GENESYS: A model of the effects of cropping system on gene flow from transgenic rapeseed

N Colbach
Unité d'Agronomie, INRA; 17 rue Sully, BV 1540, 21034 DIJON Cedex, France

J M Meynard, C Clermont-Dauphin
Laboratoire d'Agronomie, INRA-INAPG, 78850 THIVERVAL-GRIGNON, France

A Messéan
CETIOM, 174 avenue Victor Hugo, 75116 PARIS, France

ABSTRACT

The aim of the model is to evaluate the influence of cropping systems on transgene escape from rapeseed crops to rapeseed volunteers in time and space. The model input variables are the regional field pattern, crop succession and cultivation techniques. The main output variables are, for each year and plot, the number of individuals per m^2 and the genotype proportions of the adult rapeseed plants, the newly produced seeds and the seedbank. The model comprises an annual life-cycle for volunteer and cropped rapeseed plants simulated for each plot and year; the relationships between the various life-stages depend on crop type and management. Pollen and grain exchanges between plots depend on distance between plots and cropping system. With the help of the simulations performed with the model, it is possible to identify low-gene-flow cropping systems or the minimum distance between rape plots to avoid contamination of the harvest product, to estimate the consequences if one farmer does not manage his rape volunteers correctly etc.

INTRODUCTION

In the current discussion on transgenic herbicide-tolerant rapeseed varieties, the risk of gene transfer to rape volunteers is often brought up (Messéan, 1996). It is therefore essential to rapidly evaluate this risk and identify means to reduce it. Long-term field trials were set up by French technical institutes on several locations to evaluate this risk (Champolivier *et al.*, 1997), but besides being too slow, these trials only comprise a limited number of cropping systems. Therefore, we built a model of the effects of cropping systems on gene flow from transgenic rapeseed varieties to rapeseed volunteers in neighbour plots and following crops; the first version of the model concerns herbicide tolerance transgenes. Hybridisation between rapeseed and *Brassica* weeds, though possible (Lefol *et al.*, 1996; Chèvre *et al.*, 1997), were not integrated. In this paper, only the general structure of the model is presented; details are given by Colbach and Meynard (1996), Clermont-Dauphin *et al.* and Colbach *et al.* Simulations performed with the model contribute to determine the effect of field plan, crop rotation and management on gene flow, to identify low- and high-risk situations, to propose agricultural practices minimising gene flow etc.

MODEL STRUCTURE

The input variables of the model are: (i) the field plan of the region, comprising cultivated fields and field-edges or waysides (henceforth "borders") consisting of spontaneous vegetation; (ii) the crop rotation of each field (Table 1) and (iii) the cultivation techniques applied to each crop (Table 2). The type of the transgene, either a dominant allele *A* or a recessive *a*, and of the rapeseed varieties must also be chosen. All examples presented in this paper are based on a dominant allele *A*, genotype *AA* for the transgenic and genotype *aa* for the classic variety. The major output variables are, for each field and year, the number per m^2 and the genotype proportions of adult rapeseed plants, of seeds produced and of the seedbank containing the viable rape seeds in soil.

Table 1. Example of crop successions of a simulated agricultural region.

Spatial unit	Type	Crop succession				
		year 1	year 2	year 3	year 4	year 5
1	border	border	border	border	border	border
2	field	transgenic rape	winter crop	spring crop	unsown set-aside	classic rape
3	field	winter crop	sown set-aside	classic rape	winter crop	spring crop

Table 2. Example of cultural practices used to manage the simulated crops

Crop	Cultural techniques										
	stubble breaking	soil tillage	sowing date	sowing density (seeds/m^2)	herbicide[a]				cutting[b]		harvest seed loss rate
					sowing		spring				
					C	T	C	T	1st	2nd	
transgenic rape	yes	plough	31/08	70	90	0	0	0	[c]		10%
winter crop	yes	chisel	4/10	350	90	90	90	90			100%
unsown set-aside							0	0	3	no	
border							0	0	3	no	

[a] Mortality rates (%) for classic (C, of genotype *aa*) and transgenic plants (T, of genotype *Aa* or *AA*).

[b] Date of cutting in number of days after rape flowering onset.

[c] This technique cannot be used for this crop.

Description of the temporal sub-model

Figure 2 shows the general organisation of the annual rape life-cycle simulated for each plot and year. For each life-stage such as *seedlings*, *adults* etc, the model calculates the number of individuals per m^2 and the proportion of the three possible genotypes *AA*, *Aa* and *aa*. The life-cycle is modulated slightly according to the crop grown on the simulated field; for instance, the compartment *sown rape seeds* only exists in rape fields and simulates the arrival of rape seeds at the sowing of the crop. The relationships between the various life-stages depend on (further details are given in Table 3):

- on the crop grown in the plot. For instance, rape volunteers growing in a spring crop cannot complete their cycle because of insufficient vernalization and/or time and produce neither

flowers nor seeds (relations 8 and 9).

- on the cultural techniques applied to the crops. For instance, the vertical seed distribution in the pre-sowing seedbank depends on soil tillage (relation 3); ploughing mixes seeds of various layers whereas chiselling little disturbs the initial seed distribution.
- on rapeseed characteristics. For instance, plant mortality after herbicide treatments strongly depends on plant genotype (relations 5 and 7); if the applied herbicide is the one against that the transgene confers tolerance, then the plants of genotypes *Aa* and *AA* survive whereas most of those of genotype *aa* die; for any other herbicides however, mortality is independent of genotype.

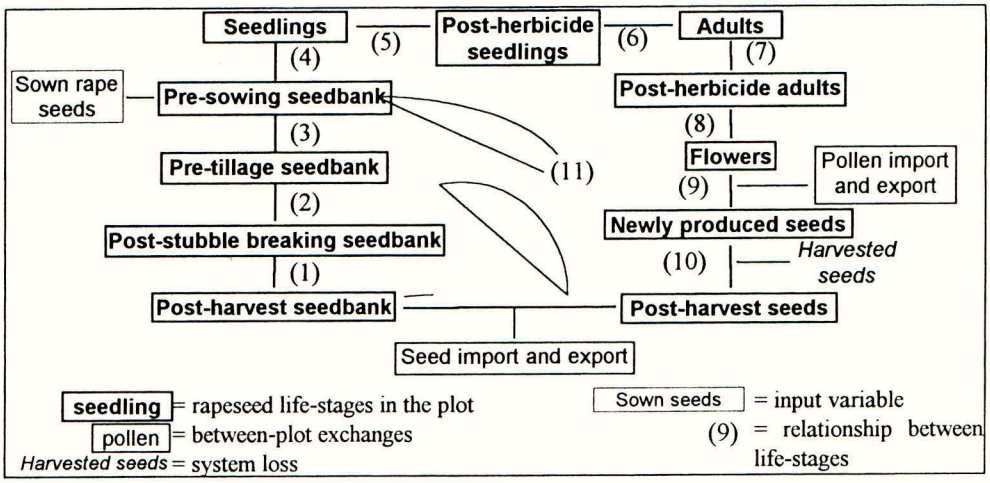

Figure 2. Annual rape life-cycle (for cropped and volunteer plants) simulated for each plot and year

