PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2005) **272**, 1909–1915
doi:10.1098/rspb.2005.3166
Published online 9 August 2005

Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*)

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A series of rotation experiments at five sites over four years has explored the environmental and agronomic implications of growing herbicide tolerant oilseed rape and sugar beet. This paper reports on the population dynamics of volunteer rape (*Brassica napus*). The experiments compared four winter oilseed rape (WOSR) cultivars: a conventional cultivar (Apex) and three developmental cultivars either genetically modified (GM) to be tolerant to glyphosate or glufosinate, or conventionally bred to be tolerant to herbicides of the imidazolinone group. Seed losses at harvest averaged 3575 seeds m^{-2} but ranged from less than 2000 up to more than 10 000 seeds m^{-2}. There was a rapid decline in seed numbers during the first few months after harvest, resulting in a mean loss of seeds of 60%. In subsequent seasons, the seedbank declined much more slowly at four of the five sites (*ca* 20% per year) and the models predicted 95% seed loss after approximately 9 years. Seed decline was much faster at the fifth site. There were no clear differences between the four cultivars in either the numbers of seeds shed at harvest or in their subsequent persistence. The importance of the persistence of GM rape seeds, in the context of the coexistence of GM and non-GM crops and the role of good management practices that minimize seed persistence, are discussed.

Keywords: genetically modified crops; herbicide tolerant crops; oilseed rape; *Brassica napus*; seed persistence

1. INTRODUCTION

Oilseed rape (*Brassica napus* L.) has been grown extensively in the United Kingdom for the last 25 years and is now the most widely grown non-cereal arable crop. One of the features of this crop is a propensity for the pods to shatter prior to and at, harvest. This leads to the presence of considerable numbers of seeds in the field after the harvest of the crop (Price *et al.* 1996). These seeds can persist in the soil and subsequent volunteer plants have frequently posed problems to growers (Lutman 1993), as the plants can be difficult to control and are very aggressive in less competitive crops such as onions or sugar beet. The issue of the persistence of oilseed rape seeds has received greater attention in recent years in relation to the commercialization of genetically modified oilseed rape. Clearly, the persistence of seeds provides an opportunity for temporal gene flow. However, much less emphasis has been put on studies of the behaviour of seeds, compared with studies of the spatial element of gene flow, via pollen dispersal (Ramsey *et al.* 1999; Thompson *et al.* 1999; Eastham & Sweet 2002; Rieger *et al.* 2002).

Fresh oilseed rape seeds as they fall off the plant are not dormant and will germinate immediately if given adequate moisture, but a combination of environmental stress conditions including darkness can induce secondary dormancy in the seeds (Pekrun *et al.* 1997*a*), so that they can persist for some years. Data on seed persistence are limited and conflicting. Schlink (1998) showed that approximately 1% of undisturbed seeds could survive for 10 years and Lutman *et al.* (2003) reached similar conclusions. In contrast, Hails *et al.* (1997) found persistence to be very short-lived. Persistence data for disturbed habitats, reflecting the annual cultivations normally used in arable fields, are even less common, though Lutman *et al.* (2003) showed that 5% of seeds survived a maximum of 3–4 years in England and Gulden *et al.* (2003), in Canada, showed 1.4% surviving for two winters. Thus, survival in cultivated conditions may be less than in undisturbed situations.

As data on the survival of rape seeds in the soil are so limited, it is unsurprising that little has been reported on the survival of seeds of genetically modified oilseed rape. Petri-dish studies reported by Gulden *et al.* (2004), showed no link in dormancy attributes between genetically modified (GM) herbicide tolerant (HT) cultivars of spring

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Received 15 February 2005
Accepted 21 May 2005

rape in Canada and conventional ones. Some work in Germany has monitored the survival of seeds of glufosinate tolerant rape and found no seed survival after 6 years (Beismann *et al.* 2003) and few seeds 2 years after harvest, but this work includes no direct comparison of the behaviour of GM and non-GM cultivars.

