

Journal Pre-proofs

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, Michael B. Bonsall

PII: S0022-5193(20)30369-6
DOI: <https://doi.org/10.1016/j.jtbi.2020.110514>
Reference: YJTBI 110514

To appear in: *Journal of Theoretical Biology*

Received Date: 3 April 2020
Accepted Date: 5 October 2020



Please cite this article as: T.R. Brewer, M.B. Bonsall, Combining refuges with transgenic insect releases for the management of an insect pest with non-recessive resistance to Bt-crops in agricultural landscapes, *Journal of Theoretical Biology* (2020), doi: <https://doi.org/10.1016/j.jtbi.2020.110514>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.

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

4 Tom R. Brewer^{a,b,*}, Michael B. Bonsall^{a,b}

5 ^aDepartment of Zoology, Mathematical Ecology Research Group, University of Oxford, Zoology Research and Administration
6 Building, 11a Mansfield Rd, Oxford OX1 3SZ

7 ^bSt. Peter's College, New Inn Hall Street, Oxford OX1 2DL United Kingdom

8 Abstract

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

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

29 1. Introduction

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

39 The strong, continuous selection pressure imposed by the toxin(s) promotes the evolution of resistance,
40 defined as a genetically-based reduction in the susceptibility of insects to Bt-toxins (Tabashnik et al., 2009;
41 Tabashnik and Carrière, 2019), threatening the long-term efficacy of these technologies (McGaughey and
42 Whalon, 1992; Tabashnik et al., 2013). The primary mechanism for managing resistance has been the High-
43 Dose / Refuge (HDR) strategy. Assuming the toxin dose is sufficient to eliminate resistant heterozygotes
44 (RS), rendering resistance functionally recessive, only homozygous-resistant (RR) individuals survive on Bt
45 crop. A sacrificial, non-toxic refuge crop is planted in close proximity to promote the survival of susceptible
46 (SS / RS) insects. If the resistance allele is rare the population of resistant homozygotes in the Bt crop will
47 be small, enhancing the probability of matings with susceptible insects from the larger refuge population.
48 Any resulting heterozygous offspring that feed on the Bt crop are eliminated before they can reproduce,
49 thereby diluting resistance. The absence of widespread resistance to Bt crops is indicative of the success of
50 the HDR strategy in delaying the evolution of Bt resistance (Huang et al., 2011; Tabashnik and Carrière,
51 2019; Tabashnik et al., 2013).

52 Despite this success, the number of cases of Bt resistance is rising while the time between the commer-
53 cialisation of new Bt crop strains and the emergence of resistance is decreasing, largely attributable to cases
54 in which the assumptions of the HDR strategy are not met. A recent census identified nineteen cases of
55 field-evolved resistance linked to either cross-resistance between Bt strains or Bt crops that failed to meet
56 the high-dose requirement (Tabashnik and Carrière, 2017, 2019). The evolution of field-evolved resistance
57 has also been linked to cases of non-recessive resistance (Campagne et al., 2013; Zhang et al., 2012), which

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

58 greatly reduces the efficacy of refugia. Another issue is refuge non-compliance due to an unwillingness to
59 sacrifice crop yields, resulting in the early development of resistance in South Africa (Kruger et al., 2009,
60 2012), India (Naik et al., 2018), and the United States (Reisig, 2017). To maintain Bt crops as a viable
61 technology it would be prudent to develop additional resistance management tools to reinforce refuges,
62 particularly for scenarios in which assumptions of the HDR strategy are violated.

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

72 Early evidence from spatially-implicit models indicated that combining the HDR strategy with releases
73 of transgenic insects can suppress target populations, delay or reverse resistance evolution, and reduce the
74 proportion of refuge crop required (Alphey et al., 2007, 2009; Harvey-Samuel et al., 2015; Zhou et al.,
75 2018). Promisingly, these findings have been validated experimentally in single-population cage experiments
76 (Harvey-Samuel et al., 2015; Zhou et al., 2018). However, cases of field-evolved resistance are often linked to
77 violations of the HDR assumptions (Tabashnik and Carrière, 2017, 2019), and ascertaining to what extent
78 these population and resistance management benefits are robust to low toxin doses, non-recessive resistance,
79 and refuge non-compliance remains an important task.

