benefit against those infections already present and protection against other diseases to which the location is at risk. It would also reduce the biting nuisance, which may have further economic implications. Model assumptions and parameter values might also need to be revised; for example, one study found that the extrinsic incubation period for dengue in mosquitoes that are co-infected with microfilariae was approximately half the duration in mosquitoes infected with dengue alone (Vaughan et al. 2009).

I focused on dengue because it is a prime candidate for genetic vector control for the reasons given in §6.1.1 above. Malaria generally attracts more attention, partly because of greater mortality. But policies that improve health without affecting the length of life may actually create greater economic benefits. Some studies have shown that substantial increases in life expectancy in developing countries reduced the per capita income, or had

a delayed low positive effect on it, because the population increased quickly but the consequent decline in birth rates took decades, while other work shows that health improvements that do not extend life can nonetheless increase productivity (Bleakley 2006, Acemoglu and Johnson 2007, Ashraf et al. 2008, The Economist 2008, Bleakley 2009).

Although there has been a focus on economic arguments for control of vector-borne diseases, there are wider potential benefits to society, such as environmental improvements from reduced insecticide use. A case can be made for using public funds to finance interventions that benefit the whole community and are important for health and environmental quality (World Bank 1993). Householders tend to undervalue investments such as area-wide pollution abatement, vector control involving actions within households and research and development, so there may be grounds for public subsidy or other interventions in these circumstances. Funding, technical and other support from governments and international agencies is likely to figure prominently in any successful large-scale elimination of vector-borne disease. Tackling infectious diseases relates directly to three of the UN's Millennium Development Goals, to reduce child mortality, improve maternal health and combat diseases such as malaria, and is closely involved in two others, to eradicate extreme poverty and hunger and achieve universal primary education (May 2007). The recent involvement of large philanthropic organisations such as the Bill and Melinda Gates Foundation has brought not just significant additional funding and increased publicity, but underlined the recognition that control of diseases such as dengue and malaria is also a humanitarian goal that addresses the basic human right to a healthy life.

7 General Discussion

The Sterile Insect Technique (SIT) has been used very successfully against a range of agricultural insect pests that damage crops or livestock (Dyck et al. 2005). By applying modern genetics, especially recombinant DNA methods, techniques such as the RIDL system could potentially provide several improvements to SIT (see §1.2), to increase its cost-effectiveness and safety, extend the range of suitable species to include vectors of disease, and aid the transfer of research and development between species. I have developed theoretical frameworks and mathematical models to address questions about the effect of applying RIDL-based techniques to control insect populations in various scenarios, and explored economic aspects of these novel genetics-based interventions.

I began by considering the release of a RIDL strain of insects, not as a population control method in its own right, but as a potential tool to improve another genetic method of controlling agricultural crop pests. This approach aims to help manage resistance to the insecticidal toxins expressed by some engineered strains of crop plants. This strategy could capitalise on the genetic advances inherent in the RIDL technology that enable the expression of the engineered dominant lethal effect to be controlled, unlike sterilisation by irradiation which induces lethal genetic mutations at random. For this purpose (managing resistance) the important feature is that the lethality is female-specific, as this permits genes from released insects, including genes conferring natural susceptibility to the plant-incorporated toxins, to be introgressed into the wild population through the male line.

Chapter 2 sets out a theoretical framework for use in analysing the gene frequency evolution of resistant or susceptible alleles at a single genetic locus in a habitat comprising the engineered crops and a refuge of non-transgenic plants. This work complements

published studies on broad ecological models concerning the stable maintenance of genetic polymorphism in an arbitrarily compartmentalised habitat, and sophisticated computer models simulating resistance allele frequency evolution in target pests mostly in specific crop settings. Analysis of that simple model showed that there are critical threshold proportions for dividing a habitat that determine the equilibrium allele frequencies, and that the nature of the outcomes at these thresholds depends on the relationships between the relative genotype fitnesses (i.e. the magnitude and dominance of resistance and of fitness costs) in the two environments. This finding, the existence of critical values of key parameters with significant threshold effects, occurs repeatedly in other Chapters.

Chapters 3 and 4 present and analyse a population genetic model, based on the framework in Chapter 2, for exploring the effect of releasing males of a female-lethal RIDL strain on the gene frequency evolution of resistance to the toxins in engineered crops. The results show that such a release programme could form an effective component of a resistance management strategy by substantially slowing or reversing the spread of resistance and reducing refuge sizes. Chapter 4 incorporated a population dynamics model and showed that the RIDL releases also had a beneficial effect on the number of insects in the pest population, and so could act in synergy with the engineered crops to reduce the population directly (through the lethality of the RIDL construct) and indirectly (by managing resistance). Chapter 4 also discusses issues relevant to the economic assessment and decision-making regarding the use of RIDL strategies as a component of crop pest management regimes.

