

## Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity

David Claessen, Christopher A. Gilligan, Peter J. W. Lutman and Frank van den Bosch

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Transgenes in plants affect life history traits including seed survival and germination. With stochastic matrix models we predict population-level consequences of transgene induced life history changes. We assess systematically which changes in life history traits, resulting from genetic modification, may increase the risk of invasion and persistence of feral crops or increase fitness in case of introgression from arable fields into conspecific, feral populations. We apply our method to feral populations of oilseed rape. Like many annual weeds, oilseed rape depends critically on disturbance; in undisturbed habitats it is generally outcompeted by perennials. The associated inherent variability and unpredictability render deterministic models inappropriate. With a stochastic matrix model we study population growth rate, elasticities and quasi-extinction times. Our results indicate that changes in survival in the seed bank impact population growth and persistence most. Less important are dormancy, fecundity and seedling survival. The predicted distribution of extinction times is highly skewed, with some patches persisting for decades.

*D. Claessen, IBED/Population Biology, Univ. of Amsterdam, Kruislaan 320, NL-1098 SM Amsterdam, the Netherlands, P. J. W. Lutman and F. van den Bosch, Rothamsted Research, Harpenden AL5 2JQ, UK (claessen@science.wa.nl). – D. Claessen and C. A. Gilligan, Dept of Plant Sciences, Univ. of Cambridge, Downing Street, Cambridge, CB2 3EA, UK.*

There is much concern on spread of genetically modified (GM) plants and their introduced foreign genes ('transgenes') into the environment. Spread of transgenes from crops can originate from hybridisation of transgenic pollen with wild or cultivated relatives, or from transgenic seeds that disperse and potentially establish a persisting feral population of transgenic plants (Wolfenbarger and Phifer 2000). In either case, however, invasion and persistence of a transgene will depend on the effect it has on the fitness of the emerging transgenic feral population, be it the crop or a wild relative (Cummings et al. 2002). The likelihood of invasion and the expected persistence time of a transgenic feral population are crucial in a risk assessment of the environmental impact of a transgene.

One of the concerns about transgenic crops is that a GM crop may be more invasive than the original

crop and hence constitute a persistent weed problem (Bullock 1999). In order to assess environmental risks of adopting GM crops it is crucial to link transgene induced life history changes (Table 1) to population dynamics. Field-release experiments such as described by Crawley et al. (1993, 2001) and Hails et al. (1997) are the most reliable way to assess the fate of specific GM plants in cultivated or natural environments. Yet, such experiments cannot be used to predict systematically which changes in life history are more likely to produce persistence than others. A useful tool for this purpose is the analysis of matrix population models (Bullock 1999, Caswell 2001).

Previous studies have used deterministic models to estimate the population growth rate of feral transgenic crops (Crawley et al. 1993, Parker and Kareiva 1996, Bullock 1999). However, both routes of spread of

Table 1. Life history traits, model parameter values, and transgene induced changes of these traits. Subscript D refers to disturbed (feral) or favourable (volunteer) habitat (see text), subscript U to undisturbed (feral) or unfavourable (volunteer) habitat. Effect of GM indicates an increase (+) or decrease (-) with respect to conventional variety. Transgenes are: OS = high stearate, OL = high laurate, Bt = Bt insect resistance, HT = herbicide tolerance (glufosinate). Significant effects of the oil-modified traits (OS and OL) were found under limited conditions.

Life history traits	Symbol	Volunteer	Feral	Ref	Effect of GM	Ref
Germination	$G_D$	0.8	0.8	a	}+(OS) }-(OS, OL)	k
	$G_U$	0.7	0.5	a		m
Seedling survival	$S_D$	0.3	0.3	b	}±(HT)	n
	$S_U$	0.001	0.01	b, c		
Seeds per plant, good year	$F_G$	5000	3000	d	}+(Bt)	p
Seeds per plant, bad year	$F_B$	2000	1000	d		
Emigration	$\mu$	0.01	0.01	e		
Probability of dormancy	$d$	0.25	0.04	f	+(OS, OL)	m
Winter survival seedling	$\sigma_1$	0.01	0.001	g		
Summer survival in layer 2	$s$	0.5	0.5	h	}+(OS) }-(HT)	q
Winter survival in layer 2	$\sigma_2$	0.6	0.6	h, i		n, r
Annual survival in layer 3	$\sigma_3$	0.8	0.8	i		
Ffraction staying in layer 2	$\delta_{22}$	0.02	0.98	j		
Fraction staying in layer 3	$\delta_{33}$	0.71	0.99	j		

References: a Schlink (1994) and Squire et al. (1997); b Crawley et al. (1993), control genotype; c effect of weed control in volunteer habitat; d Mendham et al. (1981); e no data available, low value implies poor disperser; f volunteer: seed burial experiment, fraction viable seeds after six months (P. Lutman, unpubl.), Pekrun et al. (1997), Lutman (1998), feral: Hails et al. (1997), based on annual survival of conventional seeds; g no data available but generally assumed low; h Schlink (1994); i last three years of a 53 months long seed burial experiment with volunteer oilseed rape (P. Lutman, unpubl.); j Cousens and Moss (1990), Mertens et al. (2002), much less movement in feral patches (Willems and Huijsmans, 1994). Note that  $\delta_{23} = 1 - \delta_{33}$  and  $\delta_{32} = 1 - \delta_{22}$ ; k Parker and Kareiva (1996); m Linder (1998); n Crawley et al. (2001); p Stewart et al. (1997); q Linder and Schmitt (1995); r Hails et al. (1997).

transgenes (via pollen or seed) are subject to environmental stochasticity. Many crops are annuals derived from weedy ephemeral plants such as oilseed rape and sunflower (Linder and Schmitt 1995), combining a high potential reproductive output with low competitive ability. Their strong dependence on disturbance implies that variability and unpredictability are essential features of populations of such plants, which renders deterministic models inappropriate to describe their dynamics. Here we show how to incorporate environmental stochasticity to allow for disturbance of patches and good and bad years for seed production into a parsimonious model that relates simple life history traits to the population dynamics of transgenic plants from which it is possible to assess the risk of invasion and persistence of these plants. We parameterise the model for oilseed rape (*Brassica napus* L.), which is at the focus of current discussions on GM crops, mimicking two common habitats: (i) a feral population outside arable fields and (ii) a volunteer population inside an arable field (Lutman 1993, Crawley and Brown 1995, Pessel et al. 2001).