80 Furthermore, while the effects of landscape structure and pest ecology on the efficacy of the HDR
81 strategy have been explored extensively (Peck et al., 1999; Storer et al., 2003; Huang et al., 2017; Vacher
82 et al., 2003), how transgenic releases could be integrated into landscape-level management strategies is less
83 well characterised. Sterile Insect Technique, the forerunner of modern transgenic release technologies, was
84 typically deployed in area-wide eradication schemes. However, these programmes are typically expensive
85 and require coordination across stakeholder groups over large areas (Dyck et al., 2006). If population
86 suppression and resistance management rather than eradication is an acceptable outcome, localised releases
87 targeted at population hotspots could provide an effective alternative to area-wide release programmes Zhou
88 et al. (2019). The smaller-scale and enhanced flexibility afforded by localised releases recommend them as

89 a tool in resistance management scenarios in which violated HDR assumptions render refuge-only strategies
 90 vulnerable, but more work is required to characterise the short and long-term population and evolutionary
 91 dynamic consequences of these strategies.

92 Here, we developed an existing spatially-implicit population genetics and dynamics model (Alphey et al.,
 93 2009) into a metapopulation model for the investigation of pest management strategies combining transgenic
 94 insect releases with refuges in agricultural landscapes. We used this model to simulate a generic agricultural
 95 pest with non-recessive resistance under a range of management strategies. Our objectives were to: 1) assess
 96 whether supplementing refuges with transgenic insect releases represents a viable strategy when the "high-
 97 dose" assumption is not met; 2) compare the dynamics observed when refuges are paired with area-wide and
 98 localised release regimes; and 3) determine the effect of refuge non-compliance on the efficacy of refuge and
 99 release strategies. In addressing these objectives, we aimed to expand on work investigating the potential of
 100 transgenic insect releases as a resistance management tool by considering their use in scenarios linked with
 101 cases of field-evolved resistance.

102 2. Methods

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

108 2.1. Landscape Model

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

$$F_{xy}^{Bt} + F_{xy}^{Ref} = 1 \quad (1)$$

113
 114 where F_{xy}^{Bt} and F_{xy}^{Ref} represent the proportions of Bt crop and refuge crop in the xy^{th} field respectively. The
 115 proportion of Bt crop and refuge crop planted in each field is determined by the farmers compliance with

116 the mandated refuge (C_{xy}). We assume farmers adopt one of two actions: i) comply with the mandated
 117 refuge proportion ($C_{xy} = 1$), or ii) fail to comply with the mandated refuge and plant their entire field with
 118 Bt-crop ($C_{xy} = 0$), such that the proportion of Bt crop and refuge crop in the xy^{th} field are calculated as
 119 follows:

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

120

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

121

122 where ϕ represents the mandated refuge crop proportion. The landowner's planting choice is assumed to be
 123 independent and invariant through time such that a proportion of fields in the landscape, \bar{C} , are compliant
 124 with the refuge. Toroidal boundaries are modelled to avoid edge-effects and approximate the dynamics
 125 observable on larger landscape lattices on a smaller, less computationally-expensive system (Ives et al.,
 126 2011).

127 2.2. Population Dynamics Model

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

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

$$N'_{xy,t} = \sigma_{xy,t} N_{xy,t} \quad (4)$$

137 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

138 proportion of larvae in the xy^{th} field that survive the combined effects of their genotype and natal crop type
 139 respectively. After the genotypic fitness costs have been applied, the surviving larvae experience density-
 140 dependent, intraspecific competition within their natal fields. We adopt a flexible, two-parameter form of
 141 density-dependence (Bellows, 1981; Maynard Smith and Slatkin, 1973):

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

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

$$K = \frac{\beta \sqrt{R_0 - 1}}{\alpha}. \quad (6)$$

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

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

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

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

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

151 2.3. Population Genetics Model

152 2.3.1. Resistance to Single-Toxin Bt Crops

153 Resistance to the Bt crop is conferred by a resistance allele, denoted R, at a single, autosomal, diallelic
 154 locus (herein to referred to as the S/R locus). The second allelic variant, denoted S, renders carriers
 155 susceptible to Bt crops. The frequencies of the resistance and susceptibility alleles in the xy^{th} field in the t^{th}
 156 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,
 157 i , has three possible allelic combinations, SS, RS, and RR, with each individual's probability of surviving
 158 from larvae to adulthood in Bt or refuge crop influenced by its genotype at the S/R locus. This genotype-

