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Botanical and rotational implications of genetically modified herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT Project)

by

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BRIGHT PROJECT

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BRIGHT REPORT

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1. ABSTRACT

The four year BRIGHT project was initiated in autumn 1998 and the research was conducted by NIAB, Broom's Barn, Rothamsted Research, Morley Research Centre and the Scottish Agricultural College (Aberdeen) in a consortium with Agrovista, BASF, Bayer, BBRO, HGCA and Monsanto sponsored by Defra and SEERAD through the Sustainable Arable LINK programme.

The BRIGHT project had the objective of determining the implications of growing HT crops both for agriculture and the environment by simulating different rotational scenarios, at a number of sites. In the BRIGHT project herbicide tolerant (HT) winter oilseed rape (WOSR) and sugar beet were grown in four year arable rotations with cereals and other crops. Cultivars of sugar beet and WOSR genetically modified to be tolerant to glyphosate and glufosinate were compared to conventional cultivars. Additionally, in years 1 and 2 a cultivar of WOSR resistant to the imidazolinone herbicides, bred by conventional breeding techniques, was compared to the other three. The research programme encompassed whole crop rotations, studying weed control in the non-HT crops as well as the HT crops. The programme included rotations that were perceived to be best practice and worst case scenarios (where potential impact of the use of HT cultivars might be expected to be highest). Thus, two included only oilseed rape or only sugar beet and one was based on a sequence of rape and sugar beet. HT crops were mainly grown twice in each rotation and all possible sequences of HT and conventional cultivars were compared.

The herbicide tolerance systems were effective and flexible, achieving similar or greater levels of weed control, compared to conventional practice, depending on site, season and other factors. The performance of the conventional and HT weed control systems rarely approached total weed control. No significant decreases in botanical (species) diversity were observed. Weed seed banks increased in both WOSR and sugar beet rotations between the start and end of the 4 year project, with no differences detected between treatments. At most sites weed control was more effective in the cereal component of the rotation than it was in the oilseed rape or sugar beet.

Seed banks of volunteer oilseed rape seed left in the field after harvest initially declined rapidly but then persisted at moderate levels with little apparent difference between WOSR varieties. The field studies and Petri-dish tests confirmed that the HT cultivars were no more persistent than the conventional cultivar (Apex). However, appreciable numbers of seeds remained in the seed bank at some sites up to 3 years after the harvest of the WOSR crops.

No direct impact of the transgenes themselves or the transgenic crops on crop production and botanical diversity was observed in these experiments. The only differences observed were due to the different herbicide programmes.

Outcrossing between oilseed rape varieties declined with increasing distance between plots and resulted in combined herbicide tolerance in some instances. Levels of outcrossing matched those reported in other studies in the UK.

Herbicide tolerance in WOSR and sugar beet increased options for the control of weeds in these crops. They gave greater flexibility of timing and management. In most rotations they had little effect on weed management in subsequent crops. However when the same HT was used in sugar beet, following HT WOSR, additional herbicides were needed to control volunteer rape.

The use of HT crops reduced the amount of herbicide active ingredient applied to sugar beet. In both crops the costs of glufosinate, and particularly glyphosate were lower than those of conventional broad-leaved weed and graminicide treatments. Other advantages were greater control of weeds closely related to the crops which are more difficult or expensive to control (e.g. weed beet in sugar beet).

Data from BRIGHT will be used to contribute to guidelines on the management of genetically modified herbicide tolerant crops.

The report recommends further studies to refine the management of HT crops in order to achieve environmental targets as well as agronomic objectives.

2. SUMMARY

The BRIGHT project was initiated in Autumn 1998 and the research was conducted by NIAB, Broom's Barn, Rothamsted Research, Morley Research Centre and the Scottish Agricultural College (Aberdeen) in a consortium with Agrovista, BASF, Bayer, BBRO, HGCA and Monsanto, sponsored by Defra and SEERAD through the Sustainable Arable LINK programme.

The BRIGHT Project had the following objective:
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.

The principle products were:

1. A report on the methods, results and conclusions of the research project, including recommendations for the management of GMHT crops, and an executive summary containing the main findings, conclusions and recommendations.
2. Guidelines for the appropriate management of GMHT crops in arable rotations.

Table 2.1 BRIGHT Rotations

Year	Rotation 1	Rotation 2	Rotation 3	Rotation 4	Rotation 5
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

* Cereal under-sown with GM rape or conventional beet seed

Dark shaded areas represent crops where HT and conventional types were compared.

The four year project consisted of a series of 5 rotation experiments which included three herbicide tolerant (HT) winter oilseed rape (WOSR) varieties (glyphosate, glufosinate, imidazolinone tolerant) and two GM HT sugar beet varieties (glyphosate, glufosinate tolerant), together with conventional varieties for

comparison. Rotations 1, 2 and 3 (see Table 2.1) included two crops where the herbicide tolerant cultivars could be studied. Three of the rotations (1, 3 and 4) included the different oilseed rape varieties and were designed to investigate the effects of growing herbicide tolerant rape in both normal farm rotations and in worst case scenarios (e.g. high levels of oilseed rape in the seedbank). One of these rotations (3) studied WOSR in year one followed by the different sugar beet varieties in the third year, so that interactions with the previous GM rape varieties could be studied. The other two rotations (2 and 5) contained the different varieties of sugar beet and examined weed control and interactions between the different varieties. In Rotations 1, 2 and 3 the second broad-leaved crop was grown in randomised sub-plots of the original plots so that all possible sequences could be studied.

The main results are summarised as follows:

i. Impact of HT crops:

a. No direct impacts of the HT varieties, or crops (transgenic and non-transgenic) themselves, on the botanical diversity, or the agronomic systems studied in these experiments, were observed. The GM varieties had very similar agronomic characters to the conventional varieties used in the study. However, the non-GM imidazolinone tolerant variety of WOSR had a lower vernalisation requirement than the other varieties and tended to flower and ripen earlier than the other varieties, especially in the trials in England.

b. There were no observed differences in the establishment, vigour and ground cover of the beet and WOSR varieties in this study. Yields were taken to confirm that management of the crops was realistic and that they had reached acceptable standards. The yields of the varieties and treatments were in line with those described in other programmes which have studied these varieties, bearing in mind that for regulatory reasons the sugar beet had to be harvested early.

c. It appeared that all differences in weed populations and diversity were due to the management of the HT crops and varieties and were not directly due to their genetic make up.

ii. Crop Safety:

a. No treatment produced any lasting phytotoxic effects on their respective WOSR types. Conventional and glufosinate treatments occasionally caused some chlorosis, respectively on conventional and glufosinate tolerant sugar beet, which temporarily checked their vigour during early growth. Glyphosate produced no adverse effects on glyphosate tolerant beet.

b. All tested varieties not tolerant to glyphosate were sensitive to glyphosate and all tested varieties not tolerant to glufosinate were sensitive to glufosinate.

