Combining refuges with transgenic insect releases for the management of an insect pest with non-recessive resistance to Bt-crops in agricultural landscapes

Tom R. Brewer[a,b,*], Michael B. Bonsall[a,b]

[a] *Department of Zoology, Mathematical Ecology Research Group, University of Oxford, Zoology Research and Administration Building, 11a Mansfield Rd, Oxford OX1 3SZ*
[b] *St. Peter's College, New Inn Hall Street, Oxford OX1 2DL United Kingdom*

Abstract

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

Keywords: Crop pest, insecticidal crops, self-limiting insects, metapopulation, population dynamics

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1. Introduction

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

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

Despite this success, the number of cases of Bt resistance is rising while the time between the commercialisation of new Bt crop strains and the emergence of resistance is decreasing, largely attributable to cases in which the assumptions of the HDR strategy are not met. A recent census identified nineteen cases of field-evolved resistance linked to either cross-resistance between Bt strains or Bt crops that failed to meet the high-dose requirement (Tabashnik and Carrière, 2017, 2019). The evolution of field-evolved resistance has also been linked to cases of non-recessive resistance (Campagne et al., 2013; Zhang et al.,

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*Corresponding author
Email address: `tom.brewer@zoo.ox.ac.uk`

2012), which greatly reduces the efficacy of refugia. Another issue is refuge non-compliance due to an unwillingness to sacrifice crop yields, resulting in the early development of resistance in South Africa (Kruger et al., 2009, 2012), India (Naik et al., 2018), and the United States (Reisig, 2017). To maintain Bt crops as a viable technology it would be prudent to develop additional resistance management tools to reinforce refuges, particularly for scenarios in which assumptions of the HDR strategy are violated.

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

Early evidence from spatially-implicit models indicated that combining the HDR strategy with releases of transgenic insects can suppress target populations, delay or reverse resistance evolution, and reduce the proportion of refuge crop required (Alphey et al., 2007, 2009; Harvey-Samuel et al., 2015; Zhou et al., 2018). Promisingly, these findings have been validated experimentally in single-population cage experiments (Harvey-Samuel et al., 2015; Zhou et al., 2018). However, cases of field-evolved resistance are often linked to violations of the HDR assumptions (Tabashnik and Carrière, 2017, 2019), and ascertaining to what extent these population and resistance management benefits are robust to low toxin doses, non-recessive resistance, and refuge non-compliance remains an important task.

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

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

2. Methods

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

2.1. Landscape Model

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

$$F^{Bt}_{xy} + F^{Ref}_{xy} = 1 \tag{1}$$

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

$$F_{xy}^{Ref} = \begin{cases} \phi, & C_{xy} = 1 \\ 0, & C_{xy} = 0 \end{cases} \tag{2}$$

$$F_{xy}^{Bt} = \begin{cases} 1-\phi, & C_{xy} = 1 \\ 1, & C_{xy} = 0 \end{cases} \tag{3}$$

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

2.2. Population Dynamics Model

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

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

$$N'_{xy,t} = \sigma_{xy,t} N_{xy,t} \tag{4}$$

where $N_{xy,t}$ represents the number of eggs in the xy^{th} field, $N'_{xy,t}$ and $\sigma_{xy,t}$ represents the number and proportion of larvae in the xy^{th} field that survive the combined effects of their genotype and natal crop type respectively. After the genotypic fitness costs have been applied, the surviving larvae experience density-dependent, intraspecific competition within their natal fields. We adopt a flexible, two-parameter form of density-dependence (Bellows, 1981; Maynard Smith and Slatkin, 1973):

$$\frac{1}{1 + (\alpha N'_{xy,t})^{\beta}} \tag{5}$$

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

$$K = \frac{\sqrt[\beta]{R_0 - 1}}{\alpha}. \tag{6}$$

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

$$N''_{xy,t} = \frac{\sigma_{xy,t} N_{xy,t}}{1 + (\alpha \sigma_{xy,t} N_{xy,t})^{\beta}} = \frac{N'_{xy,t}}{1 + (\alpha N'_{xy,t})^{\beta}}. \tag{7}$$

Survivors mature and proceed to mate. Each female mates with a single male and produces 2R$_0$ eggs, where R$_0$ represents the number of female offspring produced per adult female. Mating is panmitic and produces male and female offspring with equal probability. The number of offspring produced during the mating step is given by:

$$N_{xy,t+1} = 2R_0 F''_{xy,t} \tag{8}$$

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

2.3. Population Genetics Model

2.3.1. Resistance to Single-Toxin Bt Crops

Resistance to the Bt crop is conferred by a resistance allele, denoted *R*, at a single, autosomal, diallelic locus (herein to referred to as the S/R locus). The second allelic variant, denoted *S*, renders carriers susceptible to Bt crops. The frequencies of the resistance and susceptibility alleles in the xy^{th} field in the t^{th} generation are given by $p_{xy,t}$ and $q_{xy,t}$ respectively, where $p_{xy,t} + q_{xy,t} = 1$. The genotype at the S/R locus, *i*, has three possible allelic combinations, *SS*, *RS*, and *RR*, with each individual's probability of surviving from larvae to adulthood in Bt or refuge crop influenced by its genotype at the S/R locus. This genotype-and host crop-dependent survival occurs after density-dependent mortality and is captured by the relative fitness of each genotype *i* at the S/R locus, $\Omega_{xy,t,i\cdot}$, calculated as:

$$\Omega_{xy,t,i} = F^{Bt}_{xy,t}\omega_i + F^{Ref}_{xy,t}\upsilon_i \tag{9}$$

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

2.3.2. Transgenic Construct

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

$$\Omega_{xy,t,j} = (1 - \epsilon_j). \tag{10}$$

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

$$\Omega_{xy,t,ij} = (1 - \epsilon_j)[F^{Bt}_{xy,t}\omega_i + F^{Ref}_{xy,t}\upsilon_i]. \tag{11}$$

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

Table 1: Summary of the complete set of model parameters, their descriptions, and the values or value ranges used in this paper.

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

2.4. Releases of Transgenic Males

Transgenic males, homozygous for the transgene construct (ij = *SSLL*) are released into wild adult populations at a ratio of ρ transgenic males to males in the target population. Localised release policies are simulated by targeting transgenic releases at populations which exceed a fixed threshold ($N_{thresh} < N_{xy,t}$).

Insects are released into the populations prior to the mating phase and transgenic males mate with females with equal probability as wildtype males. The number of transgenic males released in the xy^{th} field in the t^{th} generation ($T_{xy,t}$) when these conditions are met is calculated by:

$$T_{xy,t} = \rho M''_{xy,t} \tag{12}$$

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

2.5. Dispersal

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

$$D_{xy,t} = \kappa F''_{xy,t}. \tag{13}$$

We implemented a dispersal mechanism that approximates continuous dispersal in discrete-space landscapes (Slone, 2011). In the absence of a good approximation for an agricultural pest insect dispersal distance kernel, for each dispersing female a dispersal distance and angle (θ) is generated from an inverse Gaussian distribution ($\mu = 0.4$, $\lambda^2 = 0.2$) and uniform distribution ($0 \leq \theta \leq 2\pi$) respectively. This parameterisation reflects our assumptions that dispersal direction is unbiased, the vast majority of individuals disperse short distances in agricultural landscapes with plentiful resources, while a minority of individuals undertake longer flights. Following dispersal, all females oviposit $2R_0$ eggs. All adult individuals then die, signalling the end of the generation and beginning of the next.

2.6. Simulations

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

We model a pest in which resistance is non-recessive ($\omega_{rs} > 0$) using, where available, published estimates for ecological and relative genotypic fitness parameters on single-toxin Bt-crop and refuge crop for the Diamondback moth, *Plutella xylostella L.*, a major pest of Brassica crops worldwide (Table 2). In the absence of published estimates for dispersal proportions, we assumed a constant dispersal proportion (κ) of 0.1. Estimates for carrying capacities and density-dependence parameters in agricultural landscapes are notoriously difficult to obtain, so we imposed an arbitrary field-level carrying capacity of 10,000 individuals to limit the computational cost of individual simulations. Simulations were run for 30 generations and 20 iterations were performed per parameterisation.

Table 2: Summary of ecological and relative fitness parameters for which estimates for the Diamondback moth (*Plutella xylostella*) were derived from the literature.

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

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

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

3. Results

Figure 1 presents the number of landscape-level pest population (Fig. 1a) and resistance allele (Fig. 1b) extinction events observed for a subset of the refuge and release parameters simulated. The across-field mean resistance allele frequency, across-field mean population size, and landscape colonisation dynamics are presented in Figures 2-5. Figures 5-7 show the distribution of across-field mean resistance allele frequency, across-field mean population size, and landscape colonisation proportions after 30 generations. To ease legibility, the figures presented in the main text show the results for a subset of the parameter combinations. Figures presenting the results for the full suite of parameter combinations modelled are available in the supplementary materials.