159 and host crop-dependent survival occurs after density-dependent mortality and is captured by the relative
 160 fitness of each genotype i at the S/R locus, $\Omega_{xy,t,i}$, calculated as:

$$\Omega_{xy,t,i} = F_{xy,t}^{Bt} \omega_i + F_{xy,t}^{Ref} \nu_i \quad (9)$$

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

163 2.3.2. Transgenic Construct

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

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

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

$$\Omega_{xy,t,ij} = (1 - \epsilon_j)[F_{xy,t}^{Bt} \omega_i + F_{xy,t}^{Ref} \nu_i]. \quad (11)$$

177 Thus, when $\epsilon_j = 1$, $\Omega_{xy,t,ij} = 0$. Though in reality there may be additional energetic and fitness costs
 178 associated with the lethal allele that affect important ecological pest parameters (e.g. fecundity, dispersal
 179 distance, longevity), we opt to model the simpler scenario in which the lethal allele incurs no additional
 180 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
\bar{C}	Proportion of farmers compliant with the mandatory refuge	$0 \leq \bar{C} \leq 1$
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	$0 \leq \omega_i \leq 1$
ν_i	Relative fitness of genotype i on refuge crop	$0 \leq \nu_i \leq 1$
$\Omega_{xy,ij}$	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	

181

182 2.4. Releases of Transgenic Males

183 Transgenic males, homozygous for the transgene construct ($ij = SLL$) are released into wild adult
184 populations at a ratio of ρ transgenic males to males in the target population. Localised release policies are

185 simulated by targeting transgenic releases at populations which exceed a fixed threshold ($N_{\text{thresh}} < N_{xy,t}$).
 186 Insects are released into the populations prior to the mating phase and transgenic males mate with females
 187 with equal probability as wildtype males. The number of transgenic males released in the xy^{th} field in the
 188 t^{th} generation ($T_{xy,t}$) when these conditions are met is calculated by:

$$T_{xy,t} = \rho M''_{xy,t} \quad (12)$$

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

192 2.5. Dispersal

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

$$D_{xy,t} = \kappa F''_{xy,t}. \quad (13)$$

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

207 2.6. Simulations

208 This system was used to simulate the control of an agricultural pest using strategies combining refuges
 209 with transgenic insect releases under a challenging scenario in which Bt crops fail to eliminate 99.9% of

210 heterozygote resistant individuals, thus violating the "high-dose" assumption of the HDR strategy. The
 211 central field was initiated with a population of 1000 individuals with an even sex-ratio and an initial resistance
 212 allele frequency of 0.5% ($p_0 = 0.005$), with all resistant individuals homozygous for the resistance allele
 213 (RRWW). The remaining fields begin uncolonised and the landscape is naive to transgenic insects ($L_0 = 0$).

214 We model a pest in which resistance is non-recessive ($\omega_{rs} > 0$) using, where available, published estimates
 215 for ecological and relative genotypic fitness parameters on single-toxin Bt-crop and refuge crop for the
 216 Diamondback moth, *Plutella xylostella* L., a major pest of Brassica crops worldwide (Table 2). In the
 217 absence of published estimates for dispersal proportions, we assumed a constant dispersal proportion (κ)
 218 of 0.1. Estimates for carrying capacities and density-dependence parameters in agricultural landscapes are
 219 notoriously difficult to obtain, so we imposed an arbitrary field-level carrying capacity of 10,000 individuals
 220 to limit the computational cost of individual simulations. Simulations were run for 30 generations and 20
 221 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	-
v_{rr}	0.29	Liao et al. (2019)
v_{rs}	0.77	Liao et al. (2019)
v_{ss}	1	-
R_0	10	Liao et al. (2019)

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

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

234 control strategies.

