The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops

Final report of the DEFRA project: Consequences for Agriculture of the Introduction of Genetically Modified Crops, RG0114.

August 2003

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SUMMARY

1. This project examined whether oilseed rape could persist in the environment, as feral (or volunteer) weeds, for long enough and in high enough number to cause impurities in later crops. Particular attention was given to the possibility of genetically modified (GM) ferals being impurities in later non-GM crops.
2. The life-cycle biology of oilseed rape was reviewed (section 2.1). Seed shed, secondary (inducible) dormancy and persistence of seed were the main physiological factors causing oilseed rape feral weeds to become part of the seedbank of arable fields. Non-GM and GM varieties differed little in these traits.
3. The presence and abundance of feral oilseed rape were then examined by re-analysis of published data. Feral oilseed rape was not detected in arable seedbanks before the 1970s (2.2), but became common following expansion of the cropped area of oilseed rape in the 1970s and 1980s. Ferals appear to have persisted in some fields for at least 10 years after an oilseed rape crop had been grown (2.3).
4. Would these populations contribute an impurity? The typical seedbank population density of feral oilseed rape is 100 m^{-2}, small compared to the total weed seedbank in an arable soil (commonly 1000 to 10,000 m^{-2}) but similar to the established stand density of oilseed rape crops. In cereal-based rotations in GB, oilseed rape is commonly grown as a 'break' crop every two to four years. Even if only $1/100^{th}$ of the feral seedbank germinated in the break year, it would have a large impact as an impurity in the oilseed rape crop. (The EU impurity threshold for GM in non-GM is 0.9%.)
5. Little scientific information is yet available for the persistence time and population density of ferals arising from GM varieties. Therefore to explore possibilities, a generic, stage-structured, life-history model of oilseed rape population dynamics was developed to predict percentage impurity (3.1, 3.2). The model was used to examine the effect of various management practices on impurity in the seedbank, emerged feral plants and harvested yield.
6. In a simulation based on two years of winter wheat and one year of winter oilseed rape, with no attempt to control the feral population, it took 16 years after harvesting of the original crop for impurity in yield to fall below 1%.
7. Simulations indicated only the most rigorous field management would meet an impurity threshold of 1% within five years. Given that impurities also arise through sown seed and by geneflow between fields, thresholds of this order will be difficult to attain in general farming practice.
8. The model is now being tested and refined against data collected in fields in which GM crops have been grown, notably the Farm Scale Evaluations in GB.

1. BACKGROUND AND RATIONALE

Introducing GM plants into commercial agriculture presents several problems to an agricultural enterprise. GM cropping, that is the GM plant and associated field practice, might affect biodiversity and food webs in farmland, while the GM plants themselves might bring impurities to other crops, either through movement of pollen or persistence of feral weeds in fields. This project concentrated on the persistence of feral weeds, and thereby complemented DEFRA's wider portfolio of GM research, which includes the Farm Scale Evaluations of GM herbicide tolerant crops (Firbank *et al*., 2003) and cross-pollination (Current geneflow research 2003).

Aims and purpose

The aim of the project was to assess whether GM plants could persist in the environment for long enough and in high enough number to cause impurities in later crops. Much of the evidence on persistence of feral plants had been obtained for non-GM varieties. The relatively little evidence for GM varieties in the UK and the rest of Europe was somewhat fragmented (e.g. Norris & Sweet, 2001). The use of models was recognised by DEFRA as a way to bring together and make better use of such information. The present project had to develop preliminary ideas in modelling into a more flexible, predictive framework.

The scientific aims of the project were (briefly), to -

- consult widely with industry to define the perceived implications of introducing GM crops;
- develop and demonstrate a workable model framework for feral weeds;
- adapt an existing life cycle model and operate it with realistic parameters for a chosen plant or system;
- produce model output to show change in % GM impurity in the seedbank, emerged plants and harvested yield over a series of years;
- compare the results of the model with measurements on feral populations in fields;
- transfer modelling concepts to other studies such as the BRIGHT project, which aims to examine ways to manage impurities caused by GMHT crops (BRIGHT 2002).

Following early consultation, it was agreed the most pressing concern was the degree to which oilseed rape (*Brassica napus* L.), as feral or 'volunteer' populations, might introduce impurities to later crops of oilseed rape. The modelling therefore concentrated on the in-field populations of this species, but was designed to be generic, so that it could be applied equally to wayside populations of oilseed rape (Anon, 1999) or to populations of other feral species. The project developed an existing life-cycle model (Squire et al., 1997) which provided output in the form of the abundance (individuals in a unit area) of emerged plants, seed at harvest or seed in the buried seedbank. Existing information was reviewed in detail to form a basis for the new life cycle model.

The report first presents a summary of the review of oilseed rape as an in-field feral (volunteer) plant, then describes the construction, and demonstrates the use of the model to address questions of population dynamics, persistence and impurity.

2. REVIEW AND SYNTHESIS OF INFORMATION ON FERAL OILSEED RAPE

Most of the crop grown in the UK as oilseed rape, or rapeseed, is the species *Brassica napus* L. Some areas, particularly in the north, are grown with a similar crop, the cultivated oilseed form of the turnip, *Brassica rapa* L. In a major botanical survey carried out in the 1990s (Preston, Pearman and Dines, 2002) the feral descendents of both *Brassica napus* L. and *B. rapa* L. were widespread in GB at the 10 km scale of recording. However, the full status of these species requires measures of abundance and longevity, neither of which had been well documented before this project. Accordingly, the available information on feral *Brassica* was reviewed as part of the process of constructing the model.

2. 1 The physiological basis of persistence in the seedbank

Rapeseed has become a widespread feral plant because (i) a proportion of seed is shed at harvest of a crop or falls from agricultural vehicles, (ii) some of this seed enters the soil, (iii) plants emerge, reproduce and drop seed back to the soil, and (iv) seed remains in a dormant state in the soil. Except in a future crop of oilseed rape, the third of these processes can be prevented in principle, especially in cereal crops where they can be controlled with herbicide. The fourth – involving dormancy and longevity – is the process that brings most uncertainty to the status of feral *Brassica*.

Dormancy in oilseed rape is mostly induced by specific conditions of temperature or dryness. Induced (or secondary) dormancy is not usually noticeable at normal testing temperatures of 15 to 20^{o}C. Studies based at Rothamsted (Pekrun *et al*., 1997; 1998) and SCRI (Marshall *et al*., 2000; Squire, 1999; Squire *et al*., 1997) showed that a proportion of the seed of most oilseed rape varieties is inducible in some conditions (e.g. by low temperature, dryness or darkness). The feral problem therefore has its origin in the physiological (genetically based) properties of seed stocks.