3.1. *The effect of refuge implementation and transgenic insect release regime on the frequency of population and resistance allele extinctions*

Area-wide releases ($\rho > 0$, $N_{thresh} = 0$) successfully eliminated the pest population across all release and refuge parameter combinations, except when the largest refuges ($\phi > 0.45$) were deployed with the lowest release ratio ($\rho = 0.5$). Localised releases also eliminated the pest population for all release ratios when

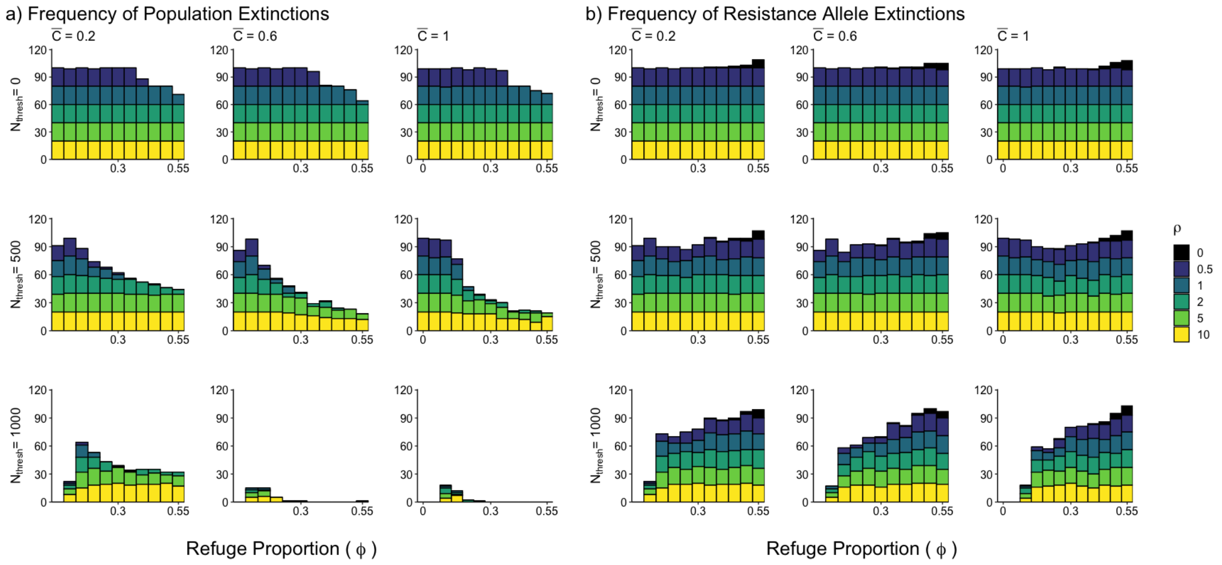

Figure 1: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level resistance allele and pest population extinctions. Individual plots within each panel present, for a subset of the combinations of release threshold (N_{thresh}), refuge compliance proportion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

N_{thresh} = 500 and $\phi < 0.1$. However, as the refuge proportion increased, a $\rho \geq 5$ was increasingly required to reliably achieve population extinction. The frequency of landscape-wide population extinctions declined dramatically when $N_{thresh} \geq 1000$ and $\bar{C} \geq 0.6$, but increased as $\bar{C}$ decreased for $\rho \geq 5$. Refuge-only strategies ($\rho = 0$) were insufficient to eliminate the pest population across all simulations.

Resistance allele extinction events were rare under refuge-only strategies, but increased in frequency as ϕ increased from 0.2 to 0.55 (Fig. 1b). Combining refuges with transgenic insect releases drove the resistance allele to extinction across the full combinatorial suite of refuge compliance proportions, refuge proportions, and release ratios for both area-wide ($N_{thresh} = 0$) and localised releases with an N_{thresh} of 500 (Fig. 2a, Fig. S2). When $N_{thresh} \geq 1000$, transgenic releases achieved landscape-wide resistance extinction when ≥ 0.35. However, when $\phi \leq 0.3$, the frequency of resistance extinctions declined with decreasing ρ and increasing N_{thresh}. Decreasing $\bar{C}$ had little effect on the frequency of resistance allele extinction.

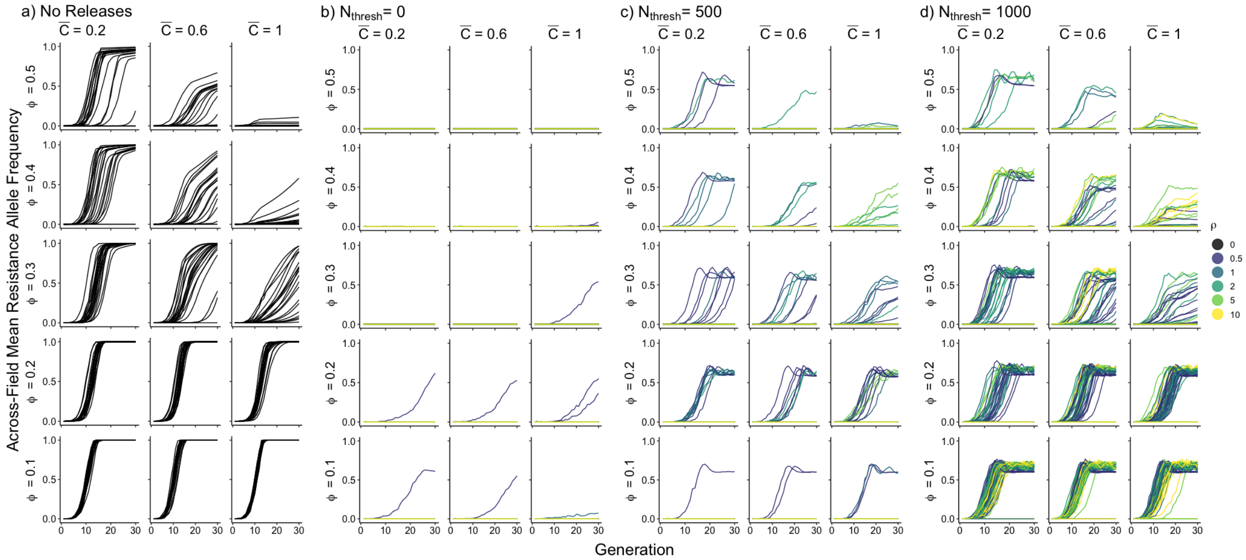

Figure 2: Across-field mean resistance allele frequency dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the across-field mean resistance allele frequency dynamics observed under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the across-field mean resistance allele frequency through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 iterations were performed per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10{,}000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

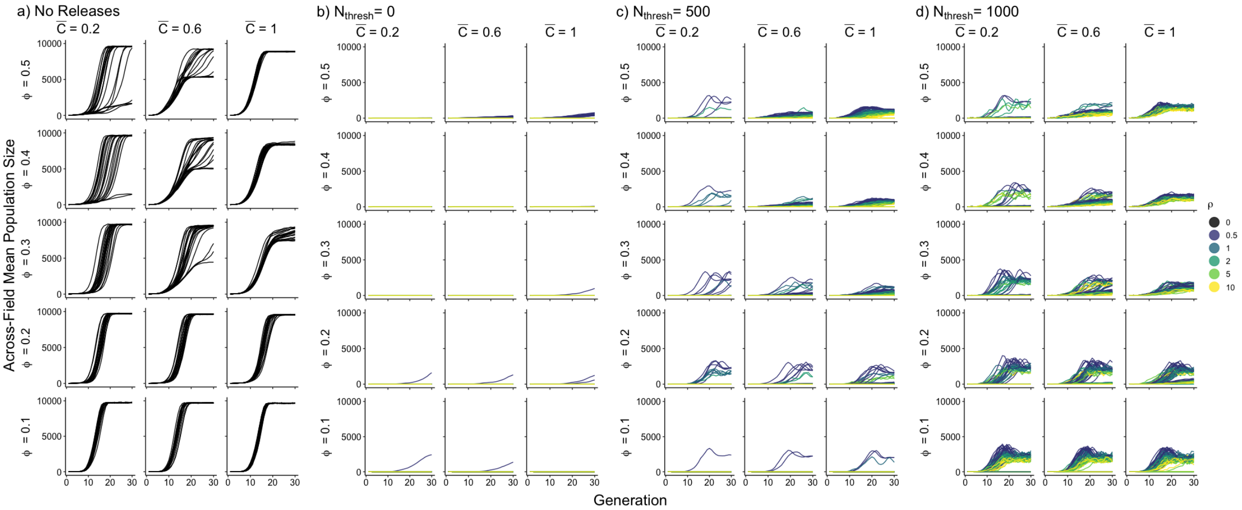

Figure 3: Across-field mean population size dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the across-field mean population size dynamics observed under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the across-field mean population size through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 iterations were performed per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: p_0 = 0.005, R_0 = 10, κ = 0.1, K = 10,000, W_{rr} = 0.82, W_{rs} = 0.205, W_{ss} = 0, V_{rr} = 0.29, V_{rs} = 0.77, V_{ss} = 1.

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

Under refuge-only strategies, the resistance allele sweeps rapidly to fixation when $\phi \leq 0.2$. Increasing ϕ slows the evolution of resistance and eventually maintains the resistance allele at low frequencies. Decreasing $\bar{C}$ when $\phi \leq 0.3$ greatly increased the rate of resistance evolution, enabling the resistance allele to reach fixation for values of ϕ which prevented it's spread in fully compliant landscapes ($\bar{C}$ = 1), but had little effect when $\phi \geq 0.2$

When insufficient to drive resistance to extinction, the degree to which releases enhanced resistance management depended on the refuge proportion and refuge compliance proportion. In landscapes dominated by Bt crop ($\bar{C} \leq 0.6$ and/or $\phi \leq 0.3$), supplementing refuges with transgenic insect releases slowed the rate of evolution (Fig. 2) and maintained the mean resistance allele at substantially lower frequencies (Fig. 5). However, as refuge crop becomes more prevalent in the landscape ($\bar{C} > 0.6$ and/or $\phi > 0.3$), the benefits to resistance management benefits offered by transgenic insects diminish. Increasing N_{thresh} increased both the rate of resistance evolution and the terminal mean resistance allele frequency, while increasing ρ slowed the rate of resistance evolution (Fig. 2), but increased the terminal mean resistance allele frequency.