235 3. Results

236 Figure 1 presents the number of landscape-level pest population (Fig. 1a) and resistance allele (Fig.
 237 1b) extinction events observed for a subset of the refuge and release parameters simulated. The across-field
 238 mean resistance allele frequency, across-field mean population size, and landscape colonisation dynamics are
 239 presented in Figures 2-5. Figures 5-7 show the distribution of across-field mean resistance allele frequency,
 240 across-field mean population size, and landscape colonisation proportions after 30 generations. To ease
 241 legibility, the figures presented in the main text show the results for a subset of the parameter combinations.
 242 Figures presenting the results for the full suite of parameter combinations modelled are available in the
 243 supplementary materials.

244 3.1. The effect of refuge implementation and transgenic insect release regime on the frequency of population 245 and resistance allele extinctions

246 Area-wide releases ($\rho > 0$, $N_{thresh} = 0$) successfully eliminated the pest population across all release and
 247 refuge parameter combinations, except when the largest refuges ($\phi > 0.45$) were deployed with the lowest
 248 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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

249 $N_{thresh} = 500$ and $\phi < 0.1$. However, as the refuge proportion increased, a $\rho \geq 5$ was increasingly required
 250 to reliably achieve population extinction. The frequency of landscape-wide population extinctions declined
 251 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
 252 ($\rho = 0$) were insufficient to eliminate the pest population across all simulations.

253 Resistance allele extinction events were rare under refuge-only strategies, but increased in frequency as ϕ
 254 increased from 0.2 to 0.55 (Fig. 1b). Combining refuges with transgenic insect releases drove the resistance
 255 allele to extinction across the full combinatorial suite of refuge compliance proportions, refuge proportions,
 256 and release ratios for both area-wide ($N_{thresh} = 0$) and localised releases with an N_{thresh} of 500 (Fig. 2a, Fig.

257 S2). When $N_{thresh} \geq 1000$, transgenic releases achieved landscape-wide resistance extinction when ≥ 0.35 .
 258 However, when $\phi \leq 0.3$, the frequency of resistance extinctions declined with decreasing ρ and increasing
 259 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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

260 3.2. Combined release strategies provided enhanced resistance management in Bt crop dominated landscapes

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

266 When insufficient to drive resistance to extinction, the degree to which releases enhanced resistance
 267 management depended on the refuge proportion and refuge compliance proportion. In landscapes dominated
 268 by Bt crop ($\bar{C} \leq 0.6$ and/or $\phi \leq 0.3$), supplementing refuges with transgenic insect releases slowed the rate
 269 of evolution (Fig. 2) and maintained the mean resistance allele at substantially lower frequencies (Fig. 5).
 270 However, as refuge crop becomes more prevalent in the landscape ($\bar{C} > 0.6$ and/or $\phi > 0.3$), the benefits to
 271 resistance management benefits offered by transgenic insects diminish. Increasing N_{thresh} increased both the
 272 rate of resistance evolution and the terminal mean resistance allele frequency, while increasing ρ slowed the
 273 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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

274 3.3. Population suppressive effect of localised releases enhanced with low release thresholds and higher 275 release ratios

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

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

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

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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

291 -sive effect of combined management strategies were maximised by reducing N_{thresh} and increasing ρ (Fig
292 3b-d, Fig 4b-d, Fig. 5, Fig. 6). As in the refuge-only strategy, the magnitude of the suppressive effect of
293 combined strategies was dependent on the extinction status of the resistance allele. Reducing \bar{C} increased

294 the suppressive effect of localised releases of transgenic releases when the resistance allele had been driven to
 295 extinction. When releases failed to eliminate resistance, the pest density increased as \bar{C} declined (Fig. 3b-d,
 296 Fig. 6b-d). When $\bar{C} \geq 0.6$, releases consistently provided superior suppression relative to the HDR-only
 297 strategy. In landscapes with $\bar{C} \leq 0.4$, when resistance remained within the population the mean population
 298 density differed little between the refuge and combined strategies (Fig.6).

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

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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

302 0.4 (Fig. 7). When releases failed to eliminate resistance, the rate of landscape colonisation differed little
 303 between the refuge-only and refuge and releases strategies as \bar{C} decreased and the pest colonised the entire
 304 landscape within the simulated number of generations (Fig. 7).

305 4. Discussion

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

318 pyramided crops, gene drive systems).