This paper reports results taken from the UK 'Botanical and Rotational Implications of Genetically modified Herbicide Tolerance' project (BRIGHT) (Sweet *et al.* 2004), which comprised a series of large plot rotation experiments at five sites, investigating the environmental and agronomic implications of growing GM herbicide tolerant oilseed rape and sugar beet, starting in 1998. This work has included investigations into the short and longer-term behaviour of seeds of oilseed rape shed from the crop at harvest. The experiments included conventional WOSR and three cultivars developed to be herbicide tolerant. Seed losses at harvest were quantified and compared to crop yields. Seed decline for all four cultivars over the following 3–4 years was monitored. Where possible, regression models have been fitted to the data to predict future seed numbers and to assess the importance of cultivar on the decline rates.

2. MATERIAL AND METHODS

(a) *General*

All crops included in the BRIGHT rotations were grown, as far as possible, in a similar way to commercial crops, with the exception of the herbicide regimes used when oilseed rape and sugar beet were grown. The experiments included four WOSR cultivars: the conventional cultivar Apex and developmental cultivars produced by GM to be tolerant to glyphosate (Monsanto, Roundup Ready) and glufosinate (Bayer Crop. Science, Liberty Link) and by conventional breeding techniques to tolerate herbicides of the imidazolinone group (Cyanamid (now BASF)). The latter was only available for the first 2 years of the project. The appropriate herbicide was applied to the tolerant cultivars and commercially available products were applied to the conventional cultivar. There were no links in the genetic background of the four oilseed rape cultivars, so the research is simply comparing the behaviour of four distinct cultivars, three with herbicide tolerance and one without.

Oilseed rape was grown at all five sites in season 1998/99 (Yr 1) (Rothamsted (RES), NIAB, Scottish Agricultural College (SAC), Broom's Barn (BB) and Morley Research Centre (now The Arable Group) (MOR)). At three of these (RES, NIAB 99a and SAC) a second crop of oilseed rape was grown 2 years later, in 2001/02 (Yr 4), on the same plots as those planted in 1998/99. Further experiments were sown with the four oilseed rape cultivars at NIAB, RES and SAC in autumn of the season 1999/2000 (Yr 2). The sites at NIAB (NIAB 99b), BB and MOR grew herbicide tolerant and conventional sugar beet in 2001 (Yr 3). Winter cereals were grown in all the years when rape or sugar beet was not planted. A high level of control of volunteer rape plants was achieved at all sites in the other crops, using relevant standard herbicides, except Year 3 cereals at SAC, where uncontrolled volunteer plants produced some seed. All crops in all years were sown following ploughing of the stubble from the previous crop, except at NIAB in year 4, where the crop was established following non-inversion cultivation. The oilseed rape crops at RES, MOR, BB and NIAB in Year 4 were harvested by direct cutting, while crops at SAC and at NIAB (Years 1 and 2) were harvested after swathing. The experiments were of a randomized block design with the four treatments being either replicated twice (experiments in season 1998/99 (Yr 1)), three times (season 1999/2000 (Yr 2)) or two or four times (season 2001/02 (Yr 4)). The limited replication in the first season was due to: (i) restrictions on the area that could be sown with GM rape, (ii) a wish to reflect field practice and thus a need for large plots and (iii) the intention to create a series of four sub-plots in each plot in later years, thus increasing replication in 2001/02 (Yr 4). Statistical analyses showed that this subdivision of the plots does not impact on the seed data reported in this paper.

More details of the materials and methods can be found in Sweet *et al.* (2004).

(b) *Assessments*

During the winter or early spring, while the oilseed rape crops were growing in 1998/99 (Yr 1) and 1999/00 (Yr 2), 20–48 round soil cores 2.5 cm diameter and *ca* 25 cm deep were taken from each plot to assess the background numbers of oilseed rape seeds in the soil. Full details of techniques are given in Sweet *et al.* (2004). When possible, the seeds were immediately separated from the soil by washing out the soil sample, using 4 and 1 mm sieves. Otherwise, the sample was frozen and then defrozen and washed out as above at a later date. Whole seeds were then removed from the contents of the 1 mm sieve. The seeds were squeezed to test their viability, as described by Sawma & Mohler (2002) (healthy seeds were resistant to squeezing and had yellow embryos, non-viable seeds were soft when squeezed and the embryos were brown) and viable seed numbers were recorded.