In Chapter 5 I built on this understanding of resistance evolution, but moved away from the scenario of genetically engineered crops, and assessed potential resistance to the lethal

mechanism of the RIDL system itself in the general context of using RIDL strategies to control populations of pests of importance in agriculture or public health. As with any control measure, the emergence of heritable resistance would be a potential threat to the success of a RIDL programme. Resistance would reduce lethality and so resistant alleles could be passed on through surviving progeny, whether using female-lethal or bisex-lethal genetic constructs. Modelling population genetics and population dynamics with monogenic biochemically-based resistance, I showed that releasing RIDL insects that are homozygous susceptible to the lethal genetic construct would have a built-in element of resistance dilution. This effect could prevent or limit the spread of resistance to RIDL constructs, but that is subject to a complicated array of competing selective forces.

Chapter 6 focussed on public health settings and the control of infectious diseases, using the arbovirus dengue and the vector mosquito *Aedes aegypti* as an example. I combined vector population dynamics and epidemiological models with techniques for assessing cost-effectiveness of RIDL vector control methods to disrupt the transmission of human diseases. My results predict that this strategy could effectively remove this disease from a human population in a short time scale (of the order of several months), either by eliminating the local vector population or by suppressing it to a level below the entomological threshold for sustained disease transmission. I also concluded that eliminating the disease locally should be achievable at a lower cost than the direct and indirect costs of disease that are averted, even without accounting for further indirect benefits to the economy.

The framework presented in Chapter 2 has wider potential applications than the use to which it was put in Chapters 3-4. For example, similar principles and methods could be

used to explore the evolution of anti-malarial drug resistance, modelling three types of environment (untreated hosts, treated hosts complying with the drug regimen and non-compliant treated hosts), in a haploid rather than a diploid organism. My later Chapters are more specific in their application, and future research should focus on extending and building on those models and results to answer further questions about the implementation of RIDL strategies or other genetic insect control methods.

The rationale for the proposed genetic strategies is fundamentally based on mathematical modelling. Models can be parameterised using data as it emerges from testing the strains and genetic constructs that are at various stages of development. In turn, this theoretical analysis will highlight critical performance characteristics of new strains to be measured in the lab, and key factors to be measured in field trials and full implementation. It is important for mathematical modellers to maintain close contact with experimental biologists and ecologists in this field.

One area where models have highlighted a need for more data is in characterizing the population dynamics of disease vectors. Density-dependent mortality of larvae can be a significant driver of mosquito population dynamics, so models are strongly influenced by these non-linear processes. Dye's (1984) model of *Ae. aegypti* population dynamics (see §6.2.1), based on field data from Southwood et al. (1972), has been widely used. However, as Legros et al. (2009) point out, Dye's mathematical description of density dependence relies heavily on an assumption that all mortality at first and second instar larval stages is density-dependent, which fixes the value of one parameter so that two remaining parameters can be estimated by fitting to the data. Using an alternative two-step approach to estimating the three necessary parameter values, those authors show that a

different function provides a very similar fit to the data in the range of the dataset, but that their function predicts very different dynamics from Dye's original model at low densities (which could be important for modelling a vector control programme if the transmission threshold is in that low range). Although existing field studies on density dependence (discussed in Legros et al. 2009) provide some insights into the relationship between larval density and survival, these studies are not of natural populations, are not in natural settings and conditions, confound density-dependent and density-independent factors, are inconsistent, and/or lack sufficient data points to build a full understanding of the density dependence involved. Comprehensive field studies would be needed to construct a detailed characterization of the relevant density-dependent processes.

As well as this density-dependence affecting the vector population, vector-pathogen-host interactions are complex and involve non-linear processes affecting multiple organisms, often with differing ecologies, over time and space. A theoretical (applied mathematical) approach is essential for designing control strategies and exploring the impacts of novel interventions on transmission dynamics, as well as trying to predict related outcomes such as their cost-effectiveness. For human and animal health, the objective of control strategies is not to eliminate the vector population *per se*, but to suppress it below the threshold needed to sustain disease transmission, so epidemiological models must be coupled with entomological models to study these systems. Generally these models cannot be studied entirely analytically because of the complexity of interacting processes and so numerical simulation is essential. Some analytical results can be obtained, as limiting cases or with simplified assumptions (for example, as in §6.3.1-2), which can help with interpretation. The approach I have taken is to develop models of intermediate complexity, which can be expressed as systems of explicit equations but incorporate more

biological detail than the classical vector models of the Ross-Macdonald school. These models are strategic tools to explore different processes to inform the development of novel strategies. For the tactical design of interventions being implemented in the field highly sophisticated stochastic simulation models may be required.