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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

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

339 When insufficient to eliminate the pest population, reinforcing refuges with localised releases of transgenic
 340 insects enhanced population suppression and slowed landscape colonisation relative to refuge-only strategies
 341 across the full combinatorial range of refuge proportions, refuge compliance proportions, release ratios,
 342 and release thresholds simulated (Figs 3-4, Figs S3-S6). Recently, Zhou et al. (2019) showed, using a
 343 system of three cage populations of diamondback moth linked by manual dispersal, that targeting the
 344 cage with the largest resistant population significantly reduced the total pest population size across the
 345 three-cage metapopulation relative to no-release strategies within three generations. Our theoretical work

346 builds on their results to show that the suppressive benefits they observed in the short-term are likely to
347 be maintained over longer-time frames, even as resistance becomes prevalent in the population (Figs. 2-3,
348 Fig. S3-S6). Furthermore, localised releases targeted at population hotspots (Pulliam and Danielson, 1991)
349 greatly enhances suppression even when non-recessive resistance reduces the contribution of toxic crops
350 to pest mortality. We also found that the most effective method for enhancing the efficacy of localised
351 release strategies is to reduce the release threshold, while increasing the release ratio also enhances their
352 suppressive effect. Taken together, our results support the conclusion of (Zhou et al., 2019) that localised
353 releases could represent a viable alternative to area-wide release regimes when population suppression, rather
354 than eradication, is an acceptable outcome.

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

366 Management strategies combining refuges with release policies based on local population densities pro-
367 vided mixed results for the management of resistance to a single-toxin Bt crop (Fig. 1b, Fig. 2, Fig. S2,
368 Fig. S4). When refuges were reinforced with transgenic insect releases, the resistance allele was driven to
369 extinction when $N_{thresh} \leq 500$, or when $N_{thresh} \geq 1000$ was combined with large refuges ($\phi \geq 0.3$). However,
370 combined strategies provided only a moderate delay to resistance evolution relative to refuge-only strate-
371 gies when resistance was not driven to extinction and, although they did prevent resistance reaching from
372 fixation, were unable to maintain resistance below 50%. Furthermore, increasing the release ratio had the
373 undesirable consequence of increasing the mean resistance allele frequency. This phenomena was an artefact
374 of the population-dependent release strategy; greater release ratios suppressed the population further below
375 the threshold, increasing the amplitude and wavelength of population oscillations and therefore the number
376 of generations between releases. The decreased frequency of releases reduced the introgression of suscep-

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

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

396 Availability of Data and Materials

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

398 Declaration of Competing Interests

399 The authors declared that they have no competing interests.

400 CRediT authorship contribution statement

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

404 Acknowledgements

405 We thank Catherine Reavey and Neil Morrison for their valuable contributions during the conceptu-
 406 alisation and model development phases, and their insightful comments on the draft manuscript. T.R.B
 407 is supported by the BBSRC Industrial CASE studentship BB/R505067/1. M.B.B. was supported by two
 408 BBSRC grant nos. (BB/H01814X/1 and BB/L00948X/1).