Description of the spatial sub-model

The annual life-cycle of Figure 2 is simulated independently on each plot, but on two occasions, the various spatial units connect by exchanging pollen and seeds that are illustrated by the "pollen or seed import and exports" compartments. These exchanges are mostly caused by wind. The amount of pollen or seeds dispersed from plot *i* to plot *j* results from multiplying the amount produced (and not exported by harvest-combines in the case of seeds) in plot *i* with the proportion of pollen or seeds dispersed from *i* to *j*. This proportion is deduced by quadruple integration from equations giving the proportion of pollen or seeds as a distance from the parent-plant; it increases with plot areas and decreases with the distance between the two units. The determination of pollen exchanges is a little more complicated as the pollen must not only arrive in plot *j*, but plot *j* must also present flowering rapeseed plants ready for pollination. Therefore, besides calculating the number of pollen-producing or receiving flowers, it is also necessary to determine when these flowers are open. The flowering dates depend on the emergence date of the seedlings which depend, in the case of cropped rape plants, on the sowing date of the rape crops or, in the case of rape volunteers, on the sowing date of the crops sown in the plot where these volunteers emerge.

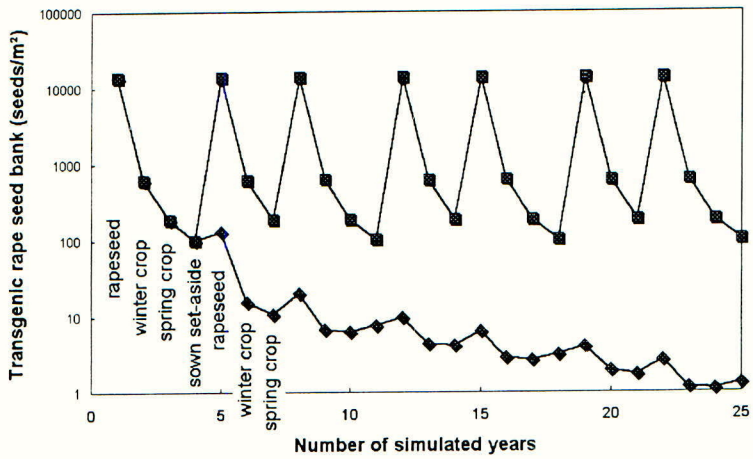

Figure 3. Evolution of transgenic rape seedbank of a field cultivated once with the transgenic, herbicide-tolerant rapeseed variety (◆) or every four to five years (■).

MODEL EVALUATION

GENESYS is currently evaluated, using surveys of farmers' plots for the dynamic part and observations from the long-term experiments of the technical institutes for the genetic and dispersal part. Preliminary results (Couturaud, 1998) show that the model correctly ranks cropping systems for rape volunteer infestation, but underestimates pollen and seed dispersal, probably because dispersal by insects and animals is insufficiently integrated into the model.

SIMULATIONS

Effect of cropping system in a single field

Figure 3 illustrates the effect of crop rotation on the evolution of the post-harvest transgenic seed bank. The basic rotation is the same for both simulations: rape/winter crop/spring crop/ set-aside/rape/winter crop/spring crop. The systematic growing of transgenic herbicide-tolerant varieties (of genotype *AA* with *A* conferring tolerance) results, of course, in the largest transgenic seed bank, with substantial increases after rape crops, because of seed loss before or during harvest, and reductions after winter and spring crops. If, except for the first year, only classic varieties are grown, then the seed bank decreases with time, with again slight increases after rape crops, despite these being classic varieties. Set-aside also increases seed bank because of conditions favouring seed production. After 20 years of simulation, there are still a few transgenic seeds left, despite there having been no transgenic variety since the first year.

The transgenic seed bank after four years is, of course, more important. If a classic variety is to be grown at the fifth year, minimising this seedbank is essential to limit the emergence of transgenic volunteers in the classic crop and thus avoid the pollution of the classic harvest by the unwanted gene which could lead to a refusal of a "non-transgenic" quality label for the harvest

or to a decrease in price if certain fatty acids were required. The simulations show that set-aside management is crucial: sowing a set-aside field in spring (system 2 of Table 4) instead of leaving it unsown (system 1) considerably decreases the transgenic seed bank as rape volunteers cannot produce seeds in spring crops. Frequent cutting of set-aside plots is also necessary: a single cutting (system 3) only delays seed production without reducing it and thus increases the seed bank 40 times compared to two successive cuttings (system 1). Seed banks can also be regulated via winter and spring crop management. Tilling with a chisel (system 4) instead of a mouldboard plough (system 1) decreases the seed bank because most freshly produced seeds are left close to soil surface where seed survival rates are low. Delaying sowing (systems 5 vs. 1) also decreases the seed bank via a reduction of both the viable seed bank at sowing and subsequent seedling emergence rates. Larger sowing rates (systems 6 vs. 1) limit rape seed production in winter crops by favouring interspecific competition between rape volunteers and cropped plants.

Table 4. Effect of cultural techniques for set-aside and winter and spring crops on transgenic post-harvest seedbank after four simulated years with a transgenic rapeseed/winter crop/spring crop/set-aside rotation.

System	Set-aside management		Winter and spring crop management			Post-harvest transgenic seed bank	
			soil tillage	sowing date	sowing density (seeds/m²)	seeds/m²	relative evolution
	sowing	cutting					
1	none	2	plough	Oct./Feb.[a]	350	14150	100
2	spring	2	plough	Oct./Feb.	350	69	0.49
3	none	1	plough	Oct./Feb.	350	570435	4031
4	none	2	chisel	Oct./Feb.	350	2296	16
5	none	2	plough	Nov./March	350	3661	25
6	none	2	plough	Oct./Feb.	400	4405	31

[a] The first sowing date concerns winter crops, the second spring crops.

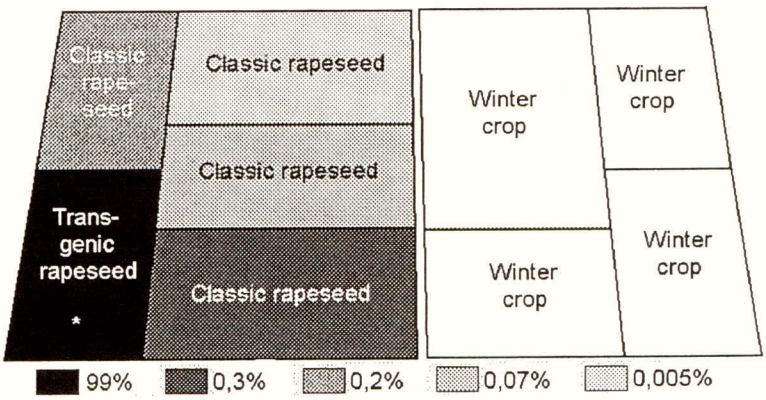

Figure 4. Proportion of newly produced seeds in rape crops carrying one or two copies of the transgene *A* coding for a fatty acid after five simulated years in a region cropped with a rape/ winter crop/spring crop/set-aside/rape/winter crop/spring crop rotation, transgenic varieties (of genotype *AA*) in field * and classic varieties in all other fields.