Once in the soil, the seed germinates or is eventually eaten, invaded by saprophytes or dies of age. Many experiments have been conducted to find out how long seed remains alive in soil. Generally, the seed of *Brassica* crop varieties was buried in the soil in pots or bags so that it could not emerge even if it germinated. The pots or bags were unearthed after different intervals and the seed tested to see if it was alive and capable of germinating. A representative sample of such experiments is summarised in Table 1. Great variation in seed persistence was observed, from the 16 years found for a *B. napus* swede variety to around 1 year for several other seed lots. When GM and non-GM seed were compared, there was little difference in longevity. Wild relatives (e.g. wild *B. rapa* and *Hirschfeldia incana*) usually persisted at greater percentage.

In most of these experiments, seeds were in stable environments, different from the highly disturbed soil of an arable field. Two of the experiments released seed into more or less natural agronomic conditions and estimated decline and longevity by re-sampling the soil (Chadoeuf *et al*., 1998; Lutman *et al*., 2002). Both prevented re-seeding of emerged plants and found the original seed persisted for (variously) three to 6 years, albeit at <1% of the original population.

This body of research implies that most buried *Brassica* seed dies rapidly, but a small fractions (less than 1%) becomes dormant and remains alive in soil for several years, especially if buried at depth (e.g. 15 to 20 cm). The time of persistence varies with the variety and also the conditions of burial. Notably, the one study in Table 1 to examine depth of burial found persistence in much greater percentage at depth than near the surface.

2.2 Review of *Brassica* species in the seedbank literature

Evidence was then examined for the presence and abundance of feral *Brassica* under normal agronomic practice A review was completed of the literature on the arable seedbank in the UK, partly under this project and partly under the remit of a DEFRA desk study on weed diversity (Marshall *et al*., 2003). The detailed analysis is presented in the paper by Squire *et al*. listed under 'Outputs'. The review ranged from the first studies at Rothamsted in 1915 to the DEFRA-funded work of the 1990s leading up to, but not including, the Farm Scale Evaluations of GMHT crops (Table 2). The various authors estimated seedbanks by one or both of two techniques. In the emergence technique, soil is removed from sites and laid in trays to encourage seeds to germinate; taxa are identified as seedlings. In the extraction technique, seeds are removed from soil usually by sieving and flotation; taxa are identified as seeds. The two techniques general produce similar estimates of presence and abundance for the common arable weed species in the UK.

The review concentrated on the main Cruciferous weeds, and specially the plants of the genus *Brassica* and its close relatives. Species of *Brassica* can be distinguished from most other Cruciferae as either seedlings or seeds. Species within the genus *Brassica* (e.g. *B. rapa* and *B. napus*) can usually be distinguished as seedlings but are very difficult to distinguish as seeds. The possibility of misidentification is taken into account in the following summary.

Among the studies in Table 2, the only Cruciferous weed that was widespread and abundant in the seedbank throughout the period was *Capsella bursa-pastoris* (Shepherd's purse). Other *Cruciferae* outwith the *Brassica*, such as *Arabidopsis thaliana*, *Thlaspi arvense* and *Sisymbrium officinale* were locally abundant. Of the genus *Brassica* and related species, *Sinapis arvensis* (charlock) was the most common, occurring in the top 25% of species ranked by frequency in several broad surveys. *Raphanus raphanistrum* (wild radish) and *Brassica rapa* (wild or feral turnip) were far less common and

abundant. No other related species was among the commonest 50 or so weeds in any of the major surveys.

Feral oilseed rape (*B. napus*) was not reported in this work before the 1980s. If residual populations of this species persisted from cultivation of rapeseed in previous centuries (Thirsk, 1997), then they must have been present in the seedbank either at a very much lower frequency than most of the common arable weeds, or in areas of the country that were not sampled in seedbank surveys. Widespread surveys in the 1980s were confined to Scotland, where *Brassica* seeds were found in a small proportion of sites: for instance, in 7 out of 100 fields in the 1982 survey by Warwick (1984), and in only 2 out of 98 cereal fields in the 1987 survey by SCRI (Table 1). Where feral *Brassica* was detected, it occurred in low abundance. However, B*rassica* was much more frequent in a range of agronomic experiments carried out during the 1990s. Most of these plants were considered to be feral *B. napus* descended from recent oilseed rape crops. For instance, in-field and wayside plants were traced to recent *B. napus* varieties by molecular fingerprinting (Anon 1999).

2.3 Feral Brassica in 1990s cereal rotations

From the late 1980s up to 1997, agronomic experiments were funded by DEFRA, SEERAD and the Home Grown Cereals Authority to examine the effects, variously, of herbicide usage, rotations and set-aside on the management or biodiversity of arable fields. The sites were located between north-east Scotland and the south of England and were managed by four organisations – the Agricultural Development and Advisory Service (ADAS), the Department of Agriculture for Northern Ireland (DANI), the Scottish Agricultural College (SAC) and Rothamsted Research (Table 3). The seedbank was assessed by SCRI in all these experiments using the extraction approach (primarily) in the same laboratory. None of the experiments aimed specifically to detect or examine oilseed rape. Nevertheless, they provide retrospectively, possibly the most comprehensive survey of *Brassica* sp. in cereal rotations during the 1990s. The following is a summary of these experiments; further details are given in the paper by Squire *et al*. (see *Outputs*).

Feral *Brassica* was found frequently, occurring at 18 out of 23 sites. It was one of nine species to occur in all five sites in an experiment on set-aside and one of four species to occur at all three *Talisman* sites (Squire et al., 2001; Young *et al*. 2001). Where it occurred, it usually remained detectable throughout the experiment, in many instances for at least 7 years between the first and final measurements. Definitive information on the likely origin of the feral *Brassica* before the experiment was not always available, and its introduction to the field by farm machinery was always a possibility. However, its presence for up to 12 years after a previous crop was confirmed in several of the experiments. The extent to which a population persisted as original dormant seed or as a result of emergence or reseeding was generally not known. However, in one experiment when re-seeding was prevented (Lutman *et al*., 2002), buried seed and emerged plants were detected over at least 4 years. After steep initial declines in seed abundance, the

seedbank populations persisted at densities of 10 to 100 m^{-2}, while emergence of *B. napus* continued at between 1 to 10 m^{-2} each year (Fig. 1).

The *Talisman* experiment (Young *et al*., 2001) illustrated several important features of feral *Brassica* dynamics. There were two rotations: one that introduced oilseed rape at the beginning of the experiment and one that did not grow oilseed rape at any time, in which case any feral *Brassica* seed would have come from a previous oilseed rape crop. The abundance of *Brassica* seed differed greatly between sites at the beginning of the experiment. Several years after harvest of the source crop, it had usually declined to <1% of the total population of the seedbank. Despite these declines in abundance, it occurred for long periods after the previous crop at all sites: eight years in the low input rotation at Boxworth and at least 12 years in the low input rotation at ADAS High Mowthorpe. It was most abundant at ADAS Boxworth, where the declines were continuous, but average populations were still above 100 m^{-2} after 6 to 8 years (Fig. 2). Eventually, some populations declined below the detection threshold (around 28 m^{-2} in this experiment), as at High Mowthorpe and ADAS Drayton. The disappearance of *Brassica* from the seedbank sampling scheme did not mean it necessarily disappeared from a site. In one instance where it was not detected at the general sampling intensity used in the experiment, it was detected when the sampling intensity was raised three-fold.

