An analytical model assessing the potential threat to natural habitats from insect resistance transgenes

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We examine the role of ecological interactions on effective gene flow from genetically manipulated plants to their wild relatives. We do so by constructing and applying to oilseed rape (OSR) an analytical model for interaction between plants with and without an insect resistance (IR) allele in natural communities, incorporating documented levels of herbivore variability. We find that with reasonable values of advantage to the IR allele, little concomitant disadvantage (physiological costs of the allele) restricts it to low proportions of the natural population for large numbers of generations. We conclude that OSR IR transgenes are unlikely to pose an immediate threat to natural communities.

Our model identifies those factors best able to regulate particular transgenes at the population level, the most effective being impaired viability of seeds in the period between production and the following growing season, although other possibilities exist. Because solutions rely on ratios, limiting values of regulating factors are testable under controlled conditions, minimizing risk of release into the environment and offering significant advancement on existing testing programmes. Our model addresses folivory but is easily modified for herbivory damaging the seed or directly affecting seed production by infested plants, or for pathogens altering seed survival in the seedbank.

Keywords: genetically modified organisms (GMOs); genetically manipulated plants; temporal niche axis; invasion ecology; invasive genomes; effective gene flow

1. INTRODUCTION

Insect resistance (IR) transgenes offer significant advantages to agriculture and the economic pressure to use them is increasing. They additionally have the advantage of reducing pesticide use and thus reducing the impact of agriculture on the natural environment (Dale *et al.* 2002; Stewart *et al.* 2003). However, there is concern that where close relatives of crop species occur in nearby natural communities, IR transgenes may ‘escape’ through gene flow from crop into native plants and disrupt normal community function (Raybould & Gray 1994; Arnaud *et al.* 2003; GM Science Review Panel 2003). Effective gene flow from a crop into a non-crop population is a composite of the compatibility of crop and non-crop types, the availability of pollen and ecological interactions of non-crop individuals with and without the ‘new’ genes that have been gained through crop pollen and possibly seed. Although ecological interactions may be the most important driver predicting the spread of transgenes (Rieseberg & Burke 2001), the focus thus far has been primarily from a population genetics perspective (e.g. Arias & Rieseberg 1994; Whitton *et al.* 1997; Linder *et al.* 1998; Haygood *et al.* 2004). To better weight the balance of understanding,

we here turn our attention to the ecological action of transgenes in a natural community.

The details of the ecological processes involved in gene flow are generally ‘black-boxed,’ as the relative difference in seed set of individuals with and without crop genes. However, a single such ‘snapshot’ may not be sufficient to predict the long-term behaviour of the interaction, through a lack of all the relevant determinants of fitness (Crawley 1999). It has been shown in a rapidly increasing number of ecological studies that the relative success of competing plant types may vary with a periodicity longer than the duration of such studies, or relative advantage may play out over an arena greater than that measured by seed set alone, or both (Chesson 2003). Seed set alone could be an accurate estimator of the relative role of ecology in determining gene flow, but that must be determined, not assumed.

Our model addresses the behaviour of the transgene in the natural habitat in terms of the costs and advantages incurred by possession of the transgene. With our model, it is possible to assess the potential that plants with an insect resistance Bt (IRBt) allele may have for displacing untransformed plants in the natural community, making it an important tool in evaluating the potential of IR transgenes to disrupt wider foodweb dynamics. If plants with the IRBt allele do not persist or do not form a large part of the population of host plants used by dependent herbivores, then a large impact of the transgene on the

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Table 1. Guide to terms.

y_i	number of adults of variety i per adult site
z_i	useful variable which equals y_i when things are changing slowly (in [quasi-]equilibrium)
x_i	number of seeds of variety i per adult site in the seed bank before germination
E_i	fraction of seeds germinating
s_i	fraction of seeds in the seed bank (left after germination) which survive until next year
Y_i	seed yield per adult, measured as viable seeds in the next season
Y^*	ratio of seed yields Y/Y_{wvw}
p_i	probability that a plant will be pollinated by variety i
η_i	amount of pollen produced by variety i
η^*	ratio of pollen production η/η_{wvw}
α_i	probability of a germinating seed reaching the adult stage in the absence of competition
β_i	seedling competitive factor
K_i	long-term rate at which seeds are lost from the seed bank
a^*	relative advantage of the transformed over the untransformed type
f	fraction of time herbivory is low (at other times it is high)
L	a measure of the fractional increase of the genetic modification, averaged over many steps

general community is unlikely. Our model describes change over time, allowing more realistic, dynamic estimations of the direct and indirect impact of transgenes on higher trophic levels as well as on herbivores. The model can be used easily in risk assessment frameworks, answering recent criticism on the lack of quantitative predictions in previous assessment practices; its parameters are defined and measurable, relating to ecological endpoints and ‘trigger values.’