Effect of regional cropping system

Figure 4 gives the proportion of seeds produced by rape crops and carrying one or two copies of a transgene *A* coding for a fatty acid. Basic rotation is the same as in the previous simulations and again identical for all plots. In this example, all rape crops of a given year were concentrated in the left-hand part of the simulated region; in the second sub-region, rape crops were grown a year later. Only one field was cultivated with the transgenic variety *AA*, but the classic varieties of the neighbour fields also produced seeds carrying the transgene. Furthermore, less than 100% of the seeds produced by the transgenic variety carried the gene. Removing the classic varieties to the right-hand sub-region to increase the distance to the transgenic variety divides the pollution of the classic harvest by at least three.

CONCLUSION

Despite the consistent results of the first evaluation and simulation steps, GENESYS is not yet ready to be used as a decision aid tool. The major sub-models needing improvement concern pollen dispersal processes and rape volunteer demographics in borders. Furthermore, the effect of rape genotype which is presently restricted to herbicide tolerance and selfpollination rates, should be extended to other rape characteristics (*e.g.* pod shattering, pollen production) to identify those features able to limit gene flow. Moreover, even though GENESYS was developed for herbicide tolerance dispersal, the model can be used for other genes, such as those coding for fatty acid content. The model therefore constitutes a reflection tool for those concerned with production, transformation and marketing of rapeseed, thus permitting a prospective analysis of the impact of changes in agricultural practices.

REFERENCES

Champolivier J; Messéan A; Gasquez J (1997). Crop management of transgenic rapeseed: risk assessment of gene flow. *Bulletin GCIRC* **14**, 63-66.

Chèvre A M; Eber F; Baranger A; Renard M (1997). Gene flow from transgenic crops. *Nature* **389**, 924.

Clermont-Dauphin C; Colbach N; Meynard J M. Modelling the influence of cropping system on gene escape from herbicide resistant rapeseed crops to rape volunteers. I. Temporal dynamic and genetic structure of the rape volunteer population in a field.

Colbach N; Meynard J M (1996). Modelling the influence of cropping system on gene flow for herbicide resistant rapeseed. Presentation of model structure. *10e Colloque International sur la Biologie des Mauvaises Herbes, Dijon 11-13 septembre 1996*, 223-230.

Colbach N; Clermont-Dauphin C; Meynard J M. Modelling the influence of cropping system on gene escape from herbicide resistant rapeseed crops to rape volunteers. II. Spatial dynamic and genetic structure of the rape volunteer population in a small region.

Couturaud M C (1998). Effet des systèmes de culture sur les risques de dissémination d'un transgène de colza dans l'environnement: validation et utilisation du modèle GENESYS. *Rapport d'ingénieur agronome INA-PG*, 50 p.

Lefol E; Danielou V; Darmency H (1996). Predicting hybridization between transgenic oilseed rape and wild mustard. *Field Crops Research* **45,** 153-161.

Messéan A (1996). Impact des plantes résistantes à un herbicide. *OCL* **3**, 5-9.

Regional patterns of gene flow and its consequence for GM oilseed rape

C E Thompson, G Squire, G R Mackay, J E Bradshaw, J Crawford and G Ramsay.
Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.

ABSTRACT

The imminent commercialisation of genetically modified oilseed rape varieties requires accurate quantification of transgene movement via pollen within realistic agricultural contexts. This study measured gene flow on a regional and local scale. A 70 square km area west of Dundee was used, in which oilseed rape crops were common, but no GM OSR was grown. Fifty-two sites were selected at distances between zero m and 4000 m from the nearest non-GM spring oilseed rape crop. At each site male-sterile bait plants were placed for a 14 day period and airborne pollen density measured. Pollination occurred at all sites, even those at the greatest distance from flowering oilseed rape fields. Although airborne OSR pollen was recorded at all sites, the density declined rapidly with distance from source. There was also evidence that bees may be important pollen vectors over a range of distances. Additional sites 100 to 900 m from a GM spring oilseed rape crop in a second study area were also investigated. Despite the predominance of non-GM OSR crops in the immediate locality, all sites were pollinated by a mixture of GM and non-GM sources. The results suggest that the farm to farm spread of OSR transgenes will be widespread.

INTRODUCTION

Accurate quantification of transgene escape from and movement within realistic agricultural contexts is required now that transgenic oilseed rape (*Brassica napus* L.) is being grown experimentally. Oilseed rape (OSR) is notable among GM crops as having a widespread occurrence of feral and volunteer weedy populations. Gene flow via pollen movement is particularly hard to quantify as it depends on several elements outwith the control of the experimenter. For example as well as the size of pollen source, the competitiveness of the GM pollen, and meteorological and environmental factors, gene flow will depend on the regional pattern of OSR crops and weedy populations.

Early trials with GM OSR imposed artificial experimental designs to reduce the possibility of gene flow, and many used relatively small plots of GM OSR as pollen sources. Both may lead to an underestimation of the frequency of gene flow. The aim of this study is to measure gene flow through pollen dispersal in a realistic agricultural context. On a regional scale this was done by looking at pollination of male sterile plants in an area with a heterogeneous range of pollen sources. By taking a similar approach in a second study area including a transgenic crop, it was possible to measure gene flow from a single source on a more local scale.

MATERIALS AND METHODS

An area west of Dundee, approximately 70 square km, was selected for study. The acreage of OSR has increased locally over the past decade and volunteer OSR populations are widespread. The cytoplasmic male-sterile component of the cv. Triolo (kindly donated by CPB, Twyford, Cambridge, UK) were used as bait plants to measure gene flow via pollen dispersal. Ten such plants (area = $0.15m^2$) were used to form a bait colony *in situ* for 14 days during the main spring OSR flowering period in the region. Passive pollen traps (double-sided adhesive tape on microscope slides) were used at each site for seven days to measure the density of airborne pollen. Motorised Burkard pollen traps (Burkard Manufacturing Co Ltd, Rickmansworth, UK) were operated at five of the sites, measuring pollen density continuously throughout the two week period.

