





An analysis of the findings of the BRIGHT trials with GM herbicide tolerant crops in relation to environmental impact

A report for GeneWatch UK, the Five Year Freeze and Friends of the Earth

Ву

RJ Turner & W Bond IOR-HDRA, Ryton Organic Gardens, Coventry, CV8 3LG, UK BD Pearce IOR-EFRC, Hamstead Marshall, Newbury, RG20 0HR, UK

March 2005



Contents

| 1Executive summary | . 2 |
|--|-----|
| 2Introduction | |
| 3The BRIGHT trial experimental design | 6 |
| 3.1Main study | |
| 3.2Additional studies of intrinsic dormancy of OSR seed | . 7 |
| 3.3Additional studies of cross pollination between herbicide tolerant and | |
| conventional OSR cultivars in 1999 | 7 |
| 3.4Comments | |
| 4Parameters measured in the BRIGHT trial | . 9 |
| 4.1Methodology | . 9 |
| 4.2Comments. | |
| 5BRIGHT trial data analysis | 11 |
| 5.1Methodology | |
| 5.2Comments. | |
| 6BRIGHT trial findings | 13 |
| 6.10verall levels of weed control and performance of the herbicide treatments in | |
| the herbicide tolerant crops | |
| 6.1.10ilseed rape | .13 |
| 6.1.2Sugar beet | |
| 6.2Timing of weed control | 14 |
| 6.3Rotational implications of weed control in HT crops | 14 |
| 6.4Overview of weed seedbank data | 14 |
| 6.5 Oilseed rape yields and harvest losses | |
| 6.6Post-harvest germination of oilseed rape seeds on stubble | 15 |
| 6.70ilseed rape seed persistence in the soil | 15 |
| 6.8Oilseed rape seedling emergence from the seedbank | 15 |
| 6.9Overall impact of oilseed rape volunteers | 15 |
| 6.10Crop yields | |
| 6.11Economics of HT oilseed rape and sugar beet | |
| 7BRIGHT trial conclusions | |
| 7.1Conclusions and comments | 17 |
| 8Conclusions of this review | 20 |
| 9References | 21 |
| 10Annex 1: Parameters measured and data analysis | 22 |
| 11Annex 2: BRIGHT trial results in detail | 25 |

1 Executive summary

This report considers the findings of the BRIGHT project (Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet), a four year study, jointly funded by Government and industry, which was intended to consider the agronomic and environmental issues of growing genetically modified herbicide tolerant crops. The final report of this study was published in November 2004 and the results headlined by the BBC on 29th November 2004 as 'Study finds benefits in GM crops. GM crops are no more harmful to the environment than conventional plant varieties, a major UK study has found'.

This report addresses whether such headlines were justified and whether, under the experimental approach used in the study, it is even possible to draw meaningful conclusions about the environmental impacts of growing GMHT crops. This report also considers the quality of the economic analysis and identifies potential environmental questions raised by the study.

Objectives and experimental design

The stated objectives of the BRIGHT trials were to determine the agronomic and environmental implications of growing GMHT winter oilseed rape and sugar beet in arable rotations and make recommendations to farmers on how to grow these to optimise agricultural benefits, while minimising their effects on the environment. However, while the experimental design is adequate for carrying out a basic herbicide evaluation trial within an arable rotation, there is insufficient replication to determine effects on biodiversity in what is very a varied environment. Therefore, the results should be considered primarily in an agronomic context.

Methodology

In relation to the determination of environmental impact, only a very restricted range of parameters were measured - weedseed bank size and species. There were no measurements of invertebrates, soil microflora, gene flow to wild species, or field margin effects.

Even though weed seedbank measurements formed the main assessment of environmental impact tested in the experiments, the weed seed data and subsequent analysis was inadequate to draw firm conclusions on the changes in the composition of the weed seedbanks or the impact of these changes in terms of biodiversity. To draw such conclusions would have required much greater resources. There were insufficient samples taken at each sampling event, and they were not take frequently enough during the rotation.

Only a fraction of the soil in each sample was processed, reducing the precision of the analysis even further. As a result, a reliable estimate of seed density can only be made for weed species with seeds present in relatively high numbers. Even for these species, there can be rapid changes in numbers over a single season, depending on when the samples are taken. When weeds are present in low numbers in the soil seedbank, such a high number of soil cores are needed for a reliable estimate that the sampling becomes impracticable. Finally, while it is true that any plant species can add to biodiversity a large proportion of the weed seed-bank increase appears to be attributable to the high number of volunteer crop seeds.

Another limitation is that the study did not compare the effect of different types of cultivation on the results. All the fields in the study were cultivated by ploughing, and so the impact of GMHT crops in other systems, such as minimum tillage or no tillage,

was not considered in the experiment. In addition, all trials were based at intensively farmed sites, which have species diversity levels far too low to be representative of the average farm. The soil types at the trial sites were also very similar.

Statistical analysis

The statistical analyses do not appear able to account for, or overcome, the inherent variability of the existing weed populations at the trial sites. This indicates that little confidence can be placed in the reasons suggested for the changes in weeds and their seeds. There seemed to be a struggle to find appropriate statistical tests from the range that were tried. Ideally, the analysis methods should have been determined at the planning stage as part of the experimental design. This raises serious questions about the robustness of the analysis and conclusions.

Economic analysis

The economic analysis includes far too many speculative variables to be meaningful. It also considered a very narrow range of costs and excluded others such as: removal of bolters in beet; more complex spray programmes to control weeds not controlled by the main herbicide; extra cultivation to manage seed shed at OSR harvest; possible loss of GM free status; insurance required to grow GM crops; and risks associated with being tied to a single supplier of seed and herbicide.

Environmental issues arising from the research

Whilst the trials could not investigate environmental effects properly, several issues emerged which could be of future concern including:

- the potential for increased use of more toxic herbicides to control weeds poorly controlled by the broad spectrum herbicides used with GMHT crops in the trial. *Viola arvensis* was poorly controlled by glufosinate and *Urtica urens* poorly controlled by glyphosate;
- that gene flow from GM to non-GM oilseed rape and gene stacking (where more than one GM trait is acquired), is a real risk. Monitoring of out crossing between the various oilseed cultivars was limited to the experimental plots which meant cross pollination rates were only recorded for relatively short distances (up to 91 metres). The data showed out crossing occurred at all sites and average rates for conventional varieties ranged from 0.3% to 0.9% with peaks of 4.2%. When the predominantly male sterile variety Synergy was tested the highest level found was 9.7%;
- that volunteer oilseed rape is likely to persist as a source of future GM contamination. Following OSR crop harvest, appreciable numbers of seeds were predicted to persist at trial sites and further monitoring of the sites is required;
- the possible emergence of herbicide tolerant weed beet and associated need for increased herbicide use. The emergence of weed beet itself is a much more complex process than the production of herbicide tolerant oilseed rape volunteers, but once HT weed beet begins to appear, continued use of the herbicide that it can tolerate will encourage it to multiply further. Herbicide tolerant weed beet are a potentially serious problem as current control relies mainly on glyphosate or expensive hand pulling;
- that difficult-to-control volunteer oilseed rape weeds which have multiple herbicide resistance may well occur if management guidelines are not followed. The BRIGHT researchers concluded that using HT crops would have little direct impact on subsequent crops but there are management issues where crops grown later in the rotation share the same herbicide tolerance and where conventional crops that follow GM ones risk seed contamination

Conclusions

To fully answer the trial objectives in relation to understanding the environmental effects of GMHT crops was impossible with the level of funding and experimental design. Environmental (botanical) impacts could not be extensively investigated in the trials which were designed to meet a primarily agronomic objective. Therefore, the trials should be seen as a small piece in a large jigsaw puzzle aimed at understanding potential effects of introducing GM technology into British farming systems. Headlines that suggest that the BRIGHT trials showed that GMHT crops do not harm the environment, misrepresent the results of a complex four-year study and do an injustice to scientists who have undertaken the research.

2 Introduction

The BRIGHT project (Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet) was a four year study, jointly funded by Government and industry, which was intended to consider the agronomic issues of growing genetically modified herbicide tolerant crops. The project started in April 1999 under the Sustainable Arable LINK programme but was initiated in autumn 1998 with pre-LINK funding to allow winter oilseed rape to be established. Field studies were completed in January 2003 and a final report was published in November 2004 (Sweet *et al.*, 2004). The results were headlined by the BBC on 29th November 2004 as 'Study finds benefits in GM crops. GM crops are no more harmful to the environment than conventional plant varieties, a major UK study has found' (http://news.bbc.co.uk/2/hi/science/nature/4046427.stm). The issue we address here, is whether such headlines were justified and whether the experimental objective in relation to investigating environmental impact was possible with the experimental design used.

The BRIGHT project objective was:

"To determine the agronomic implications and the environmental impact (especially botanical effects) of herbicide tolerant oilseed rape and sugar beet grown in a range of rotations, so that guidelines for the agronomy of these crops can be produced to enable farmers to fully exploit these crops while minimising their environmental impact" (Sweet *et al.*, 2004).

The scientific objectives were to:

- 1. Determine the effect of different HT systems on weed species and number in the HT crop.
- 2. Determine the effect of HT systems on weed species and number in subsequent rotational crops.
- 3. Determine the longer-term implications for arable plant diversity by studying the composition of seedbank populations at the beginning and end of the rotations.
- 4. Determine the principle factors involved in the evolution of HT and multiple HT volunteers.
- 5. Develop strategies for preventing build up and the control of HT volunteers in different crops.
- 6. Determine the agronomic benefits of growing HT crops.
- 7. Identify the most appropriate management systems for HT crops.
- 8. Identify snags and problems that can arise and ways to avoid or recover from them.
- 9. Develop strategies for the appropriate management of HT crops that optimise environmental and agronomic impact.
- 10. Provide information of value for evaluating the risks associated with the release of these crops.
- 11. Provide information which will contribute to developing systems of post marketing monitoring and risk management of GMHT crops.

3 The BRIGHT trial experimental design

3.1 Main study

In the BRIGHT project, two cultivars of sugar beet and two of winter oilseed rape, genetically modified to be tolerant to glyphosate or glufosinate, were compared with conventional cultivars. The GMHT crops were grown with weed control by the chemical they were tolerant to. The conventional crops were treated with a standard programme of herbicides approved for these crops. In addition, for the first two years of the study, a winter oilseed rape cultivar bred by conventional breeding techniques to be resistant to the imidazolinone herbicides was included in the comparison. The herbicide tolerant crops were grown in plots alongside conventional crops and all plots had the same management except for the herbicide treatments.