409 References

- 410 Ali, M., Bari, M., Haque, S., Kabir, M., Afrin, S., Nowrin, F., Islam, M., Landis, D., 2019. Establishing next-generation pest
 411 control services in rice fields: eco-agriculture. *Scientific Reports* 9 (1), 1–9.
- 412 Alphey, L., 2014. Genetic control of mosquitoes. *Annual Review of Entomology* 59, 205–224.
- 413 Alphey, N., Bonsall, M. B., Alphey, L., 2009. Combining pest control and resistance management: synergy of engineered insects
 414 with Bt crops. *Journal of Economic Entomology* 102 (2), 717–732.
- 415 Alphey, N., Bonsall, M. B., Alphey, L., 2011. Modelling resistance to genetic control of insects. *Journal of Theoretical Biology*
 416 270 (1), 42–55.
- 417 Alphey, N., Coleman, P. G., Donnelly, C. A., Alphey, L., 2007. Managing insecticide resistance by mass release of engineered
 418 insects. *Journal of Economic Entomology* 100 (5), 1642–1649.
- 419 Ant, T., Koukidou, M., Rempoulakis, P., Gong, H.-F., Economopoulos, A., Vontas, J., Alphey, L., 2012. Control of the olive
 420 fruit fly using genetics-enhanced sterile insect technique. *BMC Biology* 10 (1), 51.
- 421 Bellows, T., 1981. The descriptive properties of some models for density dependence. *The Journal of Animal Ecology*, 139–156.
- 422 Campagne, P., Kruger, M., Pasquet, R., Le Ru, B., Van den Berg, J., 2013. Dominant inheritance of field-evolved resistance to
 423 Bt corn in *Busseola fusca*. *PLoS One* 8 (7), e69675.
- 424 Carpenter, J. E., 2010. Peer-reviewed surveys indicate positive impact of commercialized GM crops. *Nature Biotechnology*
 425 28 (4), 319.
- 426 Carriere, Y., Crowder, D. W., Tabashnik, B. E., 2010. Evolutionary ecology of insect adaptation to bt crops. *Evolutionary*
 427 *Applications* 3 (5-6), 561–573.
- 428 Dainese, M., Martin, E. A., Aizen, M. A., Albrecht, M., Bartomeus, I., Bommarco, R., Carvalheiro, L. G., Chaplin-Kramer,
 429 R., Gagic, V., Garibaldi, L. A., et al., 2019. A global synthesis reveals biodiversity-mediated benefits for crop production.
 430 *Science Advances* 5 (10), eaax0121.
- 431 de Valdez, M. R. W., Nimmo, D., Betz, J., Gong, H.-F., James, A. A., Alphey, L., Black, W. C., 2011. Genetic elimination of
 432 dengue vector mosquitoes. *Proceedings of the National Academy of Sciences* 108 (12), 4772–4775.
- 433 Dyck, V. A., Hendrichs, J., Robinson, A. S., 2006. Sterile insect technique: principles and practice in area-wide integrated pest
 434 management. Springer.
- 435 Edgerton, M. D., Fridgen, J., Anderson Jr, J. R., Ahlgrim, J., Criswell, M., Dhungana, P., Gocken, T., Li, Z., Mariappan, S.,
 436 Pilcher, C. D., et al., 2012. Transgenic insect resistance traits increase corn yield and yield stability. *Nature Biotechnology*
 437 30 (6), 493.
- 438 Hackett, S. C., Bonsall, M. B., 2019. Insect pest control, approximate dynamic programming, and the management of the
 439 evolution of resistance. *Ecological Applications* 29 (2), e01851.