The 52 bait colonies were sited from zero to approximately 4000 m from the nearest identifiable pollen source. After two weeks the plants were removed to a glasshouse, subsequent flower buds removed, and the seeds grown to maturity. The percentage level of pollination (i.e. the percentage of flower buds forming siliquae during the experimental period) was counted for each bait colony. Also the average number of seeds set per siliqua was calculated for each plant, based on counts from five siliquae evenly distributed along the primary stem. Mature seed was harvested from a number of the bait plants, germinated and DNA extracted from green leaf samples. DNA profiling using anchored microsatellites (Charters *et al.*, 1995) was used to identify the source of pollen where the genotypes of possible sources were known.

As a special case, a similar experimental approach was taken to measure gene flow from an 11 ha field of high laurate transgenic OSR in Perthshire. The OSR carried a thioesterase gene from *Umbellularia californica* and a *npt*II marker gene conferring kanamycin resistance. Bait colonies were placed from 100 m to 900 m from the field. Several other OSR crops were being grown in the same area, the nearest to the field being some 320 m away. To detect trangenic progeny among the bait plant progeny, seeds were germinated on selection media containing kanamycin ($300mgl^{-1}$). To confirm the scoring of bait plant progeny, a subsample were grown to seedling stage and DNA extracted. PCR was used to detect the *npt*II gene (Hamill *et al.*, 1991), and further primers were designed to amplify the thioesterase transgene (EMBL M94159). For the analysis of the general relationships between pollination and source, results from these bait colonies were pooled with the rest.

RESULTS

Pollen density profile

Four of the Burkard traps at sites in a transect at zero, five, 170 and 400 m from edge of a 55 ha field were used to measure daily pollen counts. Average pollen counts over the two week period show a steep decline with distance with levels at 170 m from the field being less than 25% of that at the edge of the field. The three nearest pollen traps show

similar peaks during the time period, while levels at the most distant site are relatively constant. Although 'clumps' of OSR pollen grains (up to 50 grains) were infrequently detected up 700 m from source, over 82% of pollen recorded more than 100 m from source was present as single grains.

Regional scale gene flow

Pollination occurred at all sites, ranging from 88.4% of flowers buds being pollinated at 1 m from a field edge, to 5% at extreme distances from source. Pollination declined with distance from source ($r^2 = 0.5178$, $P < 0.0001$), under a Gaussian distribution of values (Figure 1). The density of airborne pollen, as measured on microscope slides, also declined but with a pronounced leptokurtic distribution and values in the second phase of the graph (distance greater than 150 m from source) were not correlated with distance (Spearman $r = -0.2956$, $P = 0.1427$).

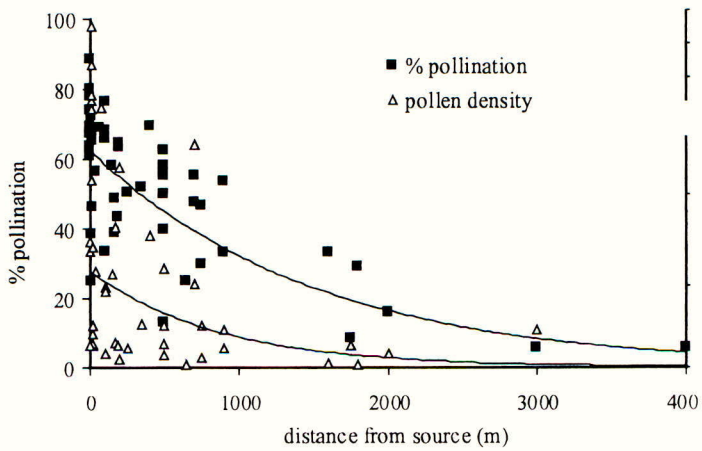

Figure 1. Decay curves of % pollination and airborne pollen density with distance from nearest pollen source.

At one of the bait sites, with a pollination rate of 33%, a sample of bait plant progeny were used for DNA profiling. The majority of the sample (>80%) were shown to have been fertilised by pollen from the nearest crop, 900 m distant. However, seeds from one siliqua sampled were fertilised by pollen of a second genotype whose nearest *known* source is some 4 km away. There was little pollen recorded at the Burkard trap at this site (<1 grain m^{-3} per day) suggesting that such longer range pollination events could have been mediated by insects.

The numbers of seed set per siliqua in bait plants close to a pollen source were identical to typical values within the actual crop (Figure 2). Values then declined over the first hundred metres, but changed relatively little thereafter. At 400 m from source, a mean of 9.8 seeds (SD ± 8.1) seeds per siliqua was recorded. At such extreme distances the large range of values (1 to 24 seeds per siliqua), despite the predominance of isolated

airborne pollen grains, suggest that pollination is not wholly due to wind dispersal, and may therefore be due to (occasional) visits by pollinating insects.