Specifically we use the model to ask the following questions. (a) How does disturbance affect the population growth rate and hence the probability of invasion? Is there a minimum rate of disturbance for population increase? (b) Which components of the crop life history have most effect on population growth rate? How is this affected by environmental stochasticity? (c) What is the distribution of extinction times for feral and volunteer patches? How is this affected by changes in life history trait? (d) For feral patches typical of those arising from

seed spill from lorries, what is the effect of initial conditions (seed number) on patch persistence and the chance that patches will be extinct after five, ten or 20 years? (e) How robust are our results to parameter uncertainty? The results are discussed in the context of risk assessment and experimental work on the spread of transgenes.

Our intention is to provide a theoretical framework to complement the earlier, deterministic studies of an ecologically important and pressing problem. Our focus on the implications of environmental variability, inherent in the dynamics of ephemeral crop plants, and transient dynamics provides a new and relevant background to evaluate the risks of adopting transgenic crops. The relevance of using a stochastic approach can be deduced from the list of questions above: they can only be studied in a framework that incorporates environmental variability.

## Model and methods

We use a stochastic matrix model to project the state of the population from one year to the next (Caswell 2001). The state of the population in year  $t$  just before seed shed is represented by the number of flowering adults,  $n_1(t)$ , the number of seeds in the top layer of the seed bank,  $n_2(t)$ , and the number of seeds in the deep layer of the seed bank,  $n_3(t)$ . The top layer of the seed bank is defined as the range of depths from which germination can result in successful establishment; for oilseed rape

this means the deep layer starts at a depth of approximately 9 cm (Lutman 1993). Our assumptions on the demographic processes within a single patch of an annual plant with a structured seed bank are summarised schematically in Fig. 1. The values of the parameters for oilseed rape in feral and volunteer habitats are listed in Table 1.

We assume that environmental stochasticity affects fecundity, germination and seedling survival; we use subscript  $t$  to identify these stochastic processes. First, in most plants fecundity depends on weather factors (Harper 1977) and we incorporate this variability in a simplified way by assuming that the number of seeds per plant,  $F_t$ , is high ( $F_G$ ) in some years (referred to as good years) and low ( $F_B$ ) in others (bad years). Second, the fraction of seeds that germinate,  $G_t$ , and the proportion of seedlings that survive to become flowering plants,  $S_t$ , depend on whether the habitat is disturbed ( $G_D$  and  $S_D$ ) or undisturbed ( $G_U$  and  $S_U$ ) in year  $t$ . In feral patches a disturbance should be thought of as a temporary reduction of interspecific competition, such as the removal or cutting of perennial grasses by mowing or the activity of rabbits. This is assumed to increase both germination and seedling survival (i.e.  $G_D > G_U$  and  $S_D > S_U$ , Table 1). We assume that a disturbance has no effect on plants beyond the seedling stage. In volunteer patches where disturbance occurs by cultivation the success of volunteer oilseed rape depends on (i) weed control and (ii) the current crop, both affecting seedling survival. Common crops in rotation with oilseed rape are cereals and field beans. In cereal fields, volunteer oilseed rape seedlings perish due to competition and the possibility of herbicide application, while in field beans or oilseed rape crops they are likely to survive and set seed. Seedling survival is hence high only if the volunteer patch escapes weed control and the current crop is noncereal. We refer to this combination of factors as a 'favourable opportunity' and model it analogously to a disturbance in feral patches (Table 1).

We denote the probability of a disturbance (feral habitat) or favourable opportunity (volunteer habitat) by  $p$  and the probability of a good year by  $q$ . The two sources of environmental stochasticity are assumed

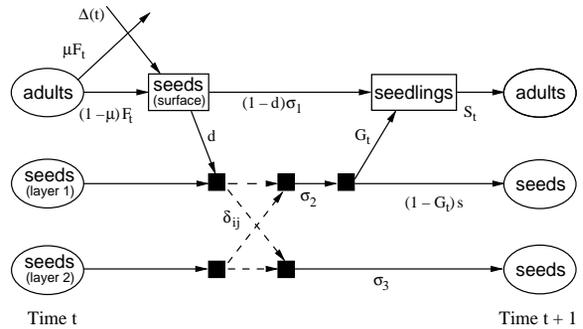


Fig. 1. Life cycle diagram of an annual plant with a structured seed bank. Ellipses represent state variables. Adults produce  $F_t$  seeds per plant. A fraction  $\mu$  of these seeds disperse.  $\Delta(t)$  seeds immigrate into the focus patch. Fraction  $d$  of seeds on the surface is incorporated in the top layer of the seed bank. Fraction  $\sigma$  of remaining seeds survives the winter as seedlings. The dashed arrows indicate vertical seed movement in the seed bank (by cultivation or the activity of earthworms etc); the fraction of seeds that moves to layer  $i$  from layer  $j$  is denoted  $\delta_{ij}$ . Annual survival rate in the seed bank is  $\sigma_3$  in the deep layer and the product of winter survival,  $\sigma_2$ , and summer survival,  $s$  in the top layer. In spring a fraction  $G_t$  of seeds in the top layer germinates and becomes seedlings, of which a fraction  $S_t$  reaches the adult flowering stage. Stochastic transitions are indicated with a subscript  $t$  (i.e.  $F_t$ ,  $G_t$  and  $S_t$ ).

tic process is independent and identically distributed (IID). We have no good estimate of  $p$ , but it is thought to be low in both habitats. We will vary  $p$  and study its effect on population dynamics. The probability of good years is assumed to be  $q=0.5$ , but preliminary sensitivity analysis shows that our results are qualitatively robust to variation in  $q$ ; the value of  $q$  affects the dynamics mainly through its effect on mean fecundity.