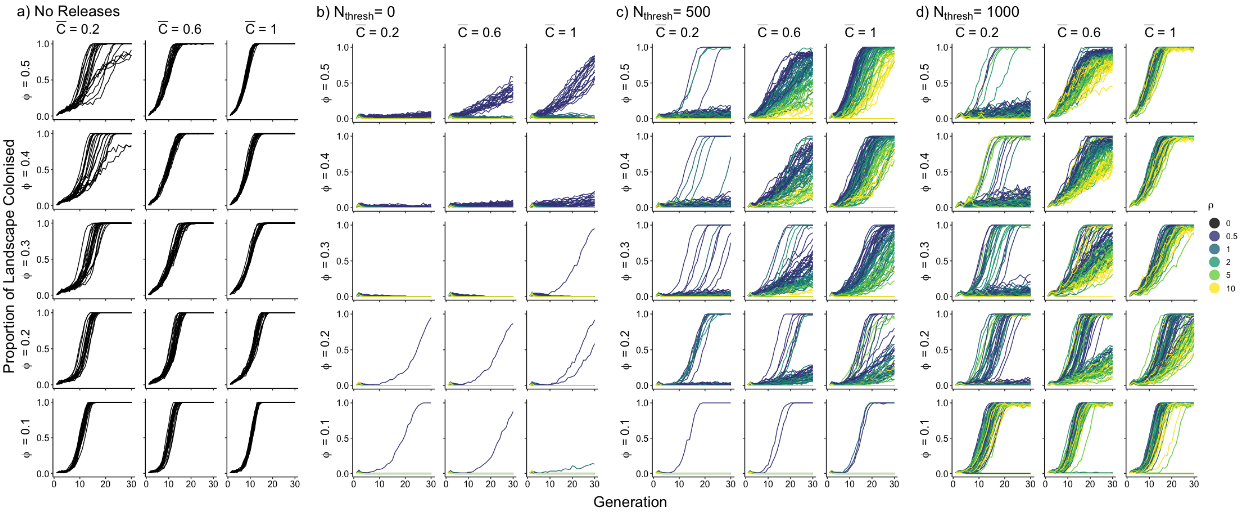

Figure 4: Landscape colonisation dynamics over 30 generations in agricultural landscapes under a range of refuge and transgenic insect release management strategies. Panel a) presents the proportion of the fields in the landscape populated through time under refuge-only management strategies, while panels b)-d) show the impact of supplementing refuges with releases of transgenic insects using release thresholds (N_{thresh}) ranging from 0 to 1000. Individual plots within panels show the colonisation dynamics observed under different scenarios of mandated refuge proportion (ϕ), proportion of fields in compliance with the mandated refuge ($\bar{C}$), and release ratio (ρ). Each line represents the proportion of the landscape colonised through time for a single simulation run, with line colour indicating the release ratio (ρ) employed. 20 iterations were performed per combination of release threshold, release ratio, refuge proportion and refuge compliance proportion. All simulations were performed on an 11x11 cell agricultural landscape lattice with the following static parameter values: p_0 = 0.005, R_0 = 10, κ = 0.1, K = 10,000, W_{rr} = 0.82, W_{rs} = 0.205, W_{ss} = 0, V_{rr} = 0.29, V_{rs} = 0.77, V_{ss} = 1.

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

Refuge-only control strategies provided little protection against the pest population which, for both the largest ($\phi \geq 0.4$) and smallest ($\phi \leq 0.15$) refuge proportions proliferated rapidly to a stable mean population equilibria that approached the fixed carrying capacity (K = 10,000)(Fig. 3a, Fig. 6a), and rapidly colonised the entire landscape (Fig. 4a, Fig. 7a). Planting intermediate refuge proportions ($0.2 \leq \phi \leq 0.3$) increased the frequency of heterozygotes, which had the lowest mean fitness across the two crop types, slowing the rate of both population growth and landscape colonisation (Fig. 3), but was insufficient to prevent the populations achieving high densities and colonising the entire landscape (Fig. 7).

Decreasing $\bar{C}$ had little effect on the population dynamics in refuge-only strategies when $\phi \leq 0.2$. However, for $\phi \geq 0.3$, when the resistance allele was extant, decreasing $\bar{C}$ increased the rate of population growth and terminal across-field mean population size. Conversely, when resistance was absent from the population, decreasing $\bar{C}$ slowed population growth (Fig. 3a) and maintained the populations of susceptible insects at increasingly lower densities (Fig. 6a).

Supplementing refuges with releases of transgenic insects dramatically decreased the population growth rate and maintained the across-field mean population at substantially lower sizes relative to refuge-only strategies across all combinations of ϕ, $\bar{C}$, N_{thresh}, and ρ simulated (Fig. 3, Fig. 6). The population suppres

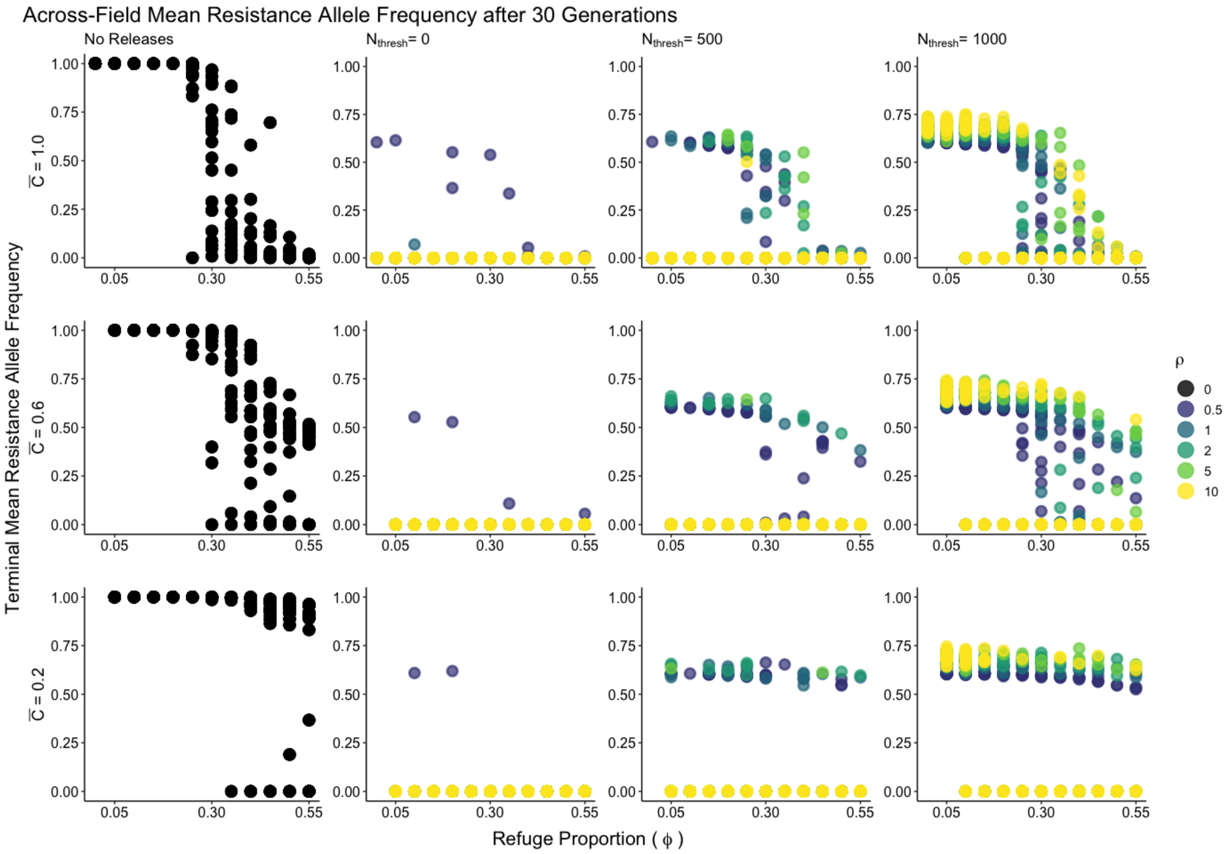

Figure 5: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean resistance allele frequency. Individual panels present the across-field mean resistance allele frequencies after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 iterations performed per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: p_0 = 0.005, R_0 = 10, κ = 0.1, K = 10,000, W_{rr} = 0.82, W_{rs} = 0.205, W_{ss} = 0, V_{rr} = 0.29, V_{rs} = 0.77, V_{ss} = 1.

-sive effect of combined management strategies were maximised by reducing N_{thresh} and increasing ρ (Fig 3b-d, Fig 4b-d, Fig. 5, Fig. 6). As in the refuge-only strategy, the magnitude of the suppressive effect of combined strategies was dependent on the extinction status of the resistance allele. Reducing $\overline{C}$ increased the suppressive effect of localised releases of transgenic releases when the resistance allele had been driven to extinction. When releases failed to eliminate resistance, the pest density increased as $\overline{C}$ declined (Fig. 3b-d, Fig. 6b-d). When $\overline{C} \geq 0.6$, releases consistently provided superior suppression relative to the HDR-only strategy. In landscapes with $\overline{C} \leq 0.4$, when resistance remained within the population the mean population density differed little between the refuge and combined strategies (Fig.6).