iii. Weed control in winter rape and sugar beet

a. No herbicide tolerant or conventional treatment achieved complete weed control although some approached it at some sites. All treatments gave commercially acceptable weed control in most situations.

b. Overall in WOSR, glyphosate tended to be most effective but there were site/years when other treatments were more active. For example, glufosinate treatment was superior in SAC Aberdeen, Rotation 1 and the conventional treatment was most effective at Morley, Rotation 3. Weed control from imazamox in the imidazolinone tolerant rape was the least effective herbicide in more situations than the other treatments.

c. In beet, the differences between treatments were less clear but again glyphosate tended to give higher levels of weed control in more situations. In 50% of the comparisons there were no clear differences between the treatments; all were equally effective.

d. In most WOSR situations one application of glufosinate or glyphosate was used. In sugar beet a mean of 1.3 applications of glyphosate and 1.7 applications of glufosinate were used to give effective weed control. One or two herbicide products were normally used in conventional WOSR and a mean of 2.7 applications (each including several products) were used in sugar beet.

e. In both beet and WOSR the timing of glyphosate and glufosinate application was more flexible than that of the conventional treatments, which, in WOSR, tended to be based around metazachlor, for broad-leaved weed control. They gave better control of larger weeds and thus were applied 2-8 weeks later. Conventional treatments either had optimum application times at pre- or very early post-emergence, or when applied later had a limited spectrum of activity. This differential was much less acute for grass weed control as conventional treatment with specific graminicides can be applied over a wider range of growth stages in both crops.

f. Glufosinate was less effective on older weeds, particularly grass weeds, than glyphosate, and this has implications for timing of these herbicides. Poor control of *Viola arvensis* by glufosinate was recorded on several sites.

g. Herbicide treatments of WOSR rarely affected weed populations in subsequent cereal crops. This only occurred when the herbicide treatments in the oilseed rape gave large differences in weed control (or failure to control). This was particularly the case with *Alopecurus myosuroides* control in WOSR at NIAB in Rotation 1, where reduced control was apparent in subsequent cereal crops.

h. In WOSR, there was no indication from the analysis of the weed species numbers in spring of year 4 that any particular treatment consistently produced a lower number of species, so that there is no indication that any one treatment reduced botanical diversity more than any other. The weed species which survived a treatment seemed to be dependant on local conditions, the timing of the herbicide application and thus weed escape and survival. In sugar beet, the numbers of surviving weeds in all treatments were very low so that no statistical analyses of effects on botanical diversity were conducted.

i. Weed seedbanks generally increased in the rotations that included oilseed rape and there were a few significant differences due to the earlier treatments. These tended to be linked to marked differences in weed biomass in the relevant years. The high variability between seedbank samples severely reduced the sensitivity of this test masking some potential differences. This increase in the seedbank was in spite of generally high levels of weed control in the cereal crops (NB. poor weed control in barley at SAC in Year 3). The increases detected were much greater than could be attributable to the presence of rape seeds in the seedbank at the end of the rotation, which were not present at the start. In sugar beet, weed seedbanks at Broom's Barn increased as a result of inadequate control of *Chenopodium album.* In Rotation 3, where both WOSR and sugar beet were grown, the seedbank increases due to growing oilseed rape were not offset by cultivating sugar beet in year 3. There were no clear differences between treatments.

iv. Gene Flow

Outcrossing occurred between plots of WOSR at frequencies that matched those from other studies (Eastham & Sweet 2002). Levels decreased exponentially with distance from pollen source. The varietal association Synergy (included as an extra conventional cultivar at NIAB) was pollinated at a higher frequency than other varieties due its low male fertility. Outcrossing between different HT varieties produced seeds with combinations of herbicide tolerance.

Reproductive shoots (bolters) were removed from all beet before they flowered in order to minimise pollen production in the beet experiments. This was a requirement of the Release Consent from DEFRA.

v. Rotational implications

a. In only a few situations was there an influence of herbicides applied to the first rape and sugar beet crops in a rotation, on the weeds and weed control in the second rape/beet crops three years later (Year 4). This suggested that there is often little cumulative effect of the same or different herbicides on weed control. However, this conclusion may have been influenced by the fact that all sites used ploughing as the main method of cultivation. Minimum tillage or non-inversion cultivations could have caused a different effect, but were not tested in BRIGHT. A greater frequency of detectable carry over effects was noted in Rotation 3, where the second treatment year was in Year 3, but the main effects recorded were still those applied in Year 3. These carry-over effects were primarily linked to very poor weed control in the first year treatments (see iii g above).

b. HT oilseed rape volunteers were controlled in oilseed rape and beet possessing a different herbicide tolerance. Where HT rape volunteers appeared in sugar beet with the same HT, then additional herbicides tank mixed with glufosinate and glyphosate were used to control them.

c. Both glufosinate and glyphosate gave good control of non-HT volunteer and weed beet in respective HT beet crops.

d. In cereal crops, the commonly used weed control programmes gave good control of HT oilseed rape including volunteers with more than one HT trait (as a result of gene flow). Overall weed control in the cereals was generally higher than that recorded in the WOSR and sugar beet.

vi. Volunteer rape

a. Over 3500 rape seeds/m^2 were lost at harvest and remained on the soil surface. Seed losses differed slightly between varieties and were not clearly related to yield.
b. The studies showed that GM oilseed rape has similar seed survival characteristics in soil to conventional oilseed rape. Management of the shed seed, seedbank and volunteers post harvest and in intervening crops reduced numbers of volunteers. However, considerable numbers of seeds survived over the 4 year crop rotation (mean 1000 seeds/m^2), supplying a potential source of volunteers to grow and provide admixture in subsequent rape crops.
c. The recommended procedures for reducing the volunteer rape seedbank: delaying post-harvest cultivation until after rainfall, destruction of germinated seedlings through cultivation or herbicide treatment, destroying volunteers in cereal stubble, and control of rape volunteers in cereal crops all helped to minimise numbers of WOSR volunteers.

vii. Costs of weed control

a. A full cost benefit analysis was not possible in BRIGHT, as crop yields do not fully reflect the likely yields of commercial HT cultivars and the sugar beet was harvested atypically early. Provisional costs of weed control were estimated on the basis of herbicide costs and the anticipated technology fee for the glyphosate tolerant cultivars.
b. Mean costs of conventional weed control in WOSR was £60/ha, based on one or two products/crop. Overall, glyphosate was applied only once/crop and glufosinate 1.1 times. This gave costs of £18/ha for glyphosate and £40/ha for glufosinate, but the Monsanto technology fee anticipated to be £20-30/ha needs to be added to the herbicide cost of glyphosate treatment. Thus, both HT treatments were appreciably cheaper than the conventional. This assumed that the HT seed was no more expensive than conventional.
c. The cost of conventional weed control in sugar beet, arising from a mean of 2.7 applications of up to eight herbicides was £84/ha. In contrast the cost of glyphosate treatment (1.3 applications/crop) was £21/ha and was £63/ha for 1.7 applications of glufosinate. Again the cost of the glyphosate technology fee needed to be added to the cost of glyphosate. These calculations indicated that there was an appreciable cost saving of £20-30/ha arising from the planting of both HT sugar beet cultivars.

viii. Discussion and conclusions

a. The herbicide tolerance systems were equally, or more, effective than conventional treatments for weed control. They did not apparently decrease the seed bank, or the species diversity in winter oilseed rape.