The three variables  $n_1(t)$ ,  $n_2(t)$  and  $n_3(t)$  together make up the population vector  $n(t)$ . The transition of the population from one year to the next, as outlined in Fig. 1, can be summarised as a vector-matrix product (Caswell 2001):

$$n(t+1) = X_t n(t) \quad (1)$$

where  $X_t$  is a stochastic,  $3 \times 3$  projection matrix. From Fig. 1 we can derive the elements of the matrix  $X_t$ :

$$X_t = \begin{pmatrix} (1-\mu)F_t[(1-d)\sigma_1 + d\delta_{22}\sigma_2G_t]S_t & \delta_{22}\sigma_2G_tS_t & \delta_{23}\sigma_2G_tS_t \\ (1-\mu)F_t d \delta_{22} \sigma_2 (1-G_t) s & \delta_{22} \sigma_2 (1-G_t) s & \delta_{23} \sigma_2 (1-G_t) s \\ (1-\mu)F_t d \delta_{32} \sigma_3 & \delta_{32} \sigma_3 & \delta_{33} \sigma_3 \end{pmatrix}$$

to be independent of each other, such that the environment may be in one of four states at each time step. We assume that there are no temporal correlations in the condition of the environment, i.e. that the stochas-

At each time step the values for  $F_t$ ,  $G_t$  and  $S_t$  are selected corresponding to the current state of the environment. This approach is equivalent to selecting entire matrices at each time step (Caswell 2001).

The deterministic equivalent model uses the mean matrix  $U$  (weighted by  $p$ ) at each time step:

$$n(t+1) = U n(t), \quad (2)$$

with  $U = \varepsilon(X_t)$  where  $\varepsilon(X_t)$  denotes the expected value of the stochastic matrix  $X_t$ .

## Population growth rate and fitness

In a deterministic matrix model, the population growth rate equals the logarithm of the dominant eigenvalue of the (mean) matrix  $U$ , denoted  $\log \lambda_1$  (Caswell 2001). Note that  $\log$  denotes the natural logarithm throughout this article. In a stochastic environment the most relevant measure of population growth is the mean long-term population growth rate,  $\log \lambda_s$  (Tuljapurkar 1997, Caswell 2001), which can be estimated as

$$\log \lambda_s = \frac{1}{T} \sum_{t=0}^{T-1} r_t \quad (3)$$

from a time series with any initial condition, where  $r_t$  is the growth factor at time  $t$ , defined as  $r_t = \log(N(t+1)/N(t))$  and  $N(t) = n_1(t) + n_2(t) + n_3(t)$ . We estimated  $\log \lambda_s$  with simulations of  $T = 50\,000$  time steps. In density-independent population models, such as Eq. 1, individual fitness is equivalent to the population growth rate.

## Elasticity analysis

Elasticity analysis is used to identify components of the life cycle to which the population growth rate is most sensitive (de Kroon et al. 1986). The elasticity of a life history parameter is defined as the proportional increase in the population growth rate in response to a proportional increase in the parameter. It is thus a form of sensitivity. The elasticity to, for example, parameter  $d$  is therefore

$$e_d = \frac{\partial \log \lambda_s}{\partial \log d} \quad (4)$$

Hence an elasticity of  $e_d = 0.2$  means that if  $d$  were increased by, say, 3%, then  $\lambda_s$  would increase by  $0.2 \times 3\% = 0.6\%$ . (Note that this is true only by approximation, as the relation between  $\log \lambda_s$  and the vital rates is generally nonlinear). By calculating the elasticities to all life history parameters, we can identify the ones that, if changed, have the largest effect on the population growth rate. Altering such parameters by GM is likely to have most impact on population dynamics (Bullock 1999). There is no a priori reason to believe that the effect of GM is best described by proportional changes of the vital rates. Yet elasticities can even be used to assess the effect of any experimentally measured change in a vital rate on the absolute change in

population growth rate using the relation  $\frac{d\lambda_s}{dx} = e_x \frac{\lambda_s}{x}$ , where  $x$  is the vital rate and  $e_x$  denotes its elasticity. We focus on elasticity analysis because it offers more straightforward comparison of the different vital rates than sensitivity analysis.

Contrary to reports by Caswell (2001) and others, elasticities in stochastic models can be very different from elasticities in deterministic models (Tuljapurkar et al. 2003). Below, we therefore present results based on the full, stochastic model, but for comparison we present results of the mean matrix model as well. We estimated elasticities with time series of  $T = 50\,000$  time steps, using the method described by Caswell (2001).

Note that we use the kind of stochastic elasticity which assumes a proportional perturbation of both the mean and standard deviation of a matrix element, and which is denoted by  $E^S$  by (Tuljapurkar et al. 2003). This is equivalent to assuming that the value of a vital rate is perturbed by the same proportion in each possible state of the environment. This kind of stochastic elasticity is the one most commonly used (Caswell 2001).

## Quasi-extinction times

In our model the number of individuals can become trivially small without ever becoming zero because we do not incorporate demographic stochasticity. However, we can specify a critical abundance, below which we consider the population extinct; this approach is referred to as quasi extinction (Caswell 2001). We consider a local population extinct if the total number of individuals drops below one,  $n_1(t) + n_2(t) + n_3(t) < 1$ . We record the time to extinction,  $t_q$ , in 20,000 runs with a fixed initial condition of 100 individuals in the shallow seed bank, that is,  $n(0) = (0, 100, 0)^T$ , where  $T$  denotes transpose. The smoothness of the estimate of  $t_q$  versus  $p$  (Results) suggests that 20,000 runs is sufficient.