Oilseed rape seed yields were recorded at harvest, on all sites and in all years and assessments were made at or immediately post-harvest, of the total numbers of seeds shed before and during harvesting. Sites at RES, SAC and NIAB recorded 5–15 10×10 cm quadrats per plot, immediately post-harvest, while those at MOR and BB were based on three or four plastic rain gutters (0.22 m^2) placed in each plot immediately prior to harvest. At RES and SAC, further counts using the same quadrats were made over the following month to monitor the fate of seeds (in the absence of post-harvest cultivations).

Every winter after the harvest of the rape crops (November–early April), a further 24–80 soil cores per plot were taken to assess the number of rape seeds in the seedbank (using the same corers as used in the initial sampling). The first samples were taken in the winter after the harvest of the first rape crop and final samples were taken in winter 2001/02 (Yr 4) at all sites except BB, where an additional sample was collected in winter 2002/03. Core numbers per plot varied slightly between experiments and were increased in later years as seed numbers declined.

Thus, each of the experiments harvested in 1999 had values for seed losses at harvest and then data on the soil seedbanks after 4–8 months (mean = 6), after 16–20 months (mean = 18) and after 28–31 months (mean = 30). One extra data set was collected at BB after 43 months. The experiments, where the rape was sown 1 year later (Yr 2) had only two sets of soil cores. Seed losses at harvest were recorded on the crops harvested in 2002 (Yr 4).

(c) *Statistical analysis*

Analysis of variance by GenStat *for Windows* (Payne *et al.* 2003) was used to assess differences between treatments for estimates of yields and seed losses at harvest and for preliminary investigation of the changes in seedbanks in subsequent years. Full analyses of the declines in the soil seedbanks were only done for the sites with four seasons of data (those started at harvest 1999). The number of data points for crops harvested in later years was inadequate to assess the true nature of the decline responses. All the seed persistence results from these experiments growing rape in 1998/99 were analysed by GenStat, using generalized regression techniques, assuming a Poisson distribution for the observed numbers of seeds. The fitted model was as follows: $Y = N \times P_1 \times P_2^{(T-0.5)}$, where N is the number of seeds shed at harvest, P_1 is proportion of the seeds remaining after the first six months and P_2 is the proportion remaining after each subsequent year. T is the time in years. Sequences of analyses were fitted to assess whether the three parameters of the model differed significantly either between treatments or between the individual plots of each experiment. The optimum model was selected for each set of data and used to determine the years to 95 and 99% predicted seed loss. The decline in the first six months $(1-P_1)$ represents the initial seed loss, prior to the seeds developing dormancy, while the P_2 parameter predicted the persistence rates of the dormant buried seeds.

3. RESULTS

(a) *Background oilseed rape seedbank*

At all sites, the numbers of oilseed rape seeds in the soil cores taken in the oilseed rape crops sown in 1998 and 1999 were generally low. At several of the sites, no oilseed rape seeds were detected (BB, SAC in 1998/99, RES in 1998/99, NIAB in 1999/2000). At the others mean seed numbers were less than 150 seeds m^{-2}, except at SAC in 1999/2000, where there was a mean of 350 seeds m^{-2}.

(b) *Seed yields and seed losses at harvest*

The mean yields from all site/years was 3.16 t ha^{-1} with an indication of slightly lower yields from the imidazolinone tolerant cultivar (Apex = 3.35; imidazolinone tolerant = 2.77; glufosinate tolerant = 3.43; glyphosate tolerant = 3.09 t ha^{-1}; s.e.d. 0.117). However, it must be noted that this comparison is not fully orthogonal, as the imidazolinone tolerant rape was not grown in year 2001/02. Differences between the other three cultivars were small. Overall seed losses were in the region of 4000 seeds m^{-2} (grand mean 3575 seeds m^{-2} (s.e. 400)). However, seed losses ranged from less than 2000 to more than 10 000 seeds m^{-2} (figure 1). The high losses at two sites (BB 99, RES 00) on the glyphosate plots, tended to be associated with increased pigeon attack prior to harvest and were not thought to be linked to any intrinsic characteristic for shattering in the cultivar concerned. There were some evidences that seed losses were lower on the swathed experiments (NIAB and SAC) than on those that were direct cut. Although there were significant differences between treatments recorded at some sites (figure 1), there was no overall consistent difference in seed losses at harvest, between the four cultivars. It was also clear that there was no obvious link between the overall yield and the level of shedding, as the lowest seed losses tended to occur with the high yielding Apex and the lower yielding imidazolinone tolerant cultivar.