Each GM crop was included in three of a series of five different four year arable rotations with cereals and other crops. The rotations included some perceived to follow best practice and others that represented worst case scenarios, where the impact of the HT cultivars might be expected to be greatest.

The trials were conducted at the research farms at NIAB, Rothamsted, Broom's Barn, Morley Research Centre and the Scottish Agricultural College (Aberdeen). A maximum of three of the five rotations was grown at each site (see Table 1)

Under the GM deliberate release approvals, the area of the GMHT crops that could be grown at the start of the project was restricted. There was a choice between greater plot size with fewer replicates or greater replication but with smaller plots. It was decided to have large plots to simulate 'normal' field responses while keeping replication to an 'acceptable' minimum. Rotations 1a, 2 and 3 had only two replications, while the smaller plot rotations 4 and 5 had three and six respectively. The consortium was aware of the limitations of the replications in rotations 1, 2 and 3. At Rothamsted and SAC rotations 1 and 3 were combined after Year 1, while they were identical, to become 1a and 1b thus providing two sites with four replications of Rotation 1.

| Year | Rotation 1a 1b | | Rotation 2 | Rotation 3 | Rotation 4 | Rotation 5 |
|-------|-----------------------|-------------------|---------------------------|-----------------------------------|-------------------------------------|---------------------------|
| Sites | NIAB Roth'd SAC | Roth' d SAC | Broom's Barn Morley | Broom's Barn Morley NIAB | NIAB Rothamsted SAC | Broom's Barn Morley |
| 1 | winter rape | | sugar beet | winter rape | winter cereal | winter cereal |
| 2 | cereal | | cereal | cereal | winter rape | cereal |
| 3 | cereal | | cereal | sugar beet | cereal | sugar beet |
| 4 | winter rape | | sugar beet | cereal | cereal | cereal |
| | | | | | onal crops were on HT rape or conve | |

| Table 1. | BRIGHT | rotations | design an | d partici | pating sites | |
|----------|--------|-----------|-----------|-----------|--------------|--|
| | | | | | | |

The soil type at four of the sites was a clay loam, at Broom's Barn it was a lighter sandy loam. Crop management followed normal practice, although sowing dates of the test crops were sometimes delayed due to problems with the supply of seed. Most sites were ploughed after crop harvest.

In August 1998, prior to drilling the winter cereal in Year 1 of Rotation 4, the plots were broadcast with oilseed rape seeds (LL, RR, IMI, CONV varieties) that were ploughed under to simulate seeds shed from a previous crop and to establish a seedbank of potential volunteers. In August 1998, prior to drilling the winter cereal in Year 1 of Rotation 5 at Morley, the plots were broadcast with weed beet seeds (non-GM) that were ploughed under to simulate seeds shed from a previous crop and to establish a seedbank of potential volunteers. At Broom's Barn, the experiment was sited on an area believed to be infested with weed beet, although this proved not to be so and additional weed beet seed was sown just prior to drilling the sugar beet in Year 3.

Decisions on herbicide applications to the cereal crops and to the conventional sugar beet and oilseed rape were made by experienced managers at each site. Herbicide applications to the herbicide tolerant crops followed the recommended rates and timings given by the relevant agrochemical companies.

In Year 1, the plots in each rotation that included oilseed rape were:

- Glyphosate tolerant variety Roundup Ready (RR)
- Glufosinate tolerant variety Liberty Link (LL)
- Imazamox tolerant variety (IMI)
- Conventional variety (CONV)

When oilseed rape was sown again in Year 3 or 4 of the rotation, the original plots were split into 4 sub-plots and the varieties randomly assigned to these so that the first year treatments were followed by each of the subsequent oilseed rape treatments. The IMI oilseed rape variety was withdrawn from the project after Year 2 and was replaced by a second conventional variety (CON*).

In the year that sugar beet plots were included in the rotation these were:

- Glyphosate tolerant variety Roundup Ready (RR)
- Glufosinate tolerant variety Liberty Link (LL)
- Conventional variety (CONV)

Where the herbicide tolerant sugar beet varieties followed herbicide tolerant oilseed rape, additional herbicides were included in tank mixes with the glyphosate and the glufosinate to control HT oilseed rape volunteers from the first year. When the experimental layout allowed, herbicide treatments in the years when GMHT crops were grown had an adjacent untreated area, in order to assess the weed potential in each plot.

3.2 Additional studies of intrinsic dormancy of OSR seed

At Rothamsted and Broom's Barn, seed was harvested from the four oilseed rape cultivars in July 1999 to test for intrinsic seed dormancy. Seeds were stored at 20°C after harvest until ready for use.

3.3 Additional studies of cross pollination between herbicide tolerant and conventional OSR cultivars in 1999

Field studies of cross pollination between herbicide tolerant and conventional OSR varieties were made at NIAB, Rothamsted and SAC in plots separate from the crop rotation studies. At NIAB, two blocks of three herbicide tolerant and two conventional winter oilseed rape varieties were established in adjacent areas of 92 m x 92 m in a 10 ha field in autumn 1998. One of the conventional varieties was a varietal association cultivar, Synergy, which has 80% male sterile plants and with greater outcrossing potential. The other was Apex, a standard open pollinated cultivar. At Rothamsted and at SAC, four blocks of three herbicide tolerant and a

conventional winter oilseed rape variety were established in areas of 24 m x 120 m in autumn 1998. The aim at the three sites was to provide data on the dispersal of transgenes between plots.

3.4 Comments

The objective of the BRIGHT trials was to determine the agronomic and environmental implications of growing GMHT winter oilseed rape and sugar beet in arable rotations. However, as seen in the following section, only a limited range of botanical effects were assessed and no other biodiversity or other environmental parameters were measured. For a fuller assessment, measurements of invertebrates, soil microflora and boundary effects would have been needed.

The secondary nature of the intention to measure environmental impact is revealed by the methodolgy used. Untreated plots were not included in the experimental design because on a commercial conventional farm, herbicides would usually be sprayed on crops and, therefore, the BRIGHT report states an untreated control is not needed for comparison purposes. However, to investigate the environmental (botanical) effects, untreated plots of each variety would seem a requirement. This raises the question of how the impact of the GM crop alone, rather than in tandem with its herbicide programme, can be evaluated, and how are the factors of variety and chemical to be separated? It is understandable that there were concerns that having untreated plots was unrealistic or would cause problems later in the rotation. However, the inclusion of plots of the HT cultivars treated with the conventional herbicides would have allowed at least some separation of the effects of the cultivars and their associated herbicides. This was a limitation also identified in the methodologies used within the Farm Scale Evaluation (FSE) trials.

However, the FSE trials did have a design much better suited to determining environmental impact. The FSE trials had a simple experimental design, 2 treatments, conventional and GM, allocated to half fields at one site. Each farm chosen represented a single replicate with a target of 60-75 farms for each crop type, and although there was no replication at an individual farm there was considerable replication over the three year period. FSE farms were selected to represent the range of geography and intensiveness across Britain with a positive discrimination to include a higher proportion of less intensive farms. The aim was to ensure a high level of biodiversity that would help magnify any changes (Perry *et al.*, 2003). In contrast, the BRIGHT trials where all the sites were intensive and had low biodiversity.

As far as it goes, the BRIGHT research has been carried out in a professional manner, but the trials had a very practical remit, to determine management programmes of GM crops for farmers, not to establish the environmental impact of their use in agricultural. To satisfactorily address the environmental objectives requires a considerable amount of further research. The authors of the BRIGHT report recommend that 'if biodiversity impact is to be a major factor in decision making on weed control in arable cropping systems, there is a need to look at the subject holistically across crop rotations and address the potential impact of all crops, not just those potentially including HT systems'.

4 Parameters measured in the BRIGHT trial

4.1 Methodology

The basic parameters measured in the BRIGHT trials were:

- Weed species and numbers
- Weed seedling numbers
- Weed biomass
- Visual assessment
- Yield
- Weed seed bank
- Oilseed rape loss at harvest
- Oilseed rape in seedbank
- Intrinsic dormancy
- Cross pollination between HT and conventional OSR in one year

Details of how these were conducted are given in Annex 1.

4.2 Comments

There is a clear deficiency in the scope of the parameters measured in relation to the determination of environmental impact. In addition, in relation to one of the measurements which does have relevance to environmental impact assessment, weed seedbank, there are limitations to the approach adopted in the BRIGHT trials.

To compare changes in the weed seedbank over time, reliably and in any detail requires intensive sampling which demands resources well beyond those of the BRIGHT study. Weed seed distribution in soil is notoriously patchy even after many years of cultivation. While some horizontal and vertical movement of seeds occurs, distinct patches of weeds are likely to be visible for many years after a significant seed shedding event. Soil sampling methodology is fraught with difficulties both in terms of sampling pattern and sampling intensity (Forcella, 1984). This is compounded by the resources needed to carry out the extraction and identification of seeds in even a minimum number of samples. Much has been written about the problem of determining the number of sample cores required to give a reasonable estimate of the seedbank (Roberts, 1981; Benoit *et al.*, 1992). Jones (1998) suggests that 60-100 soil cores are needed to accurately estimate the seedbank with 5 or 6 sub-samples needed from each bulked sample when sub-sample variability is high. A bulked sample of only 25 cores is inadequate to describe the seedbank using random sampling.

It is usually recommended that preliminary samples are taken to gain an idea of the likely number of seeds and weed species present and base the sampling strategy on this (Champness, 1949). The BRIGHT report does not indicate how the researchers decided on the sampling strategy that was used, and there is no description of preliminary sampling. Recent research has suggested that where there is spatial variability, a systematic approach to sampling, rather than a random approach, reduces the sampling size needed to achieve the same level of precision (Ambrosio *et al.*, 2004). In the study to determine the number of oilseed rape seeds remaining each year, it is not clear why the researchers varied the number of soil cores taken. Nor is it stated whether the whole soil sample was processed.