From simulations we compute the elasticity of extinction time to the life history parameters as:

$$e_x = \frac{x}{\hat{t}_q} \frac{\Delta \hat{t}_q}{\Delta x} \quad (5)$$

where  $x$  represents the considered life history parameter,  $\hat{t}_q$  the mean extinction time in 10,000 runs,  $\Delta \hat{t}_q$  the difference in mean extinction time obtained from simulations with the values  $x$  and  $x + \Delta x$ , respectively.

## Effect of initial conditions

To study the effect of seed spills, we calculate the short-term prevalence and persistence times for a range of initial conditions. The initial conditions are meant to mimic a lorry seeding event; seeds are deposited on the

surface, some of which will germinate immediately, and some of which will be incorporated into the top layer of the seed bank. Assuming that  $\Delta(0)$  seeds are deposited in year  $t=0$  in an empty patch ( $n(0)=0$ ), the population vector in year  $t=1$  is defined according to Fig. 1; for example, the number of seeds in the shallow seed bank will be  $n_2(1)=\Delta(0) d \delta_{22} \sigma_2(1-G_0)s$ . The vector  $n(1)$  was used as initial condition in Eq. 1. Results are based on 10 000 runs per initial condition.

### Robustness to model assumptions

We test the robustness of model results to parameter uncertainty. In principle the results will depend on the specific values for the life history parameters (Table 1) and it is therefore important to assess the sensitivity of our results to unavoidable uncertainty in these estimates. To systematically test the robustness we created 1000 different parameter sets by choosing all parameter values randomly from a uniform distribution ranging  $\pm 25\%$  around the original estimate for feral habitat (Table 1). For each random parameter set, stochastic elasticities were computed as outlined above.

In a second test of robustness we relaxed the assumption of constant movement in the seed bank. If vertical seed movement is related to the occurrence of disturbance, we expect more seeds to move in disturbed habitat. We checked this by reducing  $\delta_{22}$  and  $\delta_{33}$  by 80% or 50% in years with a disturbance.

## Results

### How does disturbance affect population growth rate?

The stochastic growth rate is always lower than (or equal to) the deterministic growth rate for both volunteer and feral populations (Fig. 2), as predicted by general theory (Tuljapurkar 1997). A volunteer population is predicted to increase ( $\log \lambda_s > 0$ ) only if the probability of a favourable opportunity ( $p$ ) exceeds 9% (Fig. 2a). The fact that  $\log \lambda_s < 0$  for small  $p$  shows that cultivation can be an effective method to control volunteer oilseed rape. A feral population is predicted to grow if the disturbance rate exceeds 28% (Fig. 2b). For very low  $p$  there is no difference between  $\log \lambda_s$  and  $\log \lambda_1$  which is due to the absence of variability in the deep layer of the seed bank.

In the absence of reliable estimates for the disturbance rate  $p$  we can obtain a rough estimate for feral patches based on Crawley and Brown (1995). They recorded oilseed rape density (discretised into eight density classes) in each 100 by 1 m road verge along the M25 motorway (UK) in 1993 and 1994. They note that almost 20% of sites that were empty in 1993 were occupied in 1994. Assuming (i) that establishment requires disturbance and (ii) the presence of a seed bank (i.e. no seed limitation), then 20% of sites must have been disturbed and a first estimate is hence  $p \approx 0.2$ . A second and independent estimate can be made based on the assumptions that only sites which are occupied in 1993 and which are subject to a disturbance, will produce a higher density in 1994. Using Table 1 in Crawley and Brown (1995), by summing the probabilities of all

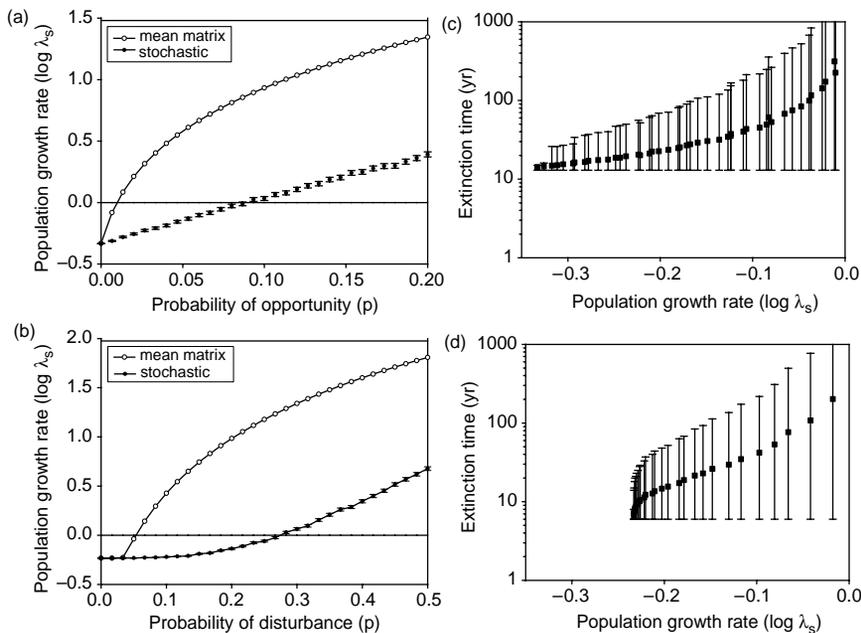


Fig. 2. The effect of changing  $p$ , the probability of a favourable opportunity (volunteer patch) or a disturbance (feral patch) on the population growth rate. (a): volunteer patch. (b): feral patch. Open symbols:  $\log \lambda_1$  of the mean matrix model (Eq. 2). Filled symbols:  $\log \lambda_s$  of the stochastic matrix model (Eq. 1). Error bars: 95% confidence interval of  $\log \lambda_s$ , estimated with the method of Caswell (2001). (c) and (d): distribution of quasi extinction times; mean (filled symbols) and 95% range, based on initial condition of  $n(0)=(0, 100, 0)^T$ , where  $^T$  denotes transpose. (c): volunteer habitat. (d): feral habitat. Parameters as in Table 1 and  $q=0.5$ .

transitions from density classes 1–7 in 1993 to a higher density class in 1994, we obtain  $p=0.11$ . We thus conclude that realistic values of  $p$  are likely to be smaller than the extinction threshold ( $p < 0.28$ ). This result is consistent with the general perception that most feral oilseed rape populations are ephemeral (Crawley and Brown 1995).