If feral Brassica *persists, does it dominate seedbanks?*

Sampling intensity was great enough in all the studies listed in Table 2 to detect generally the 15 most common species in each field. In work after 1989, when sampling intensity was usually greater, more than 25 species and as many as 50 or 60 species were recorded in a field. However, feral *Brassica* had not become among the most abundant weed species in any of the experiments in Table 3. Two or three years after the source crop was grown, it generally constituted less than 1% of the total seedbank, and even where weed control was relaxed (e.g. the low input plots in *Talisman*, and natural regeneration treatments in set-aside) feral *Brassica* failed to increase in abundance as did many other species. It was low ranking in the seedbank, but frequent in that it occurred at many sites.

Despite its low abundance relative to other species, its actual abundance in the seedbank was typically around 100 m^{-2} at many of the sites in Table 3 (estimated in a soil depth of 20 cm). This abundance is comparable to the established stand density of oilseed rape crops. To what extent did feral *Brassica* emerge unseen within these crops? There is in fact little systematic evidence of the density at which a seedbank contributes feral oilseed rape to subsequent crops of oilseed rape. Since most sown crops and ferals look very similar, it is often difficult to tell the feral and crop seedlings apart. Nevertheless, experience indicates that such a seedbank might generate an emerged population of at least 1 m^{-2} seedlings in any year (e.g. Fig. 1). In summary, therefore, the impact of feral *Brassica* through competition with other weed populations was probably small in these studies, but its potential impact as an impurity in later oilseed rape crops was large. The model developed in the project aimed to explore its potential as an impurity.

3. A MODEL OF FERAL POPULATION DYNAMICS

3.1 Origins and general structure

The model was constructed to investigate the processes responsible for the persistence of feral oilseed rape and to predict the resulting impurity in subsequent crops. A detailed description of the model and its application in realistic rotations is described in the paper by Begg *et al*. (see *Outputs*). The aim of the model was to consider the presence of GM seed, but the approach was designed to be generic and so applicable to any type of genetic impurity (GM or not) in any type of oilseed rape crop. A stage-structured, life-history approach was taken in which the population dynamics of oilseed rape was modelled in terms of the density of individuals at three stages of the plant's lifecycle: seed in the seedbank, emerged plants, and seed on mature plants at harvest. The effect of various management practises on persistence and contamination were incorporated. The oilseed rape is subject therefore to a set of life-history processes and events associated with the management of an arable rotation that together dictate the growth rate of the feral population.

3. 2 Biological basis and interactions with environment and agronomy

Models had already been developed at SCRI (Squire *et al*., 1997), and in France (Colbach *et al*., 2001). However, neither approach was considered (by the present authors) to be flexible enough to incorporate several important factors that appeared to influence seedbank density. Accordingly, the prototype was improved as follows.

- Multiple populations having different life-history characteristics (e.g. GM versus conventional) could be modelled simultaneously.
- Several depth strata were distinguished (6 in this instance) within the seedbank, allowing the effect of the depth at which the seeds reside on their viability, germination and emergence to be introduced.
- Seeds could be rearranged among the depth strata, thereby distinguishing a number of different soil-cultivation techniques.
- Induced dormancy was included, in which the germination profile of a population is modified according to changes in environmental conditions, represented by temperature and depth.

The structure of the model and the point of action of the life-history processes and management events are represented as a flow-diagram in Fig. 3. The main features are as follows.

Feral populations are represented by the density at 3 stages of the oilseed rape life-cycle; the seedbank, emerged plants and seeds on plants.

The life-history processes incorporated in the model are mortality, germination, and seed production. At each stage, the population is subject to mortality that is treated as a constant daily rate in each case. Germination is a complex process controlling the flow of individuals from seedbank into the pool of emerged plants. Induced or secondary dormancy is represented in the model by imposing temperature-dependence on the cumulative proportion of germinating seeds plus a depth-dependent reduction in the germination rate. Mortality in the seedbank is also depth-dependent. The per capita rate of seed production is determined by a density dependent function that incorporates the effect of intra and interspecific competition. All the life history traits and the range of values used in the simulations were obtained from studies of either crops in the field or seed and seedlings in highly controlled conditions (laboratory, seed burial experiments). They were not obtained from measurements of ferals in their in-field or wayside habitats. An important purpose of the modelling was to see if this basic data on the crop plant gave rise to reasonable estimates of the density and persistence of feral populations.

The management events implemented within the model are sowing, cultivation, herbicide application and harvesting. At sowing, seeds are introduced to the depth strata of the seedbank at densities which can be varied to represent different sowing practises. Cultivation redistributes seeds between the depth strata representing a range of cultivation types (sprint tine, power harrow, rotovator, spader, shallow cultivation) while removing emerged plants and returning seeds on plants to the seedbank. Harvesting acts to remove emerged plants and returns a proportion of seeds to the seedbank. The application of herbicide increases the daily mortality rate of the emerged plants for a defined duration.

The model also allows for the simultaneous development of multiple populations and competitive interactions within and between populations including crops other than oilseed rape.

3.3 Simulation of decline over time

The behaviour of the model is shown here by a simple demonstration (the paper includes a wider range of simulations). Any rotation or combination of crops may be considered, but for simplicity, the analysis used a rotation in which winter oilseed rape is sown in the first year followed by winter wheat in the two subsequent years. The model was parameterised with values representative of a typical winter oilseed rape variety, using sources of information on life history traits given in Squire *et al*. (1997), supplemented by new information gained in this project. The first crop is considered to be the one introducing impurity into later crops and feral populations. For illustration, the analysis concentrates on an arbitrary impurity threshold of 1% and simulations are shown for the seedbank only. Since – in this set of simulations – ferals and sown crop are given the same emergence traits and have the same growth characteristics, values of percentage impurity shown in the figures refer also to percentage impurity in yield.

In the first example, no attempt was made to control ferals (Fig. 4). The seedbank persisting from the initial crop declined (linearly when abundance is plotted on a log

scale as in Fig. 4A) despite seed being returned to the seedbank at each oilseed rape harvest in the rotation (the sharp peaks in the trace in Fig 4A). The corresponding decline in percentage impurity, expressed as the presence of feral seed from the first crop as a fraction of all seed (ferals and crops), is given in Fig. 4B. The decline was slow taking several years to dip below 10%. When the sixth oilseed rape crop was harvested in year 16, impurity had fallen below the 1% threshold for the first time, reaching a level of 0.9%.

Persistence was shown to be sensitive to some aspect of each life-history process. For example, mortality at each stage of the life-cycle acted to reduce persistence, with seedbank mortality having the greatest effect. Despite this, even a high mortality rate of 1% per day failed to reduce contamination below the 1% threshold until ten years after sowing of the original crop. Persistence was also sensitive to changes in all parameters of the seed production function, i.e. maximum seed production, density thresholds, and the strength of competition. In particular loosening the density dependent constraint on seed production (described in the paper) had a large effect on persistence - a doubling of the density thresholds resulting in a contamination of 10% after 18 years. Of the parameters associated with germination, sensitivity was only demonstrated towards the fraction of seed induced into dormancy. The base-line parameterisation assumed a maximum germination value of 90%. Reducing this by 10% resulted in an increase in impurity from 0.9% to 10% after 16 years.