- 440 Harvey-Samuel, T., Morrison, N. I., Walker, A. S., Marubbi, T., Yao, J., Collins, H. L., Gorman, K., Davies, T. E., Alphey,
441 N., Warner, S., et al., 2015. Pest control and resistance management through release of insects carrying a male-selecting
442 transgene. *BMC Biology* 13 (1), 49.
- 443 Hendrichs, J., Ortiz, G., Liedo, P., Schwarz, A., 1983. Six years of successful medfly program in Mexico and Guatemala. *Fruit*
444 *Flies of Economic Importance*. AA Balkema, Rotterdam, The Netherlands, 353–365.
- 445 Huang, F., Andow, D. A., Buschman, L. L., 2011. Success of the high-dose/refuge resistance management strategy after 15
446 years of Bt crop use in North America. *Entomologia Experimentalis et Applicata* 140 (1), 1–16.
- 447 Huang, Y., Qin, Y., Feng, H., Wan, P., Li, Z., 2017. Modeling the evolution of insect resistance to one-and two-toxin Bt-crops
448 in spatially heterogeneous environments. *Ecological Modelling* 347, 72–84.
- 449 Ives, A. R., Glaum, P. R., Ziebarth, N. L., Andow, D. A., 2011. The evolution of resistance to two-toxin pyramid transgenic
450 crops. *Ecological Applications* 21 (2), 503–515.
- 451 Kakinohana, H., Kuba, H., Kohama, T., Kinjo, K., Taniguchi, M., Nakamori, H., Tanahara, A., Sokei, Y., 1997. Eradication
452 of the melon fly, *Bactrocera cucurbitae* (Coquillett), by mass release of sterile flies in Okinawa Prefecture, Japan. *Japan*
453 *Agricultural Research Quarterly* 31, 91–100.
- 454 Kruger, M., Van Rensburg, J., Van den Berg, J., 2009. Perspective on the development of stem borer resistance to Bt maize
455 and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection* 28 (8), 684–689.
- 456 Kruger, M., Van Rensburg, J., Van den Berg, J., 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and
457 reports of stem borer resistance in South Africa. *Journal of Applied Entomology* 136 (1-2), 38–50.
- 458 Leftwich, P. T., Koukidou, M., Rempoulakis, P., Gong, H-F., Zacharopoulou, A., Fu, G., Chapman, T., Economopoulos, A.,
459 Vontas, J., Alphey, L., 2014. Genetic elimination of field-cage populations of mediterranean fruit flies. *Proceedings of the*
460 *Royal Society B: Biological Sciences* 281 (1792), 20141372.
- 461 Liao, J., Xue, Y., Xiao, G., Xie, M., Huang, S., You, S., Wyckhuys, K. A., You, M., 2019. Inheritance and fitness costs of
462 resistance to *Bacillus thuringiensis* toxin Cry2Ad in laboratory strains of the diamondback moth, *Plutella xylostella* (L.).
463 *Scientific Reports* 9 (1), 6113.
- 464 Maynard Smith, J., Slatkin, M., 1973. The stability of predator-prey systems. *Ecology* 54 (2), 384–391.
- 465 McGaughey, W. H., Whalon, M. E., 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science* 258 (5087),
466 1451–1455.
- 467 Naik, V. C., Kumbhare, S., Kranthi, S., Satija, U., Kranthi, K. R., 2018. Field-evolved resistance of pink bollworm, *Pectinophora*
468 *gossypiella* (Saunders)(Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (Bt) cotton expressing crystal 1Ac
469 (Cry1Ac) and Cry2Ab in India. *Pest Management Science* 74 (11), 2544–2554.
- 470 Parisi, C., Tillie, P., Rodríguez-Cerezo, E., 2016. The global pipeline of GM crops out to 2020. *Nature Biotechnology* 34 (1),
471 31.
- 472 Peck, S. L., Gould, F., Ellner, S. P., 1999. Spread of resistance in spatially extended regions of transgenic cotton: implications
473 for management of *Heliothis virescens* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 92 (1), 1–16.
- 474 Pulliam, H. R., Danielson, B. J., 1991. Sources, sinks, and habitat selection: a landscape perspective on population dynamics.
475 *The American Naturalist* 137, S50–S66.
- 476 Reisig, D. D., 2017. Factors associated with willingness to plant non-Bt maize refuge and suggestions for increasing refuge
477 compliance. *Journal of Integrated Pest Management* 8 (1), 1–10.
- 478 Romeis, J., Naranjo, S. E., Meissle, M., Shelton, A. M., 2018. Genetically engineered crops help support conservation biological