### Which components of the life history have most effect on population growth rate?

The elasticities to the life history parameters in a feral, stochastic habitat (i.e. Eq. 1) are plotted in Fig. 3a for a range of  $p$ -values. The analysis shows that survival in the seed bank is the most important life history aspect, although the emphasis shifts from the deep ( $\sigma_3$ ) to the shallow ( $\sigma_2$ ) layer of the seed bank as  $p$  increases. For very low disturbance rates,  $\sigma_3$  is practically the only parameter of importance. The increase with  $p$  of the elasticity of  $\sigma_2$ , as well as of dormancy ( $d$ ), fecundity ( $F_t$ ) and seedling survival ( $S_t$ ), coincides with a shift from a declining population to a growing population (Fig. 2). The elasticity of  $\mu$  is negligibly small and not shown in the figure. It should be noted, however, that the importance of seed dispersal cannot be assessed with

our model since dispersing seeds are disregarded (below).

If we ignore environmental stochasticity by using the mean matrix model (Eq. 2), we find a very different pattern of elasticity (Fig. 3b). As in the stochastic model, for  $p < 0.05$ , the highest elasticity is found for seed bank survival  $\sigma_3$ . But for  $p > 0.05$  high elasticity is found for  $\sigma_2$ , dormancy  $d$  and seedling survival  $S_t$  (Fig. 3b). The mean matrix model also identifies germination  $G_t$  as an important parameter. By ignoring stochasticity one thus reaches a different conclusion about which traits are essential for feral oilseed rape dynamics. For a given value of  $p$ , comparison of panels a and b in Fig. 3 shows that the parameters which are subject to stochastic variation ( $F_t$ ,  $S_t$  and  $G_t$ ) have a much lower elasticity in the stochastic matrix model than in the mean matrix model. This illustrates the more general finding that increasing the variability in a life history parameter reduces its elasticity (Caswell 2001).

In volunteer habitat with  $p=0.1$  the stochastic elasticities are, in decreasing order:  $\sigma_3$  (0.76),  $\sigma_2$  (0.22),  $d$  (0.2),  $S_t$  (0.12),  $F_t$  (0.11),  $G_t$  (0.06),  $s$  (0.02),  $\sigma_1$  (0.02) and  $\mu$  ( $-0.002$ ). The order of elasticities is the same across the whole range of  $p$  and the elasticities do not change much with  $p$  (data not shown). Again, survival in the seed bank is most important, followed by dormancy, seedling survival and fecundity.

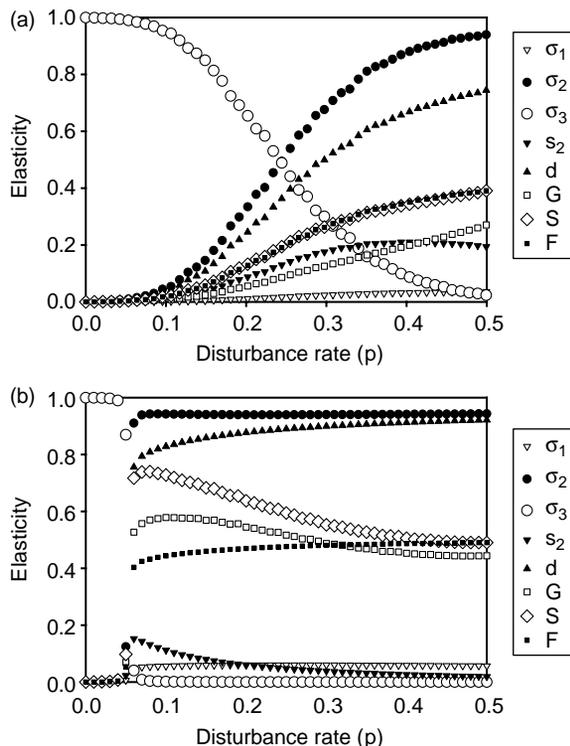


Fig. 3. Elasticities, broken down to life history parameters, depending on  $p$ . Parameters are for a feral patch (Table 1) and  $q=0.5$ . (a) Stochastic matrix model (Eq.1). (b) Mean matrix model (Eq. 2).

### What is the distribution of extinction times for feral and volunteer patches?

This question is relevant since feral and volunteer patches are predicted to go extinct for low  $p$  (Fig. 2). For the same range of  $p$  values as in Fig. 2a and b the extinction times are plotted against the corresponding value of  $\log \lambda_s$  in Fig. 2c and d. The constant values of the lower limit of the 95% range of the distribution correspond to the ‘worst case scenarios’ in feral and volunteer patches, respectively. That is, they represent the minimum time to extinction, obtained by selecting the worst growing conditions each year. The figure illustrates a number of results. First, the distribution of extinction times is highly skewed; most patches go extinct quickly, but some persist for decades. Second, in the range  $-0.2 < \log \lambda_s < 0$  the mean extinction time is approximately the same for feral and for volunteer populations. These two observations confirm the theoretical prediction that the quasi-extinction time approaches an inverse Gaussian distribution, the mean of which depends on  $\log \lambda_s$  only, not on the specific life history parameters (Caswell 2001).