The main conclusion from simulations of this type – in which there is no control of the initial population except through competition and 'dilution' by subsequent populations - was that a threshold of impurity of 1% was unlikely to be met in any reasonable timescale. This simulated management regime is unrealistic, because broadleaf weeds (which includes ferals) would normally be reduced or eliminated in cereals. The traces in Fig. 4 are intended as an upper reference line.

3.4 Using the model to explore scenarios to meet thresholds

In the second set of examples, practices were introduced to control ferals. For instance, the rotation was modified to incorporate herbicide treatment and to increase the years between oilseed rape crops. The control of loss of seed to the soil at harvest was also simulated.

Herbicide acts by increasing the mortality of the emerged plant and as such had a similar affect on persistence as underlying plant mortality. The complete eradication of emerged plants and the consequent prevention of seed production during the two cereal crops resulted in a reduction in seedbank contamination to 0.01% by the 16^{th} year and to below the 1% threshold by the 10^{th} (Fig. 5). Extending the period between oilseed rape crops from 2 to 4 years, while simulating 100% effective herbicide treatment, had little further effect on persistence.

A typical value of 5% seed loss at each harvest was assumed in the base-line parameterisation of the model. Reducing this figure to 1% resulted in the 1% contamination threshold being achieved by the time the third oilseed rape crop was harvested in year 7 (Fig. 6). Eradicating harvest losses altogether in years after the initial crop resulted in seedbank density declining more rapidly still but had little further impact on the rate at which impurity declined, the 1% threshold again being achieved by year 7.

The simulations described above represent rigorous management scenarios, i.e. 100% effective herbicide treatments during winter wheat crops and the complete control of harvest losses in oilseed rape. Under these circumstances, the initial feral population was reduced to between 1 and 10 m^{-2} within a few years in the more suppressive treatments in Fig. 5 and Fig. 6. These are low absolute values for seeds in the seedbank, a small proportion (say, 1 in a 1000) of the seeds of all species. However, they are still quite high when considered as a percentage impurity of all oilseed rape seeds in the seedbank. The main conclusion that follows from this is that a threshold of impurity of 1% in the seedbank or harvested yield would be difficult to achieve within a 5-year timescale.

4. CONSEQUENCES FOR AGRICULTURE

Do measured and modelled persistence agree?

The model of oilseed rape persistence was developed and parameterised using experimental information on the physiology of oilseed rape, derived mainly from measurements on crop plants, not ferals *in situ* (Squire *et al*., 1997, and amendments). The decline rates and other standard parameters used in the model were judged to be intermediate, that is neither high nor low within the observed ranges. The values of absolute seedbank density predicted by the model – 10 to 100 m^{-2} several years after the source crop – are consistent with the absolute values of *Brassica* seedbank density measured at least three years and sometimes up to 12 years after a crop (e.g. those in Table 3, Fig 1, Fig. 2). The difficulty of reducing a feral population in the model (based on the general life cycle biology of the species) is therefore consistent with their observed persistence and abundance in cereal rotations towards the end of the 20th century.

What are the consequences of persistence?

The persistence of feral *Brassica* at these densities in the seedbank has different implications for food purity and the resilience of arable food webs.

The typical population density of ferals in the seedbank – around 100 m^{-2} – is high enough to bring substantial **impurities to yield**. There is no documented reason why ferals should be less adept at capturing resource and reproducing than crop plants. So even if only 1% of the feral seedbank germinates within a crop of oilseed rape sown at a density of 100 m^{-2}, it should generally contribute about 1% to yield. At present, there appear to have been few accurate measurements of impurity caused by ferals in oilseed

crops. However, if the feral is different to the crop variety, for instance if the one is GM and the other not, then such impurities should be readily detectable by molecular screening techniques.

The feral population density, though high immediately after harvest, declines in both models and reality to a low value similar to or lower than that of many other common weeds. The ferals, over a wide range of experimental and commercial fields and treatments, had conservative seedbanks that did not rise when weed management was relaxed. On present evidence, the **ecological impact** of feral *Brassica* itself appears to have been small, e.g. through competitive interactions with other plants.

Would this still be true if genetically-modified herbicide tolerant crops give rise to ferals? If ferals had traits such as GM herbicide tolerance, they would only be advantaged over other ferals and weeds if the specific herbicide to which they were tolerant was used in a later crop. If this was so, more ferals in the seedbank would become GMHT for as long as those GMHT crops continued to be grown in the field. Impurities introduced accidentally, e.g. glyphosate-tolerant impurities in imported rapeseed, would be advantaged if glyphosate were applied to the field without knowledge that the feral population contained resistant individuals. This might occur since glyphosate was the second most widely used agricultural herbicide in the latest reported pesticide survey (for 2000). The model has further potential use in estimating the control of one type of herbicide tolerance by using a GMHT crop tolerant to a different herbicide!

Are thresholds of impurity attainable in farm practice?

If GM oilseed rape had <u>not</u> been grown previously in the field, and farm machinery and source seed had been rigorously segregated from GM sources, then a threshold of 1% should be achievable, since geneflow from any surrounding GM fields would contribute impurity at around 1 in a 1000 (0.1%). The ferals arising from non-GM oilseed rape crops grown in that field would swamp any such GM ferals. This conclusion might not hold if the recipient field was partly male sterile, when geneflow itself might be great enough to exceed a 1% threshold. Very few male sterile varietal associations are now grown in the UK, though the usage has been higher in Scotland which grows about 10% of UK rapeseed, and of that about 10% is male sterile. Research is underway to estimate geneflow to fields of varieties having different degrees of male sterility (Current geneflow research 2003).

If GM oilseed rape had been grown previously in the field, the model of feral population dynamics predicted that only rigorous and suppressive management strategies would keep impurities in yield caused by ferals below 1%. Given that impurities will also arise through the sown seed and through geneflow from neighbouring fields, a threshold of around 1% (of adventitious presence) in yield would be achieved only with difficulty in normal commercial practice if oilseed rape had been grown in the past few years. Moreover, the position with home-saved seed is unclear. Current informed estimates suggest home-saved seed is used in at least 20% of rapeseed crops, possibly more in

some areas. Impurities through home-saved seed on farms that have grown GM crops may be higher than from seed bought from a seed merchant: this needs quantifying.