The effect of $\overline{C}$ on landscape colonisation rate also depended on the resistance allele status (Fig. 4b-d, Fig. 7). When releases drove the resistance allele to extinction, decreasing $\overline{C}$ and increasing ρ slowed the rate of landscape colonisation (Fig. 5), preventing the pest from colonising the entire landscape when $\overline{C} \leq$

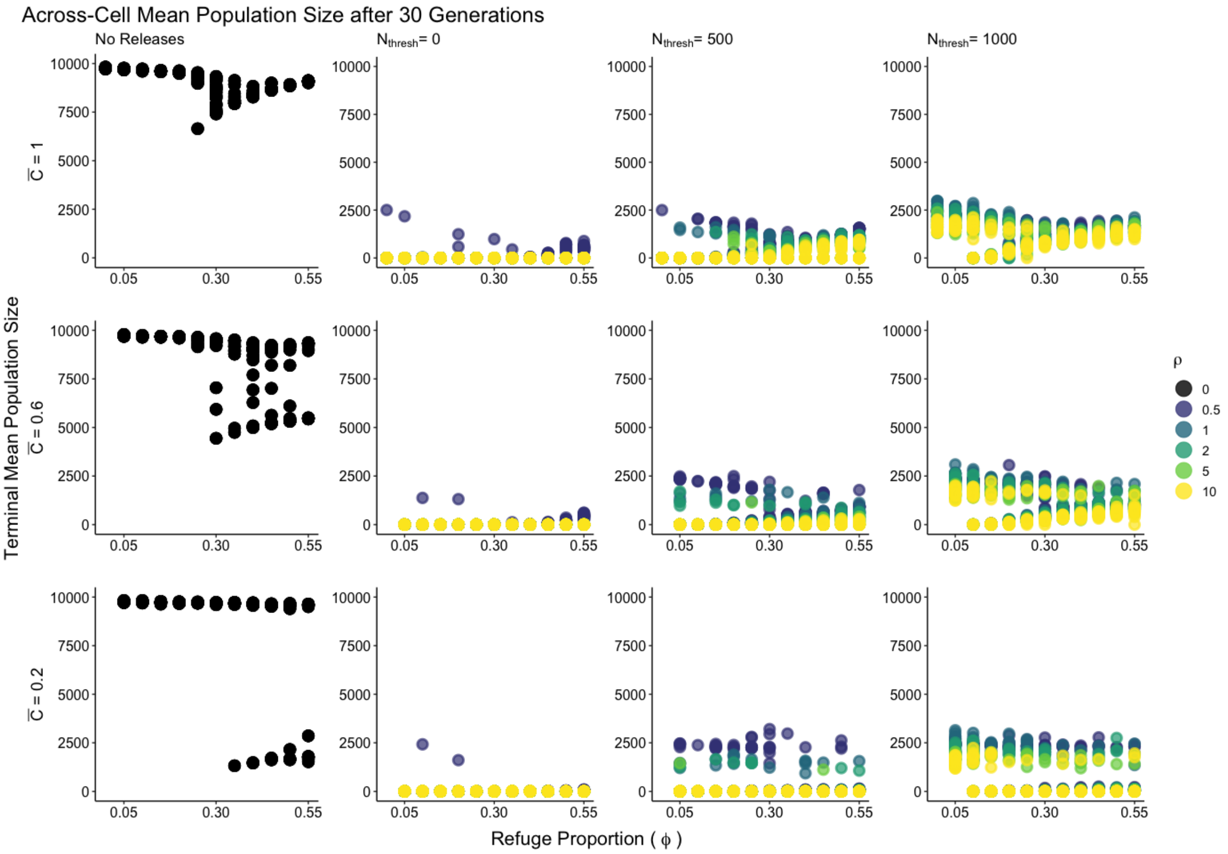

Figure 6: The effect of refuge implementation and transgenic insect release regime on the terminal across-field mean population size. Individual panels present the across-field mean population sizes after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 iterations performed per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: p_0 = 0.005, R_0 = 10, κ = 0.1, K = 10,000, W_{rr} = 0.82, W_{rs} = 0.205, W_{ss} = 0, V_{rr} = 0.29, V_{rs} = 0.77, V_{ss} = 1.

0.4 (Fig. 7). When releases failed to eliminate resistance, the rate of landscape colonisation differed little between the refuge-only and refuge and releases strategies as $\overline{C}$ decreased and the pest colonised the entire landscape within the simulated number of generations (Fig. 7).

4. Discussion

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

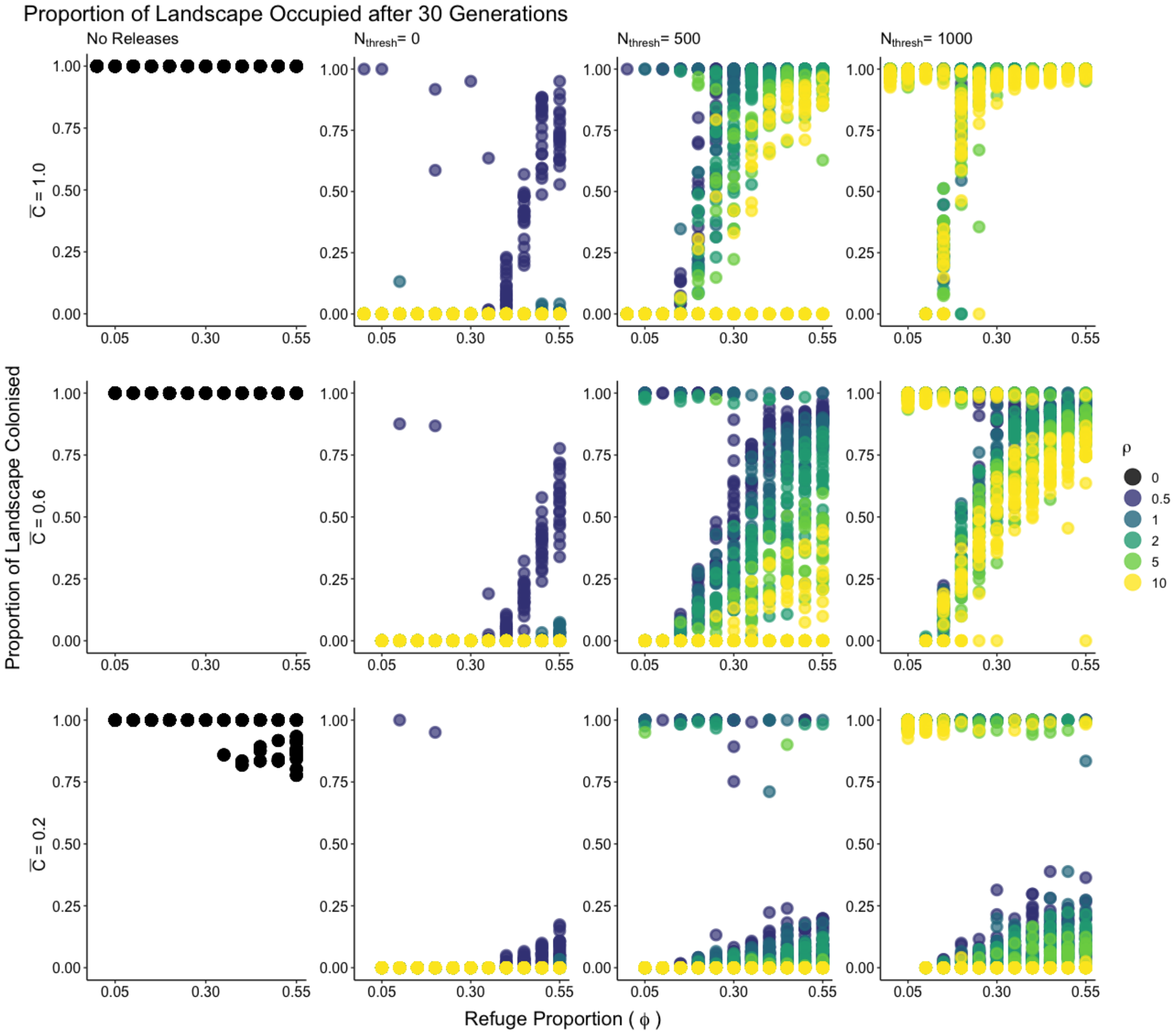

Figure 7: The effect of refuge implementation and transgenic insect release regime on the terminal proportion of the landscape colonised. Individual panels present the proportion of fields in the landscape colonised after 30 generations for all refuge proportions (ϕ) and release ratios (ρ) simulated for a subset of the release threshold (N_{thresh}) and refuge compliance proportion ($\bar{C}$) parameter combinations. Each point represents a single simulation run, with 20 iterations performed per parameter combination. All simulations were run on an 11x11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, K = 10,000, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

pyramided crops, gene drive systems).

As anticipated, combining refuges with area-wide releases (N_{thresh} = 0) eliminated the pest for the majority of refuge proportion and release ratio combinations tested (Fig. 1a, Fig. S1). That continuous releases of self-limiting, female-lethal transgenic males provides effective pest suppression, particularly when combined with an insecticide treatment or Bt crops, is theoretically (Alphey et al., 2009, 2011) and empirically (Ant et al., 2012; Leftwich et al., 2014; Harvey-Samuel et al., 2015; Zhou et al., 2018) well established. We also made some simplifying assumptions that favoured the efficacy of transgenic releases, including that the transgene works with perfect efficiency ($\epsilon_j = 1$), incurs no fitness cost to the carrier, and that released males mate with equal probability as wildtype males. However, recent evidence from laboratory and field trials suggests that self-limiting strains appear to exhibit dispersal ability, survival, and mating competency equal to that of a wildtype strain (Shelton et al., 2020). Though, in our simplified system, area-wide releases with low release ratios relied to some extent on the additional suppressive power of the transgenic crop, as evidenced by the failure of the lowest release ratios with the largest refuges to achieve eradication, eradication was always achieved when deployed with sufficiently large release ratios. Given that area-wide applications of SIT have been used successfully to eradicate pest insects, including screwworm (*Cochliomyia hominivorax*) from the southern United States (Scott et al., 2017), melon fly (*Bactrocera cucurbitae*) from the Okinawa island chain (Kakinohana et al., 1997), and medfly (*Ceratitis capitata*) from Mexico and Guatemala (Hendrichs et al., 1983), despite radiation-sterilised insects typically suffering greatly reduced competence, our results are consistent with the expectation that area-wide releases of transgenic insects are likely to be an effective technology for landscape-wide eradication programmes, particularly if integrated with additional management tools.

When insufficient to eliminate the pest population, reinforcing refuges with localised releases of transgenic insects enhanced population suppression and slowed landscape colonisation relative to refuge-only strategies across the full combinatorial range of refuge proportions, refuge compliance proportions, release ratios, and release thresholds simulated (Figs 3-4, Figs S3-S6). Recently, Zhou et al. (2019) showed, using a system of three cage populations of diamondback moth linked by manual dispersal, that targeting the cage with the largest resistant population significantly reduced the total pest population size across the three-cage metapopulation relative to no-release strategies within three generations. Our theoretical work builds on their results to show that the suppressive benefits they observed in the short-term are likely to be maintained over longer-time frames, even as resistance becomes prevalent in the population (Figs. 2-3, Fig. S3-S6). Furthermore, localised releases targeted at population hotpots (Pulliam and Danielson, 1991) greatly enhances suppression even when non-recessive resistance reduces the contribution of toxic crops to pest mortality. We also found that the most effective method for enhancing the efficacy of localised release strategies is to reduce the release threshold, while increasing the release ratio also enhances their suppressive effect. Taken together, our results support the conclusion of (Zhou et al., 2019) that localised releases could represent a viable alternative to area-wide release regimes when population suppression, rather than eradication, is an acceptable outcome.