There remain many paths to follow to continue my work on modelling release of genetic autocidal insect strains. One area for early investigation is to expand the vector control models to assess interactions with other disease control methods. For example, insecticide-treated bednets (a key measure against malaria) currently use only a single class of chemicals (pyrethroids) and resistance is beginning to be observed (John et al. 2008). Modelling the interaction of RIDL mosquito releases with bednets, would unite the vector control and resistance management themes of my thesis. As candidate dengue vaccines progress to clinical testing (Whitehead et al. 2007), there is interest in models of the interaction of RIDL mosquito release and vaccination. With the recent publication of comprehensive and consistent global studies of the burden of disease in terms of the World Bank / WHO standard measure, Disability Adjusted Life Years (Suaya et al. 2009), there is scope to couple the output from entomological-epidemiological models with more sophisticated health economics models to examine the costs and benefits of novel vector control strategies. These could be assessed alone or in conjunction with other measures, to investigate what combinations of interventions are worth exploring in particular contexts to find an integrated approach that could be considered socially and economically optimal.

So far I have focussed on population reduction (suppression or elimination) strategies, because those are closer to field use than population replacement strategies. Mathematical models are also needed in conjunction with the latter, in which the insect vector population

is converted, by spreading a genetic construct through it, into a "refractory" strain that is unable (or less able) to transmit the disease. There is a growing literature on the molecular biology and mathematical models of refractory insects and the gene drive systems that will be needed to work against natural selection to achieve population replacement. Further variations on these approaches are also likely to emerge. For example, a "death on infection" approach has been posited. Here a genetic construct is inherited by progeny of released males and engineered conditional lethality is only triggered when a vector mosquito becomes infected, killing the vector or significantly reducing the vector's lifespan and thus its ability to incubate and transmit the pathogen. This selective killing of infected vectors is a mixture of the population replacement and population reduction strategies (Terenius et al. 2008).

With various stages of trials and regulatory approvals under way for crop pests and mosquitoes, the prospect of adding release of RIDL insects to the collection of tools available for pest management is nearing realisation. As other strategies continue to develop, in time further options will reach the stage of trials (e.g. strain evaluation in large outdoor enclosures) and open releases, at field sites with appropriate pest/vector ecology and (where applicable) disease epidemiology. These present a wide range of issues to be considered by the parties involved and challenges to be addressed, especially in the case of vector control.

Technological challenges include minimising the loss of insect fitness caused by the transgenes and, in the case of refractory strategies, the development of a suitable stable and effective genetic drive system and research into the evolutionary response of the pathogen to the anti-pathogen trait (Knols et al. 2007, Sperança and Capurro 2007). Regional

guidelines for importing and confined field releases of transgenic plant pests have been agreed for North America (North American Plant Protection Organization 2007). Guidance for genetically modified mosquito trials has been published, covering scientific recommendations on matters such as pre-trial testing, assessing risks and hazards, containment and trial conduct (Benedict et al. 2008), and ethical, legal, social and cultural considerations for site selection (Lavery et al. 2008). Potential environmental hazards and public health risks must be considered. Genetic vector control interventions will be area-wide programmes, so community consent should be obtained, and community and wider public engagement are recommended, not least because genetic engineering is controversial (Knols et al. 2007, Benedict et al. 2008, Lavery et al. 2008). The uncertainty surrounding novel technologies inevitably leads to greater regulatory scrutiny; the development of regulatory guidance specific to genetically engineered insects (e.g. to obtain permits for importation, experimental use or pilot releases) is still relatively early in its development, and the relevant regulatory authorities may not yet have been determined (Wozniak 2007). Research, including mathematical modelling, is needed to inform these decisions, particularly to aid risk assessment and evaluation of future public health benefits.

Although it has been known for over a century that mosquitoes are vectors of pathogens that cause human disease, global mortality and morbidity from such infections remain high. Dengue viruses transmitted by *Aedes* mosquitoes, the protozoan malaria parasites transmitted by anopheline mosquitoes, leishmaniasis by phlebotomine sandflies, trypanosomiasis by (*Glossina*) tsetse flies or triatomine bugs, and lymphatic filariasis by mosquitoes of several genera, in aggregate impose a major economic and social burden. As well as the search for effective new ways to deal with familiar existing arthropod-borne

diseases, the (re-)emergence of vector-borne diseases (e.g. blue tongue virus in British livestock, recent chikungunya epidemics in Reunion and Italy) continues to create new focuses for research. Genetics-based vector control is at the cutting edge of biological research, and as new experiments are performed and new ideas emerge, there is a continuing need for mathematical models to inform laboratory research, field trials, and regulators.

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