The difficulty of meeting the threshold arises because the return of seed from the first oilseed rape crop is high compared to the seed density sown in subsequent crops, so that whatever happens subsequently has only a relatively small influence on percentage impurity. The only factors that would seem to reduce impurity in seed yield more rapidly than indicated by the simulations described above are a catastrophic decline of seed populations in the soil (much higher than most measured rates), non-emergence, for whatever reason, of seed in the seedbank during subsequent oilseed rape crops, and low competitive ability of ferals compared to crop plants. The crucial factor is reducing the seed that enters the soil. A method of control proposed by Pekrun and Lutman (references cited), and widely adopted in GB, in which seed is left to germinate on the soil surface after harvest, then killed before the next crop is sown, can bring about a massive fall in population. However, not all such seed germinates and some enters the soil: it is still not certain that such practice will reduce impurities below 1%. Lower seed production in ferals might sometimes occur, as when a spring feral emerges in a winter oilseed rape crop, but otherwise there is little information to show that seed production of ferals is less (plant for plant) than crop plants emerging at the same time.

Uncertainties and further research

The collation and comparison of experimental information during the project revealed a substantive baseline in feral population biology that has not yet been thoroughly explored and quantified. It includes, as well as the experiments in Table 3, many trial sites managed by NIAB and other organisations in which GM varieties have been grown, and the Farm Scale Evaluations conducted at >250 sites across the UK. These data should be analysed to provide answers to the following.

- The large observed variation between sites in the feral population densities they support is largely unexplained. Different ways of treating the soil surface after harvest could be partly responsible (see findings of Pekrun and Lutman, references cited). Further sampling across a wide range of sites should provide insights on the influence of soil type, weather and treatment of the soil. The analysis of feral *B. napus* the Farm Scale Evaluations will be invaluable.
- The low population density of the ferals means that reliable estimates of population size are difficult to make – this makes it difficult to manage ferals to achieve thresholds. There was also evidence (not presented in the Project) that the low populations are spatially clumped within fields. Further work is required to quantify and understand these low density distributions and how they change over time.
- The link between the physiological properties of the source variety and the persistence of feral populations is not understood. While varieties are known to differ in inducible dormancy, it is unclear, whether varieties differ also, for example, in persistence once in the soil. It would be feasible, however, to trace the origin of feral populations using molecular markers (e.g. Anon, 1999) and to compare the physiological traits of source varieties having different persistence.

- Ferals come into close contact with some wild relatives such as *Raphanus raphanistrum*: their role in exchanging genes, albeit at very low frequency, with wild relatives needs further study.

The process of constructing the enhanced model and running simulations revealed deficiencies in data, the most notable being the impact on feral *Brassica* by the various herbicides used in cereal rotations. Discussion with representatives of the agrochemical industries indicates such information exits in companies' archives, but in a largely uncoordinated form. There is the scope therefore to collate this information and incorporate it within the model.

Development of a management aid

The project established and maintains close contact with those undertaking the BRIGHT trials and other research on the management of GM ferals in the UK. More widely, the model offers a means by which the knowledge of feral oilseed rape can be used to advise policy makers and growers on setting and achieving thresholds of impurity and on safeguarding general weed populations as food sources for invertebrates, mammals and birds. The model will now be rigorously tested against independent data in a new project. If deployed at specific sites, the model would require some limited, on-site 'calibration', for example, more specific information of the origin of the seed (e.g. whether bought from a merchant or farm-saved), its likely percentage impurity, and counts of feral populations or simple seedbank estimates. However, the development of a management aid is now feasible.

Acknowledgements

The authors are grateful to the support from Defra under project RG0114. We are pleased to acknowledge our colleagues Sian Hockaday, who initiated the new life cycle model and Gladys Wright for maintaining seedbank records. John Crawford developed the equations describing the time-dependence of emergence on temperature. Peter Lutman and Jeremy Sweet provided advice throughout the project, while a Focus Group of industry representatives gave their valuable time and experience. We thank anonymous referees for their comments.

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Current geneflow research 2003. Projects funded by Defra, contact g.squire@scri.sari.ac.uk; or Roger Daniels red@ceh.ac.uk . Project funded by SEERAD, contact g.ramsay@scri.sari.ac.uk.

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Outputs from the Project

Begg, Hockaday, McNicol, Askew & Squire. (submitted for peer review 2003). Modelling the persistence of feral oilseed rape.

Squire, Begg et al. (submitted for peer review 2003). Status and persistence of feral oilseed rape in the arable seedbank.

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Begg GS, Hawes C, Marshall B, D'Hertefeldt T, Ramsay G, Young M, Squire GR, Wright G. (2002) Dispersal and persistence of feral oilseed rape: mechanisms and consequences. European Science Foundation Working Group Meeting: Estimating and managing gene flow and dispersal in GM crops. University of Lille, 2-3 July 2002.

Squire GR, Begg G, Askew M, Young M. 2002. Persistence and effect of GM and non-GM feral OSR as arable seedbank and wayside plants. Presentation at conference: G*MOs: ecological dimensions.* Association of Applied Biologists, Reading, UK.

Begg GS, Young M, Hawes C, McNicol J, Squire GR. 2003. Modelling feral (volunteer) oilseed rape in relation to thresholds of impurity. Paper to be given at a meeting to be held on coexistence of GM and other crops in Denmark, November 2003.

Table 1. Examples of experiments on persistence of buried seed of *Brassica napus, Brassica rapa* and certain hybrids, where re-seeding was prevented, to illustrate range of approximate percentage survival under different circumstances.

Reference	*Species/variety/source*	*Seed viability*		*Method of burial*
		Year	%	
Kjaer (1940) Kjaer (1948) Madsen (1962)	(i) *B. napus* L. var. *napobrassica* (swede), well-germinating commercial seed (ii) *B. campestris* (=*B. rapa*) L. var. *rapifera* Metzg., turnip	16 16	1 1	porous clay pots, 20cm deep.
Chadoeuf *et al*. (1998)	(i) *B. napus* L., oilseed rape (ii) *Hirschfelda incana* L., hoary mustard	3 3	<1 50	openwork nylon bags, 30cm deep in undisturbed soil.
Hails *et al.* (1997)	*B. napus* L., oilseed rape cultivar 'Westar' for both conventional and transgenic lines (i) Conventional (ii) Kanamycin resistant (iii) Kanamycin resistant and glufosinate resistant	 2 2 1	 0.5 0.1 0.4	nylon mesh bags buried at 2cm or 15cm, in three regions of the UK, in different habitats; data given here are the mean per genetic line per year, for both depths, all locations and habitats.
Schlink (1994, 1995)	*Brassica napus* L., oilseed rape, cv. Rubin (i) depth 3 cm (ii) depth 27 cm	 4.5 4.5	 0.1 23	in nylon sachets up to 0.27 m deep in soil.
Linder and Schmitt (1994)	(i) *B. rapa*, cultivar Tobin *(ii) B. rapa*, wild species (iii) cultivar (mother) x wild (iv) wild (mother) x cultivar	1 1 1 1	7 80 33 67	seeds buried in soil.
Linder and Schmitt (1994) Linder and Schmitt (1995)	*B. napus,* oilseed rape: (i) control without transgene (null segregant) (ii) transgenic stearate (higher concentration of stearic acid)	 1.6 1.6	 2.5 2.5	buried in woven polyester bags, 10cm deep; data for Georgia site.

Table 2. Studies of the UK arable seedbank, 1915 to 1996, representative of those examined for the presence and abundance of Cruciferous weeds, especially members of the genus *Brassica* and related species.