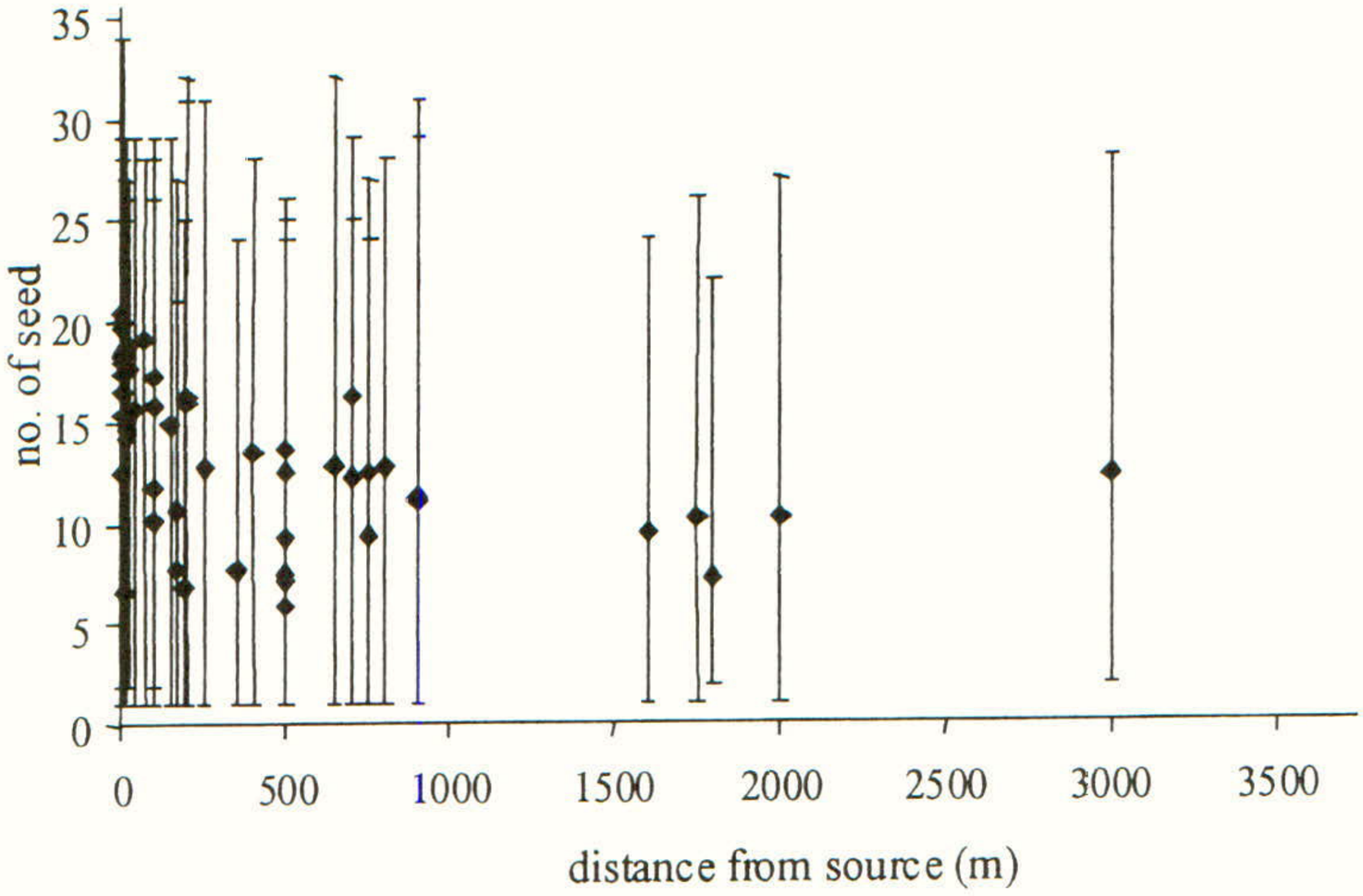

Figure 2. Mean number of seed set per siliqua and distance from source. Bars indicate maximum and minimum values

Measurement of gene flow from a single GM source

Fertilisation by GM and non-GM pollen was detected at all bait colony sites around the transgenic field (Table 1). While the level of total pollination remained relatively constant, the percentage of seeds set that proved to be transgenic was highest at the site closest to the GM field, and lower at more distant sites or those nearer to a non-GM crop.

Table 1. Pollination profiles of GM and non-GM pollen sources

	Distance from nearest crop (m)		% total pollination	% of GM seed set
	GM	non-GM		
site 1	100	1160	65.6	74.7
site 2	580	220	61.2	12.4
site 3	670	900	58.0	30.3
site 4	800	500	49.3	31.1
site 5	930	1200	54.8	10.3

DISCUSSION

The experimental approach described here is highly realistic in that it uses existing field crops of OSR as sources to measure the potential transfer of transgenes. We have also demonstrated that DNA profiling techniques can be used to determine the source of pollen. The use of male-sterile bait plants was essential to measure gene flow on such a large scale, but the results obtained represent only the *potential* for gene flow. While we may anticipate that pollination decay curves would be similar, pollination levels for male-fertile plants are likely to be much lower. However, the popularity of varietal hybrids in which the majority of plants are male-sterile (e.g. cvs. Triolo and Synergy) has important consequences for gene flow, and these crops are more likely to be pollinated by external sources.

Both levels of pollination and airborne pollen density declined with distance in a similar manner to that found by Timmons *et al.* (1995). In that study, pollen density at 360 m was found to be 10% of that found at the field margin, compared to approximately 5% at 400 m in this study (at both Burkard and slide traps). However the frequency of pollination events (0.8% at 2500 m from source) reported in Timmons *et al.* (1995) has been easily exceeded here. Notably, petals were removed from the bait plants used by Timmons *et al.* (1995), in order to make them less attractive to honeybees which maybe in part responsible for the lower levels found in that study. OSR pollen dispersal by bees has been previously reported (e.g. Mesquida *et al.*, 1988; Bilsborrow *et al.*, 1998), and the behaviour of honeybees in this respect is reported elsewhere (G. Ramsay *et al.*, these proceedings). The relative importance of wind and insect pollination is difficult to examine in field conditions, however indirect evidence suggests bee involvement. For example high numbers of seed set per siliqua occurred at distant sites, despite an overall low frequency of pollination events. This, and the occurrence of pollination events in the absence of high levels of airborne pollen, suggest that insects play an important role and could mediate gene flow over a range of distances.

This study also shows bait plants pollinated over both short- and long-range distances by a mixture of pollen sources indicating that their regional distribution is an important factor in contributing to overall pollination levels. This is also apparent from the variation in pollination levels at any one particular distance from source. For example, pollination in bait colonies 500 m from the nearest source ranged from 13.0% to 57.9% of flowers. This may be due in part to the microgeographical habitat, (the lowest value came from a sheltered site), but may also be due to the clustering of crop sources irrespective of the distance to simply the nearest source.

Reported gene flow levels from transgenic OSR plots in the presence of normal pollen competition (e.g. Scheffler *et al.*, 1993; Paul *et al.*, 1995) conflict with results reported by Timmons *et al.* (1996), and by implication with the results from this study. Since 1993 over 60% of winter OSR crops in the wider Tayside area were grown within 100 m of another crop simultaneously in flower, and at least 20% within 100 m of a feral population (DETR, 99). In the current study, an average of 61% of flowers on male-sterile bait plants were pollinated at 100 m from source with 9.5 seed set per siliqua, and 49% of seed set on male-sterile plants at 100 m from a GM crop were

transgenic. Despite the over-estimation of gene flow levels detected by the approach used here, the results have implications for growing non-traditional OSR types. The slow decline in pollination levels with increasing distance from source illustrates the potential distance OSR transgenes may move, and that background gene flow occurs across substantial distances from the more intensely farmed areas. Thus potential exists for a continuous network of cross-pollination of OSR across any region growing the crop. This far-reaching gene flow, even if in reality less frequent than these results suggest, will lead to volunteers and feral populations acting as gene pools for introduced genes coming from adjacent and even distant farms.

ACKNOWLEDGMENTS

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REFERENCES

Bilsborrow P E; Evans E J; Bowman J; Bland B F (1998). Contamination of edible double-low oilseed rape crops via pollen transfer from high erucic cultivars. *Journal of the Science of Food and Agriculture*, **76**, 17-22.

Charters Y M; Robertson A; Wilkinson M J; Ramsay G (1996). PCR analysis of oilseed rape cultivars (*Brassica napus* L. spp. *oleifera*) using 5' anchored simple sequence repeat (SSR) primers. *Theoretical and Applied Genetics* **92**, 442-447.

DETR (1999). *Investigations of feral oilseed rape populations. Research Report No. 12.* Department of the Environment, Transport and the Regions: London.

Hamill J D; Rounsley S; Spencer A; Todd G: Rhodes M J C (1991). The use of polymerase chain reaction in plant transformation studies. *Plant Cell Reports* **10**, 221-224.

Mesquida J; Renard M; Pierre J S (1988). Rapeseed (*Brassica napus* L.) productivity: the effect of honeybees (*Apis mellifera* L.) and different pollination conditions in cage and field tests. *Apidologie* **19**, 51-72.