For feral patches with low disturbance rates ( $p \approx 0.05 - 0.1$ ) the expected time to extinction is 6–10 years, while 95% of patches will go extinct within 20–40 years (Fig. 2d). This is longer than the estimates that patches

are unlikely to persist for more than two years given by Crawley et al. (2001), but the discrepancy may lie in the working definitions used for extinction. If, for example, we define extinction as two consecutive years without adult plants, that is,  $n_1(t_q) < 0.5$  and  $n_1(t_q + 1) < 0.5$ , the predicted mean extinction time is below two years for  $p = 0.1$ . However, for management and risk assessment purposes the presence of transgenic seeds in the seed bank should be taken into account (Pessel et al. 2001) and therefore we prefer to define extinction as presented in Fig. 2.

For volunteer patches it is predicted that it takes at least 10 years before the seed bank drops below the extinction threshold. Even with infrequent favourable opportunities (e.g.  $p = 0.02$ ;  $\log \lambda_s = -0.26$ ) a volunteer patch may persist for up to several decades (Fig. 2).

We examined the effect of changes in the life history parameters on the distribution of mean extinction time  $t_q$  for a limited set of parameter values (feral patch:  $p = 0.1$ ,  $p = 0.2$  and  $p = 0.25$ ; volunteer patch:  $p = 0.05$ ). The ranking of life history parameters according to this elasticity was consistently:  $\sigma_3$  (8.25),  $\sigma_2$  (1.27),  $d$  (0.82),  $S_t$  (0.54),  $s$  (0.43),  $F_t$  (0.41),  $G_t$  (0.18),  $\sigma_1$  (0.06),  $\mu$  (0.01), where values in parentheses are the elasticity values for  $p = 0.1$  in feral habitat. With the exception of  $s$ , the ranking is identical to that of elasticity of population growth rate (above). We hence found that the elasticity of  $\log \lambda_s$  was a good predictor for the relative magnitude of the elasticity of  $t_q$ .

### What is the fate of patches founded by seed spills?

Although seeds are dispersed by birds or by other natural mechanisms, it is generally thought that the main origin of feral patches of oilseed rape is seed spill from farm machinery and from lorries during transport from farms to seed processing plants (Crawley and Brown 1995). Here we ask the questions: how does initial density of seeds affect the fate of a newly seeded patch? What is the probability that a patch will still be

occupied a number of years later? What kind of abundances can we expect with which frequency?

For a fixed disturbance rate ( $p = 0.1$ ) but a range of initial conditions, Fig. 4a shows the probability of reaching 1, 10 or 100 adult plants within 10 years after the seeding event. It shows that if fewer than  $10^3$  seeds are spilled, the establishment of a patch of 10 plants is unlikely, although a single plant may be observed. Only spills of at least  $10^4$  seeds are likely to result in a patch with more than 100 plants. Another way to characterise short term dynamics is the probability of extinction within a fixed number of years. Figure 4b shows that half of patches seeded by  $10^3$  seeds are extinct within five years but nearly all are extinct in 20 years. Spills of more than  $10^4$  seeds persist at least 10 years and possibly longer. For a volunteer patch with  $p = 0.03$  results very similar to Fig. 4a are found, but extinction probability is much lower than in Fig. 4b, as can be expected from Fig. 2c.

### How robust are our results to parameter uncertainty?

A first indication of the robustness of our results is that despite substantial differences between parameters for volunteer and feral habitat the ranking of life history parameters according to their elasticities are remarkably similar (above).

The results of the systematic test of robustness to parameter uncertainty are given in Fig. 5 and Table 2 for  $p = 0.15$ . The large uncertainty in parameter values results in considerable variation in elasticities, as illustrated by the frequency distribution of elasticities found for the seed bank survival parameters  $\sigma_2$  and  $\sigma_3$  (Fig. 5a) and dormancy  $d$  and seedling spring survival  $S$  (Fig. 5b). Table 2 lists the frequency of rankings of the parameters according to their elasticity from highest (rank 1) to lowest (rank 8). Despite large variation in parameter values, the qualitative pattern of elasticity in terms of ranking is remarkably robust, with parameters  $\sigma_3$ ,  $\sigma_2$  and  $d$  occupying the first, second and third place

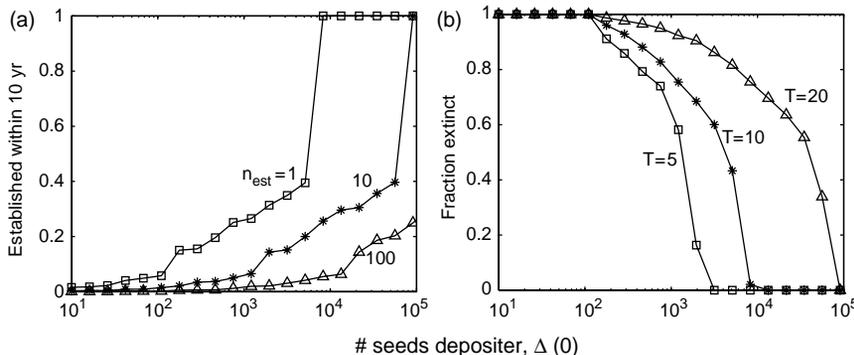


Fig. 4. The short term fate of a newly founded feral patch as a function of the initial number of founding seeds, for  $p = 0.1$  and  $q = 0.5$ . (a) The probability of establishment within 10 years, defined as the fraction of runs in which the number of flowers exceeds a threshold value:  $n_1(t) > n_{est}$ , in at least one year, with respectively  $n_{est} = 1$  ( $\square$ ),  $n_{est} = 10$  (\*),  $n_{est} = 100$  ( $\triangle$ ). (b) The probability of extinction, defined as the fraction of runs in which  $n_1 + n_2 + n_3 < 1$  within  $T$  years, for respectively  $T = 5$  ( $\square$ ),  $T = 10$  (\*),  $T = 20$  ( $\triangle$ ).