(c) *Seed losses in the first autumn*

The studies of the behaviour of the seeds immediately post-harvest were only done at RES and SAC. A sample of data from RES 2000 (Yr 2), reflecting the responses at the other sites is given. Further data are presented in Sweet *et al.* (2004). Seed losses at harvest of the rape plots reached 10 000 seeds m^{-2} for the glyphosate resistant cultivar but were significantly lower for the other three cultivars (figure 2: 1 day). After 8 days, most seeds remained on the plots, though a small minority had germinated, despite the lack of appreciable rainfall post-harvest. There was 14 mm of rain on days 8 and 9 and this seems to have been sufficient to cause the majority of seeds to germinate. A further 40 mm on day 14 ensured that most seeds germinated by day 22. There was still a small minority of ungerminated seeds (*ca* 200 seeds m^{-2}) at day 22, indicating that even in wet conditions some seeds could still be incorporated into the seedbank and thus persist. There is also evidence of a decline in total seed and seedling numbers over time, suggesting that there was a modest level of seed predation.

(d) *Seed losses from the soil in succeeding years*

Seed numbers in the soil seedbank in the first winter (4–8 months after the rape harvest), were much lower than the numbers present immediately after harvest. The decline rate of the seeds in subsequent years tended to be slower. Variations in the rates of seed decline between sites are clearly apparent in figure 3. Decline appears rapid at MOR 99, but at SAC (SAC99a and b) after the initial decline over the first six months, there appeared to be little further decline. This may have been caused by the failure to control volunteer rape plants in the barley crop in year 3, causing an increase in the seedbank after 30 months. Significant seeding by volunteer rape did not occur at any other site. Appreciably more seeds were present after 6, 18 and 30 months on the RES and SAC experiments '99b', than on experiments '99a'. This was because experiments 'b' were ploughed immediately after harvest, incorporating virtually all the shed seeds into the seedbank, while experiments 'a' were left for approximately four weeks before cultivation, permitting appreciable seed losses prior to cultivation (see above §3*c*). The 'immediate' ploughing on experiments 'b', prevented assessments being made on seed shedding at harvest, but as the two rotations were adjoining there is no reason to think that seed losses in Rotations 99a and 99b would have been very different.

The data presented in figure 3 do not provide any information on the relative persistence of the different cultivars. This was analysed using generalized regression analyses of the relative behaviour of the seed populations over the course of the programme. These analyses were done on the experiments, where the rape was harvested in 1999 and where there were at least four sets of data (seeds shed, seedbank after *ca* 6, 18, 30 months (+43 months at BB)). The data from SAC 99a failed to fit the decline model, due to the probable increase in the seedbank in year 3, but satisfactory fits were achieved for RES 99a, NIAB99a, NIAB99b, BB 99 and MOR 99. Parameter values from the models fitted for the five sites are given in table 1.