Paul E M; Thompson C; Dunwell J M (1995). Gene dispersal from genetically modified oil seed rape in the field. *Euphytica* **81**, 283-289.

Scheffler J A; Parkinson R; Dale P J (1993). Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Transgenic Research* **2**, 356-364.

Timmons A M; O'Brien E T; Charters Y M; Dubbels S J: Wilkinson M J (1995). Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. *Euphytica* **85**, 417-423.

Timmons A M; Charters Y M; Crawford J W; Burn D; Scott S E; Dubbels S J; Wilson N J; Roberston A; O'Brien E T; Squire G R; Wilkinson M J (1996) Risks from transgenic crops. *Nature* **380**, 487.

Modelling the potential for gene escape in oilseed rape via the soil seedbank: its relevance for genetically modified cultivars

C Pekrun
Institute Agronomy Grassland (340), University Hohenheim, 70593 Stuttgart, Germany

P W Lane, P J W Lutman
IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK

ABSTRACT

A simple mechanistic model is presented which describes population dynamics of volunteer oilseed rape within a field. The model calculates the number of volunteers appearing in each crop and the seedbank after each crop. The main input variables are harvesting losses when the crop is oilseed rape, crop rotation, soil cultivation, moisture distribution in soil and the level of volunteer control in each crop in the rotation. The impact of the amount of harvesting losses and of the level of volunteer control are tested and presented in this paper. Simulations of the effects of replacing oilseed rape with linseed are shown. Sensitivity analysis revealed that the relationship between the number of volunteer seedlings and the size of the seedbank in the absence of direct control measures was of particular importance and further research is necessary to improve the definition of this relationship.

INTRODUCTION

From an ecological point of view the soil seedbank of a species ensures genes for the future. Oilseed rape, more than many other crops, has the potential to develop large and persistent seedbanks (Schlink, 1994, 1998). At the same time oilseed rape is very amenable to genetic engineering and is the subject of work relating to herbicide resistance, oil quality and to resistance against pests and diseases. The ability of oilseed rape to persist in soil may cause agronomic problems and is of concern for ecological reasons. If volunteers confer a different oil quality to that of the crop sown, impurities and thus quality reductions may be the result. If volunteers are resistant to glyphosate or glufisonate, herbicide programmes based on either of these herbicides will not be effective in their control. Oilseed rape can hybridise with several wild crucifers and hence genes can be transferred into the genome of wild plants of the agro-ecosystem (Mikkelsen *et al.*, 1996; Chèvre *et al.*, 1997). This again may result in enhanced weed problems both within and outside fields.

There are no data available on the long-term behaviour of seedbanks of oilseed rape under arable conditions. To date, in the limited number of field experiments which have studied seedbank dynamics, data are only available for a maximum of three and a half years (Lutman *et al.*, 1998; Chadoeuf *et al.*, 1998). So, the only way to study seedbank dynamics of volunteer oilseed rape over a longer period of time is by modelling. There are data available on most steps of the life-cycle of volunteer rape and so a synthesis of the different pieces of information into a simulation model provides a mechanism for further insight into the problem. In this paper we present a simple deterministic model of the life-cycle of volunteer oilseed rape along with the output of some simulation runs.

PRESENTATION OF THE MODEL

The basic structure of the model is presented in Fig. 1. The boxes represent the state of some aspect of the seeds or plants at some time during the year and the valves represent factors that influence the transition from one state to the next. Boxes indicating the seedbank in the soil are divided into four sections representing layers of the soil profile: 0 – 5 cm, 5 – 10 cm, 10 – 15 cm and 15 – 20 cm.

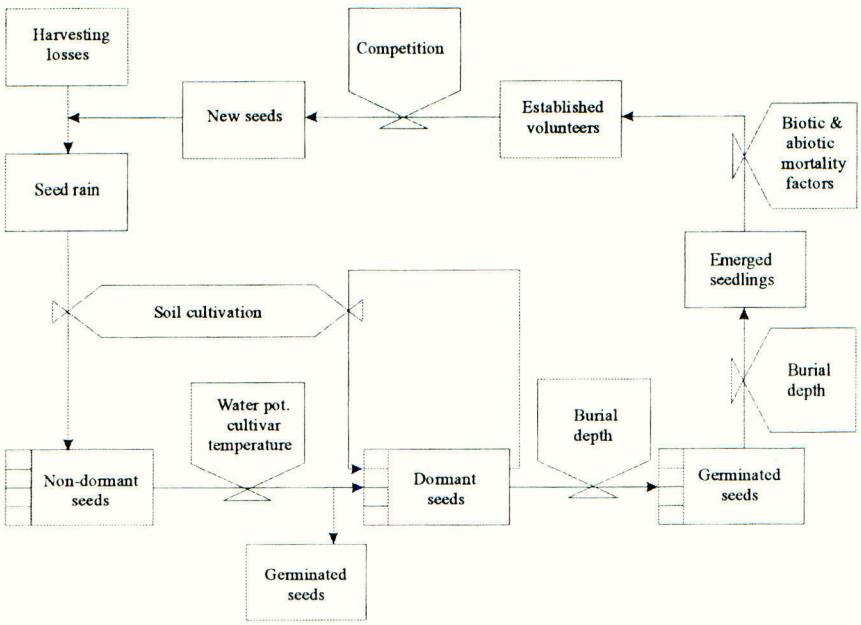

Fig. 1: General structure of the model. Boxes represent individuals (seeds or volunteer plants). Valves represent factors that influence the transition from one step to the next.

The formula for the model is:

$$s_{t+1} = D_j\,(C_j\,(I\text{-}G)\,s_t + d\,C_j\,\{l_t + p\,e\,G\,s_t\,(b_0 + b_C\,(p\,e\,G\,s_t + n_X\,e_X))^{-1}\}\,),\quad j = 1 \ldots m$$

s_t matrix for seedbank in year t.

C_j, D_j matrices for seed movement within the soil profile caused by soil cultivation j ($j = 1 \ldots m$). C_j represents the first cultivation after harvest, D_j all the following cultivations. Matrices for ploughing and spring-tining were taken from Cousens & Moss (1990). For discing an additional matrix was constructed by extending the matrix for spring-tining.

I identity matrix when no redistribution between layers occurs.

d proportion of seeds developing dormancy in year t after first soil cultivation. A logistic curve was derived from an experiment by Pekrun *et al.* (1998a) with water potential as the explanatory variable and the proportion of dormant seeds as response variable.

l_t harvesting losses in year t.