In Bt-dominated landscapes with small refuges and/or low refuge compliance, the resistance allele evolved rapidly to fixation in the absence of releases. The efficacy of refuges for resistance management is greatly diminished when the HDR assumptions of recessive-resistance, rare resistance, and random mating are violated (Campagne et al., 2013; Carriere et al., 2010) and, as expected, large refuges ($\phi \geq 0.3$) were required to delay or prevent the rapid evolution of non-recessive resistance, with even the largest refuges insufficient to drive resistance to extinction (Fig. 1b, Fig. 2, Fig. S2, Fig. S7). While increasing the abundance of refuge crop represents one potential solution to enhance resistance management (Tabashnik and Carrière, 2019), particularly when refuges are compromised by non-recessive resistance, this may result in economically unacceptable yield sacrifices. To facilitate the planting of smaller refuges, strategies that incorporate additional insect control measures, such as releases of transgenic insects, are likely to be necessary to protect both short-term yields and the longer-term efficacy of transgenic crops (Alphey et al., 2009).

Management strategies combining refuges with release policies based on local population densities provided mixed results for the management of resistance to a single-toxin Bt crop (Fig. 1b. Fig. 2, Fig. S2, Fig. S4). When refuges were reinforced with transgenic insect releases, the resistance allele was driven to extinction when $N_{thresh} \leq 500$, or when $N_{thresh} \geq 1000$ was combined with large refuges ($\phi \geq 0.3$). However, combined strategies provided only a moderate delay to resistance evolution relative to refuge-only strategies when resistance was not driven to extinction and, although they did prevent resistance reaching from fixation, were unable to maintain resistance below 50%. Furthermore, increasing the release ratio had the undesirable consequence of increasing the mean resistance allele frequency. This phenomena was an artefact of the population-dependent release strategy; greater release ratios suppressed the population further below the threshold, increasing the amplitude and wavelength of population oscillations and therefore the number of generations between releases. The decreased frequency of releases reduced the introgression of susceptibility alleles and subjected the population to longer periods of selection for resistance between releases, enabling the resistance allele to reach greater frequencies. While our model shows that population-centred release strategies can enhance resistance management efforts, even when assumptions of the HDR strategy are violated, developing more nuanced management programmes that find optimal solutions which minimise both the short-term crop losses and longer term resistance costs (Hackett and Bonsall, 2019) would greatly enhance the benefits to both population and resistance management benefits of transgenic insect releases.

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

Availability of Data and Materials

All model and simulation scripts can be made available upon request.

Declaration of Competing Interests

The authors declared that they have no competing interests.

CRediT authorship contribution statement

Tom Brewer: Conceptualisation, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - original draft, Writing - review and editing, Visualisation. **Michael Bonsall**: Conceptualisation, Methodology, Writing - review and editing, Supervision.

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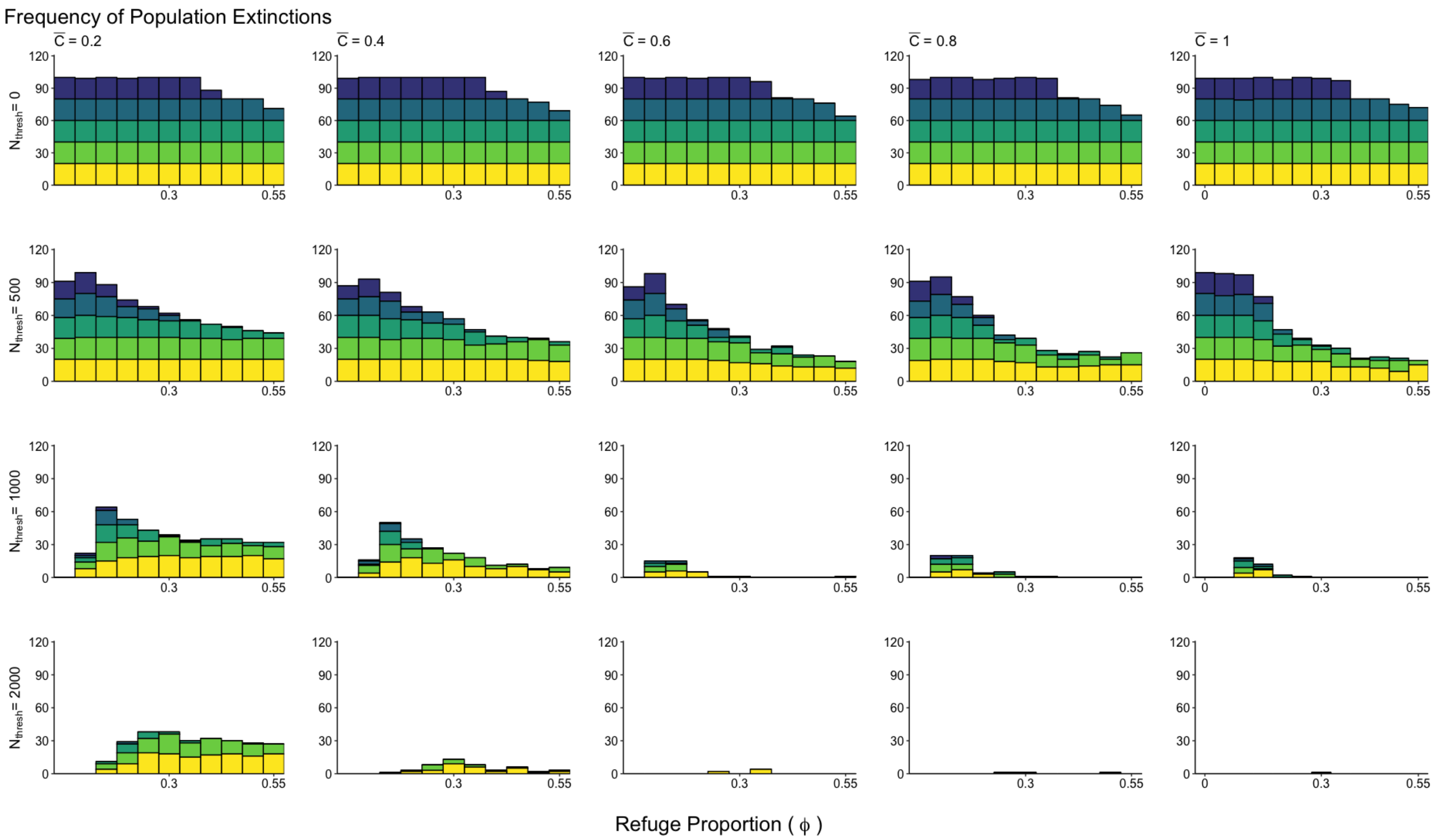
Highlights

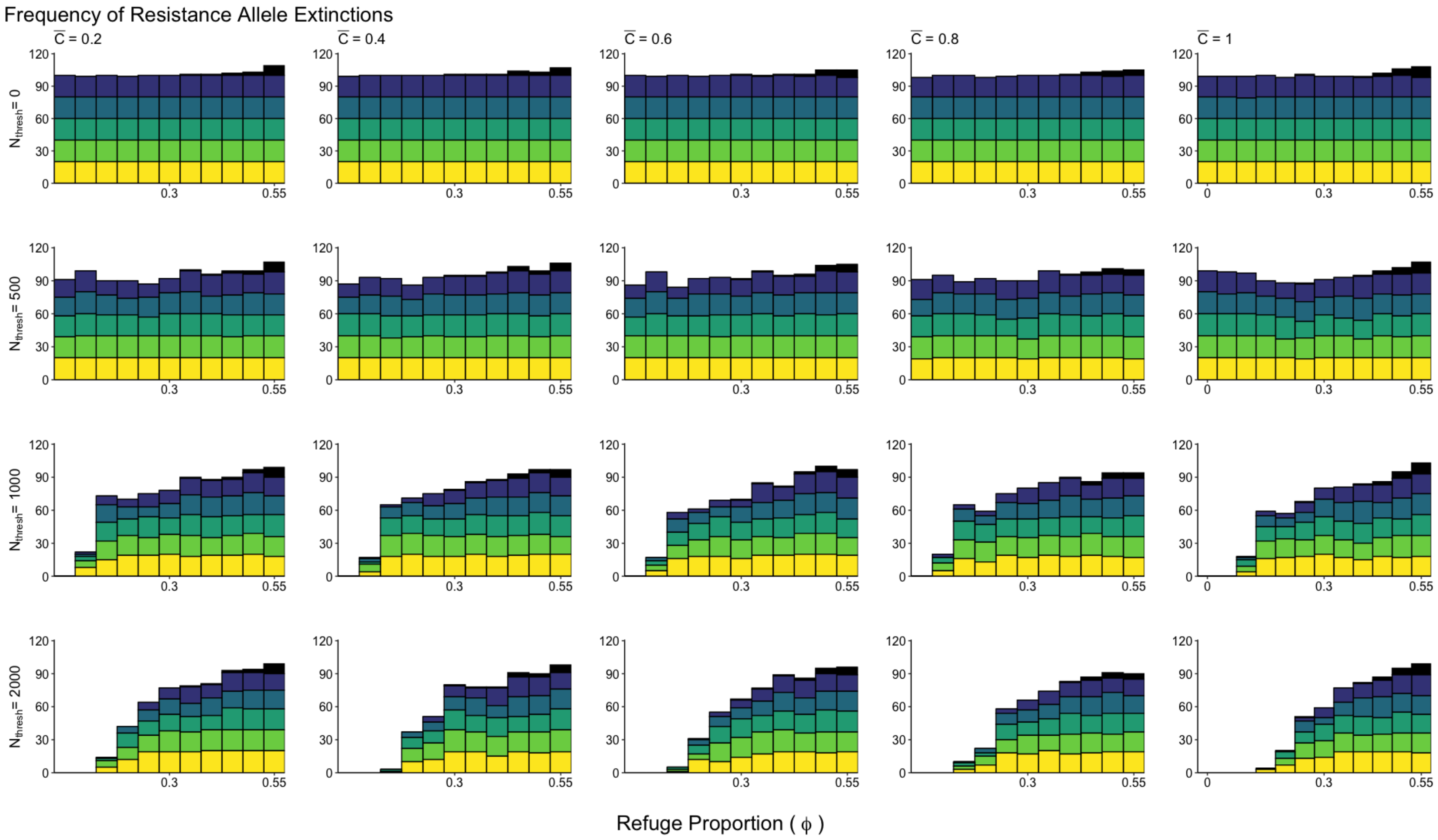
- Modelled management strategies combining transgenic insect releases with refuges
- Combining refuges with area-wide releases effectively managed pest with non-recessive resistance
- Area-wide releases reliable eradicated the pest from the landscape
- Targeting population sources achieved effective pest suppression and resistance management