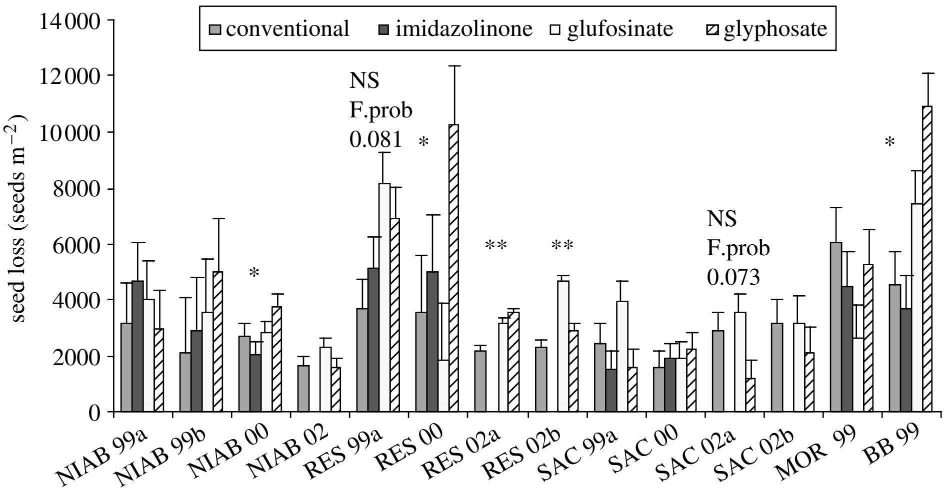

Figure 1. Overall effect of oilseed rape cultivar (conventional: glufosinate, glyphosate and imidazolinone tolerant) on seed losses at harvest in 14 studies (vertical bars, 1×s.e.d.; significance of differences between cultivars within sites, *$p<0.05$, **$p<0.01$).

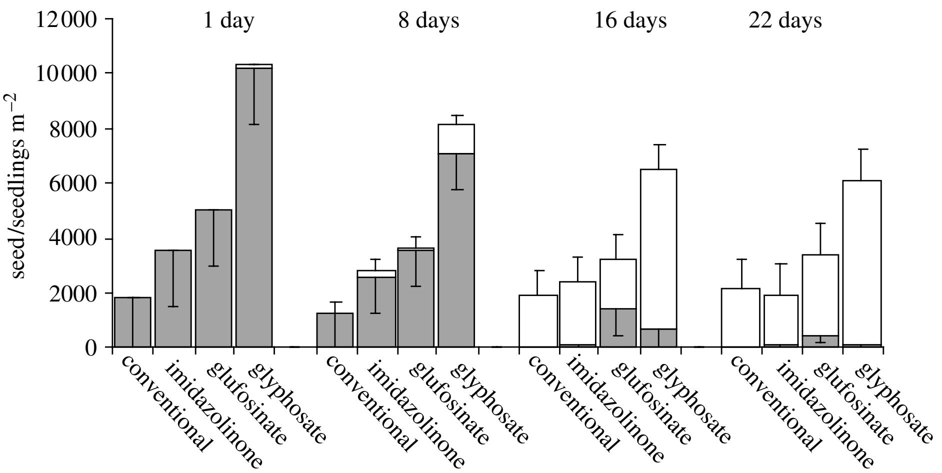

Figure 2. Germination behaviour of rape seeds post-harvest at RES in July–August 2000. Effect of rape cultivar (conventional: glufosinate, glyphosate and imidazolinone tolerant) and time on the number of rape seeds post-harvest (1 day) and on their subsequent germination after 8, 16 and 22 days (vertical bars, 1×s.e.d.; seeds, dark columns; seedlings, white columns).

There was a rapid decline in the first six months at all sites and at four of them, this decline decreased markedly in the subsequent 24 months (figure 4). At MOR 99, the decline rate differed from the other four as it continued to be high, such that few seeds survived to the fourth year. At the other four sites there were appreciable numbers of seeds left at the time of the final sample. The models predicted overall 95% seed loss in 9 years (range 3–20) and 99% loss in 15 years (range 5–34). However, as there were only four points on most decline curves the accuracy of these predictions must be treated with caution and more data are needed to confirm the final shapes of the curves. At three of the five sites, the analyses concluded that there were no detectable statistically significant differences in the decline rates (P_1 and P_2 parameters) between the four cultivars tested. In the other two experiments, treatment differences were identified. Interestingly, these differences manifested themselves only in the declines in the initial six months and no cultivar differences were detected in the subsequent decline (parameter P_2). However, the main differences between cultivars seemed to be in the initial numbers of seeds shed rather than in the rates of decline. The differences between the cultivars were not large but it appeared that the conventional was the most persistent and the imidazolinone tolerant cultivar, the least (table 1). In three of the sites, the parameter N (initial seed loss) differed between the replicates within the experiments. For simplicity, table 1 presents only a common estimate for each treatment at these sites. Similarly, the points in figure 4 were rescaled, to remove these plot-within-treatment effects, by dividing the data values from each plot by its own estimate of N and multiplying by the common estimate N for the relevant treatment.