Our model explicitly includes temporal variability in herbivore levels, a well documented phenomenon but one rarely incorporated in analytical treatments of the impact of herbivory. In the simplest case, if the IRBt allele were to provide a consistent benefit of IR to its carrier, it would exclude the wild-type allele. However, year-to-year fluctuation in numbers of insect pests is an innate property of both crop and natural systems and the advantage of resistance to herbivores will vary over time (Price 1997; NERC Centre for Population Biology 1999). The effect on plants of temporal fluctuation in a selective agent is described by storage dynamics (Chesson & Huntly 1989; Chesson 1990, 2003; Kelly & Bowler 2002, 2003, 2005). ‘Storage’ refers to recovery of a population from a period of low recruitment being ‘stored’ in the reproductive capacity of long-lived individuals or in resting propagules. Storage theory tracks the impact of a selective factor on recruitment; this makes it especially well suited for herbivory, where the probability of mortality from herbivory is likely to be greater at the seedling than the adult stage. In the storage model of Kelly & Bowler (2002, 2005), competitors are differentially sensitive to a controlling environmental factor (Fenner *et al.* 1999; Hanley & Lamont 2001; Kelly & Hanley 2005), as would be the case for plants with and without the IRBt allele. Our model follows Kelly & Bowler (2002, 2005), incorporating a modification of the differential sensitivity model so that population persistence rests upon ‘storage’ of seeds in the soil seed bank *sensu* Chesson & Huntly (1989) and Chesson (1990). We have applied our model to oilseed rape (OSR) and its herbivore diamondback moth (*Plutella xylostella* syn. *P. maculipennis*). We have selected this system because the majority of IR genes that have been commercialized are lepidopteran-specific and because year-to-year data on population levels of the pest are readily available.

2. THE MODEL

Ours is a single-locus, diploid model with a genetically manipulated allele (g) and a ‘gap’ (w) analogous to a wild-type allele determining anti-herbivore defence levels. We suppose that at some stage a genetically modified crop has successfully crossed with a wild variety, and denote individuals without the transgene (wild-type) by wvw , individuals carrying one copy of the modified allele (hemizygous) by wg and plants carrying two copies of the modified allele by gg . As a result of repeated crosses between wild and wild–hybrid individuals, the transgene will be operating within the genetic background of the native species. The model assumes that seedling establishment happens in a single wave at the start of the growing season, when the initial proportion of seedlings of each type present is determined by the proportion of available seeds germinating. As the season progresses, seedlings are differentially eliminated by insect herbivores (Brown & Gange 1989; Hanley *et al.* 1995) or through competition (Ford 1975). At the end of the season all suitable sites are filled by mature plants producing seeds, which enter the seed bank to germinate in following seasons.

The IR allele may drive the wild-type allele extinct or the IR allele may merely become so common as to threaten the stability of those herbivores depending on the plant. Both possibilities are of interest. The speed and extent to which the transgene may displace the wild-type allele depends on the proportion of seasons that herbivory is low (f) or high ($1-f$), and the relative advantage/disadvantage of the modified and unmodified types at high and low levels of herbivory. This relative relationship is largely embodied as a single quantity a^* ; a^* is the time-dependent relative advantage of the transgene lineage over the unmodified lineage under target conditions and a *ratio* of several competitive factors (presented in equation (2.6)). When herbivory is low, the advantage is represented as a^{*+} , i.e. the cost of resistance for the resistant form. When herbivory levels are high and have a high impact on seedling mortality, a^{*-} indicates the relative advantage of the protected form of the plant (see table 1).