Code	Sample year(s)	Sites	Number of fields	Sample depth (m)	Total soil volume processed (l)	Author
1	1915	arable fields at Rothamsted Experiment Station: Geescroft Field, New Zealand Field, Long Hoos, Agdell, Barn Field.	6	0.15	50	Brenchley, 1918
2	1929-31	fields classed as 'pasture formerly arable', in Wales	5	0.30	67	Chippendale and Milton, 1934
3	1940	fields classed as arable, ley, ploughed pasture, in Warwickshire at locations specified	11	0.175	u.	Milton, 1943
4	1944	lowland arable fields (previously pasture) in England, locations unspecified	20		120	Champness and Morris, 1948
5	1958-62	Vegetable fields in 23 counties of England	58	0.15	286	Roberts and Stokes, 1966
6	1968-75	vegetable fields in Lancashire, Yorkshire, East Anglia and elsewhere	89	0.15	uncertain	Roberts & Neilson , 1982
7	1972-77	unspecified locations in Oxfordshire and Warwickshire	64	0.15	226	Roberts and Chancellor, 1986
8	1972-78	unspecified locations throughout Scotland	>300	(0.20)	(110)	Warwick, 1984
9	1977-79	fields in Berkshire and Oxfordshire	4	0.20	7	Froud-Williams, Chancellor & Drennan, 1983
10	1982	fields in Scotland	100	0.20	100	Lawson, Wright & Smoktunowicz, 1988
11	1987	cereal fields in Scotland	98	0.20	98	SCRI seedbank records
12	1982-88	fields on one farm, Boxworth in Cambridgeshire	11	0.20	52	Marshall & Arnold, 1994
13	1990-96	experimental fields of the Agricultural Development and Advisory Service (TALISMAN experiment)	3	0.2	128	Squire, Rodger & Wright, 2000

Table 3. Agronomic experiments between 1987 and 1997 in which evidence of feral *Brassica* (probably ***B. napus***) in the seedbank was looked for retrospectively. Experiments were hosted and managed by the Agricultural Development and Advisory Service (ADAS), Scottish Agricultural College (SAC), Department of Agriculture for Northern Ireland (DANI) and Rothamsted Research (RR). Analysis of the frequency and abundance of feral *Brassica* at these sites is given in a paper in preparation (Squire *et al*.).

Experiment / site	Starting year	Ending year	Total taxa recorded at site	Site manager
Herbidide dose trials				
Cambridge	1987	1991	17	ADAS
Bridgets	1987	1991	19	ADAS
Drayton	1987	1991	21	ADAS
Gleadthorpe	1987	1991	19	ADAS
Rosemaund	1987	1991	30	ADAS
Strangford	1987	1991	26	DANI
Niddrie Mains	1987	1991	28	SAC
Smiths	1987	1991	18	SAC
Gleghornie	1987	1991	16	SAC
Remote	1998	1992	12	SAC
Time and depth of soil disturbance				
Rothamsted	1991	1995	n.a.	RR
Long Ashton	1991	1995	n.a.	RR
Fallow (set-aside)				
Boxworth, Cambridgeshire	1989	1996	40	ADAS
Bridgets	1989	1996	62	ADAS
Drayton	1989	1996	44	ADAS
Gleadthorpe	1990	1997	50	ADAS
High Mowthorpe, Yorkshire	1989	1996	39	ADAS
Hillfield	1989	1994	33	SAC
Organic (grass/cereal/root)				
Tulloch, Aberdeenshire	1991	1999	25	SAC
Aldroughty, Aberdeenshire	1991	1999	37	SAC
Rotation ./ herbicide (TALISMAN)				
Boxworth	1990	1996	28	ADAS
Drayton	1990	1996	27	ADAS
High Mowthorpe	1990	1996	31	ADAS

n.a. This study estimated decline rates of several sown species, including ***B. napus***; the existing seedbank was therefore uncertain.

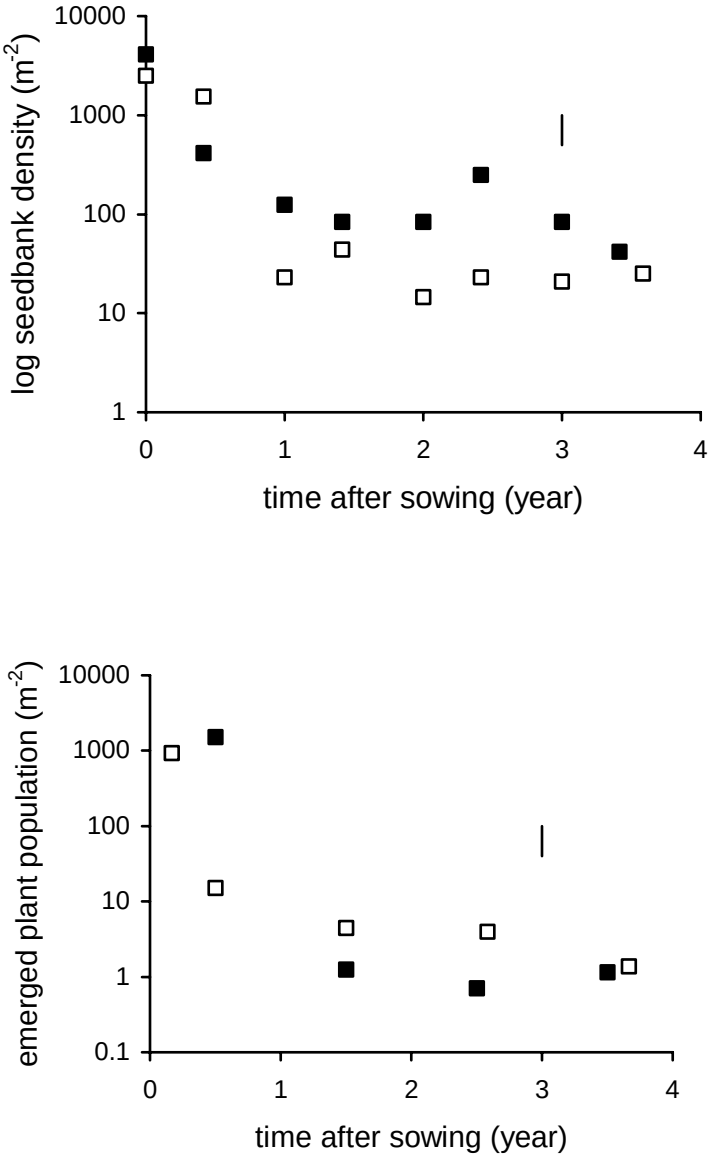

Figure 1. Density of oilseed rape seeds in the seedbank (top) and emerged seedlings (bottom) after controlled sowing of seeds in the field at Rothamsted (open square) and Long Ashton (closed square). Data shown on $\log_{10}$ scale. Vertical bars show typical standard errors. The field continued after time 0 under wheat cropping, in which emerged seedlings were prevented from flowering. Compiled from data, averaged across treatments, in the experiment described by Lutman et al. (2002), with acknowledgements to P J Lutman for supplying emergence counts.