4. DISCUSSION

The results of these experiments confirm that appreciable numbers of seeds of oilseed rape can be left in the field after harvest. The mean value of 3575 seed m^{-2} found in this work is somewhat lower than that reported by earlier studies which indicated that losses of winter rape seeds would be in the region of 7500 seeds m^{-2} (Price *et al.* 1996; Lutman *et al.* 1998) but is similar to the conclusions of Gruber *et al.* (2004) who reported losses to be between 3000 and 3500 seeds m^{-2}, in Germany. There was no strong evidence that any of the HT cultivars were more prone to shattering and consequent seed loss, than the conventional one. More specific targeted studies would be needed to confirm this, as the regulatory constraints on the

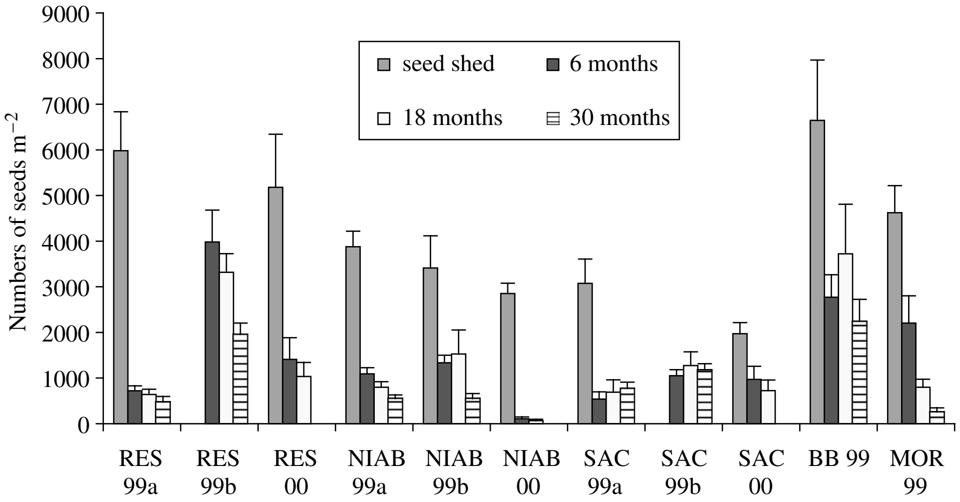

Figure 3. Overall decline rates for oilseed rape after *ca* 6, 18 and 30 months following seed shed at harvest at 11 sites. (RES, NIAB, SAC, BB, MOR = sites; numbers refer to harvest years and letters to different rotations within one site; vertical bars, 1×s.e.m.).

Table 1. Parameter values for the optimum regression decline models, predicting the decline in the oilseed rape seedbanks and estimates of the numbers of years to achieve 95 and 99% seed loss.
(Numbers in parentheses are the standard errors of the values.)

site	treatment	parameters of regression lines			number of years to achieve seed losses of	
		N	P_1	P_2	95%	99%
BB 99	conventional	3779 (665)				
	imidazolinone	3900 (659)	0.486 (0.0729)	0.886 (0.0595)	20	34
	glufosinate	8789 (1155)				
	glyphosate	8378 (1068)				
MOR 99	all treatments	4624 (575)	0.465 (0.1020)	0.299 (0.0774)	3	5
NIAB 99a	conventional	3137 (418)	0.412 (0.0829)		8	12
	imidazolinone	4581 (506)	0.231 (0.0462)	0.713 (0.0608)	6	11
	glufosinate	3982 (472)	0.359 (0.0680)		7	12
	glyphosate	2934 (403)	0.334 (0.0730)		7	12
NIAB 99b	all treatments	3413 (549)	0.456 (0.127)	0.702 (0.148)	8	12
RES 99a	conventional	3653 (453)	0.217 (0.0498)		9	17
	imidazolinone	5072 (534)	0.074 (0.0209)	0.817 (0.0892)	3	11
	glufosinate	8089 (673)	0.145 (0.0273)		7	15
	glyphosate	6632 (621)	0.085 (0.0199)		4	12
SAC 99a	no fit					

BRIGHT project meant that at most sites all cultivars were harvested on the same date, not at the individually optimum times.