We suppose that at some stage a genetically modified crop has successfully crossed with a wild variety and consider the evolution of the population composed of three different single-locus genotypes. Reproductive capacity is stored in a seed bank and at a given time the number of seeds of genotype i present in an area which can

Figure 1. Rate of change of wild-type (black circle), homozygous GM (blue asterisk) and hemizygote (red triangle) genotypes when herbivory levels are high 35% (=observed) and 65% of years (left and right columns, respectively) under differing levels of IR advantage. The relative advantage of resistance when herbivory is high is represented by a^{*-} ; the relative advantage of the IR allele when herbivory is low, i.e. the cost of the IR allele, is a^{*+} . (a), (b) The effect of levels of advantage from protection from *Plutella* herbivory recorded for crop systems. a^* values are calculated as the ratio of seed set of oilseed rape with and without protection from herbivory. In both cases it is assumed that there is no disadvantage to the IR allele when herbivory is low ($a^{*+} = 1$; Stewart *et al.* 1997; Brown *et al.* 1999). (c–e) Effects of differing levels of cost to carrying IR alleles when herbivory is low (a^{*+}) projected for non-crop species. Here, the IR advantage at high levels of herbivory (a^{*-}) is assumed to be 1.2 (i.e. at high herbivory, the wild-type plant produces 80% the number of seed per seed sown of individuals carrying the IR allele). In all cases, $K_{wv} = K = 0.3$ (K = long-term rate at which seeds are lost from the seed bank; Roberts & Boddrell 1983).

sustain one adult plant is x_i . Its value x_i^+ at the next time step depends on the loss of seeds from the seed bank and the recruitment of seeds following seed set in adult plants. Genotype i sets Y_i seed per adult plant and the probability

that an adult of type i occupies a suitable site is represented by y_i . Vegetation is dense and we impose $\sum y_i = 1$; all available places are taken by an adult of one of the three genotypes.

The loss of seeds from the seed bank in one time step is $x_i K_i$ where

$$K_i = 1 - \{1 - E_i\} s_i, \quad (2.1)$$

with E_i the fraction of seeds germinating in any one year and s_i the number surviving to the next season.

Without interbreeding the evolution equations for the seed bank would be

$$x_i^+ = (1 - K_i)x_i + Y_i y_i, \quad (2.2)$$

where the second term on the right is recruitment to the seed bank in terms of the probability of an adult of genotype i and fecundity Y_i . With interbreeding the recruitment is not so simply described. The evolution equations, which describe a drift through the Hardy–Weinberg landscape under ecological pressures, become

$$\left. \begin{aligned} x_{ww}^+ &= (1 - K_{ww})x_{ww} + [Y_{ww}y_{ww} + 0.5Y_{wg}y_{wg}][p_{ww} + 0.5p_{wg}] \\ x_{wg}^+ &= (1 - K_{wg})x_{wg} + [Y_{ww}y_{ww} + 0.5Y_{wg}y_{wg}][p_{gg} + 0.5p_{wg}] \\ &\quad + [Y_{gg}y_{gg} + 0.5Y_{wg}y_{wg}][p_{ww} + 0.5p_{wg}] \\ x_{gg}^+ &= (1 - K_{gg})x_{gg} + [Y_{gg}y_{gg} + 0.5Y_{wg}y_{wg}][p_{gg} + 0.5p_{wg}] \end{aligned} \right\} \quad (2.3)$$

In the equation defining x_{ww}^+ , the first term in square brackets is the number of seeds a female can endow with a w allele and the second term in square brackets is the frequency with which that female encounters w pollen, and so on through the three equations. The p_i are the probabilities of a single plant being pollinated by variety i .

Comparison of equations (2.2) and (2.3) shows that recruitment to the seed bank of any one variety is not controlled by a single fecundity parameter (see also equation (2.4) below). The total seed yield, which is the sum of the terms in square brackets in equation (2.3), has a simple form

$$Y_{ww}y_{ww} + Y_{wg}y_{wg} + Y_{gg}y_{gg},$$

which is approximately equal to

$$K_{ww}x_{ww} + K_{wg}x_{wg} + K_{gg}x_{gg},$$

if the total number of seeds is approximately constant.