The analysis of weed seeds in the BRIGHT study suffers both from a lack of samples at each sampling event and the limited number of times in the rotation that samples were taken. To determine weed seed numbers at the start of a trial and

only once again at the end provides information that is of limited value. There is no indication of what has happened to seed numbers between these two points in the rotation. The time gap is far too long to implicate any specific factor in seedbank changes be they an increase or decrease in seed numbers. Within the time scale of a single four year rotation, it is likely that the individual crops, their specific management requirements and even the season of growth will have as great an effect on the weed seed levels as the individual treatments.

In the BRIGHT seedbank study, there is no basic information on whether seed numbers presented are per weight of wet or dry soil and, when represented as seeds per m², if this value relates to the initial sampling depth. Soil cores were taken to 30 cm and homogenised when a minimum sampling requirement should ideally have been a 0-15 and 15-30 cm sample. There are no details of plough depths presented to tie in with sampling depth, and there are no dates of cultivation to establish when the weed seed samples were taken, i.e. before or after ploughing, to aid interpretation of results and whether the same approach was taken at all sites. Benoit *et al.*, (1992) record that seedbank density at sampling is likely to increase or decrease depending, in particular, on when sampled in relation to flushes of seedling emergence or seed shedding.

Four separate sets of twelve 30 cm cores were taken from each sub-plot but it is unclear whether these were treated as four separate samples in any analysis or as four sub-samples. Only the seeds from 500 g of soil from each bulked set of twelve cores would seem to have been washed out. The full weight of soil for each sample is not given and there is no indication of how the quantity of soil for processing was decided upon. The dry soil from twelve cores of just 10 cm depth is known from experience be around 1 kg in weight. Twelve cores of 30 cm depth would therefore weigh around 3 kg. Processing just 17% of the soil from what is already a relatively small sampling area is unlikely to be sufficient to give reliable results.

When weeds are present in low numbers in the soil seedbank, estimates will be unreliable unless an unrealistically high number of soil cores are taken. Seeds of species present in low numbers are likely to be missed completely, or if found will become multiplied up to an improbable number per m² by a conversion factor based on a relatively small sampling area. The figures become even more distorted if converted at a later date to seeds per ha. Only for weed species with seeds present in relatively high numbers can a reliable estimate of seed density be made but even here there can be rapid changes in numbers over a single season depending on when the samples are taken (Roberts, 1981).

In summary the main problems with the weed seed sampling were:

- no preliminary investigation to establish distribution of weed seeds
- insufficient sampling occasions
- different sampling strategies between sites
- whole soil sample not analysed
- no cultivation information presented to aid interpretation of the results
- low seedbank numbers form basis of conclusions

Due to all the factors explained it would be unsafe to draw firm conclusions on weed seedbanks from the BRIGHT trials.

5 BRIGHT trial data analysis

5.1 Methodology

All experiments were factorial in design with variable levels of treatments and replications. Rotation 1 at Rothamsted and SAC had 4 replicates, Rotation 1a at NIAB had 2 replicates. Rotations 2 and 3 all had 2 replicates. Rotations 4 and 5 had 3 replicates. The smallest trial (Rotation 2) had 18 plots in the final year giving 8 degrees of freedom in the error for comparing treatment effects. Analysis of the data took into account the hierarchical structure of rotation, replicate, plot and sub-plot. Cross site analysis was not carried out because of the variability between sites and it was considered to be beyond the scope and resources of the project.

A number of techniques were considered to assess the effect of treatments on species diversity. Simply counting species numbers per plot and carrying out a standard ANOVA suffers from the problem that it could be biased by weed density. Even using log transformed weed number per plot as a covariate (SlogN) leaves the problem that while the species number on different plots may be the same the composition of those species may be different. Other techniques for measuring species diversity include Log series α which assesses species numbers in a different way from SlogN and Berker Parker dominance which identifies the importance of the most dominant species.

Initial statistical analyses indicated that the BRIGHT data was skewed with many plots having few weeds and a minority having many. Weed counts per m² data were analysed both as raw data and transformed ($log_{10}(x+1)$) data to cater for zero counts. After consideration, the results were not adjusted using spatial aggregation covariates. Many species occurred only rarely and then at densities too low for treatment comparisons. Other species occurred in some but not all years. The main analyses focussed on a restricted number of the commoner species. Total weeds and total weed species were estimated for each plot and used to calculate the effect of treatments on botanical diversity.

5.2 Comments

Although the experimental design is adequate for carrying out a basic herbicide evaluation trial within an arable rotation, there is insufficient replication to determine treatment effects on biodiversity in what is very a heterogeneous environment. The level of precision desired should determine the replication, four to six replications being considered adequate for the evaluation of several herbicide treatments (Frans & Talbert, 1977). In studies of weed control, particularly herbicide evaluation trials. the results can consist of seed or seedling counts that are at or approaching zero together with other much higher counts. Where this is consistent with the treatments, transformation of the data may allow valid comparisons to be made of any differences between treatments. However, the spatial variability of weed distribution in arable fields means that high and low counts can occur in plots that share the same treatment regime. Where there is prior knowledge of the distribution of the weeds, as here with initial seedbank data and pre-treatment seedling counts, it may be possible to take this into account when analysing data. However, knowledge of the composition of the weed seedbank is often of limited use in predicting the likely weed emergence.

The researchers describe in some detail the lengths they went to in choosing suitable techniques to assess the effect of treatments on species diversity. All of the analyses had some limitations in dealing with the data. There are many other ways of measuring weed species diversity in crop/weed studies (Topham & Lawson,

1982). Perhaps a different analysis would be more effective and reliable over the wide range of data available. The BRIGHT trial report acknowledges that the analyses made to ascertain whether there were any major treatment effects in the rate of decline of OSR seed lost at harvest were not strong as in many data sets there were only two replicates.

6 BRIGHT trial findings

Annex 2 gives the BRIGHT project results in detail. Here we give an overview and commentary.

6.1 Overall levels of weed control and performance of the herbicide treatments in the herbicide tolerant crops

It is stated that the BRIGHT project was not to assess whether generally accepted levels of weed control were ecologically acceptable, and that the FSE trials that had the target of comparing the ecological impact of growing herbicide tolerant and conventional crops. Some information on changes in the species diversity and on the seedbank at the start and end of the project was recorded. The studies did not show a major decline in species numbers or seedbanks. However, as there were no untreated plots included in the experimental design, there was no determination of the absolute effect on weeds. This was not thought appropriate as the trials were primarily agronomic. It was proposed that such studies could be considered in future trials. In addition, the high level of weed control in most of the cereal crops had a big impact on the weed flora anyway.

The statistical analyses do not appear able to account for or overcome the inherent variability of the weed population in the study. This indicates that little confidence can be given to the suggested reasons for the changes in weeds and their seeds. At two of the trial sites, baseline monitoring of weed seedlings showed a significant effect of 'treatment' on the distribution of fat-hen (*C. album*) on plots even before the herbicide treatments were applied. As it was decided not to use the co-variate analysis, it is unclear how this 'effect' was taken into account. There seemed to be a struggle to find appropriate statistical tests from the range that were tried. Ideally the analysis methods should have been determined at the planning stage as part of the experimental design. This raises serious questions about the robustness of the analysis and conclusions.

6.1.1 Oilseed rape

In oilseed rape, all the treatments at some sites, and some years gave the best weed control. Much depended on the local weed flora and on the timing of herbicide application. Overall, glyphosate gave the best control or shared the best control in 8 of the 12 experiments. Glufosinate gave the best control or shared the best control in 5 of the trials. The conventional herbicides gave the best control or shared the best control or shared the best control in 3 experiments.

6.1.2 Sugar beet

In sugar beet, only 6 comparisons of performance were made but differences were small or there was no clear pattern of response. Comparisons were difficult as the conventional treatments were made up of different product combinations. Glyphosate tended to give the best weed control. However, metamitron was included in the sprays where herbicide tolerant volunteer oilseed rape was present.

It is said that because more weed beet was recorded on the conventional treatments while the glufosinate and glyphosate treatments had almost none, that this demonstrates the value of the two GM tolerant cultivar-herbicide combinations in controlling weed beet. However, this would not be true once weed beet developed herbicide resistance. In 2001, weed beet was recorded in over 70% of beet fields (Knott, 2002). Current control methods include hand pulling and using weed wipers to apply glyphosate to flowering stems growing above the crop.

6.2 Timing of weed control

Many of the conventional herbicides applied to winter oilseed rape and sugar beet need to be applied pre- or early post-emergence of the crop and weeds. The application time for glyphosate and glufosinate appears to be more flexible because they will control weeds at later stages. Nevertheless, the timing of glyphosate and glufosinate applications is important because they have no residual activity so weeds that emerge later will not be controlled. In addition, weeds tend to become less susceptible at later growth stages and can become protected by the expanding crop canopy if application is delayed for too long. Weeds left in the crop for too long can also begin to reduce yields.

6.3 Rotational implications of weed control in HT crops

In an arable rotation, one of the benefits of including herbicide tolerant crops with the potential to achieve high levels of weed control is the reduced need for weed control in succeeding and possibly even preceding crops. This was not explored in the BRIGHT experiments, although the importance of managing volunteer oilseed rape was studied. There was only limited evidence of any carry over of effects on the weed flora from the first GM crops grown in Year 1 to when the second GM crops were sown in Year 3 and 4. The use of ploughing as the primary cultivation method at all sites could have masked any effects. Carry over could have been more noticeable if a reduced or non-inversion cultivation system had been employed. Splitting the treatment plots to allow this comparison was considered but it was decided that the goals of the BRIGHT project would be better met by not reducing plot size. Cultivation method can also affect the persistence of volunteer oilseed rape but this was being studied elsewhere using non-GM cultivars.

6.4 Overview of weed seedbank data

The weed seedbank data was rather variable but overall trends were apparent despite the modest amount of soil that was processed. In six of the seven data sets, weed seedbanks increased between the start and end of the experiment. In some cases the increases were small, whilst in others they were huge. Variability in the data meant it was difficult to identify significant effects of treatments. Where effects were recorded there was no overall trend across sites. At most sites, weed control in the cereals was good and they had little additive effect on weed seed numbers. However, poor weed control in winter barley in year 3 was responsible for the massive increase at SAC in Rotation 1. Weed seed increases tended to be greater where sugar beet had been grown often because of inadequate control of fat-hen (*C. album*).