Timing of glyphosate and glufosinate application appeared to be much more flexible than that of the conventional treatments for both WOSR and sugar beet. They gave better control of larger weeds and thus could be applied later. Conventional treatments, especially those for broad-leaved weed control, either had optimum application times at pre- or very early post-emergence or had a limited spectrum of activity. This differential was much less acute for grass weed control. The flexibility of conventional treatments for grass weeds would have been much more restricted if they had already acquired resistance to the standard conventional herbicides.

b. Glufosinate was less effective on older weeds, particularly grass weeds, than glyphosate. Therefore it needed to be applied earlier than glyphosate. Both glufosinate and glyphosate have no soil acting residual activity, so that later emerging weeds were not affected. Where these were able to compete with the crop, then a second treatment was needed, reducing the cost benefit. This problem was less acute for glyphosate as activity was not so affected by weed size.

c. Both glufosinate and glyphosate are foliar acting. Consequently, if applications are delayed too long it is possible that crop leaves will screen the weeds, so that they are shielded from the sprays, giving poorer control. This was not explicitly studied in BRIGHT but impacted on decisions as to when to apply the herbicides.

d. Despite the flexibility of glufosinate and glyphosate, both have optimum application windows to achieve good control and without having to be applied twice. The start of the window depends on the end of weed emergence while the end depends on crop ground-cover, which in turn depends on crop vigour driven by crop emergence date and weather. The end of the application window may also be constrained by concern that the weeds will cause irretrievable yield loss. This was not specifically addressed in BRIGHT but was part of the decision making process when deciding on timing of treatments.

e. The research demonstrated some weaknesses in the spectrum of weeds controlled by the HT systems, such as grass weeds and *Viola arvensis* control by glufosinate, so that it could be anticipated that close rotational growing of glufosinate tolerant rape might result in an accumulation of these weeds. This could be overcome by using appropriate tank mixes and greater control of these weeds in other parts of the rotation.

f. Little advantage was seen in following one HT rape crop with a rape crop with a different sensitivity in order to control weeds. Because of the potential of creating volunteers tolerant to more than one herbicide, which could make their control problematic in subsequent years, it would seem appropriate to stay with the same herbicide tolerance in any one rotation. Although, this could encourage the selection of resistant weeds and a build up of HT volunteers, if rape is only grown once in four years and other weed management measures are considered, this should not create problems.

g. The data from BRIGHT enable conclusions to be drawn as to which HT system to use in WOSR. Glyphosate is extensively used for control of rape volunteers in stubbles, set aside and other parts of the rotation and for desiccation of rape crops. By contrast glufosinate is not used widely in other arable crops, but its main use is in desiccation of potatoes and oilseed rape. Thus if glufosinate tolerant rape is grown, glyphosate would still be available for the desiccation of crops and the control of the volunteer rape in stubbles. Tank mixes of other herbicides with glyphosate could be used to control glyphosate tolerant volunteers but this may not be very practical and will add costs and complexity to autumn operations at a busy time of year.

h. In sugar beet, the experiments showed that there was no need to apply pre-emergence herbicides to HT crops and weeds could be controlled at much more advanced growth stages, especially by glyphosate. However, there was also a need to protect young beet seedlings from weed competition in order to achieve rapid crop establishment so that two applications of herbicide were sometimes needed.

i. A major benefit in beet was the ability to control weed beet with a low cost chemical method. Weed beet now infests 70% of the beet area in the UK and is spreading. The main control methods are inter-row hoeing and intra-row hand rogueing, both of which are time consuming and expensive.

j. Since the beet experiments had to be harvested early (September) the full effect of the herbicides on the control of late emerging and late season weeds was not evaluated. In some instances additional treatments may have been needed if beet crops were to be harvested in November/December. In addition greater levels of weed seed return may have occurred.

k. Sugar beet has poor tolerance of weed competition and thus high levels of weed control are much more important in beet than in rape. In rotations containing both rape and beet it thus will be more effective to grow crops with different herbicide tolerances in order to facilitate volunteer rape control. The preferred option would be to utilise glyphosate tolerance in sugar beet.

l. In cereal crops, the commonly used weed control programmes gave good control of HT oilseed rape and beet volunteers, so it may be possible to reduce levels of weed control in cereal crops in the rotation especially if good control of problem weeds is occurring in the HT crops. However, in the BRIGHT cereal plots there was sometimes an appreciable late spring emergence of rape volunteers. In order to ensure that there is no seeding by HT rape volunteers in the cereals and hence carry over into subsequent rape crops, this late cohort was treated with an extra herbicide treatment. This greater use of herbicides such as the sulfonyl ureas (e.g. metsulfuron-methyl) has the potential to reduce populations of other weeds and hence impact on biodiversity in these cereal crops.

ix. Recommendations.

Recommendations for use of HT technology arising from BRIGHT

a. One application of glyphosate will give commercially acceptable levels of weed control in WOSR in most circumstances. Two treatments may be needed for glufosinate.

b. Two applications of both glyphosate and glufosinate are more likely to be needed in sugar beet, especially when the crop is harvested at a ‘normal’ time.

c. Having decided on a particular HT cultivar of WOSR, do not change to a different HT one in subsequent seasons, as this will increase the risk of gene stacking and subsequent problems over volunteer management. The same constraint does not apply to beet provided there is complete control of weed beet and removal of bolters.

d. The results from BRIGHT suggest that if successive crops of HT WOSR and HT beet are to be grown, the use of a different HT cultivar, preferably glufosinate tolerant rape and glyphosate tolerant beet would be advisable. The latter crop is less competitive and therefore would benefit from the higher reliability of glyphosate.

e. In WOSR, it is essential to maximise seed losses post harvest, by using appropriate cultivation options to minimise the size of the subsequent seedbank. A low proportion of the shed seeds will be present after 3 years, and probably longer. This has implications for the cultivar choice in subsequent rape crops. When considering the impact of cultivation of HT beet or WOSR on botanical diversity in arable fields, it is also critical to review the management and impact of cereals grown in rotation with them. It was apparent from this study (and in others) that current weed management methods in cereals often depleted weed numbers more than the treatments in WOSR and beet.

x. Research Recommendations

a. The introduction of GM HT WOSR can create new management issues in subsequent non-HT rape crops or other crops with the same herbicide tolerance. It is proposed that longer term studies of WOSR seedbank decline are conducted using the BRIGHT sites which have already been observed for 4 years, in order to supply more comprehensive information on seedbank decline rates of different HT varieties.