Equations (2.3) need to be completed by specifying the relationship between the seed densities x_i , pollination probabilities p_i and the probabilities y_i of an adult of variety i . The latter depends on x_i , the germination fraction E_i , the probability that a germinating seed produces an adult in the absence of competition with other plants α_i and on competitive factors following germination

$$y_i \propto \alpha_i \beta_i E_i x_i.$$

The effects of competition are included by writing

$$y_i = \frac{\alpha_i \beta_i E_i x_i}{\sum_j \alpha_j \beta_j E_j x_j}, \quad (2.4)$$

so that $\sum y_i = 1$. The quantities β_i are possible additional competitive factors, such as effects of overtopping of one variety by another or, in this application, the costs of producing insecticide. We are particularly concerned with the time dependent effect of herbivores on the α_i parameters. This equation also illustrates that the success of one genotype over another is governed by factors other than simple fecundity.

The pollination probabilities p_i must sum to 1 and might simply be the y_i ; we can be more general by setting

$$p_i = \frac{\alpha_i \beta_i E_i \eta_i x_i}{\sum_j \alpha_j \beta_j E_j \eta_j x_j},$$

where η_i measures pollen production for an adult plant of type i .

Given the parameters, the equations can be iterated from desired starting conditions but as they stand they are rather indigestible. We specialize to the case where wg and gg look the same to the world (i.e. g is fully dominant) and use new variables

$$z_{ww} = \frac{K_{ww}x_{ww}}{Y_{ww}}, \quad z_{wg} = \frac{K_{wg}x_{wg}}{Y}, \quad z_{gg} = \frac{K_{gg}x_{gg}}{Y}.$$

These variables are the ratio of seed loss rate to seed production per individual of that variety. They have a simple interpretation; when things are changing slowly (in [quasi-]equilibrium) they are just the fractions of available sites occupied, the quantities y_i . This follows immediately from equation (2.2). If there is no interbreeding (i.e. there are no heterozygotes) the sum of (two) z_i becomes unity after a few steps. This remains true more generally if all three Y_i are equal; if any two z_i are small the third is approximately unity. This property is useful in working out analytic approximations as in equations (2.8) and (2.9) below. The greatest advantage of these variables is that equation (2.3) can be recast in a form which depends almost entirely on ratios of parameters characterizing the two alleles, as set out in equation (2.6) below.

The equations (2.3) now become

$$\left. \begin{aligned} z_{ww}^+ &= (1 - K_{ww})z_{ww} + \frac{K_{ww} \left(\frac{z_{ww}}{a^*} + 0.5Y^* z_{wg} \right) \left(\frac{z_{ww}}{a^* \eta^*} + 0.5z_{wg} \right)}{\left(\frac{z_{ww}}{a^*} + z_{wg} + z_{gg} \right) \left(\frac{z_{ww}}{a^* \eta^*} + z_{wg} + z_{gg} \right)}, \\ z_{wg}^+ &= (1 - K)z_{wg} + \frac{K \left[\frac{z_{ww}}{a^*} \left(\frac{1}{Y^*} + \frac{1}{\eta^*} \right) + z_{wg} \right] [z_{gg} + 0.5z_{wg}]}{\left(\frac{z_{ww}}{a^*} + z_{wg} + z_{gg} \right) \left(\frac{z_{ww}}{a^* \eta^*} + z_{wg} + z_{gg} \right)}, \\ z_{gg}^+ &= (1 - K)z_{gg} + \frac{K[z_{gg} + 0.5z_{wg}]^2}{\left(\frac{z_{ww}}{a^*} + z_{wg} + z_{gg} \right) \left(\frac{z_{ww}}{a^* \eta^*} + z_{wg} + z_{gg} \right)}, \end{aligned} \right\} \quad (2.5)$$

provided K_{ww} , K , Y_{ww} , Y do not vary from step-to-step. Thus equations (2.5) apply to foliage herbivory, where plants are damaged before flowering. The equation is easily modified for herbivory which damages the seed or directly affects seed production by infested plants (Burke & Rieseberg 2003; Snow *et al.* 2003), or for pathogens that alter seed survival in the seed bank (see below). The quantity a^* is the dominant factor in the overall advantage of modified plants, carrying g , over the natural variety ww . The quantity Y^* is the ratio of seed yield Y/Y_{ww} and η^* is the ratio of pollen production η/η_{ww} . The former is also a factor in a^* , which is given by

$$a^* = \frac{\alpha \beta E Y}{K} \frac{K_{ww}}{\alpha_{ww} \beta_{ww} E_{ww} Y_{ww}}, \quad (2.6)$$

and will fluctuate with time with the ratio of any pair of parameters, such as α/α_{ww} .