In Rotation 1, it is concluded that glufosinate and glyphosate can give as good or sometimes better weed control in oilseed rape than conventional herbicides but the efficacy depends on local conditions at the time of treatment. Treatments had no clear effect on species diversity. Impacts on the seedbank seemed to reflect the variable performance of the herbicides at the different sites in different years. It is acknowledged that overall weed species numbers present on the three Rotation 1 sites were relatively low (maximum of 12 species per treatment). This is explained to be the result of the limited range of species adapted to the disturbed ecosystem of an arable field, and to the herbicide treatments. Rotation 4 had only one broad-leaved crop year with GM cultivars and it was not possible to explore the impact of a sequence of the different herbicides applied to such crops.

6.5 Oilseed rape yields and harvest losses

The potential for winter oilseed rape to establish a seedbank and provide a pathway for the persistence of herbicide tolerant rape through the rotation was predicted from existing data and was one of the prime reasons for the project. Over the four years, 12 winter oilseed rape crops were sown at 5 sites. Seed yields were in the region of 3.0 t/ha for all four varieties. Seed losses at harvest ranged from 2000 to 10000 seeds per m², with a mean of 3575 seeds per m² which equates to about 5% of the crop yield. Herbicide tolerance did not seem to have any effect on the level of seed shedding.

6.6 Post-harvest germination of oilseed rape seeds on stubble

Seed in undisturbed stubble remained ungerminated until there was appreciable rainfall. There was some predation while seeds remained on the soil surface but germination was responsible for the majority of seed losses. Some seeds remained ungerminated despite significant rainfall. The results suggest that leaving rape stubble uncultivated for 7 days after a significant rainfall event will allow any seeds that are likely to germinate to do so and be killed by subsequent cultivations.

6.7 Oilseed rape seed persistence in the soil

The oilseed rape seed shed and decline results were not consistent. Greater seed shed was found in different cultivars at different sites. Only in 9 of the 49 site/rotation/years was any significant difference detected. There was no major difference in persistence between the four cultivars. Any differences seemed to be associated with greater seed shed initially. Oilseed rape seed loss at harvest was about 4000 seeds per m². There was, on average, a 60% decline in seed numbers in the first 6 months followed by a much slower decline of 5-10% in the subsequent two years. Actual decline rates varied between rotations and sites perhaps due to soil type or climate. There may have been additions to the seedbank from surviving oilseed rape volunteers in the cereal years. Appreciably more seeds remained where the soil was ploughed immediately after harvest than when ploughing was delayed for 4 weeks. This confirmed earlier work that described the benefit of leaving rape stubble uncultivated for a period after harvest in reducing seed persistence. Petri-dish tests of persistence potential confirmed that likely persistence of herbicide tolerance cultivars was no greater than for the conventional cultivar Apex. Decline followed an exponential curve. The predicted survival at all 4 sites was more than 10% of seeds by Year 4. Many would probably survive for several more years and there is evidence that OSR can persist for over a decade.

6.8 Oilseed rape seedling emergence from the seedbank

Limited data was collected from all three sites of Rotation 1 and one of the Rotation 3 sugar beet sites. Between 1 and 5% of the seedbank emerged each year. The small number of susceptible seedlings among volunteers from glyphosate tolerant plots was explained by seed movement from susceptible OSR plots during harvest or subsequent cultivation. The explanation was similar for glufosinate except at Rothamsted where there was a link between seedling numbers and the seedbank. Some segregation of the glufosinate tolerance may have occurred in the F2 generation so that not all shed seeds were tolerant. It has been estimated that up to 14% of self-pollinated glufosinate tolerant OSR could be non-transgenic. Levels at Rothamsted were higher than this and outcrossing may have occurred but as there were only 4 data points precise estimates were difficult to make.

6.9 Overall impact of oilseed rape volunteers

Additional herbicides were required to control both conventional and herbicide tolerant volunteer OSR in sugar beet. It caused more difficulty when the volunteer OSR and the sugar beet shared the same herbicide tolerance. Seedbank

persistence would be a problem if a grower wished to change from growing herbicide tolerant OSR to growing conventional OSR. Even after a delay of 3 years, herbicide tolerant volunteers would be numerous enough to significantly affect seed purity

6.10 Crop yields

The oilseed rape yields compared favourably with the national average. Sugar beet yields were somewhat lower than the national average due to the enforced early harvest of the crop and these should be taken with some caution.

6.11 Economics of HT oilseed rape and sugar beet

A full comparison of the production costs of oilseed rape and sugar beet was not the original intention of the research. However, one of the main commercial justifications for the development of herbicide tolerant crops is that it reduces weed control costs, although this may tend to be balanced by greater seed costs and additional technology fees. Within the experiments, it was possible to compare weed control inputs between herbicide tolerant and conventional cultivars. In the OSR, the average cost of conventional weed control was £60/ha but there was a wide variation in inputs. The weed control costs for glyphosate and glufosinate were £16 and £40/ha respectively, but there may be additional seed and technology costs imposed by the companies involved. For sugar beet the average cost of conventional weed control was £84/ha and involved an average 2.7 sprays. The average cost for the glyphosate plots was £21/ha (av. 1.3 sprays) and for glufosinate plots £63/ha (av. 1.7 sprays) plus any additional technology costs. A further in depth study of costs is needed.

7 BRIGHT trial conclusions

The BRIGHT report concluded that weed control in herbicide tolerant crops was more flexible and gave similar or better levels of control than herbicide treatments in the conventional crop. It was also concluded that the increased flexibility may allow improvements in biodiversity. It is suggested that the use of HT crops could reduce herbicide inputs in other crops in the rotation. Further studies on appropriate management strategies for GM crops were proposed and that if the impact of weed control measures on biodiversity is the main issue of concern, there is a need to look at the effect of all crops in the arable rotations especially those like cereals that have the most impact on weed survival.

7.1 Conclusions and comments

The main conclusions made in the BRIGHT report are listed below (in italics) and then these are considered.

The researchers concluded that:

- weed control in herbicide tolerant crops was more flexible and gave similar or better levels of control than herbicide treatments in the conventional crop. This conclusion conceals some of the complexity and problems that may arise in future years. Weaknesses can be seen with these so called broad spectrum herbicide products and poor control of certain weeds e.g. Viola arvensis with glufosinate or control of Urtica urens with glyphosate is of major concern for the future. A recent survey by Lainsbury et al. (1999) found that Viola arvensis was the most frequent weed species present in conventional sugar beet crops surveyed in East Anglia in autumn. Urtica urens was shown to be poorly controlled by glufosinate in GMHT forage maize (Read & Ball, 1999). These are prolific arable weeds that will soon dominate in an unchallenged environment and become increasingly difficult to control. This means other herbicides will be needed to be incorporated into the spray programme to control certain weeds, moving further away from the simple one chemical system promoted. This is a scenario that has become reality in North America (Kirkwood, 2002).
- using HT crops would have little direct impact on subsequent crops. However, there are management issues where crops grown later in the rotation share the same herbicide tolerance and where conventional crops that follow GM ones risk seed contamination. Difficult-to-control volunteer weeds which have multiple herbicide resistance may well occur if management guidelines are not followed. By July 2001, there were 249 herbicide resistant biotypes (153 distinct species) recorded in 52 countries. Three biotypes (2 species) were resistant to glyphosate which was originally considered to have a low risk of tolerance developing (Moss, 2002). More recent information (www.weedscience.com) has seven species listed as resistant to glyphosate. Who will regulate any guidelines and when they are not adhered to and who will be responsible for and who will pay for the weed problems created?
- one of the main benefits of HT technology is the potential control of weed beet. However, there remains the risk that the herbicide tolerance will be introduced into the weed beet itself although this is a much more complex process than the production of herbicide tolerant oilseed rape volunteers, which occurs the first time a HT oilseed rape crop is grown. Outcrossing was only investigated in OSR as the sugar beet was harvested before flowering of weed beet and crop bolters were not permitted to flower under the conditions of the GMO consent. There are many references to the need to prevent the crop from flowering but it would

be virtually impossible for a farmer to remove all bolters. Around 70% of farms that grow sugar beet are affected by weed beet at present and around one third do not bother with control. Weed beet can evolve from the seed of any beet left to flower in the field such as weed beet, sugar beet groundkeepers, normal sugar beet that has bolted, bolters from contaminated sugar beet seed and wild beet. All variants appear inter-fertile and cross freely. In the native annual beet the gene for annual bolting is dominant and the one for bolting resistance is recessive. While it is a more complex process than the development of HT feral oilseed rape, HT weed beet would eventually appear if HT sugar beet was grown.

- there was no impact of the HT cultivars themselves on botanical diversity. Species survival depended on local conditions, timing and active ingredient but there was no indication that any particular treatment reduced biodiversity more than any other. However, apart from limited observations on some untreated areas this was not properly tested and these conclusions can not be drawn. It would have been helpful to have included GM crops treated with the conventional herbicide programme at one or more sites. Without this treatment it is difficult to separate out the effects of the GM crop and the effects of the different herbicide programme. While the GM crop plus the herbicide that it has resistance to come as a package, a positive result will imply that all GM crops are beneficial when it is simply a reflection of the herbicide regime. The conventional herbicide treatments applied to OSR differed between sites and there did not seem to be a standard comparison being made and the same applied for beet. In Rotation 3 Year 1 at Broom's Barn, where the imazamox OSR plots were given the conventional herbicide treatment it was decided that varietal effects would be small compared to herbicidal effects and data from the conventional and imazamox treatments was merged and presented as the conventional treatment. This suggests that the BRIGHT research team, like the FSE scientists, simply assumed that it was the herbicides that were the main treatment, not the GM cultivars.
- overall crop management was thought to be responsible for differences in weed populations. For the crops themselves, there were no lasting effects of the appropriate herbicide treatments applied to the different crop cultivars. Crop yields were at the levels expected given the limitations imposed.
- there was little advantage in following an earlier rape crop having tolerance to one herbicide by another later in the rotation with tolerance to the other herbicide.
- there is the potential to create volunteers with resistance to both chemicals. Over 3500 rape seeds/ha were lost at harvest and considerable numbers of these were able to survive in the seedbank under normal cropping over the 4 years of the present study. Volunteers from HT cultivars would be present in sufficient numbers at this time to breach the EU threshold in any non-GM oilseed rape crop that was grown. The recommended delay in cultivations following rape harvest reduced but did not eliminate the problem. From other research it is clear that HT oilseed rape volunteers can survive in the seedbank under normal cropping for long enough and in sufficient quantities to breach EU thresholds for GM impurities in non-GM crops that are grown later in the rotation. Recommended practices to encourage the germination of shed seeds will minimise but not eliminate the problem. It was proposed that the oilseed rape seedbanks established at the experimental sites should be monitored for 3 more years to follow any decline in numbers.
- careful consideration has to be made about the choice between glyphosate and glufosinate for crop desiccation and post-harvest volunteer rape control in

stubble. The same applies where HT sugar beet and HT rape are grown in rotation, although many beet would growers avoid growing oilseed rape because of the volunteer problem. In cereals, the herbicides used and high levels of crop competition make volunteer rape control less of a problem.