Work reported by Pekrun *et al.* (1998) indicated that the best strategy for post-harvest management in the UK to minimize seed survival was to leave the stubbles uncultivated for several weeks, to permit the seeds to germinate. Two parts of the research reported here support this conclusion. The comparisons of seed persistence on RES 99 a and b and SAC 99 a and b, showed that fewer seeds survived where ploughing was delayed, particularly at the RES site (figure 3). The detailed monitoring of the fate of seeds at the RES site in 2000 also demonstrated the high percentage germination that can occur (figure 2). The close link between rainfall and the germination of the seeds was confirmed. There were also indications that seeds and/or seedlings, were disappearing from the experiment, presumably as a result of predation by a combination of birds, rodents and invertebrates.

Despite the potential for many seeds to germinate and thus not be incorporated into the soil seedbank, the high numbers of seeds shed, combined with the uncertainty of post-harvest, pre-cultivation rainfall meant that appreciable number of seeds were detected in the soil cores sampled in the first winter after the rape harvest, on all experiments. As the background level of rape seeds in the seedbank was nil or very low, these seeds must have been derived from those shed at harvest. However, the percentage decline between the rape harvest and the date of the first soil cores was considerable. The decline curves indicated a mean 63% loss in the first few months. Similar declines have been reported for conventional cultivars in the experiments of Pekrun *et al.* (1998). The subsequent decline in seed numbers in the soil was much slower at four of the sites, with a mean annual decline rate of only *ca* 20%. At these four sites nearly 1000 seeds m^{-2} were present in the soil 3 years after rape harvest (figure 4) and the regression models predicted that it would on average take 9 years to lose 95% of seeds (table 1). This prediction needs to be treated with caution as the last data set from the trials was collected in the fourth year. It must be pointed out that even a 95% loss of the mean 3575