If a few hybrids (wg) exist in a background of wvw , the hybrid population will grow (and generate gg), provided that the dimensionless measure of the fractional increase each step

$$L = \left\langle \ln \frac{z_{wg}^+}{z_{wg}} \right\rangle_{z_{wvw} \approx 1} > 0, \quad (2.7)$$

where the average over many time steps in equation (2.7) is to allow for environmental fluctuations in, for example, the α parameters (which might be caused by varying herbivory). This is easily obtained from the second of equation (2.5), yielding the result that the transgene will spread against the wild variety provided

$$L = \left\langle \ln \left[1 - K + 0.5K \left\{ \frac{\eta^*}{Y^*} + 1 \right\} a^* \right] \right\rangle, \quad (2.8)$$

is greater than 0. If $\eta^*/Y^* \approx 1$ and K not close to 1, the condition is $\langle a^* \rangle > 1$. The parameters in equation (2.8) apply to the heterozygotic variety; This equation is not affected by the possibility that the gg homozygote might look different.

At the other end, suppose the gg variety has become prevalent, $z_{gg} \approx 1$. If the wild variety is not to be driven extinct, both z_{wvw} and z_{wg} must be able to grow when almost gone. The condition for this is approximately

$$\left\langle \frac{1}{a^*} \left[\frac{1 + Y^*/\eta^*}{2} \right] \right\rangle > 1, \quad (2.9)$$

or if $Y^*/\eta^* \approx 1$, equation (2.9) is $\approx \langle 1/a^* \rangle > 1$

The wild variety, the hybrid and the gg variety will coexist between these limits, which can be made possible by fluctuations in herbivory. The limit (2.9) is of little real interest because the time-scale for recovery is very long.

Doubling time—a useful number for assessing the rate of spread of g is the time taken for some initially small population of wg to double in a background of wvw . This is given by

$$\frac{\ln 2}{L}, \quad (2.10)$$

where L is the average defined in equation (2.8).

The doubling time equation (2.10) calculates the amount of time it takes for the proportion of hemizygotes to double (while they comprise approximately less than 20% of the population). The same information is needed for the doubling time equation and equations (2.5); if the doubling time is long, then the patterns generated by iterating equations (2.5) gives no more information than does doubling time alone.

3. EXAMPLES

We have iterated the model using various values of herbivore frequencies, transgene advantage and potential costs for the transgene in a non-agricultural background. Field estimates indicate diamondback moth levels are high in approximately 35% of the years, ranging between 20 and 40% (Harcourt 1963; Markkula 1965; Vanholder 1997). We have used high herbivore frequencies of 35 and 65% to illustrate the effect of frequency in the selective factor on progress of the transgene in the host plant population. Crop studies suggest that possessing the transgene may allow 20 times the productivity of that of untransformed plants in an agricultural context (Stewart

et al. 1997). In contrast, a study on BC_1 plants revealed advantages no greater, on average, than 1.55 and in some circumstances not significantly different from unity (Snow *et al.* 2003). We have, therefore, also examined a range of advantage levels. We have illustrated the potential impact of the transgene carrying a cost under natural conditions by applying reasonable levels for the costs of the defences that transgenes might bear (for example, through protein manufacture) in natural systems (Herms & Mattson 1992; Bergelson & Purrington 1995). For all iterations we have set η^* and Y^* unity. (These quantities do modify the effect of the dominant factor a^* —for example, male sterility in g carrying plants would correspond to very low values of η^*).

We present the results of our iterations of the model in figure 1 and table 2. Although we performed iterations with both 1 and 10% hemizygote starting proportions and give the output for both in table 2, we show, for clarity, figures for only the higher initial proportion. In figure 1, the frequency at which herbivore levels are high increases from left to right. The first two rows of figure 1 shows the case in which there is no cost to possessing the transgene; in the third through fifth rows, the cost of possessing the transgene decreases from a 20% fitness decrement to a 1% fitness decrement.