- there was little evidence of any cumulative effects of the HT oilseed rape herbicides in the rotations although there was poor control of certain weed species. In the beet crop it was the effect of poor control of fat-hen (C. album) by conventional treatments that carried over. More effects might have been noticed if reduced cultivations rather than inversion ploughing had followed harvests. Additional herbicides were required in sugar beet where the volunteer OSR was tolerant to the applied herbicide treatment. In general, to achieve similar levels of weed control, herbicide costs for HT crops were lower than those for conventional crops. However a full cost analysis was not possible.
- in the winter oilseed rape, outcrossing occurred between the different cultivars at • frequencies similar to those recorded elsewhere. However, outcrossing and gene stacking has been clearly shown in these trials. It is not possible to conclude that outcrossing of WOSR levels decreased exponentially with distance from pollen source', when at SAC, 2 out of 6 transects taken showed an increase in percentage of glufosinate tolerant seeds in seed samples. The average percentage glyphosate tolerant seeds at 56 m from the GMHT crop is over 1% in plot 5 at Rothamsted. This level of outcrossing has serious implications for control of GM crops. The furthest distance sampled was 91.5 m, far too close to represent a commercial situation. In 1999, the plot size at the three sites varied meaning that at two sites pollen movement was recorded only over a relatively short distance and there was the likelihood of greater interference from plots in other treatment blocks. Where gene stacking has occurred, more complex herbicide mixtures and sequences will be required to control weeds in subsequent crops. In addition, following this study feral oilseed rape with resistance to one or more wide spectrum herbicides is now present on the study farms and is likely to be there for the foreseeable future. Outcrossing from bolted sugar beet to weed beet was not studied here because of the limitations imposed by DEFRA.
- there may be economic benefits from growing GMHT crops. However, the economic analysis has far too many speculative variables to be of any use. It considers fairly narrow parameters and neglect other issues such as potential costs for removal of bolters in beet; costs of more complex spray programmes to control weeds not controlled by the main herbicide; extra cultivation to manage seed shed at OSR harvest; that subsequent crops may well not be pure enough to sell as GM free therefore may suffer a price reduction; that insurance may be required to grow GM crops; and that the system will be in the control of one company who supplies seed and herbicide and can raise prices at any time. Faced with competition, conventional herbicide manufacturers might lower their prices.

8 Conclusions of this review

As the BRIGHT report states, this investigation represents only one farming system, a plough based rotation. There has been no assessment of the impact of GMHT crops on other systems such as minimum tillage or no tillage. All trials have all been based at conventional intensively farmed research stations and no other sites. A maximum species diversity level of 12 in the BRIGHT trials, is too low to be representative of many British farms. Assuming that the experimental sites are conventional arable fields, and have been so for a reasonable period, the weed flora would have already become stabilised at a relatively low level of diversity. At Broom's Barn, the initial weed populations were moderate to low due to a history of good weed control. Therefore, the trials began with a somewhat depleted weed flora and it is unclear what was expected to happen over one cycle of a 4 year rotation. While any plant species can add to biodiversity, in the BRIGHT trials there appeared to be a large number of volunteer crop seeds adding to weed seedbank numbers. The results tend to reflect the species present and their response to the individual herbicides. In addition, the soil type at all sites was very similar.

The publicity that has surrounded the publication of the report has distorted its findings. All recommendations in the executive summary are for practical crop growing, the abstract has one recommendation that there are further studies to investigate the environmental impact, because it could not been prioritised in this study. There has been no investigation of any other biotic factor other than plants, no invertebrates, birds or any mammalian species. No abiotic factors have been considered. So it cannot be concluded from this study that GMHT crops have a benign environmental impact when this has not been fully investigated, only one parameter has been partially researched. To state no environmental impacts were observed, is because they were not looked for rather than researched and ruled out.

To answer fully the trial objectives in relation to understanding the environmental effects of GMHT crops, was impossible with the level of funding. Environmental (botanical) impacts could not be extensively investigated in trials designed to meet a primarily agronomic objective. Therefore, the trials should be seen as a small piece in a large jigsaw puzzle aimed at understanding potential effects of introducing GM technology into British farming systems. Headlines that suggest that the BRIGHT trials showed that GMHT crops do not harm the environment, misrepresent the results of a complex four-year study and do an injustice to scientists who have undertaken the research.

9 References

Ambrosio L, Iglesias L, Marin C, Del Monte JP (2004). Evaluation of sampling methods and assessment of the sample size to estimate the weed seedbank in soil, taking into account spatial variability. *Weed Research* **44**, 224-236.

Benoit DL, Derksen DA, Panneton B (1992). Innovative approaches to seedbank studies. *Weed Science* **40**, 660-669.

Champness SS (1949). Note on the technique of sampling soil to determine the content of buried viable seeds. *Journal of the British Grassland Society* **4**, 115-118.

Firbank LG, Heard MS, Woiwod IP, Hawes C, Haughton AJ, Champion GT, Scott RJ, Hill MO, Dewar AM, Squire GR, May MJ, Brooks DR, Bohan DA, Daniels RE, Osborne JL, Roy DB, Black HIJ, Rothery P, Perry JN (2003). An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology* **40**, 2-16.

Forcella F (1984). A species–area curve for buried viable seeds. *Australian Journal of Agricultural Research* **35**, 645-652.

Frans RE & Talbert RE (1977). Design of field experiments and the measurement and analysis of plant responses. In: *Research Methods in Weed Science*, 2nd Edition (ed. B Truelove), Southern Weed Science Society, 15-23.

Jones N (1998). The number of soil cores required to accurately estimate the seedbank on arable land. *Aspects of Applied Biology*. Weed seedbanks: Determination Dynamics & Manipulation **51**, 1-8.

Roberts HA (1981). Seed banks in soils. *Advances in Applied Biology* **6**, 1-55. **Kirkwood R C** (2002). Herbicide-tolerant crops. In: *Weed Management Handbook*, 9th Edition

(Ed. R E L Naylor), Blackwell Science, Oxford, UK, 253-279.

Knott CM (2002). Weed control in other arable and field vegetable crops. In: *Weed Management Handbook,* 9th Edition (Ed. REL Naylor), Blackwell Science, Oxford, UK, 359-398.

Lainsbury MA, Hilton JG, Burn A (1999). The incidence of weeds in UK sugar beet crops during autumn 1998. *Proceedings Brighton Crop Protection Conference – Weeds*, Brighton, UK, 817-820.

Moss SR (2002). Herbicide resistant weeds. In: *Weed Management Handbook,* 9th eedition (Ed. REL Naylor), Blackwell Science Ltd, Oxford, 225-252.

Perry JN, Rothery P, Clark SJ, Heard MS, Hawes C (2003). Design, analysis and statistical power of the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology* **40**, 17-31.

Read MA & Burn JG (1999). The control of weeds with glufosinate-ammonium in genetically modified crops of forage maize in the UK. *Proceedings Brighton Crop Protection Conference* – *Weeds*, Brighton, UK, 847-852.

SAPPIO Programme Factsheet BRIGHT: Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance (1999). Accessed 21/1/05.

http://www.defra.gov.uk/Science/LINK/publications/Publicity/SAPPIO/LK0906_Leaflet.pdf **Squire GR, Begg GS, Askew M** (2003). The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops. *Final Report DEFRA project RG0114: Consequences for Agriculture of the Introduction of Genetically Modified Crops,* DEFRA, London.

Sweet J, Simpson E, Law J, Lutman P, Berry K, Payne R, Champion G, May M, Walker K, Wightman P, Lainsbury M (2004). Botanical and rotational implications of genetically modified herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT Project). *Project Report No. 353.* HGCA.

Topham PB & Lawson HM (1982). Measurement of weed species diversity in crop/weed competition studies. *Weed Research* **22**, 285-293.

Young JEB, Griffin MJ, Alford DV, Ogilvy EE (eds) (2001). *Reducing agrochemical use on the arable farm; the TALISMAN and SCARAB projects.* DEFRA, London www.weedscience.com Accessed 14/2/05.

10 Annex 1: Parameters measured and data analysis

Weed species and numbers

Within each treatment, weed species and numbers were assessed in each year. The degree of control of herbicide tolerant and non-tolerant oilseed rape volunteers was noted in crops that followed oilseed rape. Sugar beet volunteers were assessed in Rotation 5.

Assessments followed standard operating procedures (SOPs) written by the research team and were made to determine:

- weed plant populations prior to herbicide treatment.
- weed plant populations at various times after herbicide treatment.
- weed biomass prior to harvest (at times of maximum weed growth).
- crop yield.
- weed seedbank levels at the beginning of the project prior to any herbicide treatments and at the end of the project after the final herbicide treatment and weed assessments. (i.e. the autumn of Year 1 and the spring/summer/autumn of Year 4).
- volunteer WOSR seedbanks each winter following an initial WOSR crop.

Weed seedling assessments

The timing of pre-herbicide weed counts depended on the crop and on herbicide treatment. Where possible, counts were made immediately before herbicide application. The counts were made in a series of random quadrats, the number and size of which varied between sites due to different weed infestation levels. At all sites, a minimum of twelve quadrats was counted per sub-plot. At some sites, when the test crops of sugar beet and WOSR were grown, untreated quadrats or areas of 0.5m² or 1m² were used as a comparison with the treated plots. These were counted before the first herbicide treatments were applied and again, where possible, prior to the application of later treatments. Weed counts were converted to weeds per m².

The post-herbicide weed counts were made in a series of random quadrats, the same as with pre-herbicide counts. The timing of the counts varied with the crop and with the site. For the oilseed rape crop the counts were made in early spring, in sugar beet the counts were carried out in summer, about six weeks after herbicide application. The same procedures used for weed assessment in the broad-leaved crops were followed in the cereals, care being taken to assess volunteer oilseed rape seedlings. In some cereals where weed emergence was very low in the autumn, weed counts were delayed until spring/summer.