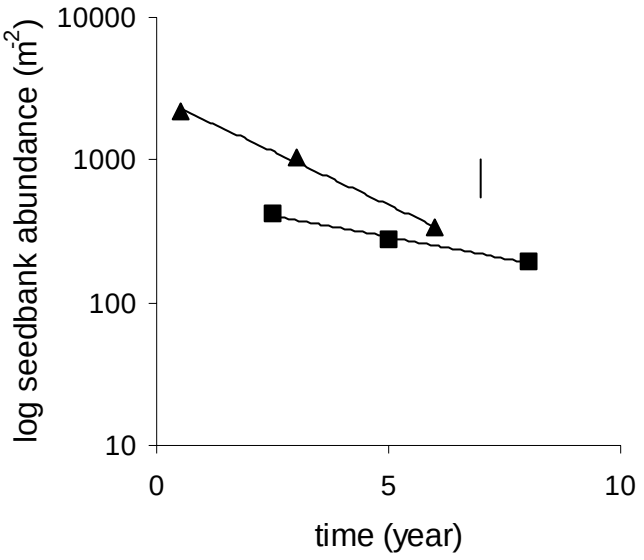

Figure 2. Density of the oilseed rape seedbank (top 0.2 m of soil) in an experiment at ADAS Boxworth, showing declines following a sown crop of oilseed rape (triangles) and of a population persisting from a crop sown about two years earlier (squares). Vertical bar shows typical standard error. For experimental details, see Cook 2001, Young *et al*. 2001; for seedbank study, Squire *et al*., 2001).

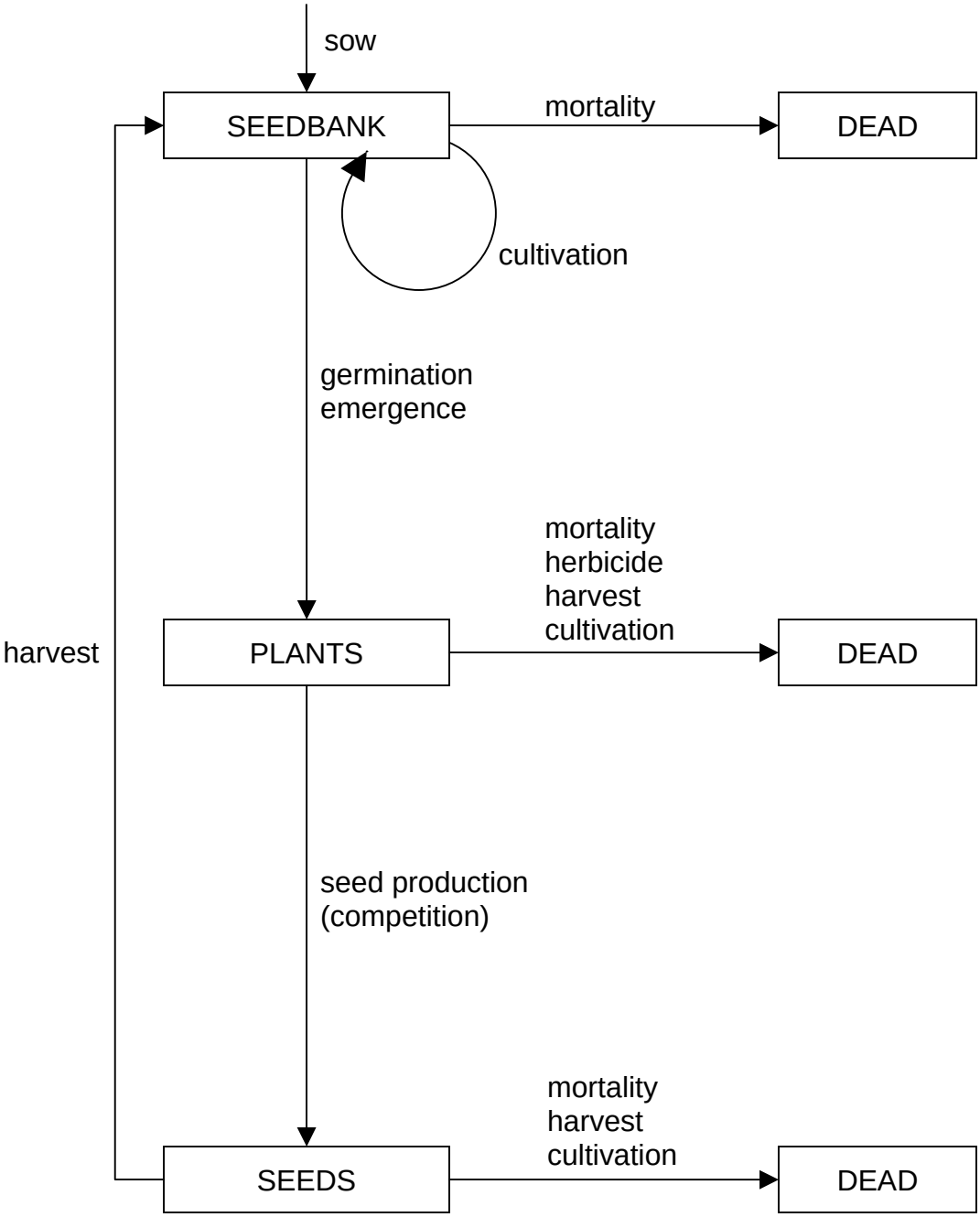

Fig. 3. Flow diagram of the structure of the model showing the relations between the life-cycle stages (upper case text) and the life-history and management processes (lower-case text).