Figure 1 shows that when there is no cost to possessing the transgene, the effect of increased frequency of high herbivore levels is primarily quantitative, speeding the rate at which transformed plants come to dominate the population. At 35% high herbivore levels, however, transgene costs at a level that have been observed in natural systems of defence (Bergelson & Purrington 1995) are sufficient, even when quite low (1%), to greatly slow the rate at which transformation of the population takes place. In at least one instance at 35% high herbivore frequency, the transformed type is driven out (figure 1c). In figure 1d, where the transgene carries a 10% fitness cost, the doubling rate of transformed plants under 35% high herbivore levels is so slow as to be indistinguishable from stable coexistence within the 200 year span of the iteration. At a greater frequency of high herbivore levels, however, all three of the iterations where transgenes carry a cost ($a^{*+} < 1$) show a steady decrease over time in the proportion of wild-type individuals.

4. USING THE MODEL TO ASSESS A TARGET TRANSGENE

In selecting a transgene for commercial use, the a^* value might be chosen to decrease the possibility of transgene takeover under natural conditions. In our model, the advantage a^* depends linearly on a number of factors (seedling resistance to herbivory α , seedling competitive ability β , viable seed production Y), but its dependence on the germination fraction E and the dormancy parameter s is nonlinear (see figure 2; equation (2.1)). Because of this nonlinearity and given the values of s that occur in wild species (Roberts & Boddrell 1983), manipulation of E and s may not have the desired effect. On the other hand, the linearity of the relationships between a^* and α , β and Y means that the degree of change in any of these factors straightforwardly predicts the degree of change in the relative competitive abilities of the two lineages. For IR transgenes, the crop will have been selected for a large

Table 2. Prevalence of genotypes in the population.
(The values shown here for 10% initial *wg* correspond to figure 1.)

a^{*-}	a^{*+}		initial % <i>wg</i> at 35% high herbivory		initial % <i>wg</i> at 65% high herbivory		doubling time ^a (generations)	
			10	1	10	1	35% high herbivory	65% high herbivory
20	1	generation at which <i>wvw</i> falls below 50% % <i>wvw</i> and <i>wg</i> at 200 generations	3	5	3	4	$\approx 1^b$	0.56^2
2	1	generation at which <i>wvw</i> falls below 50% % <i>wvw</i> and <i>wg</i> at 200 generations	20	45	11	21	7.5	4.1
1.2	0.8	generation at which <i>wvw</i> falls below 50% % <i>wvw</i> and <i>wg</i> at 200 generations	—	—	160	> 200	<i>wg</i> goes to extinction	42.5
1.2	0.9	generation at which <i>wvw</i> falls below 50% % <i>wvw</i> and <i>wg</i> at 200 generations	> 200	$\gg 200$	65	171	1150	23.7
1.2	0.99	generation at which <i>wvw</i> falls below 50% % <i>wvw</i> and <i>wg</i> at 200 generations	125	> 200	54	130	31	17.8

^a When heterozygote proportion is below approximately 20%.^b When doubling is a year or less it is only an average. What actually happens is highly dependent on herbivory levels in year 1. Figure 2. Trait manipulability. The curve shows the relationship between *E*, the germination fraction and the relevant function of *E* from equation (2.1), at various values of *s*, the fraction of seeds in the seed bank that survive until the following season (excluding the period between 0 and 1 year). Note the increasingly nonlinear relationship with increasing values of *s*. If *s* is close to 1, and *E* is greater than 0.5, *E* becomes relatively unimportant in determining a^* and so an unrewarding target for manipulation. If *E* is small, it is very important in determining a^* . If *E* is close to 1, then *s* has little effect—because nearly all seeds germinate. For non-crop Cruciferae, *s* appears to be approximately 0.9 and *E* in the range of 0.2–0.3 (Roberts & Boddrell 1983).

value of α under agricultural conditions and this parameter may not be available for further manipulation.