b. The FSE studies showed that glyphosate reduced weed biomass in beet crops. However other studies of HT beet (e.g. in Denmark by Strandberg *et al.,*2002) have contradicted this result and BRIGHT has shown no consistent differences. The increased flexibility of management of HT beet can be used to achieve biodiversity objectives, as has been demonstrated by the studies of Pidgeon *et al* (2001) with HT beet at Broom's Barn, and there is also the possibility that use of HT crops could reduce herbicide usage in cereals offsetting impacts of HT beet. It is therefore proposed that further studies are conducted to determine appropriate management strategies for HT beet and crops in rotation with them that will enhance their environmental profile.

c. In these BRIGHT studies the winter cereals often had a greater impact on the surviving weed numbers than the beet or WOSR, irrespective of whether the weed control was conventional or herbicide tolerant. Thus, other aspects of crop rotational management, apart from weed control, can have as great an impact on botanical diversity in arable systems. 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 impacts of all crops, not just those potentially including HT systems. We therefore recommend that studies are carried out to assess the impact on biodiversity of all arable cropping systems and to develop methods to achieve the desired end results.

xi. Guidelines

The use of the information from the BRIGHT project to prepare guidelines on the management of HT crops was part of the original proposal and progress has been made on devising a system to achieve this. The actual preparation and dissemination of the guidelines was without the BRIGHT project. The consortium recommends that the results and conclusions arising from BRIGHT are used, along with other existing data, and the protocols already proposed by SCIMAC, to develop management guidelines for the commercialisation of herbicide tolerant crops in the UK. These guidelines should be prepared prior to planting, but after there is legislative agreement that such HT crops could be sown commercially by UK farmers.

3. INTRODUCTION

Herbicide tolerant crops (maize, oilseed rape, fodder beet, sugar beet) are being developed for UK agriculture and are in the process of being evaluated for commercial planting. These crops are tolerant to broad spectrum herbicides such as glufosinate, imidazolinone herbicides (e.g. imazamox) and glyphosate, which have activity against a very wide range of plant species, both crop and weed. The glufosinate and glyphosate tolerant systems have been introduced into the crops by genetic modification while other systems (e.g. imidazolinone) have been produced by mutation breeding. Some of these herbicide tolerant crops are being widely grown in the USA and Canada. (James, 2003).

These systems permit the use of broad spectrum herbicides at the post- crop emergence phase and thus have the potential to allow greater targeting and control of weeds at this phase. It is important that the agronomy of these crops is understood so that information can be supplied to farmers on how best to manage and exploit these varieties and avoid problems such as volunteer build up, multiple herbicide tolerance, contamination of other crops and reductions in botanical diversity. There are concerns that the use of broad spectrum herbicides may result in much higher levels of weed control and reductions in weed abundance and diversity, which can lead to reductions in the overall biodiversity of arable ecosystems (World Wildlife Fund, 1995; Friends of the Earth, 1998; Gene Watch, 1998; English Nature, 1998; Fromwald & Strauss, 1998; Hill, 1999). The Farm Scale Evaluations (FSE) (Heard *et al* 2003) have already indicated that weed control in glyphosate tolerant beet and glufosinate tolerant spring rape can be higher than in their conventional equivalents, but the Farm Scale studies on winter rape are yet to be published. Other published data suggests that glufosinate applications in winter rape can be as effective as the more active conventional treatments (Read & Ball, 1999). Further data on the impact of HT and conventional treatments are needed, especially over a rotation, as the focus of the FSE trials was on weed control in a single season, and their results cannot be seen in the full context of rotational weed management.

Changes in visible weed populations may result in longer term effects on the soil weed seedbank. The diversity of plant species appearing in crops each year is largely dependent on existing seedbank populations and the management of the crop. In order to determine the effect of one or more herbicide tolerant crops in a rotation, weeds need to be recorded at several stages of crop development and estimates made of seed production. In addition the impact of the different rotational systems on soil seedbank reserves of weeds needs to be determined in order to measure or estimate trends in species or population changes.

It is also important to study the range of interactions that can occur between different HT crops and conventional varieties. This needs to be done in a number of crop rotations simulating anticipated standard practice and practices that would not necessarily be recommended; i.e. best and worst case scenarios. Volunteers are anticipated to be a problem and rotations should be studied where there are likely to be high numbers of volunteers so that their control can be investigated.

3.1 THE BRIGHT PROJECT

The BRIGHT project was established in 1998/9 with the objective of determining the implications of growing HT crops both for agriculture and the environment by simulating different rotational scenarios at a number of sites. In the BRIGHT project herbicide tolerant (HT) oilseed rape and sugar beet have been grown in arable rotations with cereals and other crops. Management studies therefore encompassed whole crop rotations studying the interactions between the crops at all stages of the rotation and the management of the non-HT crops in the rotation as well as the HT crops.

The BRIGHT project started in September 1998 with Pre-LINK funding from DEFRA (MAFF) allowing WOSR to be established that autumn. In spring 1999 a three-year Sustainable Arable LINK programme was agreed with all the sponsors and partners. Only three years funding was initially forthcoming from the industrial partners because they wanted to review the programme at three years in relation to the commercialisation of GMHT crops and other external developments. They also wanted to be sure that the project was addressing the correct issues in relation to the regulatory requirements for the commercialisation of GM crops. In 2001 the EU issued directive 2001/18 EU requiring that the biodiversity implications of growing GM crops be assessed, including the impact of the agronomic practices associated with a GM crop.

BRIGHT had anticipated these regulatory changes by commencing a study of the agronomic and agro-environmental implications of cropping systems containing GM herbicide tolerant crops. It was also a very useful complement to the UK Farm Scale Evaluations (Firbank *et al*., 2003), which were essentially looking at seasonal or short term impacts of GM crops on biodiversity, and to studies in other European countries on the biodiversity implications of GM cropping. In 2001 the industrial sponsors reaffirmed support for the project and a successful submission was made to SA-LINK to extend the project for a fourth year. Field studies were completed in January 2003.

Project objective

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.

Scientific objectives

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 crop rotations.
4. Determine the principal 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.

4. MATERIALS AND METHODS

4.1 Experimental Design and Site Details

Site Details

Descriptions of individual site soil types and cropping histories are detailed in Appendix 10.3.1.

Plate 4.1.1. Experimental site at Rothamsted Research with winter oilseed rape flowering in the first year of the programme.

Description of crop rotations

The project consisted of a series of large plot experiments to investigate the impact of herbicide tolerant oilseed rape and sugar beet over a series of five, four-year rotations. The herbicide tolerant crops were grown in plots alongside conventional crops and all plots had the same management, except for the herbicide treatments. There were a total of five rotation designs but only a maximum of three were grown at each site (Table 4.1.1).