Supplementary Materials

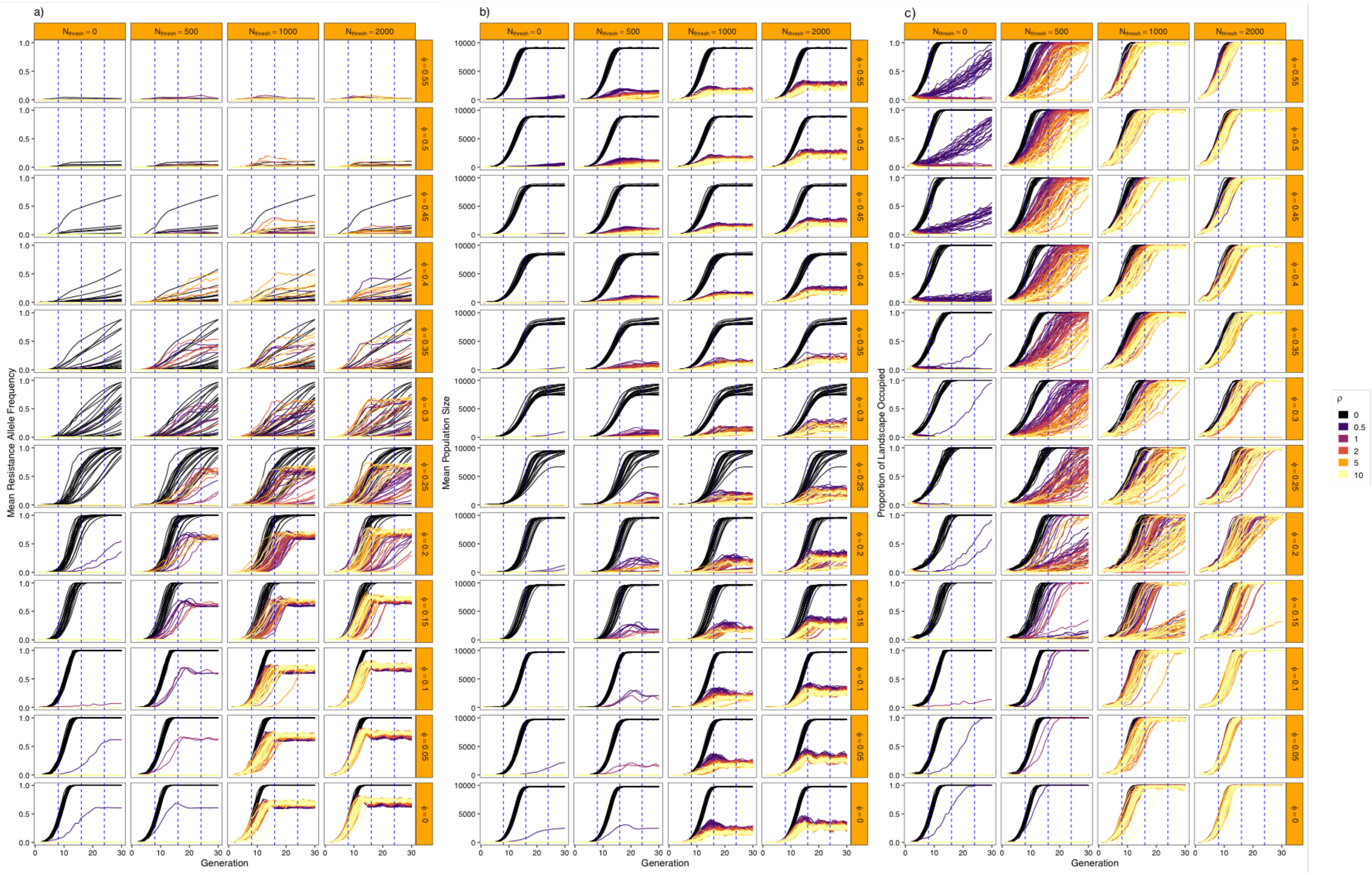
In the supplementary materials are presented versions of the figures presented in the main text with the results for the complete set of parameter combinations simulated. Note that, in Supp. Figs 3 - 6, a different colour scheme is used to enhance the legibility of the figures, but the different colours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



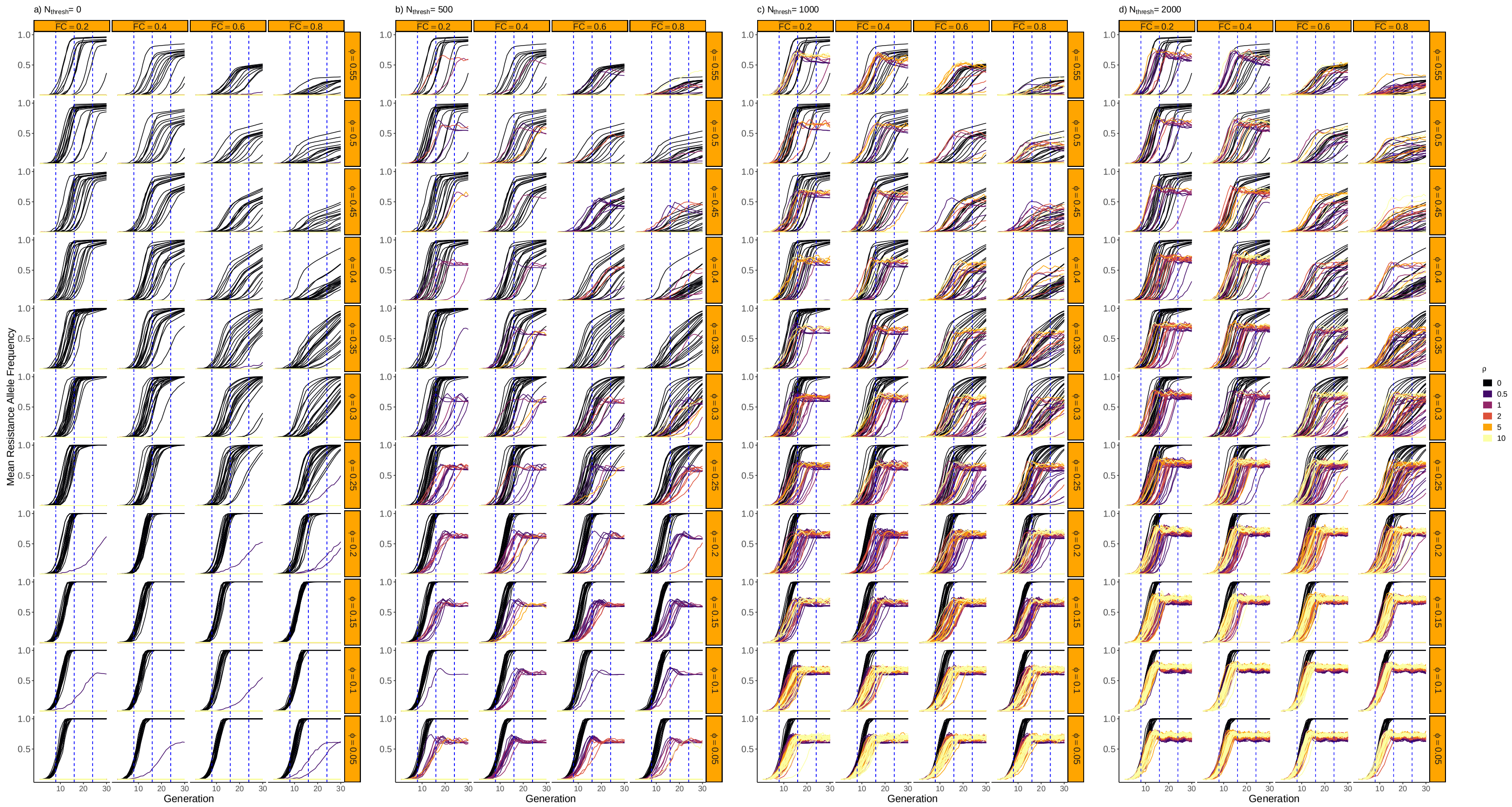
Supplementary Figure 1: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level pest population extinction. Individual plots depict the frequency of landscape-level population extinctions under the full combinatorial range of refuge proportions (ϕ),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