G yearly germination from the soil seedbank. Based on findings by Schlink (1994) and Pekrun *et al.* (1997, 1998a) germination is assumed to be 100% in the 0–5 cm layer. For

the 5–20 cm layer, seedbank data published by Lutman *et al.* (1998) were used. From this a yearly rate of decline of 0.55 was calculated, with a 95% confidence interval of 0.37 and 0.82. As this value was calculated for 0–20 cm and as the confidence interval for it was very large, 0.50 was assumed to be a reasonable value for the soil seedbank in 5–20 cm.

e emergence of seedlings in relation to burial depth derived from data by Lutman (1993).

p survival of emerged seedlings. There are no data available on this parameter in the absence of direct volunteer control. With emergence being depth dependent and $p = 0.1$ a maximum of 5 % of the seedbank was found to give rise to volunteer seedlings. This approximately reflects the situation in our own experiments (Lutman *et al.*, 1998; unpublished observations) and therefore was used in simulation runs without direct volunteer control.

e_X density equivalent for crop x - peas, beans or linseed - calculated on the basis of Lutman *et al.* (1996).

n_X density of crop x.

b_0, b_C 1/maximum seed production of a plant in isolation and 1/maximum seed production per unit area were based on data by Leach *et al.* (1994).

Sensitivity analysis on each of the parameters suggests that the proportion of seeds germinating from the seedbank each year has a particularly large impact on the output of the simulations.

SIMULATION RUNS

A number of simulations have been run with a range of different input parameters such as seed losses, rotations and levels of control of volunteers. The basic rotation tested was oilseed rape, wheat, field beans, wheat, as this is quite typical of UK rotations where farmers are trying to maximise the proportion of first wheat crops. The first simulation shown in this paper compares the impact of different levels of harvesting losses. Seed losses from oilseed rape have been found to vary between 20 and 650 kg/ha (Pekrun *et al.*, 1998b). Fig. 2 shows the effect of seed losses varying between 20 and 400 kg/ha. Assuming seed losses of 20 kg/ha and average agronomic practices the model suggests that after three rotations no volunteers appear in beans and 2 volunteers m^{-2} in oilseed rape. In a simulation which reflects a more average situation with seed losses of 200 kg/ha the model predicts 2 volunteers m^{-2} in beans and 15 m^{-2} in oilseed rape, after three rotations. Where seed losses are 400 kg/ha the model predicts 4 and 30 volunteers m^{-2}, respectively. In Fig. 3 the impact of varying levels of volunteer control in beans is shown. Seed losses are 200 kg/ha each year if oilseed rape is grown. In beans, control efficiency of volunteer oilseed rape can vary between 85 and 100 % with an average of 90 % (Knott, pers. commun.). At 100 % control no volunteers will appear in beans and approximately 2 volunteers m^{-2} in oilseed rape. When assuming 90 % volunteer control in beans about 2 volunteers m^{-2} will appear in beans and about 15 m^{-2} in oilseed rape. This will be increased again if control is decreased to 85 %, resulting in 3 and 22 plants m^{-2} in beans and rape, respectively. In Fig. 4 the effect of growing linseed instead of oilseed rape is shown. The starting seedbank is a seedbank reached after growing 4 rotations of oilseed rape, wheat, beans, wheat at average input variables and agronomic practices. After changing to a rotation with linseed, wheat, beans, wheat, the seedbank declines such that after only two rotations seedbanks are so small that less than 1 volunteer plant m^{-2} sets seed.

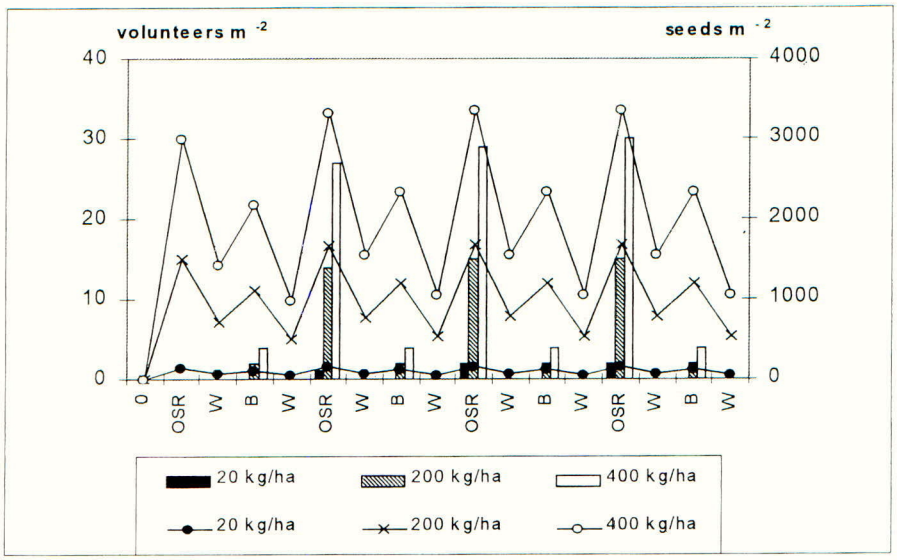

Fig. 2: Effect of varying seed losses from oilseed rape in a rotation with oilseed rape (OSR), wheat (W), beans (B), wheat (W). Seed losses are varied between 20, 200 and 400 kg/ha. Bars represent volunteers m^{-2} in each crop, lines represent seeds m^{-2} at 0-20 cm after each crop.

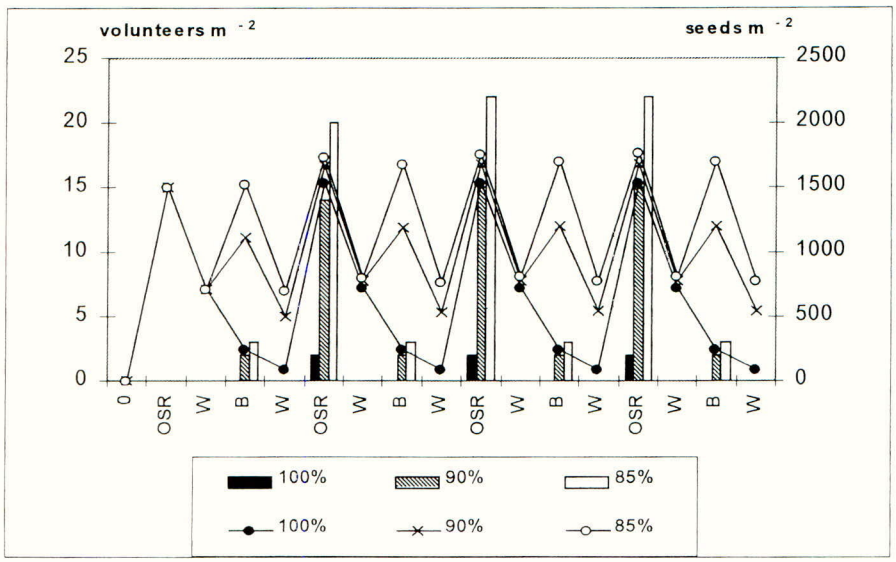

Fig. 3: Effect of varying levels of volunteer control in beans in a rotation with oilseed rape (OSR), wheat (W), beans (B), wheat (W). Volunteer control is varied between 85, 90 and 100%. Bars represent volunteers m^{-2} in each crop, lines represent seeds m^{-2} at 0-20 cm after each crop.