Deliberate release approvals at the start of the project restricted the areas that could be planted/treated and it was decided that plot size should be as big as possible and replication should be kept to an acceptable minimum, in order to simulate 'normal' field responses. Thus, Rotations 1a, 2, and 3 had only two replications, whilst the smaller plot rotations 4 and 5 had three and six, respectively. The consortium was aware of the limitations of the replication in Rotations 1, 2 and 3 but after statistical consultation concluded that it was acceptable. Even in the smallest trials (rotation 2) there was a total of 18 plots in the critical fourth year (when seeking interactions between the first and second set of treatments) and consequently 8df in the error. Because of the consortium's decision to double-check the statistical validity, Rotations 1 and 3 at Rothamsted and SAC were combined after year 1, whilst they were identical, to become 1a and 1b thus providing two sites with 4 replications of Rotation 1. Problems of achieving adequate replication in field-scale projects with large plots and limited numbers of treatments are discussed by Chapman & McIndoe (2000) in the report of the LINK IFS project. Some of the issues were similar and confirm that the consortium was right to amalgamate the two rotation 1/3 experiments at Rothamsted and SAC, to increase replication. Plot sizes for the main studies with winter oilseed rape (WOSR) (Rotations 1 & 3) initially exceeded 0.2ha but when the second set of treatments was imposed (see below) these were reduced to c. 0.05ha. For Rotation 2 which had sugar beet only, plots were smaller, initially 0.3-0.6 ha reducing to 0.08-0.23 ha in Year 4. Plots were smaller for Rotation 4 & 5. Full details are given in Appendix 10.3.1.

Table 4.1.1 Four year rotation design and participating sites

Year	Rotation 1a/1b[2]	Rotation 2	Rotation 3	Rotation 4	Rotation 5
Sites	NIAB/Roth/SAC	BB/Morley	BB/MOR/NIAB	NIAB/Roth/SAC	BB/Morley
1	Winter oilseed rape	Sugar beet	Winter oilseed rape	Winter cereal[1]	Winter cereal[1]
2	Winter cereal	Winter cereal	Winter cereal	Winter oilseed rape	Winter cereal
3	Winter cereal	Winter cereal	Sugar beet	Winter cereal	Sugar beet
4	Winter oilseed rape	Sugar beet	Cereal	Cereal	Cereal

BB= Broom's Barn, MOR=Morley Research Centre, Roth=Rothamsted Research, NIAB=NIAB, SAC=Scottish Agricultural College, Aberdeen,

[1]The two rotations designated as undersown in year 1 received rape (R.4) or beet (R.5) seeds during late summer which were ploughed under to simulate seeds shed from a previous crop establishing a seedbank of potential volunteers.

[2]NIAB - Rotation 1a only, Rothamsted Research and SAC Aberdeen Rotation 1a and 1b

Herbicide Treatments

Each treatment was replicated at least twice (see above section for details) and the plots in each of the rotations including oilseed rape were sown and treated in the first year as follows:

Glyphosate Tolerant variety, Roundup Ready (RR)	GlufosinateTolerant variety, Liberty Link (LL)	Imazamox Tolerant variety (IMI)	Conventional variety (CONV)

The subsequent broad-leaved crop plots in years 3 or 4 had the same treatments as Year 1 but these were applied to randomised subplots of the first year plots, so that first year treatments were followed by each of the subsequent treatments in oilseed rape (Figure 4.1.2). The randomised layout at each site is described in Appendix 3 in 10.3.1.

The IMI oilseed rape variety was withdrawn from the project after year two and was substituted in later years with an additional conventional variety and treatment (CON*). The herbicide treatment applied to the CON* plots differed from the other conventional treatment and this is commented on in the detailed reports from each site.

The sugar beet varieties were RR, LL and CONV in all their sowing years. However where the RR and LL sugar beet treatments followed oilseed rape RR and LL treatments, other herbicides were included in tank mixes with glyphosate and glufosinate to control tolerant oilseed rape volunteers from the first year. Where sites had been split four ways after Year 1, as occurred in Rotation 3, an additional conventional treatment (CON*) was included which used a different herbicide programme to the other conventional.

Where the experimental layout allowed, herbicide treatments in the years when GMHT crops were being grown had an adjacent untreated area, in order to assess the weed potential in each plot.

Herbicide treatments of non-GM beet and oilseed rape were according to best local practice and the weeds present at the individual sites. All cereal plots at each centre in subsequent years received similar conventional herbicide treatments appropriate to the weed infestation present.

Figure 4.1.2 The sub-division of the year 1 oilseed rape plots in years 3 or 4

RR/RR[x]	RR/LL	RR/CON*	RR/CONV
LL/RR	LL/LL	LL/ CON*	LL/CONV
IMI/RR	IMI/LL	IMI/ CON*	IMI/CONV
CONV/RR	CONV/LL	CONV/CON*	CONV/CONV

x = year 1/subsequent year treatments

4.2 Crop management

Crop management and herbicide applications

Soil types of four of the sites were relatively similar being either sandy/silty clay loams or clay loams. The soil was lighter at Brooms Barn – a sandy loam. Full details are given in Appendix 10.3.1. Normal farm practices for land preparation and crop management were followed for all crops, including fertiliser applications and use of fungicides and insecticides. Most sites were ploughed after the harvest of the test crops (rape and beet) and most were ploughed after the cereal crops. Details are given in Tables 4.3.4 - 4.3.8.

Sowing dates of the crops endeavoured to reflect normal farming practice but difficulties with accessing seeds for some of the GM cultivars meant that sowing of the WOSR and sugar beet tended to be delayed such that the WOSR was sown in early-mid September and sugar beet at the end of April (Tables 4.3.4 – 4.3.8). Despite this, the establishment of all the GM crops was satisfactory at all sites. Site managers aimed to sow the winter cereals early in October.

Herbicide applications to HT oilseed rape and sugar beet variety plots in all experiments followed the recommended rates and timings given by the agrochemical companies supplying the herbicides. Decisions on herbicide products and timings for conventional crops were made by experienced managers at each site and were selected on the basis of weed infestations present at individual sites. The site managers also made decisions on the agronomy of the cereal crops grown in the years when the GM crops were not planted.

Rotations 1 & 2

Details of herbicide applications made at each site for weed control in WOSR and sugar beet are shown in Tables 4.3.1 - 4.3.3. Herbicide applications made to cereal crops in each rotation are shown in Appendix 10.2 (Section 10.2)..

Rotation 3

In year 1 of Rotation 3 the four different oilseed rape varieties were grown and the treatments followed those used on Rotation 1. In year 3 the three different sugar beet varieties were grown. In order to control HT rape volunteers emerging in sugar beet with the same HT, mixtures of herbicides were used (Table 4.3.2). Glufosinate applied to LL sugar beet in year 3 in plots that had LL winter oilseed rape in year 1, had metamitron plus an adjuvant oil added to the first application of glufosinate and triflusulfuron-methyl to the second application to the LL beet, where necessary.