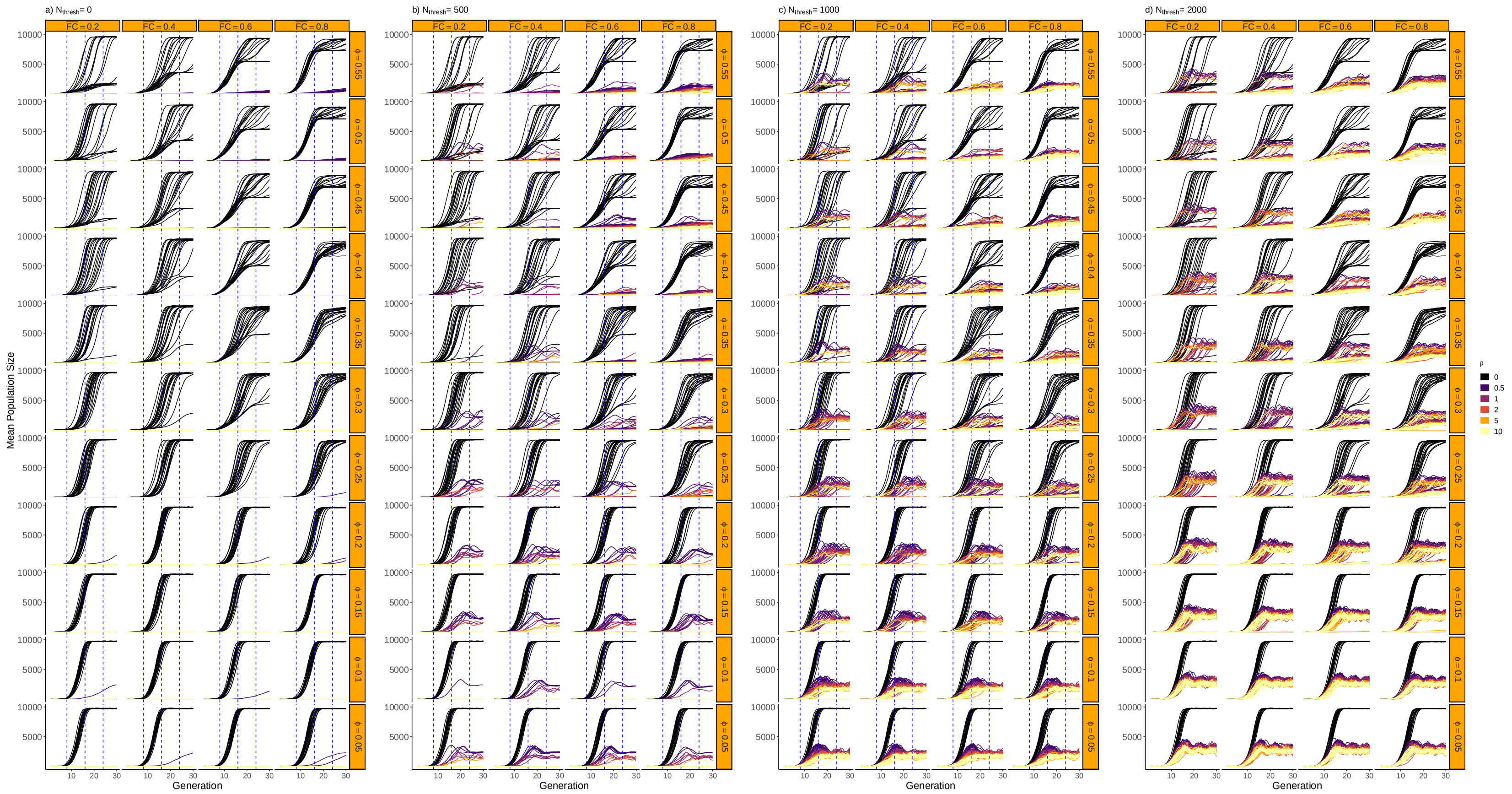
Supplementary Figure 2: The effect of refuge implementation and transgenic insect release regime on the frequency of landscape-level resistance allele extinction. Individual plots depict the frequency of landscape-level resistance allele extinctions under the full combinatorial range of refuge proportions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



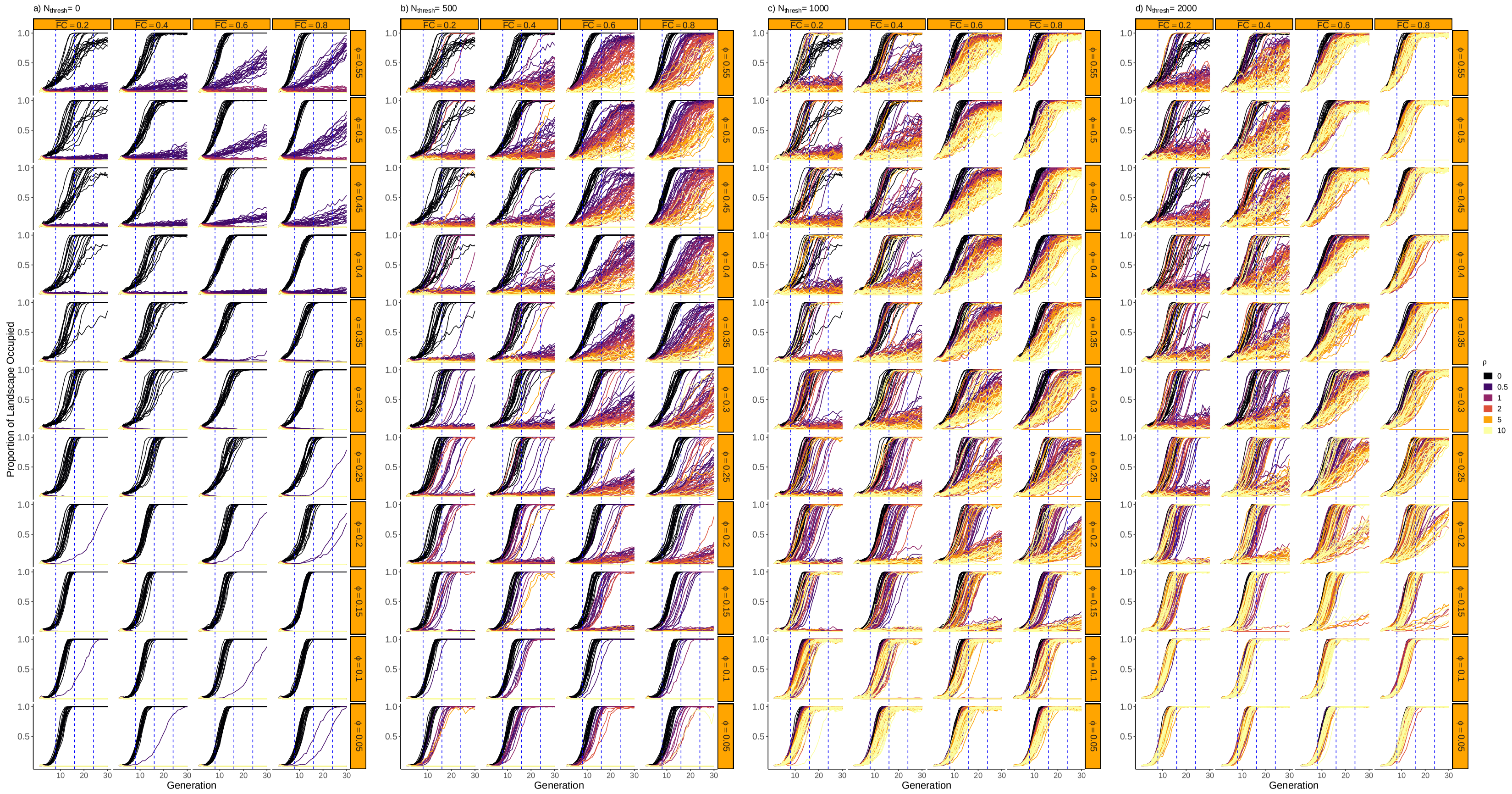
Supplementary Figure 3: Pest population and resistance allele dynamics under a range of refuge and transgenic insect release management strategies in fully-compliant agricultural landscapes. Individual panels show the dynamics of a) the across-field mean resistance allele frequency, b) the across-field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