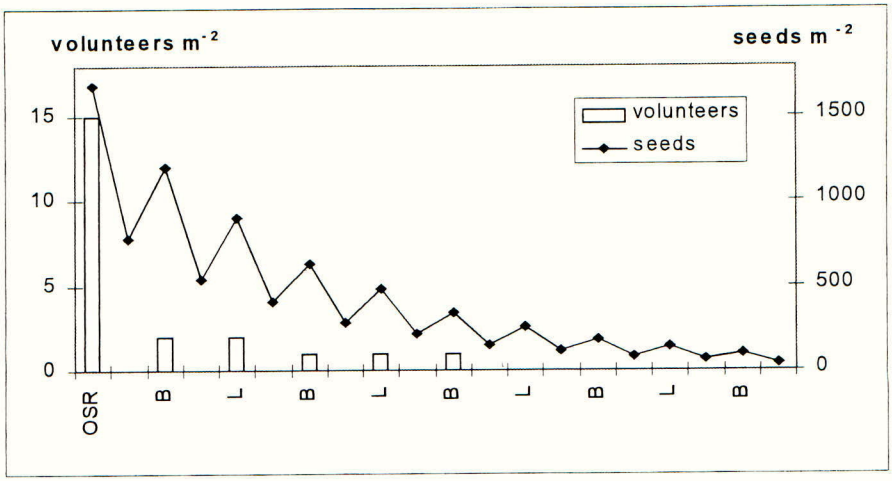

Fig. 4: Reducing the seedbank by growing linseed instead of oilseed rape. Simulations starts from an average seedbank reached after growing 4 x oilseed rape (OSR), wheat (W), beans (B), wheat (W). Bars represent volunteers m^{-2} in each crop, lines represent seeds m^{-2} at 0-20 cm after each crop

DISCUSSION

At present the model presented in this paper cannot be fully validated or improved as there is no independent data set to use for comparison. So, the model cannot be used for prediction. However, it can be used to identify major gaps in knowledge and to study the relative impact of various agronomic practices. Additionally, it helps to understand the processes underlying population dynamics of volunteer oilseed rape. Sensitivity analysis showed that the proportion of seeds germinating from the seedbank each year is a critical factor. As this parameter is based on very limited data there is a clear need for future research to define it more precisely. Also, there is a need for research to quantify survival of emerged seedlings in the absence of direct control measures. We have been unable to find any data on this parameter and therefore derived it on the basis of generating outputs that realistically describe the situation in the field.

The simulation runs presented in this paper show that the amount of seed losses and the level of volunteer control are important input variables. The former is not surprising but the latter is particularly interesting. Volunteer control in beans was varied only marginally but still had a significant impact. It has an even bigger impact when volunteer control in beans is assumed to be 0 % (not shown), a situation that could occur when herbicide programmes are based solely on glyphosate or glufosinate and the oilseed rape volunteers arise from crops resistant to these herbicides, a result also supported by the model of Squire *et al.* (1997). In all runs the model suggests relatively large volunteer densities in oilseed rape. So, there is considerable potential for contamination when the quality of volunteers differs from the sown crop. Also, it shows that if a farmer decides not to grow gm-rape any more but to return to conventional oilseed rape a relatively large hidden population of volunteer rape would still appear for a number of years, hence potentially spreading unwanted genes. In contrast, replacing oilseed rape by a crop in which reasonable volunteer control is possible (eg linseed) would result in a rapid decrease in seedbanks.

Gene escape in time via the soil seedbank is only one aspect that needs to be considered in risk assessments of genetically manipulated rape. More complex models such as the model by Colbach & Meynard (1996) are necessary to study gene flow as a function of agronomic practices and ecological factors. Our model could function as a sub-model in one of these models.

ACKNOWLEDGEMENTS

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REFERENCES

Chadoeuf R; Darmency H; Maillet J; Renard M (1998). Survival of buried seeds of interspecific hybrids between oilseed rape, hoary mustard and wild radish. *Field Crops Research* **58**, 197-204.

Chèvre, A-M; Eber F; Baranger A.; Renard M (1997). Gene flow from transgenic crops. *Nature* **389**, 924.

Colbach N; Meynard J M (1996). Modelling the influence of cropping system on gene flow for herbicide resistant rapeseed. Presentation of the model structure. *Proceedings of the Xe Colloque international sur la biologie des mauvaises herbes, Dijon*, 223-230.

Cousens R; Moss S R (1990). A model of the effects of cultivation on the vertical distribution of weed seeds within the soil. *Weed Research* **30**, 61-70.

Leach J E; Darby R J; Williams I H; Fitt B D; Rawlinson C J (1994). Factors affecting growth and yield of winter oilseed rape (*Brassica napus*), 1985-89. *Journal Agricultural Science, Cambridge* **122**, 405-413.

Lutman P J W (1993). The occurrence and persistence of volunteer oilseed rape (*Brassica napus*). *Aspects of Applied Biology* **35**, *Volunteer crops as weeds*, 29-36.

Lutman P J W; Risiott R; Ostermann H P (1996). Investigations into alternative methods to predict the competitive effects of weeds on crop yields. *Weed Science* **44**, 290-297.

Lutman P J W; Pekrun C; Albertini A (1998). Dormancy and persistence of volunteer oilseed rape. *HGCA-report No*. OS32.

Mikkelsen T R; Andersen B; Jorgensen R B (1996). The risk of crop transgene spread. *Nature* **380**, 31.

Pekrun C; Lutman P J W; Baeumer K (1997). Germination behaviour of persisting oilseed rape seeds in relation to temperature. *Weed Research* **37**, 419-431.

Pekrun C; Hewitt J D J; Lutman P J W (1998a). Cultural control of volunteer oilseed rape (*Brassica napus* L.). *Journal Agricultural Science, Cambridge* **130**, 155-163.

Pekrun C; Lutman P J W; Baeumer K (1998b). Research on volunteer rape: a review. *Pflanzenbauwissenschaften* **2**, 84-90.

Schlink S (1994). Ökologie der Keimung und Dormanz von Rapssamen (*Brassica napus* L.) und ihre Bedeutung für eine Überdauerung der Samen im Boden. *Dissertationes Botanicae* 222, Cramer, Berlin, Stuttgart.

Schlink S (1998). 10 years survival of rape seed (*Brassica napus* L.) in soil. *Zeitschrift Pflanzenkrankheiten Pflanzenschutz Sonderheft* **XVI**, 169-172.

Squire G R; Burn D; Crawford J W (1997). A model for the impact of herbicide tolerance on the performance of oilseed rape as a volunteer weed. *Annals Applied Biology* **131**, 315-338.