Of β and Y , β represents physical traits such as overtopping which confer a competitive advantage, or the costs of producing insecticide. The existence or otherwise of such costs in the *wg* variety certainly merits investigation. Y , the yield of seeds viable in year 1 per *wg* or *gg* adult, offers a trait that reliably determines the proportion of seedlings available to mature into seed-producing adults. Y appears in both a^* and Y^* in equation (2.8). It is also clear from equation (2.8) that male sterility associated with the *wg* variety substantially offsets a considerable advantage in a^* . We conclude that the necessary competitive disadvantage is accomplished most effectively through Y and/or η . The proportion of viable seeds may be manipulated through, for example, seed viability *per se*; male sterility in modified crop plants (genetic usage restriction technology; GURT) is another possibility. However, if fertility is to be manipulated, it must be tightly linked to the IR gene complex and even then will eventually become dissociated from the insecticide allele through recombination. The optimal plan to ‘control’ spread through natural populations of genes of ecological consequence may thus best combine use of GURTs with the sort of negative trade-off addressed in our model.

There are two variants of the model that allow it to be applied to transgenes developed for other sorts of natural enemies. In the first variant, seed rather than seedling survivorship in the sensitive species is more affected by herbivory, e.g. seed predation of seed on parent plant.

This means quantifying the relative differences in production of undamaged (viable) seed for the modified and unmodified lineages under high and low seed predation levels. The second variant is closer to [Chesson \(1990\)](#) in that the fraction of seeds germinating varies due to the effect of seed predation in the soil or of a soil pathogen affecting the seed. To apply the model to this variant, relative differences in germination fraction of transgene-containing and wild-type seed cohorts under high and low levels of the environmental factor must be assessed.

In the first case the Y parameters are functions of environmental fluctuations, in the second case fluctuations in the E or s parameters affect the K in equation (2.1). Equation (2.3) remains valid. Equations (2.5) requires the generic replacement

$$z^+ \rightarrow \frac{KY^+z^+}{YK^+},$$

on the left hand side (where the $+$ indicates the value at the next step). Nonetheless, equation (2.8) and the expression for the doubling time equation (2.10) remain valid.

The model is ecologically more general than this application to herbivory; the same dynamics and solutions should also apply to anti-pathogen transgenes.

5. DISCUSSION

We conclude that, in general, IR transgenes are unlikely to pose an immediate threat to natural communities. At lower frequencies of high herbivore levels, it takes very little disadvantage to slow or halt the progress of the transgene in the population, and it seems unlikely that there will be no such disadvantage under natural conditions ([Herms & Mattson 1992](#)). Population profiles for Lepidoptera available in the NERC Global Population Dynamics Database ([NERC Centre for Population Biology 1999](#)) indicate probability of high herbivory levels to be mostly less than 0.5. The only relevant data we have found show fitness advantage of an IRBt-containing plant under natural conditions to be similar to that used in our simulations ([Snow *et al.* 2003](#)).

Regardless of any general expectation, particular transgenes should continue to be assessed individually. However, our model offers the significant advantage of clarifying what needs knowing and how well it needs to be known for such assessment. It has been suggested that temporal variability in the selective factor may affect the influx of transgenes into natural populations ([Burke & Rieseberg 2003](#)); our iterations demonstrate that to be the case for herbivore resistance transgenes. Indeed, our model indicates that a failure to account for temporal variability in herbivore levels would produce at best an incomplete, and more probably a misleading projection of transgene behaviour.

Transgenes may be best manipulated through viable seed production, yet viable seed production is far from a sufficient measure of advantage. Differential seedling survival also contributes to fitness and may be expected to have an especially large role in the impact of IR transgenes. Damage from herbivory that may or may not decrease seed set in a mature plant will kill a seedling. That being said, an IR transgene in a wild-type

background is expected to impart less advantage than the same transgene in a crop genome. Non-agricultural species possess a number of mechanisms to defend themselves against herbivory ([Belsky *et al.* 1993](#)) and the transgene will be only one more; the productivity of a crop species with an IR transgene has been shown to be as much as 20 times that of the untransformed crop under the same conditions ([Stewart *et al.* 1997](#)), while the greatest relative advantage thus far shown for a transgene in a 75% wild-type background (BC_1) has been 1.55 : 1 ([Snow *et al.* 2003](#)). The two studies used different genera (*Brassica* versus *Helianthus*) and the first study encompassed seedling mortality while the second did not, but this is the best information currently available, not a test of the idea.