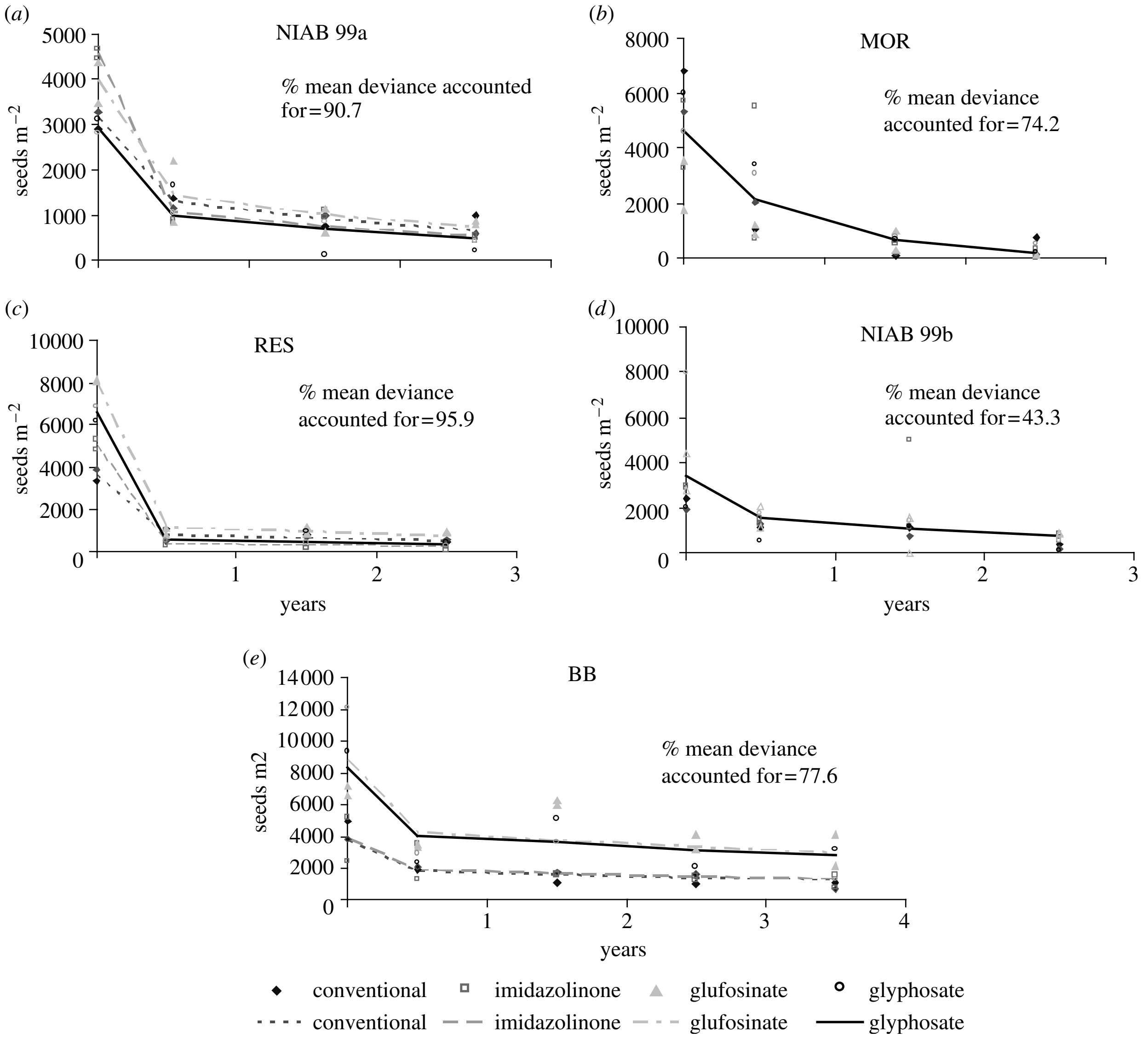

Figure 4. Modelled decline curves for four types of oilseed rape seeds (conventional (Apex) (filled diamond), imidazolinone tolerant (open square), glufosinate tolerant (filled triangle) and glyphosate tolerant (open circle)) from time of harvest for the next 30 months at five sites: (*a*) NIAB 99a (*b*) MOR 99 (*c*) RES 99 (*d*) NIAB 99b and (*e*) BB 99.

seeds m^{-2} shed at harvest, would still leave nearly 200 seeds m^{-2}. Such numbers would be highly likely to result in the presence of more than two volunteer plants per m^2 in a rape crop sown 9 years after the HT crop. This density would exceed the European Union threshold of 0.9% adventitious presence of GM seeds in a non-GM crop, if the subsequent crop was 'conventional'. The reason for the rapid loss of seeds at the fourth site is not clear, but this site had the lightest soil and other work has suggested that weed seeds persist for a shorter time on light soils (Lutman *et al.* 2002). Other studies at RES (Lutman *et al.* 2003) indicated that 95% of rape seeds would disappear in 3–4 years but in the BRIGHT experiments reported here, it appears that survival could be longer. Thus, standard rotations which tend to sow rape one year in four will have to be extended to avoid cross-contamination problems, if the grower wishes to change from growing GM rape to conventional cultivars. However, the seed persistence conclusions from the BRIGHT experiments would benefit from further sampling to confirm the shape of the tail of the decline curve. Further, soil samples are currently being taken from some of the BRIGHT experiments, as a result of securing additional funding through the European Union SIGMEA project.

There was no clear evidence in these studies that the four tested cultivars differed in their persistence, even though they came from diverse genetic backgrounds. It is known that cultivars differ in their potential to persist (Pekrun *et al.* 1997*b*; Gruber *et al.* 2004; Gulden *et al.* 2004), but it seems that the three HT cultivars were not more persistent than the conventional variety Apex. Gulden *et al.* (2004) also failed to find a link between herbicide tolerance and persistence. There was some suggestion that Apex might have been more persistent than the other treatments (table 1) and this concurs with previous work (Pekrun *et al.* 1997*b*) indicating that this cultivar appears to be one of the more persistent of conventional cultivars.

Thus, this work has shown that although HT cultivars of rape are no more persistent than conventional ones, there is a potentially serious problem associated with the temporal persistence of rape seeds in soil, in relation to the coexistence of GM and conventional rape crops at currently acceptable levels of adventitious presence. It also emphasizes the importance of minimizing the numbers of seeds incorporated into the soil post-harvest, by optimizing post-harvest management to maximize seed germination.

We thank Defra and SEERAD and the industrial sponsors of this Sustainable Arable Link project (Bayer Crop Science, Monsanto plc, BASF, HGCA, BBRO and Agrovista, UK) for their support. Rothamsted Research receives grant-aided support from BBSRC.

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