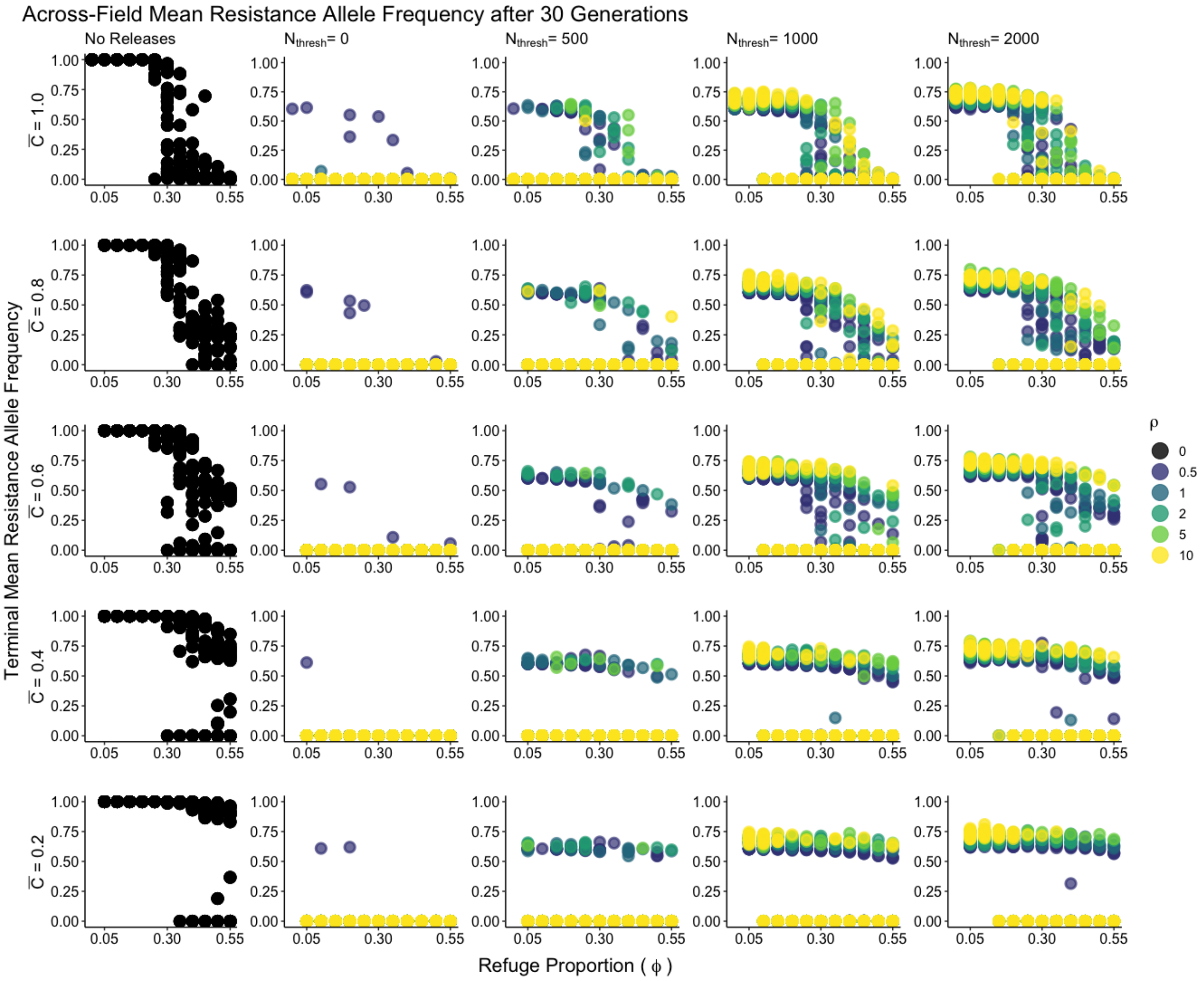
Supplementary Figure 4: Across-field mean resistance allele frequency dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels present the response of mean resistance allele frequency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



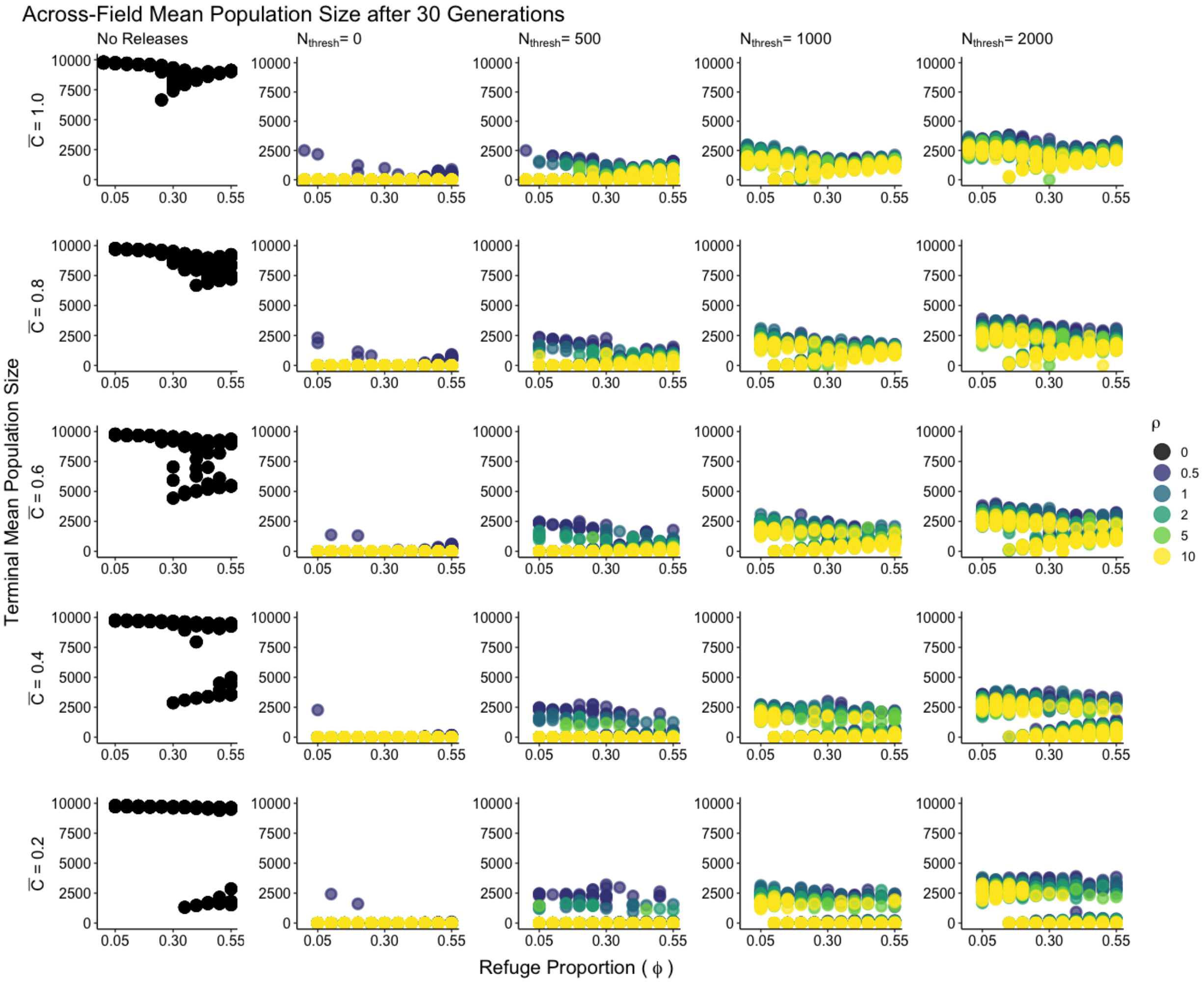
Supplementary Figure 5: Across-field mean population size dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels present the response of mean population size under a) area-wide releases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



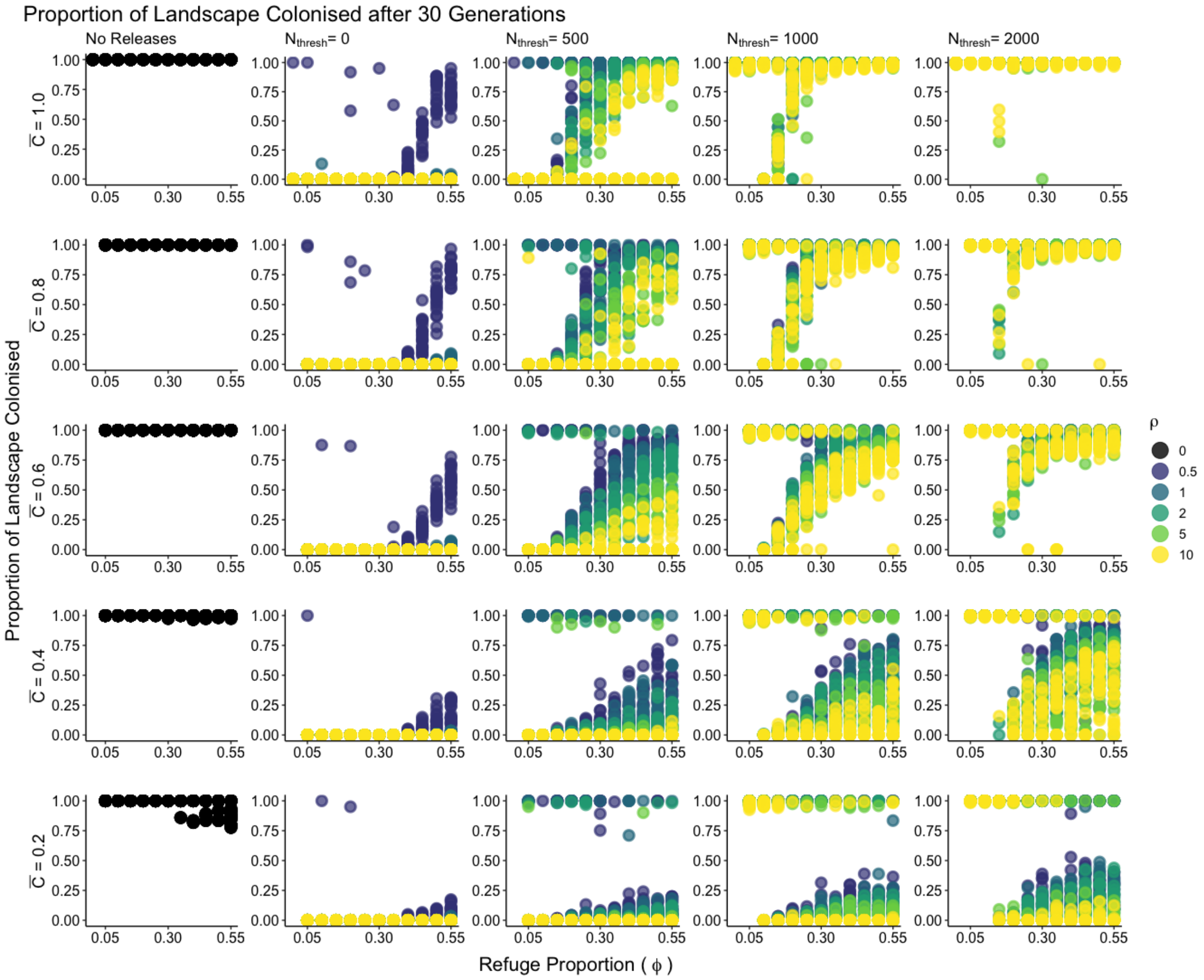
Supplementary Figure 6: Landscape colonisation dynamics over 30 generations in incompletely compliant agricultural landscapes under a range of refuge and transgenic insect release management strategies. Individual panels show the proportion of the fields in the landscape that are colonised through time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



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



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



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