A small cost to carrying the transgene can greatly influence its progress through the natural population, so it is essential that the existence and degree of any costs to possession of a transgene under natural conditions be determined with certainty. Transgene costs are more likely in the context of the complex demands of a wild-type genome operating in the low resource availability of a natural habitat ([Herms & Mattson 1992](#); [Stamp 2003](#)), and the limited data that exist do not refute cost to the transgene under natural conditions ([Snow *et al.* 2003](#)). Unfortunately, appropriate experiments have not, to our knowledge, been performed. In the best study to date, sample sizes are too small to reveal costs to transgene possession as great as 10% decrement in productivity (relative to untransformed plant). Furthermore, estimates of cost have not accounted for potential differential seedling survivorship ([Snow *et al.* 2003](#)). In the absence of herbivory, seedlings containing IRBt may be less competitive than untransformed seedlings, affecting β , and in consequence suffer higher mortality in mixed communities.

Earlier models have dealt with environmental variation by asking what might be the relative benefits of producing a cohort of highly variable offspring versus a cohort of offspring with the same geometric mean fitness but not variable, as strategies for dealing with a stochastically variable environment (e.g. [Gillespie 1972](#)). Our storage model is asking what are the relative benefits of IR with a physiological cost versus non-IR where herbivory, the off-setting benefit of the IR trait, varies over time. Thus two different things are variable: in [Gillespie \(1972\)](#), the number of offspring varies stochastically, while in our model, the environmental factor, herbivory, varies. Also, we model the variable parameter differently, in that it does not depend on a normal distribution of high-low fluctuations; it thus possesses an advantage over mean/variance trade-off models in that the distribution of fluctuations can be chosen by the researcher.

Our model as it is presented is not age-structured, although it can be made so. We have run our iterations using a constant probability of emergence from the seed bank, a reasonable approximation of the available data, but it could easily be made a non-uniform function to include age-structuring in the seed bank *sensu* [Kalisz & McPeck \(1992\)](#).

Our model indicates that natural populations may maintain a significant proportion of untransformed individuals for more than the lifetime of a transgene cultivar (approximately 12 years). These individuals could

constitute a significant 'refugia' for a target herbivore *sensu* Carrière & Tabashnik (2001), and thereby delay herbivore adaptation to the IR transgene. The possibility of this will depend on the nutritional value of the non-crop plant to the herbivore and the size of the natural population versus that of the crop. Although these are factors outside the aegis of our model, our results suggest that they may be worth investigating in the interest of IR management strategies.

We have explicitly examined the case only for simple dominance, but our model is not restricted to this assumption. The general case is contained in equations (2.3). By inserting values appropriate to the target relationship it is possible to produce predictions for other specific cases such as co-dominance or particular levels of incomplete dominance, but even qualitative results can be valuable. For example, we expect full lethality to the target herbivore from a single transformed allele (hemizygote) for commercialized IR genes; this would ensure the highest level of effectiveness of the transgene under crop conditions. A 'dosage effect' with the plant producing twice the amount of defence in the homozygote would be irrelevant to the relative advantage of the homozygote over the hemizygote: a dead herbivore is dead. On the other hand, although twice as much defensive product may have little negative effect on the plant in a highly nutrient-enriched agricultural field, it could double the expected cost for the homozygous IR individual in the more constrained budget of a plant under nutrient-poor natural conditions. Under co-dominance of this sort, the IR gene would increase even more slowly in the natural community than for simple dominance (but the doubling time from a very low level, equation (2.8), would not be affected).

When applying our model as a tool to investigate the spread of a given transgene, all the important differences controlling interactions between lineages with and without the transgene are relative (ratios), and the influence of particular trait values in potential transgene spread could therefore be evaluated under controlled conditions (Linder 1998). The similarity of controlled and real conditions may be calibrated through wild-type lineages, thus allowing appraisal of the danger of transgene runaway with minimum exposure of the transgene to the environment and the risk of unplanned release.

Our goal has been to clarify the components of fitness in the interaction between individuals with and without IRBt alleles in nature. Should it be deemed worthwhile, the action of recurrent gene flow (the repeated input of IR transgenes into natural systems) can be added to our model in future and examined explicitly in relation to the ecology of temporal variability. We have also developed an effective tool for efficiently identifying transgene types that have the ecological capability to invade wild-type communities. With an advance in risk assessment and regulation of transgenes, our model is also of significance to the basic ecology of crop-native plant communities. Its predictive power should serve to increase greatly the precision of regulation and mitigation of GM and non-GM crops with regard to risks associated with gene flow.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.