Where glyphosate was applied to plots which had RR winter oilseed rape in year 1, metamitron and adjuvant oil were added to the first RR beet application of glyphosate, and triflusulfuron-methyl to the second application to the RR beet, where necessary.

On plots where IMI and conventional WOSR were sown in year 1, the conventional treatments for beet in year 3 were based on metamitron +/- phenmedipham (cotyledon stage rape only) and/or lenacil + phenmedipham (cotyledon to two true leaves stage rape). Triflusulfuron-methyl was sometimes used on the plots to assist control of the volunteer rape.

Undersown crops

Rotation 4

Plots in Rotation 4 received 'non-dressed' oilseed rape of LL, RR IMI and CONV varieties. Seed was broadcast at a rate of 45g/m^2 (c.10 000 seeds/m^2) in August 1998 to simulate seeds shed from a previous rape crop. Seeds were spread on plots and ploughed into the soil as soon as possible afterwards. The number of volunteers arising from the artificial seedbank was recorded and characterised and their level in the seedbank recorded in winter 1998/99. Treatments in year 2 followed the same pattern as those used in the previous year (year 1) in Rotation 1.

Rotation 5

Plots in Rotation 5 received weed beet (non-GM) seed broadcast at a rate of 46/m^2 to simulate a weed beet problem. Seeds were spread on plots in late summer/early autumn and ploughed into the soil as soon as possible afterwards. The number of volunteers arising from the artificial seedbank was recorded. At Broom's Barn, the experiment was sited on an area previously infested with weed beet. The soil core assessments provided a measure of the weed beet seed abundance. Additional weed beet seed was sown just

prior to drilling the sugar beet in year 3. On 2.5.2001 seed of a Syngenta annual bolting line was broadcast across plots at a rate of 1.7 seeds/sqm onto plots prior to final seed bed preparation by power harrowing.
In year 3, HT and conventional beet was grown using the herbicide treatments specified in Table 4.3.3. The weeds were assessed as in Rotations 2 and 3 and beet seedlings emerging between the rows were included in the weed assessments.

4.3 Herbicide treatments and agronomic details for each rotation at each centre

The following tables (4.3.1 - 4.3.3) list the herbicide treatments applied when oilseed rape or sugar beet were grown in rotations to compare treatments. Full details of herbicide applications are shown in Appendix 1 (Section 10.1).

Table 4.3.1 Herbicide treatments in oilseed rape crops in Rotations 1a and 1b at NIAB , Rothamsted and SAC in years 1 and 4

Rotation	Site	Year	Treatment	Date of application	Herbicides used
1	NIAB	1	CON	11.11.98	metazachlor + fluazifop
			LL	23.11.98	glufosinate
			RR	23.11.98	glyphosate
			IMI	23.11.98	imazamox
1a/1b	RES	1	CON	28.09.98	metazachlor
			CON	11.11. 98	cycloxydim
			LL	11.11. 98	glufosinate
			RR	11.11. 98	glyphosate
			IMI	9.10.98	imazamox
1a/1b	SAC	1	CON	6.11.98	metazachlor + benazolin + clopyralid
			LL	17.11.98	glufosinate
			RR	19.11.98	glyphosate
			IMI	6.11.98	imazamox
1a	NIAB	4	CON	6.09.01	metazachlor + quinmerac
			CON	25.09.01	cycloxydim
			LL	25.09.01	glufosinate
			LL	6.11.01	glufosinate
			RR	25.09.01	glyphosate
			CON*	25.09.01	cycloxydim
1a/1b	RES	4	CON	28.09.01	metazachlor + quinmerac
			LL	1.11.01	glufosinate
			RR	2.11.01	glyphosate
			CON*	20.10.01	cyanazine
1a/1b	SAC	4	CON	5.09.01	metazachlor + quinmerac
			LL	26.11.01	glufosinate
			RR	26.11.01	glyphosate
			CON*	26.11.01	propyzamide

CON* is an alternative conventional treatment applied to replace the imidazolinone treatment (previously referred to as IMIC).

Table 4.3.2. Herbicide treatments in winter oilseed rape and sugar beet crops in Rotation 3 at NIAB, Broom's Barn and Morley

Rotation	Site	Year	Treatment	Date of application	Herbicides used
3	NIAB	1	CON	15.10.98	metazachlor + fluazifop
			LL	16.10.98	glufosinate
			RR	23.11.98	glyphosate
			IMI (Con)#	15.10.98	metazachlor + fluazifop
3	Brooms Barn	1	CON	2.02.99	benazolin + clopyralid + cycloxydim
			LL	2.02.99	glufosinate
			RR	2.02.99	glyphosate
			IMI (Con)#	2.02.99	benazolin + clopyralid
3	Morley	1	CON	12.09.98	metazachlor (pre-em)
			LL	11.11.98	glufosinate
			RR	11.11.98	glyphosate
			IMI(Con)#	11.11.98	metazachlor + benazolin + clopyralid
3	NIAB	3	CON	8.05.01	metamitron + ethofumesate
				25.05.01	triflusulfuron-methyl + desmedipham + phenmedipham
				7.06.01	triflusulfuron-methyl + desmedipham + phenmedipham
			CON*	17.04.01	chloridazon (pre-em) + same post-emergence treatments as CON
			LL	21.05.01	glufosinate (+metamitron)**
				5.06.01	glufosinate
			RR	31.05.01	glyphosate (+metamitron)**
3	Brooms Barn	3	CON	23.05.01	phenmedipham+lenacil
				12.06.01	desmedipham+ethofumesate+ phenmedipham triflusulfuron +lenacil
			LL	13.06.01	glufosinate (+metamitron)**
			RR	13.06.01	glyphosate (+metamitron)**

** LL and RR plots that followed LL and RR rape in year 1 received a tank mix of metamitron to control herbicide tolerant oilseed rape volunteers

IMI(Con) was sown with imidazolinone tolerant rape but due to approval limitations was treated with conventional herbicides.

Table 4.3.2 ***(continued)*** **Herbicide treatments in winter oilseed rape and sugar beet crops in Rotation 3 at NIAB, Broom's Barn and Morley**

Rotation	Site	Year	Treatment	Date of application	Herbicides used
3	Morley	3	CON	4.05.01	chloridazon (pre-em)
				30.05.01	Phenmedipham+lenacil
				22.06.01	Phenmedipham+lenacil
				9.07.01	phenmedipham+desmedipham + ethofumesate + lenacil
			LL	12.06.01	glufosinate (+metamitron)**
			LL	16.07.01	glufosinate (+triflusulfuron)**
			RR	12.06.01	glyphosate(+ metamitron)**
			RR	16.07.01	glyphosate (+triflusulfuron)**

** LL and RR plots that followed LL and RR rape in year 1 received a tank mix of metamitron and then one with triflusulfuron to control herbicide tolerant oilseed rape volunteers.

Table 4.3.3. Herbicide treatments in sugar beet crops in Rotations 2 & 5 at Broom's Barn and Morley.