Weed biomass assessments

Each sub-plot was sampled for weed biomass during June for the oilseed rape crops, July for cereals and August/September for sugar beet. The number (2 or 4) and size (up to $1m^2$) of quadrats sampled depended on the size of the sub-plot and the density of the weeds. All the above ground vegetation, apart from the crop, was collected by cutting at ground level. The weeds were sorted into species, washed as necessary and the dry weight of each species recorded. Dry weights were converted to g/m².

Visual assessments

A range of visual assessments was made through the growing season in each year to monitor crop and weed growth. These included percentage cover of weeds and crop, percentage control of weed species and assessment of crop damage following

herbicide treatment. The results were not analysed and are not presented in the report.

Assessment of crop yield

Crop yields were recorded for both WOSR and sugar beet. For WOSR, the harvesting method and area harvested depended on the site and the plot size. Sub-samples were taken to estimate moisture content and yields were re-calculated at 9% moisture. In sugar beet, the roots from at least 2 rows per plot were harvested, washed and weighed. Samples were extracted for sugar content determination. Yields were calculated as fresh weight, sugar content and sugar yield per hectare.

Weed seedbank determinations

To determine the soil weed seedbank composition at the start of the experiment, soil cores were taken from Rotations 1a and 1b, 2 and 3 in the autumn of Year 1 prior to any treatment of the soil. Soil cores were taken again in the same places in the spring/summer/autumn of Year 4 once all the herbicides had been applied and no further seed return from surviving weeds was possible. Each sub-plot was divided into 4 zones and their middles marked. A 2 m x 2 m square around each mid point was marked and twelve 2.5 cm diameter cores 25-30 cm deep were taken at random within each of these areas and bulked together. A total of 48 cores were taken per sub-plot, but the 12 cores from each area within a sub-plot were kept separate. The soil from each lot of 12 cores was weighed, thoroughly mixed and a sub-sample of 500 g was removed for processing. Where necessary, samples were stored frozen at -20° C prior to processing. The seed content of the soil samples was determined by a well established system of wet sieving, flotation, extraction and identification. Seeds were assumed to be viable if they resisted gentle pressure when squeezed between forceps. Seed counts per 500g soil were converted to seeds per m².

Oilseed rape seed losses at harvest

Seed losses from the oilseed rape during crop harvest were estimated by the collection of seeds in gutters at harvest (Broom's Barn, Morley), or by counting quadrats in each plot the day after harvest (NIAB, Rothamsted, SAC). Quadrat and gutter size and number varied with site and year. Numbers of quadrats in Year 4 tended to be lower than in previous years as the data were intended to confirm earlier trends and were not to form the basis of calculations of seed decline in subsequent years. Rape seeds or seedlings were counted post-harvest on Rotation 1a/b (Year 1 and 4) and Rotation 4 at SAC and Rothamsted. The quadrats were the same size as those used to count seed lost at harvest. At SAC in Year 1 and 2, two quadrats were counted per plot 19 days post-harvest. In Year 2, all quadrats were counted at 10 and 22 days post-harvest. In Year 2, all quadrats were counted at 8, 16 and 22 days after harvest. In year 4, the quadrats were counted 13 days after crop harvest, there was no explanation for the change in monitoring protocol.

Assessment of OSR in and emerging from the seedbank and its rate of decline

A substantial number of seeds were shed at oilseed rape harvest. Some became incorporated into the soil weed seedbank by post-harvest cultivations and formed the basis of a persistent reserve of volunteer oilseed rape seeds. Each winter after an oilseed rape crop, the number of oilseed rape seeds in the soil seedbank was assessed at all sites by taking 2.5 cm soil cores 25-30 cm deep. The number of cores per plot varied from 24 to 80 depending on the site and year. Soil samples were washed out by wet sieving immediately or frozen and washed out later. Seeds were extracted and tested for viability by gentle squeezing with forceps.

The number of non-herbicide tolerant volunteer oilseed rape seedlings arising from the crop grown in Year 1 of Rotation 1, was recorded following the application of

glufosinate and glyphosate to the oilseed rape drilled in Year 4. Once symptoms of herbicide damage became apparent, the surviving and the dying volunteers were counted in random quadrats on the appropriate plots at NIAB, Rothamsted and SAC. At Broom's Barn in Year 3, the number of volunteer rape plants in sugar beet (Rotation 3) was recorded prior to herbicide application. Counts were made on 25 June and 14 July. Twelve 0.5m² quadrats were counted per plot. A 3 m strip alongside each sub-plot was treated with glyphosate or glufosinate alone while tank mixtures with metamitron were applied to the actual sub-plots. Between 10 and 25 days after application of glyphosate and glufosinate, the number of rape plants that survived treatment was counted. Oilseed rape seedlings that emerged after herbicide application were counted separately to distinguish them from the rape plants that had survived treatment. The seedling numbers were compared to the estimates of oilseed rape in the soil seedbank.

The data on seed numbers from soil samples taken each winter after the first winter oilseed rape had been grown were analysed initially using standard analysis of variance to determine whether there were significantly more seeds of one rape cultivar than another. Overall means were also calculated in order to compare changes with time between the sites and rotations. Subsequently, regression analyses were performed to predict longer-term decline rates.

Additional studies of intrinsic dormancy of harvested OSR seed

The seed harvested from the four oilseed rape cultivars grown in the BRIGHT trials at Rothamsted and Broom's Barn in July 1999 was tested for intrinsic seed dormancy in two tests. In test 1, six replicates of 50 seeds of each cultivar were placed in water in Petri-dishes and incubated at 20°C in the light. Germination was recorded after 7 days. In test 2, seeds were placed in Petri-dishes with polyethylene glycol solution at a water potential of –1.5Mpa. These were incubated at 20°C in the dark. Germination was recorded after 4 weeks. The seeds were then transferred to fresh Petri-dishes containing pure water, returned to the incubators and germination recorded 2 weeks later. The experiment was repeated in January 2000. Germinated seed numbers were transformed using logit transformations, the results of the two tests were amalgamated and means and standard errors calculated.

Additional studies of cross pollination between herbicide tolerant and conventional OSR cultivars in 1999

In the cross pollination studies at NIAB, seed samples were collected at intervals along linear transects across each block of the mature oilseed rape plots. At Rothamsted and at SAC, seed samples were collected at 5 m intervals along linear transects across each block. At SAC, seed samples were taken only from the conventional variety plots and were tested for glufosinate and glyphosate tolerance. The main raceme was removed from 20 plants within a 1m² quadrat at each sampling point. After drying at ambient temperatures for 14 days the seeds were removed from the pods by crushing. At NIAB, seeds were randomly sub-sampled in order to test two replicates of 1000 seeds per sample. At Rothamsted, 100 seeds were tested per replicate.

Seed samples collected from herbicide tolerant and conventional winter oilseed rape varieties were sown in trays of peat based potting compost under glasshouse conditions. Trays sown with seed of a conventional oilseed rape variety were included as a control treatment in each test. Trays were arranged on the glasshouse bench in a randomised block design. At NIAB, plants were sprayed with either a 1% solution of glufosinate, a 0.5% solution of glyphosate or a 1% solution of imazamox plus wetter. At Rothamsted and at SAC a 1% solution of glufosinate and of glyphosate was used and no imazamox tolerance testing was carried out.

11 Annex 2: BRIGHT trial results in detail

Weed control – Rotation 1

In Rotation 1 at Rothamsted, 7 major weeds, including volunteer oats, were present together with 35 weed species recorded at low densities. Treatment differences were detected in the oilseed rape crops in Years 1 and 4. Overall, glyphosate achieved the highest level of weed control, glufosinate and the conventional treatments achieved similar levels of control, and imidazolinone when used often gave the poorest control. Weed control was uniformly high in the cereal crops of Years 2 and 3 with little evidence of any carry over from the treatments in the first oilseed rape crop. None of the treatments resulted in complete weed control but weed biomass was not great even following the poorest herbicide treatment. In the diversity analyses there was a significant effect of Year 4 treatments on the number of species present. Species numbers were low anyway but fewer species were present on the glyphosate treated plots.

In Rotation 1a and 1b at SAC, 5 major species were present together with other broad-leaved and grass weeds at varying densities. Treatment differences were detected in the oilseed rape crops in Years 1 and 4. Overall, glufosinate achieved the highest level of weed control, glyphosate and conventional treatments achieved similar levels of control, and imidazolinone gave the poorest control. No herbicides were applied to the winter barley in Years 2 and 3 and, as a result, there was some indication of the poor control with imidazolinone being reflected here. Weed biomass was appreciable in summer in the oilseed rape and in the Year 3 winter barley. This resulted in a massive increase in the weed seedbank. There was also a high level of seed return from volunteer oilseed rape in this crop that increased the numbers in the seedbank. In the diversity analyses at SAC, the main effect on species number was from the treatments applied in Year 4 but there were not many species present.

In Rotation 1 at NIAB, 6 major weeds, including volunteer wheat, were present but few other weeds. In Year 1 oilseed rape, glyphosate gave the best weed control followed by glufosinate, with the imazamox and conventional treatments giving equivalent levels of control. In Year 4, glyphosate gave the poorest control and the conventional and glufosinate treatments were the best but there were some application problems. Weed numbers were low in both cereal years. Weed biomass was related to difficulties in the control of blackgrass (*Alopecurus myosuroides*). The original distribution of this weed prior to Year 1 of the experiment caused problems in the interpretation of the results. There was some effect on weed diversity in Year 4. Fewer species were present on the glufosinate treatment and the 'alternative' conventional herbicide but species numbers were low anyway at this site.

Seedbanks – Rotation 1

The data from the weed seedbanks studies was analysed raw and after log₁₀ transformation. In Rotation 1 at Rothamsted, seeds of a total of 18 species were identified in Year 1 samples but the seedbank was dominated by a limited number of them. The overall mean was 2595 seeds per m² but the data was quite variable. In the samples taken in Year 4 the overall seed number had increased to 5773 seeds per m². Much of this was due to the addition of volunteer oilseed rape seed and to poor control of two of the main weeds, apparently during the oilseed rape crop years. Seed numbers in Year 4 were significantly higher on plots treated with glufosinate and with one of the conventional treatments.