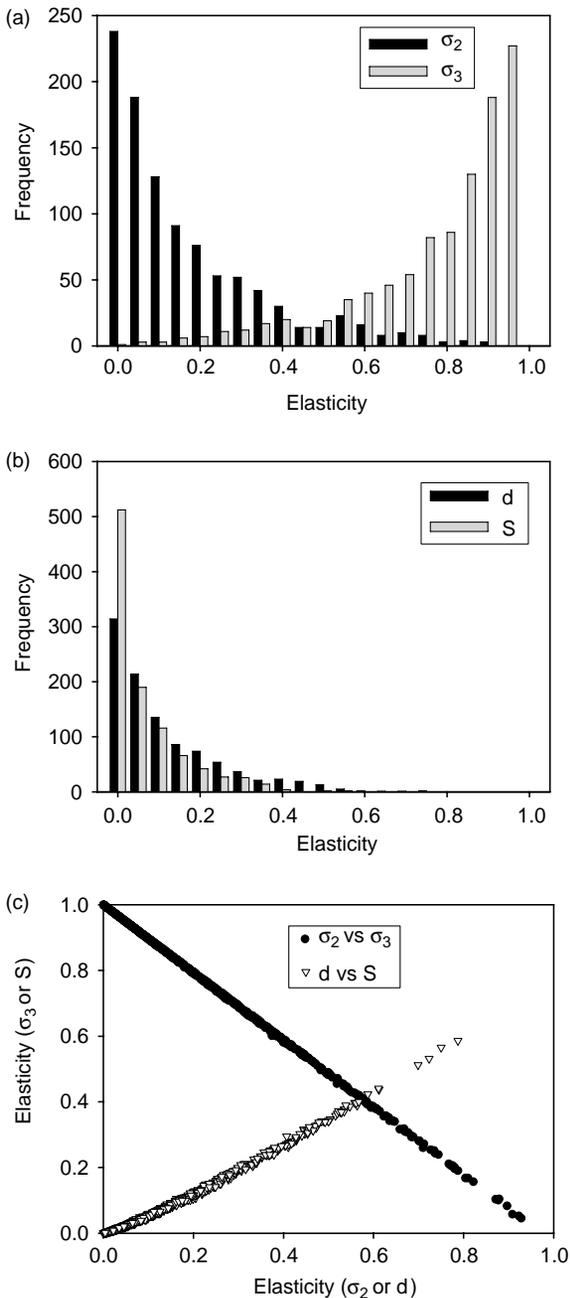


Fig. 5. Test of robustness of the pattern of elasticity to parameter uncertainty (cf. Table 2). (a) Frequency distribution of elasticities found for the seed bank survival parameters  $\sigma_2$  and  $\sigma_3$ . (b) Frequency distribution of elasticities found for dormancy  $d$  and seedling spring survival  $S$ . (c) Scatter plot of elasticity of  $\sigma_2$  versus  $\sigma_3$  (black dots); and of  $d$  versus  $S$  (open triangles).

in more than 90% of the random parameter sets. Hence the general pattern of elasticity is robust against simultaneous uncertainty in all parameters.

This robust ranking may come at some surprise when considering the observed variation in elasticity in some

Table 2. Test of robustness of the pattern of elasticity to parameter uncertainty. Computed from 1,000 random parameter sets (with each parameter drawn from the default value for feral habitat  $\pm 25\%$ , Table 1) and 10000 time steps per parameter combination, with  $p=0.15$ ,  $q=0.5$ . For each parameter, the table gives the frequency of rankings of its elasticity, in % of parameter sets.

Parameter	Rank:	1	2	3	4	5	6	7	8
	freq (%)								
$\sigma_3$		90.7	2.9	2.9	0.6	1.1	1.0	0.8	0
$\sigma_2$		9.3	90.7	0	0	0	0	0	0
$d$		0	6.4	93.6	0	0	0	0	0
$F_t$		0	0	0	86.9	12.9	0.2	0	0
$S_t$		0	0	3.3	10.3	75.7	10.7	0	0
$s$		0	0	0.2	2.2	9.9	69.4	18.3	0
$G_t$		0	0	0	0	0.4	18.7	77.9	3.0
$\sigma_1$		0	0	0	0	0	0	3.0	97.0

of the parameters (Fig. 5a, b). The consistency in ranking is explained by strong correlations between elasticities, as illustrated in Fig. 5c for  $d$  and  $S$ . It appears that the elasticities of all parameters correlate strongly ( $r > 0.84$ ) with that of  $\sigma_2$ , except that of  $\sigma_3$  which has a correlation coefficient of  $-1$  (Fig. 5c). Thus, a random parameter set which leads to a high elasticity in, for example,  $d$  is associated with a high elasticity of  $\sigma_2$  as well. These correlations maintain the ranking of the parameters in spite of variation. Strong positive correlations between all parameters except  $\sigma_3$  confirm the antagonistic relation between this parameter and the rest as observed in Fig. 3.

Relaxing the assumption of constant movement in the seed bank had only a minor, quantitative effect on elasticities but did not change the overall pattern (data not shown).

## Discussion

We have shown how stochastic matrix population models can be used to predict population level consequences of transgene-induced life history changes. For oilseed rape, seed bank survival is predicted to be the most critical aspect of the life cycle (Fig. 3). The importance of seed bank survival reflects that (i) life history parameters with little or no variability are less affected by the usually negative effect of stochasticity on elasticity; and (ii) local populations of oilseed rape function as sinks: they are expected to go extinct and the time to extinction is mainly determined by persistence in the seed bank. In more favourable habitat (i.e. high disturbance rate  $p$ ), local seed production contributes significantly to population dynamics. Even in this case, it appears that seed bank survival is the critical life history process although dormancy and, to a lesser extent, fecundity and seedling survival are important aspects as well.