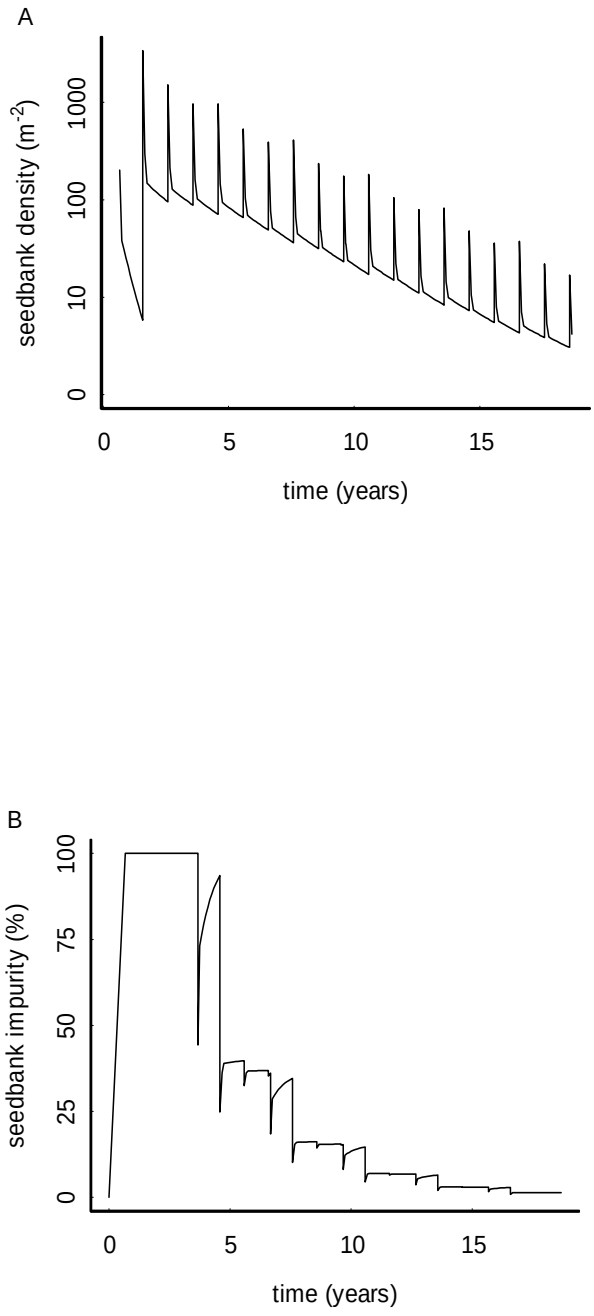

Fig. 4. Variation in the initial oilseed rape population over six cycles of the basic rotation. (A) $\log_{10}$ seedbank density. (B) percentage impurity in the total oilseed rape seedbank.

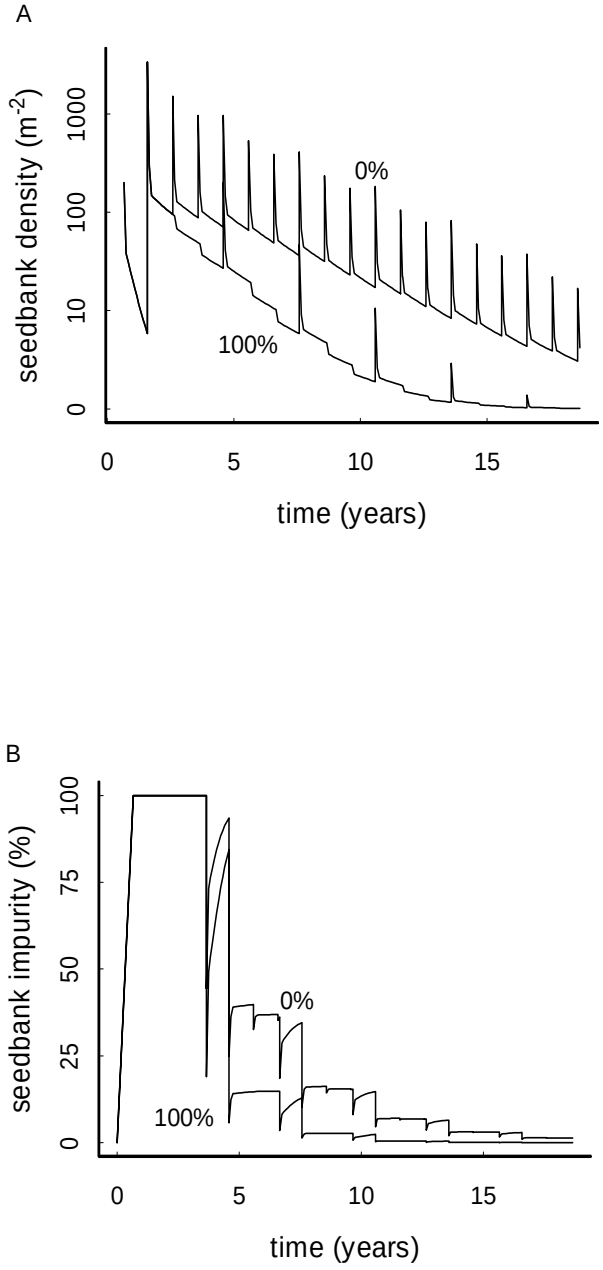

Fig. 5. Comparison of the decline in the initial oilseed rape population over six cycles of the 3-year basic rotation with 0 and 100% effective herbicide application. (A) $\log_{10}$ seedbank density. (B) percentage impurity.

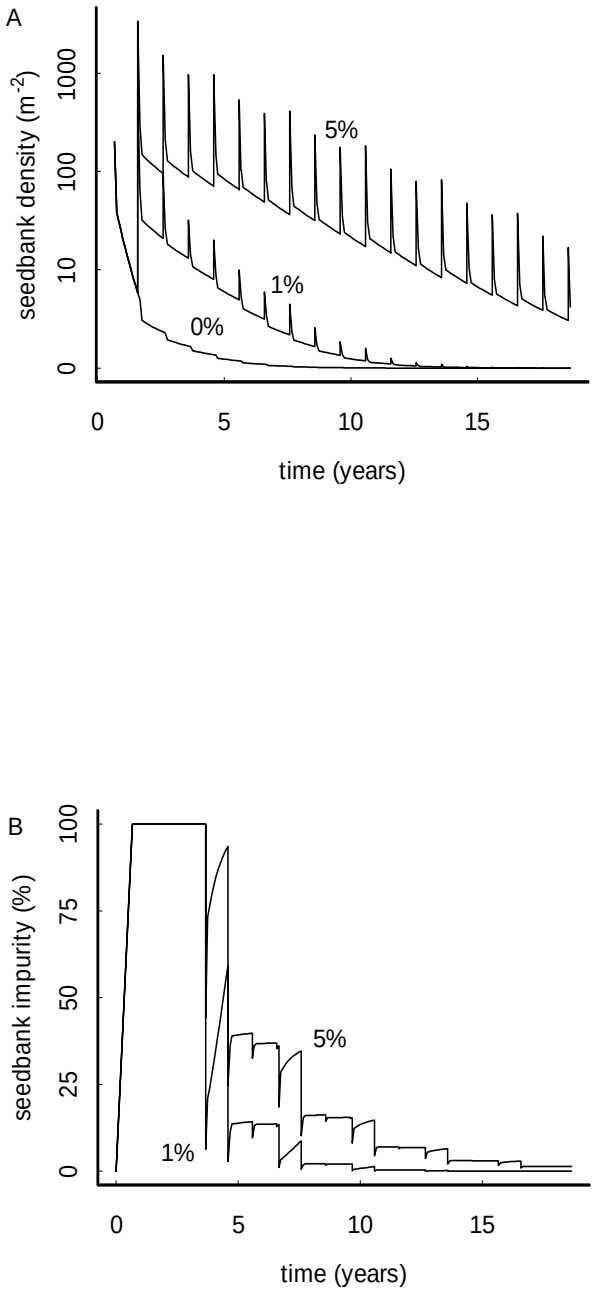

Fig. 6. Comparison of the decline in the initial oilseed rape population over six cycles of the 3-year basic rotation with 0, 1 and 5% loss of seeds at harvest. (A) $\log_{10}$ seedbank density. (B) percentage impurity (0% loss curve omitted for clarity).