In Rotation 1 Year 1 at SAC, the seedbank was dominated by 4 weed species, the overall seed number was 8239 seeds per m². There was an increase in all the species in Year 4 and the overall seed number was 89,323 per m². Oilseed rape made up 10% of the increase and poor weed control in the Year 3 cereal also contributed to much of the increase. Mayweed seeds increased in seedbank numbers. There was no clear evidence that any of the treatments applied to oilseed rape in either year had a significantly different effect on overall seed numbers. There was an indication that plots treated with glyphosate in Year 1 had more seeds overall especially annual meadow-grass (*Poa* annua) and common chickweed (*Stellaria media*). There were significantly more volunteer oilseed rape seeds on the plots treated with glufosinate.

In Rotation 1 Year 1 at NIAB, only 15 weed species were recorded from the seedbank samples with 3 main species present. Total seed numbers were 3051 per m². There was a very low number of volunteer oilseed rape seeds present. The data was rather variable and the number of fat-hen (*Chenopodium album*) seeds on plots intended for treatment with glyphosate was significantly higher than on the other plots even before treatments began. By Year 4, overall weed numbers were 8774 seeds per m² but the data was still very variable. The raw and transformed data showed no real effects of Year 1 or Year 4 treatments on species apart from blackgrass (*A. myosuroides*).

Weed control – Rotation 2

In Rotation 2 Year 1 at Broom's Barn, the weeds present in the sugar beet crop included some that had survived seedbed preparation and drilling and others that had emerged following these operations. The main weed seedling population was of fat-hen (*C. album*) and there were significantly greater numbers of these on the plots to be treated with glufosinate even before treatment. After treatment, there were no significant differences in weed number or biomass with any treatment. In the winter barley crops of Year 2 and 3, there was good control of weeds and no carry over of an effect from the sugar beet crop. In the sugar beet crop in Year 4, pre-emergence herbicide treatments on the conventional beet plots prevented pre-emergence weed counts being made. Counts were made after each post-emergence herbicide application including a second application of glufosinate. Weed control was equally good on the GM and conventional beet plots. Weed biomass was greater on the conventional plots but this was due in part to a lack of crop competition following bird and animal damage.

In Rotation 2 Year 1 at Morley, the information collected in the sugar beet crop did not match that collected at other sites and so was not comparable. Data was only presented on the Year 4 sugar beet crop. There were 7 main species present. As at Broom's Barn in Year 4, pre-emergence treatments on the conventional plots prevented weed counts on these plots prior to treatment. Weed densities posttreatment demonstrated good control of most species by the herbicides but the single application of glufosinate showed poorer control of field pansy (*Viola arvensis*) and annual meadow-grass (*P. annua*). This was reflected in higher weed biomass for this treatment.

Seedbank – Rotation 2

Twenty weed species were recorded in the weed seedbank in Year 1 but fat-hen (*C. album*) was the dominant species. The mean density was 9095 seeds per m^2 . At the end of the experiment the seedbank had increased to 21948 seeds per m^2 mainly due to changes in fat-hen seed numbers. Comparisons of the treatment effects on the main weed were difficult due to the variation in the data.

Weed control – Rotation 3

In Rotation 3 Year 1 at Broom's Barn, seven main weed species or groups of weed species were present in the oilseed rape. Herbicide applications were delayed by poor weather. Plots sown with the imazamox tolerant variety were treated with the same herbicides as the conventional plots. It was decided that varietal effects would be small compared to herbicidal effects and data from the conventional and imazamox plots was merged and presented as the conventional treatment.

The glyphosate and glufosinate treatments gave the best weed control, the conventional herbicide the poorest. The chosen conventional herbicide has a limited weed spectrum and activity was reduced further by spring application. Weed biomass was significantly greater on the conventional plots. In the Year 2 winter barley, weed control was good but there was some carry over of effect from the poor weed control in the conventional treatments of the oilseed rape crop. Low numbers of volunteer oilseed rape occurred, most were found on the glyphosate plots.

In the Year 3 sugar beet crop, initial weed density was moderate but there were high numbers of volunteer oilseed rape. Volunteer densities were more than twice as high on the glufosinate and glyphosate tolerant plots and this was related to seed shed at harvest in Year 1. Following herbicide application, weed counts were greatest on plots that had carried conventional oilseed rape in Year 1. Oilseed rape volunteers were in high numbers on the conventional sugar beet plots but herbicide tolerant volunteers were more numerous than conventional ones. The number of volunteers on the glufosinate and glyphosate resistant beet plots was greatest where the rape in Year 1 had been tolerant to the same herbicide. Late-flushes of oilseed rape seedlings also contributed to high counts with the wide germination window making volunteer oilseed rape difficult to control in sugar beet. In Year 4, in the winter barley, there were no significant carry over effects from previous crops once the standard herbicide treatments had been applied.

In Rotation 3, Year 1 at NIAB, there were five main weed species present including volunteer wheat. No imazamox treatment was applied, a conventional herbicide was applied instead. The were differences in the number of some weed species between treatments but the main difference was in volunteer wheat numbers, the highest population being recorded in the glufosinate treatment. The biomass assessments showed significantly less weed growth on the glyphosate than the other treatments. Herbicide treatments in the winter wheat were highly effective and no carry over effects from the previous treatments were recorded. In the sugar beet crop in Year 3, in addition to the herbicide programmes, the conventional plots were weeded with a steerage hoe in May 2001 due to excessive weed growth in these plots. Weeds were assessed prior to herbicide applications and hence at different times in relation to crop drilling. This and the uneven distribution of weeds contributed to the apparent differences in weed numbers prior to treatment. Post-herbicide weed assessments were carried out after all the weed control measures had been implemented. There was some carry over from the glufosinate treatment in Year 1, leading to higher numbers of annual meadow-grass (P. annua) and annual nettle (Urtica urens). In Year 3, there were higher numbers too of volunteer oilseed rape. Neither glufosinate nor glyphosate controlled annual nettle in Year 3. Overall, control of weed numbers was better on the glyphosate treated plots and poorer on the conventional plots in Year 3, although, the reverse was true for weed biomass. The volunteer oilseed rape was responsible for this despite the addition of metamitron to the glufosinate and glyphosate herbicide sprays. In the winter wheat in Year 4, the weed numbers and biomass were low following herbicide treatment and there was no effect of previous crop treatments.

In Rotation 3 Year 1 at Morley, there were two sets of conventional plots and different standard herbicide treatments were applied to each of them. Presumably one set was the imazamox tolerant cultivar. There were differences in weed counts made on plots prior to most herbicide treatments because a pre-emergence treatment had already been applied to the conventional plots. The glufosinate treatment gave the lowest weed control overall and the conventional pre-emergence appeared the most effective. In Year 2 winter wheat there was no carry over effect apparent from Year 1 following treatment with a standard pre-emergence herbicide. In the sugar beet in Year 3, metamitron was added to the glufosinate and glyphosate sprays in anticipation of the need to control HT oilseed rape volunteers. Weed control was better on the glyphosate treated plots and weed counts highest on the glufosinate plots. High numbers of volunteer oilseed rape were recorded on both these treatments despite the addition of metamitron. The conventional treatment was better than glufosinate in controlling most weed species. Glufosinate was particularly weak on red deadnettle (Lamium purpureum). In the Year 4 winter wheat, weed numbers were low but there were more seedlings of cleavers (Galium aparine) recorded on the former glufosinate plots.

Seedbank – Rotation 3

In the seedbank studies in Rotation 3 at Broom's Barn, 15 weed species were found in the seedbank of which five were the main species and others occurred only rarely. The results were rather variable but overall numbers were 5416 per m². At the end of the experiment in Year, density had increased to 13460 seeds per m² primarily due to an increase in fat-hen seeds to 11109 seeds per m². Survival of the weed in Year 3 sugar beet on the glufosinate and conventional plots appeared responsible for the increase. As in Year 1, there was considerable variability in the data.

In the seedbank studies in Rotation 3 at NIAB, 13 weed species were identified of which six were considered main species. There was some variation in seed levels across plots. The overall mean number was 14453 per m². In Year 4, the seedbank had increased to 18974 per m². There was a substantial number of volunteer oilseed rape seeds on all plots with an average density of 437 seeds per m². Although there was high variability in the data significant differences were detected in Year 4 as a result of Year 1 and 3 treatments. However the uneven distribution of weeds in the initial samples and the removal of the imidazolinone treatment plots from the analysis of Year 1 is acknowledged to have distorted the analysis when the data was expressed as log10. In fact because of the high variability, there was no particular pattern for most weeds.

In the seedbank study in Rotation 3 at Morley, high levels of 'brassica' seed were apparent in the seedbank despite no oilseed rape having been grown previously and charlock (*Sinapis arvensis*) not being a weed there. The data from this experiment was therefore not used.

Weed control – Rotation 4

In Rotation 4 Year 1, the intention was to establish seedbanks of volunteers of the four different oilseed rape cultivars prior to drilling the winter cereal crop by broadcasting the rape seeds. Unfortunately a high percentage of the seed germinated immediately and only low numbers became incorporated in the soil seedbank. Consequently, Rotation 4 was treated as an extra data set to investigate the performance of treatments applied in Year 2.

In Rotation 4 Year 1 at Rothamsted, the volunteer oilseed rape seedbank had a mean density of 119 seeds per m². The winter wheat was managed as a normal crop. In Year 2 oilseed rape pre-treatment assessments in the autumn could be made only on imidazolinone, glufosinate and glyphosate plots as a pre-emergence

treatment had been applied to the conventional plots. The principle data set assessing the effectiveness of the herbicide treatments was weed counts made in early spring. Some weeds survived on all plots but there were fewer on the conventional plots. Imidazolinone plots had the most weeds and glufosinate was poor on field pansy (*V. arvensis*). In the winter wheat grown in Years 3 and 4 there were few differences arising from the oilseed rape treatments. Volunteer oilseed rape was present in both years but more emerged in Year 4 with significantly higher numbers on plots where the glyphosate tolerant rape had been grown previously. The cereal herbicides killed most of the volunteers.