If stochastic variation of life history parameters is independent and identically distributed (IID), then the more variation a parameter has, the lower its elasticity is (Caswell 2001). This explains why  $F_t$ ,  $S_t$  and  $G_t$  have lower elasticity in the stochastic model than in the mean matrix model. It is surprising, however, that stochasticity so strongly affects the elasticity of dormancy ( $d$ ), which is assumed to be constant. The explanation is that the effect of stochasticity on life history transitions applies to the entire life history pathway to which the transition belongs (Claessen 2005). For example  $d$  is part of the annual pathway via the seed bank (i.e. new seeds that overwinter in the top layer of the seed bank and germinate the following spring). This pathway is subject to large variability which affects all parameters involved. The low elasticity of  $d$  in the stochastic case is hence the consequence of the large variability in seed production, germination and seedling survival. This result is the first demonstration of the important role of life history pathways (or 'loops', van Groenendael et al., 1994) for elasticities in stochastic matrix models (Claessen 2005).

A transgene that, intentionally or not, improves seed survival is likely to increase persistence of oilseed rape. This means that transgenic lines should be tested for seed survival. It also means that it is advisable to use cultivars with low seed survival as the basis for transgenic lines. Depending on the expected level of disturbance ( $p$ ) dormancy, fecundity and seedling survival may also contribute significantly to population growth. Yet, the low sensitivity of fecundity found for small  $p$  shown by the elasticities in Fig. 3 means that reduced fecundity of a transgenic line is no guarantee of a low fitness, as increased seed survival may outweigh the decreased fecundity.

By considering known effects of transgenes on life history in oilseed rape (Table 1), we conclude that the positive effect of transgenic oil-modifications (in particular high stearate: HS in Table 1) on seed survival and dormancy could slow population decline and thereby increase persistence of transgenic feral and volunteer populations. Bt transgenes increase performance through plant survival and fecundity (Table 1), which are predicted to have less impact on fitness. It will therefore depend to a large extent on the unintentional effects on seed survival and dormancy whether Bt transgenes increase or decrease fitness. To our best knowledge, there is currently no data available on the effect on these traits.

A central question in risk assessment of GM crops is: will a transgenic crop be able to persist as a weedy population? In many studies this question has been approached by comparing the ecological performance or fitness of the GM crop relative to the original crop or wild relatives (Crawley et al. 1993, Linder and Schmitt 1995, Fredshavn and Poulsen 1996, Hails et al. 1997, Snow et al. 1999). However, our results stress that even if

the fitness or population growth rate of a GM crop is lower than that of its conventional counterpart, or even negative, it may persist longer than is acceptable (Fig. 2). In undertaking risk assessment it is therefore essential to specify what level of prevalence is acceptable, and during what period of time. With our approach it can then be estimated whether these criteria are likely to be met.

In the case of oilseed rape, a complete risk assessment of the spread of transgenes will require knowledge of the frequency distribution of seed spills ( $\Delta(t)$  in Fig. 1). Although there is indirect evidence for seeds spill from lorries (Crawley and Brown 1995), a reliable quantification is not currently available. A reduction of seed spills from lorries may be a necessary measure to limit transgene spread.

To parameterise of our model we used estimates drawn from different literature sources (Table 1). Because experimental conditions are not constant between these different sources, it is necessary to check for the sensitivity of our results to parameter uncertainty. Although uncertainty in parameter values implies uncertainty in their elasticities (Fig. 5a, b) correlations between the elasticity of different parameters (Fig. 5c) lead to a consistent ranking of the life history parameters in terms of their elasticity despite such uncertainty. We have thus shown that our results are robust to considerable parameter uncertainty.

Our matrix model relies on two simplifying assumptions: density-independent population growth and spatial homogeneity, which allow us to use the tools for analysis described above. Density-independent population growth may be a reasonable assumption in the context of invasion and extinction since densities are expected to be low during these processes. We need, however, to check for the robustness of this assumption. Spatial structure may be an important ecological aspect for feral crop populations due to their ephemeral character. Elsewhere we report a check of the robustness of our results to relaxing both simplifications by studying a density dependent, spatially-structured population model consisting of a linear array of a large number of sites coupled by migration, thus mimicking a string of roadside populations (Claessen et al. unpubl.). With this extended model, we consistently found the highest elasticity for seed bank survival ( $\sigma_3$ ) followed by dormancy ( $d$ ), seedling spring survival ( $S_t$ ) and seed production ( $F_t$ ), independently of the size of seed spills. These results show that the ranking of most important vital rates does not depend critically on the two simplifying assumptions of the matrix model.

Although we apply our method to a crop plant, in principle the effect of transgene introgression into a wild species can be studied by our method as well. This requires parameterisation of the model for the wild species of interest, but otherwise the same analyses. The applied method cannot say anything about the

likelihood of introgression, but it can say something about the expected effect of transgenes on the fitness of individuals that harbour the transgene.

In conclusion, our study shows that environmental stochasticity has implications for GM risk analysis, because the stochastic model gives other results than the deterministic model. Stochasticity has a major impact on the contribution of life history traits to population dynamics. By ignoring stochasticity one reaches a different conclusion about which traits are essential for feral oilseed rape dynamics. We have thus shown that for risk analysis of transgenic crops with an ephemeral character it is crucial to take environmental stochasticity into account. In addition, we have demonstrated that by adopting a stochastic approach, one can address issues of particular interest to risk assessment, such as the distribution of persistence times and the probability of invasion. We performed extensive tests of robustness to make sure our claims are not sensitive to parameter uncertainty. These tests demonstrated remarkable robustness, suggesting that seed bank survival and dormancy are the most important contributors to population growth and persistence times also for other crop species with a life history comparable to oilseed rape.

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