Rotation	Site	Year	Treatment	Date of application	Herbicides used
2	Broom's Barn	1	CON	19.05.99	metamitron+phenmedipham
				14.06.99	desmedipham+ethofumesate+ phenmedipham
				14.06.99	metamitron+clopyralid
			LL	14.06.99	glufosinate
			LL	6.08.99	glufosinate
			RR	14.06.99	glyphosate
		4	CON	30.03.02	chloridazon
				16.04.02	phenmedipham + lenacil
				24.04.02	phenmedipham + lenacil
				19.06.02	ethofumesate + phenmedipham + clopyralid
			LL	8.06.02	glufosinate
			LL	12.07.02	glufosinate
			RR	8.06.02	glyphosate
2	Morley	4	CON	3.04.02	chloridazon
				10.05.02	phenmedipham + metamitron
				6.06.02	phenmedipham + lenacil
			LL	6.06.02	glufosinate
			RR	6.06.02	glyphosate
5	Broom's Barn	3	CON	23.05.01	ethofumesate: phenmedipham + metamitron
				12.06.01	ethofumesate: phenmedipham + metamitron
			LL	13.06.01	glufosinate
			RR	13.06.01	glyphosate

Herbicide treatments at Morley in Rotn 5 were similar to those at Broom's Barn and are described in Appendix 10.1.

Herbicide treatments of cereal crops

Cereal crops were grown in rotations which differed from centre to centre and received different herbicide treatments at each centre in each year. These are described in Appendix 2 (Section 10.2). Each site used treatments appropriate to their local conditions. These herbicide treatments were applied uniformly across each site in each season and rotation so that there were no different treatments.

Details of sowing cultivation and harvesting

The following tables present details of the cultivation and dates of sowing and harvest in each of the four years.

Table 4.3.4 Rotation 1a and 1b years 1-4, dates of cultivation, drilling and harvest at NIAB*, Rothamsted Research and Scottish Agricultural College Aberdeen****

Site/Year	Crop	Primary cultivation/date	Crop drilling date	Crop harvest date
NIAB year1	WOSR	Subsoil:1.09.98 Ploughed: 2.09.98	16-17.09.98	Swath: 9.07.98 Harvest:13.07.98
NIAB year 2	W. wheat	Subsoil:26.08.99 Ploughed: 31.08.99	07.10.99	28.07.00
NIAB year 3	W. wheat	Ploughed: 5.07.00	11.10.01	18.08.01
NIAB year 4	WOSR	Min till: 26.08.01	31.08.01-3.09.01	Direct cut: 16-19.07.02
Rothamsted Research year 1	WOSR	Ploughed: 1-2.09.98	4.09.98	Desiccated: 14.07.99 Harvest: 21.07.99
Rothamsted Research year 2	W. wheat	Ploughed: 24.07.99 + 25.08.99	9.10.99	16.08.00
Rothamsted Research year 3	W. wheat	Ploughed: 29-30.08.00	30.09.00-01.10.00	14.08.01
Rothamsted Research year 4	WOSR	Ploughed: 24-25.08.01	05.09.01	Desiccated: 16.07.02 Harvest: 24.07.02
SAC Aberdeen year 1	WOSR	Ploughed: 11-14.09.98	15.09.98	Swath: 30.7.99 Harvest 8-9.8.99
SAC Aberdeen year 2	W. barley	Ploughed: 10.08.99	18.09.99	07.08.00
SAC Aberdeen year 3	W. barley	Ploughed: - Sept 00	7.10.00	24.08.01
SAC Aberdeen year 4	WOSR	Ploughed: 25-28.08.01	30.09.01	Swath: 07.08.02 Harvest 18-19.08.02

*NIAB: Rotation 1a **Rothamsted Research and SAC Aberdeen: Rotation 1a and Rotation1b

Table 4.3.5. Rotation 3 years 1-4, dates of cultivation, drilling and harvest at Brooms Barn, NIAB and Morley Research Centre

Site/Year	Crop	Primary cultivation/date	Crop drilling date	Crop harvest date
NIAB year 1	WOSR	Ploughed: 20.08.98	20-27.08.98	Swath: 1.07.99 Harvest: 9.07.99
NIAB year 2	W. wheat	Ploughed: 22.08.99	11.10.99	28.07.99
NIAB year 3	Sugar beet	Ploughed: 19.12.00	12.04.01	24.09.01
NIAB year 4	W. wheat	Ploughed: 03.10.01	11.10.01	19.08.02
Brooms Barn year 1	WOSR	Ploughed: 16.09.98	21-22.09.98	23-24.07.99
Brooms Barn year 2	W. barley	Ploughed: 28.07.99	18.09.99	26.07.00
Brooms Barn year 3	Sugar beet	Ploughed: 03.10.01	09.05.01	10.09.01
Brooms Barn year 4	W. barley	Ploughed/Disc: 13.09.01	28.09.01	26.07.02
Morley Res. year 1	WOSR	Ploughed: 3.09.98	11.09.98	31.07.99
Morley Res. year 2	W. wheat	Ploughed: 20.08.99	17.03.00*	25.08.00
Morley Res. year 3	Sugar beet	Ploughed: 3.12.00	3.05.01	17.09.01
Morley Res. year 4	W. wheat	Ploughed: 3.09.98	11.10.01	06.08.02

* autumn sown wheat crop failed and site resown in spring.

Table 4.3.6 Rotation 4, Years 1-4, dates of cultivation, drilling and harvest at NIAB, Rothamsted Research and Scottish Agricultural College Aberdeen

Site	Crop	Primary cultivation/date	Crop drillling date	Crop harvest date
NIAB year 1*	W. wheat	Ploughed: 4.09.98	20.10.98	17.06.99
NIAB year 2	WOSR	Ploughed: 20.08.99	9.09.99	Swath: 7.07.00 Harvest: 18.07.00
NIAB year 3	W. wheat	Ploughed: 27.09.00	17.01.01	18.08.01
NIAB year 4	W. wheat	Ploughed: 12.09.01	17.10.01	17.08.02
Rothamsted Research year 1*	W. wheat	Ploughed: 02.09.98	16.09.98	31.07.99-1.08.98
Rothamsted Research year 2	WOSR	Ploughed: 26-27.08.99	10.09.99	Desiccation: 12.07.00 Harvest: 25.07.99
Rothamsted Research year 3	W. wheat	Ploughed: 29-30.08.00	30.09.00	14.08.01
Rothamsted Research year 4	W. wheat	Ploughed: 24-25.08.01	13.09.01	16.08.02
SAC Aberdeen year 1*	W. barley	Ploughed: 14.09.98	October 98	5.08.99
SAC Aberdeen year 2	WOSR	Ploughed: 10.08.99 Power harrow: 03.09.99	06.09.99 for Conv, RR and LL varieties 10.09.99 for IMI	Swath: 17.08.00 - 08.09.00 Harvest: 08.09.00 - 17.09.00
SAC Aberdeen year 3	W. barley	Ploughed: Oct. 00	October 00	24.08.01
SAC Aberdeen year 4	W. barley	Ploughed: Aug. 01	October 01	11.08.02