In Rotation 4 Year 1 at SAC the volunteer oilseed rape seedbank had a mean density of 350 seeds per m². The winter barley was managed as a normal crop. In the year 2 oilseed rape crop significant treatment effects were recorded in the spring but the effectiveness of the herbicides varied with the weed species. Imazamox was poorest on annual meadow-grass (*P. annua*), the predominant weed. However, counts of this species were also higher on these plots prior to treatment. Glufosinate and the conventional treatment were less effective on field pansy (*V. arvensis*). In the Year 3 winter barley, wet conditions delayed drilling, crop and weed emergence was patchy and assessments were not done until summer. The major weed was annual meadow-grass (*P. annua*) and there were significantly fewer where the glufosinate plots had been. Very low levels of volunteer oilseed rape were recorded in the biomass samples. In the Year 4 winter barley crop, establishment was better and as in the previous year annual meadow grass (*P. annua*) was the main weed and there were significantly fewer where the glufosinate plots had been. There were the glufosinate plots had been. There were very low levels of volunteer oilseed rape were recorded in the biomass samples. In the Year 4 winter barley crop, establishment was better and as in the previous year annual meadow grass (*P. annua*) was the main weed and there were significantly fewer where the glufosinate plots had been. There were very low levels of volunteer oilseed rape seedlings.

In Rotation 4 Year 1 at NIAB, no volunteer oilseed rape seeds were recorded in the soil seedbank. The site was sown with winter wheat but no data was collected. In the Year 2 oilseed rape, weed densities were low with cleavers (*G. aparine*) the dominant weed. The imidazolinone treatments were the poorest on the weeds present here. Weed biomass in June was low and cleavers (*G. aparine*) was the only surviving weed of importance. In the winter wheat in Years 3 and 4, weed numbers and biomass were low. In the final year, virtually the only weed was cleavers (*G. aparine*). Volunteer oilseed rape was present at very low levels in the winter wheat in the spring and was absent from the biomass samples as a result of the cereal herbicide.

Weed control – Rotation 5

In Rotation 5 at Broom's Barn, the experiment was placed in an area which was considered to have an existing weed beet problem, however, no seed was found when soil samples were processed. Annual beet seed was sown in 2000 to ensure the presence of some weed beet in the sugar beet crop. In the sugar beet crop, weed beet were recorded and removed in August. Data was analysed using log_{10} (x+1) due to the high number of zeros. There was significantly more weed beet recorded on the conventional treatments.

Rotation 5 at Morley followed the same protocol as at Broom's Barn with weed beet being sown into the area in 2000. Weed beet were counted and removed in early August and data was transformed to log₁₀ prior to analysis due to the number of zeros. No weed beets were recorded on the glyphosate or glufosinate treatments and 0.9 plants per m² on the conventional treatments. The herbicide tolerant beet received two applications of either glufosinate or glyphosate.

Overall crop yields

It is stated that the primary aim of the work was not to assess the relative yields of the different treatments as the cultivars were not yet fully commercialised. However, information on yields was needed to assess the level of post harvest oilseed rape seed losses and to demonstrate that the crops were being grown realistically. For sugar beet this was clearly not the case where yields were relatively low due to the early harvest date imposed by DEFRA. Where there was lower root yield this was often compensated for by a greater sugar content.

Indications were that the oilseed rape crops grown in the experiments reflected 'normal' cropping. Overall seed losses at harvest were in the region of 4000 seeds per m² but much higher losses were recorded on some plots. The normal sowing rate of an oilseed rape crop is 100 seeds per m². The seeds lost at harvest are the primary source of volunteer oilseed rape. Crop growth of the oilseed rape cultivars was similar at all sites with little effect of treatment on yields. There was some bird and rabbit damage to the crop at most sites. The sugar beet also suffered bird damage especially at Broom's Barn.

Estimated seed loss at oilseed rape harvest

At Rothamsted in 1999, most shed seeds were recorded on the glufosinate and glyphosate plots in Rotation 1a. Seed numbers were estimated at 2000 to 4000 per m^2 at harvest. Many seeds germinated after shedding and following rainfall but a minority of seeds remained ungerminated. There was also some predation. In 2002 in Rotation 1, seed losses were again 2000 to 4000 per m^2 but few remained after substantial rainfall. Less seed was shed on the conventional treatment plots. In Rotation 4 in 2000 there were more seeds shed on the glyphosate plots, up to 10,000 seeds per m^2 .

At SAC in Rotation 4 in 2000, oilseed rape seed losses varied from 1000 to 4000 seeds per m². The cultivars were harvested on different dates due to differences in time of maturity. Few seeds remained following appreciable periods of rain after crop harvest.

Decline rate of oilseed rape seed shed at harvest

A substantial number of seeds were shed at oilseed rape harvest. Some became incorporated into the soil weed seedbank by post-harvest cultivations and formed the basis of a persistent reserve of volunteer oilseed rape. At Rothamsted in Rotation 1a, significant differences were recorded in Years 3 and 4. In all years more seeds were found on the glufosinate plots. There was a marked decline in seed levels between the seed shed at harvest and the seed recorded in soil cores around 6 months later. The plots were left uncultivated for 4 weeks after harvest, as recommended, to encourage this decline. In Rotation 4, where rape was sown in Year 2, there were again significant differences between cultivars with most seeds found on glyphosate tolerant rape plots and least on the conventional plots.

At NIAB there were no detectable differences in Rotation 3. In Rotation 1, only in Year 4 was a significant effect detected when more seeds were recorded on the glufosinate and conventional plots. This was despite indications in Year 1 that more seeds had been shed by the glyphosate tolerant rape.

At SAC no treatment differences were detected in Rotations 1b and 4. In Year 1 and 3 of Rotation 1a most rape seeds were present on plots that had previously grown glufosinate tolerant rape. There was some survival of volunteer rape in the Year 3 cereal crop of Rotation 1 and seed shed by these could have increased seed numbers on the plots.

Morley and Broom's Barn grew oilseed rape only in Rotation 3. In Year 1 at Broom's Barn, significantly more seeds were present on plots that had previously grown the

glyphosate tolerant cultivar. The effect was not noted in later years. No treatment effects were noted at Morley.

Where there was 4 years data on the decline of shed oilseed rape seed, regression analyses were made to estimate decline rates. For various reasons complete data sets were available only for Rothamsted 1a, NIAB 1, NIAB 3, SAC 1a, Morley 3 and Broom's Barn 3. The data from SAC did not fit the regression and those from Broom's Barn were very variable. This left 4 data sets where decline curves could be fitted. At Rothamsted 1a and NIAB 1, there were significant differences between the four rape cultivars. At Rothamsted 1a, the highest number of seeds present throughout the experiment was on the glufosinate plots. At NIAB 1, the conventional variety Apex and the glufosinate resistant cultivar were the most persistent and the conventional cultivar Synergy the least persistent. Appreciable numbers of seeds were detected.

Estimates of the proportion of OSR in the seedbank that emerged and grew into plants

Oilseed rape volunteers were difficult to record in the cereal crops because preemergence and early post-emergence herbicides were applied across all plots. In the sugar beet crops that followed an earlier OSR crop at Morley and NIAB herbicide timings, particularly on the conventional plots, also made recording difficult. At Broom's Barn, OSR volunteers were recorded prior to herbicide applications on the sugar beet. At Rothamsted, SAC and NIAB, in the second OSR crop, volunteers could be recorded in some plots prior to herbicide application. Where volunteers were from a cultivar with tolerance to a different herbicide to the one applied, dead plants could be recorded.

At Rothamsted in Rotation 1 Year 4, appreciable volunteers were observed on plots treated with glyphosate and glufosinate where the previous OSR grown had been a different cultivar. These volunteers were killed and there was a strong relationship between seedling numbers and seedbank numbers. The emerged seedlings represented 1% of the seedbank. Where glufosinate was applied to plots that had previously grown glufosinate resistant OSR a few seedlings were killed and there was a correlation with seedbank numbers but a low slope to the regression line. Where glyphosate was applied to a previous glyphosate resistant OSR plot, a small number of seedlings were killed but there was no relation to seedbank.

At SAC in Rotation 1 Year 4, there was a similar pattern of response. Where glufosinate and glyphosate were applied to plots that had carried non-tolerant OSR previously, there was a strong correlation with seedbank numbers. Emerged seedlings represented 2.7% of the seedbank. Where glufosinate was used on potentially glufosinate tolerant volunteers a small number were killed but there was no correlation to seedbank. Where glyphosate was applied there was again no link to seedbank.

At NIAB Rotation 1 Year 4, there were fewer plots for comparisons. Where plots had a dissimilar herbicide applied previously there was a weak relationship between killed seedlings and seedbank numbers. Emerged seedlings represented around 1% of the seedbank. A few glufosinate susceptible plants were recorded on previously glufosinate treatment plots but no glyphosate susceptible plants on previous glyphosate treatment plots.

At Broom's Barn Rotation 3 Year 3, all volunteers were counted prior to herbicide application so it was not possible to determine their origins. Lower than expected numbers emerged from seedbanks with the highest seed numbers giving a curved

relationship between seedlings and seedbank. Based on the lower part of the curve, emerged seedlings represented approximately 5% of the seedbank.

Additional studies of intrinsic dormancy of harvested oilseed rape seed

In the Petri-dish tests to determine the intrinsic potential of the rape cultivars to develop secondary dormancy, the herbicide tolerant cultivars seemed marginally less likely to become dormant than the conventional variety Apex.

Additional studies of cross pollination between herbicide tolerant and conventional OSR cultivars in 1999

The results describe the level of outcrossing with the glufosinate cultivar and the testing of the resulting seed for glufosinate tolerance. At NIAB, each conventional plot was split between the two conventional cultivars. Outcrossing by the predominantly male sterile, conventional cultivar Synergy was high at 9.7% compared with the conventional open pollinated cultivar. Although outcrossing levels in the latter were lower than either the glyphosate or imidazolinone tolerant cultivars too. Excluding Synergy, outcrossing levels meaned across all transects, distances and plots was 0.3%. At Rothamsted, overall cross pollination ranged from 0-4.5% nearest the pollen source to 0-1.8% at the furthest sample point. The mean was 0.9% across all sample points. At SAC, pollination ranged from 0.3-4.2% nearest the pollen source to 0.7-4.2% at 20 m. The overall mean was 0.9%.

The results show that where conventional and/or different herbicide tolerant oilseed rape cultivars are grown in the same area, a varying proportion of seed shed by a conventional cultivar would contain a tolerant gene. Also seed shed by a tolerant OSR cultivar would have a varying proportion of seeds containing more than one herbicide tolerance gene.