- 479 control. *Biological Control*.
- 480 Scott, M. J., Concha, C., Welch, J. B., Phillips, P. L., Skoda, S. R., 2017. Review of research advances in the screwworm
481 eradication program over the past 25 years. *Entomologia Experimentalis et Applicata* 164 (3), 226–236.
- 482 Shelton, A. M., Long, S. J., Walker, A. S., Bolton, M., Collins, H. L., Revuelta, L., Johnson, L. M., Morrison, N. I., 2020.
483 First field release of a genetically engineered, self-limiting agricultural pest insect: Evaluating its potential for future crop
484 protection. *Frontiers in Bioengineering and Biotechnology* 7, 482.
- 485 Sisterson, M. S., Antilla, L., Carrière, Y., Ellers-Kirk, C., Tabashnik, B. E., 2004. Effects of insect population size on evolution
486 of resistance to transgenic crops. *Journal of Economic Entomology* 97 (4), 1413–1424.
- 487 Sisterson, M. S., Carrière, Y., Dennehy, T. J., Tabashnik, B. E., 2005. Evolution of resistance to transgenic crops: interactions
488 between insect movement and field distribution. *Journal of Economic Entomology* 98 (6), 1751–1762.
- 489 Slone, D., 2011. Increasing accuracy of dispersal kernels in grid-based population models. *Ecological Modelling* 222 (3), 573–579.
- 490 Storer, N. P., Peck, S. L., Gould, F., Van Duyn, J. W., Kennedy, G. G., 2003. Spatial processes in the evolution of resistance
491 in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt transgenic corn and cotton in a mixed agroecosystem: a biology-rich
492 stochastic simulation model. *Journal of Economic Entomology* 96 (1), 156–172.
- 493 Tabashnik, B. E., Brévault, T., Carrière, Y., 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature*
494 *Biotechnology* 31 (6), 510.
- 495 Tabashnik, B. E., Carrière, Y., 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature*
496 *Biotechnology* 35 (10), 926.
- 497 Tabashnik, B. E., Carrière, Y., 2019. Global patterns of resistance to Bt crops highlighting pink bollworm in the United States,
498 China, and India. *Journal of Economic Entomology*.
- 499 Tabashnik, B. E., Liu, Y.-B., Malvar, T., Heckel, D. G., Masson, L., Ballester, V., Granero, F., Ménsua, J. L., Ferré, J., 1997.
500 Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis*. *Proceedings*
501 *of the National Academy of Sciences* 94 (24), 12780–12785.
- 502 Tabashnik, B. E., Sisterson, M. S., Ellsworth, P. C., Dennehy, T. J., Antilla, L., Liesner, L., Whitlow, M., Staten, R. T., Fabrick,
503 J. A., Unnithan, G. C., et al., 2010. Suppressing resistance to Bt cotton with sterile insect releases. *Nature Biotechnology*
504 28 (12), 1304.
- 505 Tabashnik, B. E., Van Rensburg, J., Carrière, Y., 2009. Field-evolved insect resistance to Bt crops: definition, theory, and
506 data. *Journal of Economic Entomology* 102 (6), 2011–2025.
- 507 Vacher, C., Bourguet, D., Rousset, F., Chevillon, C., Hochberg, M. E., 2003. Modelling the spatial configuration of refuges for
508 a sustainable control of pests: a case study of Bt cotton. *Journal of Evolutionary Biology* 16 (3), 378–387.
- 509 Zhang, H., Wu, S., Yang, Y., Tabashnik, B. E., Wu, Y., 2012. Non-recessive Bt toxin resistance conferred by an intracellular
510 cadherin mutation in field-selected populations of cotton bollworm. *PLoS One* 7 (12), e53418.
- 511 Zhou, L., Alphey, N., Walker, A. S., Travers, L. M., Hasan, F., Morrison, N. I., Bonsall, M. B., Raymond, B., 2018. Com-
512 bining the high-dose/refuge strategy and self-limiting transgenic insects in resistance management—a test in experimental
513 mesocosms. *Evolutionary Applications* 11 (5), 727–738.
- 514 Zhou, L., Alphey, N., Walker, A. S., Travers, L. M., Morrison, N. I., Bonsall, M. B., Raymond, B., 2019. The application of
515 self-limiting transgenic insects in managing resistance in experimental metapopulations. *Journal of Applied Ecology* 56 (3),
516 688–698.

518 Highlights

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

523 Supplementary Materials

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

Journal Pre-proofs

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 (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 (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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_r = 0.29$, $V_s = 0.77$, $V_{ss} = 1$.

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 ($C = 1$). Line colour is used to represent the release ratio employed, with black lines showing the dynamics observed under refuge-only management strategies ($\rho = 0$). Blue dashed lines, denoting generations 8, 16 and 24, are included to aid visual comparison. 20 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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.

Supplementary Figure 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 ($N_{inrst} = 0$) and b)-d) localised releases ($N_{inrst} > 0$). 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 ($C < 1$). 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 11×11 cell agricultural landscape lattice with the following static parameter values: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_r = 0.82$, $W_s = 0.205$, $W_{rs} = 0$, $V_r = 0.29$, $V_s = 0.77$, $V_{rs} = 1$.

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 ($C < 1$). 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 ($N_{trans} = 0$ and b)-d) localised releases ($N_{trans} > 0$). 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 ($C < 1$). 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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_T = 0.82$, $W_S = 0.205$, $W_{SS} = 0$, $V_T = 0.29$, $V_S = 0.77$, $V_{SS} = 1$.

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: $p_0 = 0.005$, $R_0 = 10$, $\kappa = 0.1$, $K = 10,000$, $W_{rr} = 0.82$, $W_{rs} = 0.205$, $W_{ss} = 0$, $V_{rr} = 0.29$, $V_{rs} = 0.77$, $V_{ss} = 1$.