*In year 1 rape seeds were broadcast pre-sowing of the winter wheat crop at a rate of approximately 10 000 seeds/m^2 (varieties CONV, LL, RR, IMI)

Table 4.3.7 Rotation 2 years 1-4, dates of cultivation, drilling and harvest at Broom's Barn and Morley

Site/Year	Crop	Primary cultivation/date	Crop drillling date	Crop harvest date
Broom's Barn year 1	Sugar beet	Subsoil: 04.09.98 Ploughed: 16.09.98	30.04.99	03.09.99
Broom's Barn year 2	W. barley	Ploughed: 14.09.99	18.09.99	26.07.00
Broom's Barn year 3	W. barley	Ploughed: 03.10.00	4.10.00	28.07.01
Broom' s Barn year 4	Sugar beet	Ploughed: 18.12.01	27.03.02	30.08.02
Morley year 1	Sugar beet	Ploughed 1.11.98	29.04 99	2.09.99
Morley year 2.	W. wheat	Plough/press 16.9.99	16.9.99	25.8.00
Morley year 3	W. wheat	Plough/Press 24.10 00	24.10.00	31.08.01
Morley year 4	Sugar beet	Ploughed: 10.12.01	3.04.02	2.09.02

Table 4.3.8. Rotation 5 years 3-4, dates of cultivation, drilling and harvest at Broom's Barn and Morley

Site/Year	Crop	Primary cultivation/date	Crop drillling date	Crop harvest date
Broom's Barn year 3	Sugar beet	Ploughed:04.10.00	04.05.01	10.09.01
Broom's Barn year 4	W. barley	Ploughed/Disc:13.09.01	27.09.01	27.07.02
Morley year 3	Sugar beet	Ploughed 14.09.00	03.05.01	21-28.09.01
Morley year 4	W. wheat	Ploughed/pressed 09.10.01	9/10/01	21.08.02

4.4 Weed and crop assessment methods

Introduction

Weed species and plant numbers were assessed in each treatment in each season. Control of tolerant and non-tolerant oilseed rape volunteers was noted in crops following oilseed rape. Sugar beet volunteers were assessed in Rotation 5. Assessments were conducted to determine the following:

- weed plant populations prior to herbicide treatment.
- weed plant populations at various times after herbicide treatment
- weed biomass prior to harvest (at time 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.

Full details of the dates and methods of assessment are given in the Appendices (10.3.4). All assessments were based on agreed 'Standard Operating Procedures (SOPs) written by the the research team. Therse were collated and retained by the project co-ordinator.

Weed counts were carried out pre- and post-herbicide application. The timings of these counts varied between crop species. Pre-herbicide application counts were normally either carried out in autumn (for winter oilseed rape and winter cereals) or spring/summer (for sugar beet). These timings gave an idea of the background weed population structure.

Post-herbicide application counts were either carried out in the spring (for oilseed rape and winter cereals) or summer (for sugar beet). This timing gave an indication of weed species that were not killed by the herbicides and in the case of autumn sown crops, which species were spring germinating and therefore would not be affected by an autumn herbicide application. Weed biomass was measured on plots in late summer, at a time when the weeds had reached their maximum size (June/July for oilseed rape and cereals, August for sugar beet. This measurement was used to indicate weed survival and to provide some information on seed production on the basis that weed weight gives a reasonable indication of reproductive success (Lutman, 2002).

Pre-herbicide application weed assessments

The timing of these counts depended on the plot treatment, as glyphosate and glufosinate were applied later than the conventional herbicides, and on the crop (oilseed rape/sugar beet/cereal). Counts were carried out immediately prior to treatment, where possible. The size of the random quadrats counted varied between sites due to weed infestation levels. All sites counted a minimum of twelve quadrats per sub-plot. Details of sizes used are given in Appendix 10.3.4

Untreated fixed quadrats

At some sites when the test crops of sugar beet and WOSR were grown, untreated quadrats or areas were used as a comparison with the treated plots. These were counted pre-herbicide. The size of quadrat / assessment area depended on the site, crop and weed levels but was normally $0.5m^2$ or $1m^2$. Where possible the areas were counted again when the later treatments were applied. This gave an indication of any subsequent weed emergence**.**

Post-herbicide application weed assessment

The same number of quadrats in each sub-plot was counted as in the pre-herbicide assessment. The timing of this count varied with crop and the site. For oilseed rape the count was carried out in early spring, in sugar beet the counts were done in summer, about 6 weeks after the last herbicide application.

The same procedure was used for weed counts in the test crops of rape and sugar beet and in the cereal crops. Particular care was taken in the cereal crops to assess any volunteer rape seedlings. In some cereal crops weed emergence in autumn was very low and consequently counts were delayed until the spring/summer. Full details of dates and quadrat numbers and sizes are in Appendix 10.3.4.

Weed biomass assessment

Each sub-plot was sampled using 2 or 4 quadrats up to $1m^2$ each, during June for rape, July for cereals and August/September for sugar beet. The exact number and size of quadrats sampled depended on the density of the weeds or if the sub-plots were small. Three–sided $1m^2$ quadrats (or 2 three-sided $0.5m^2$) were used which could be slid into the crop at the desired position (the position of the fourth side was estimated). In rape the sample areas were at least 1.5m from any tramlines or pathways.

All the above ground vegetation (except the crop) was collected by cutting it at ground level with shears or knives. The weeds were sorted into species, washed if necessary and then the dry weights of each species were recorded.

Analysis of weed species diversity (Rotation 1)

There are a number of ecological techniques that can be used to explore variation in species diversity. One widely used technique to assess effects on species diversity is simply to count the number of species (S) on each plot and then do a standard ANOVA to determine any treatment effects. However, this analysis could be biased by the weed density; on the basis that the more individuals present the greater the species number is likely to be. One way of resolving this issue is to include weed plant number/plot (log transformed) as a covariate in the analysis of species number(s) (SLogN). In the BRIGHT oilseed rape experiments (Rotation 1) herbicide treatments were applied in autumn year 1 and again in autumn year 4. As the two year's of treatments were factorialised it was possible to explore the impact of different combinations of treatments on species number, provided the assessment of surviving weeds was done after the impact of the second GM crop treatment was apparent. Thus the subsequent conclusions are based on the assessment of total weed numbers and of species number in spring of year 4.

There is one consequence of this type of analysis to detect effects of sequences of treatments. If the species present on one replicate are different from those in another, but the total species number is the same (e.g. 10), the analysis will assume that there is a mean of 10 species on these two plots. In reality there may be more than 10. This cannot be resolved within the constraints of analysis of variance, which is endeavouring to detect statistically sound treatment effects. This means that total species number/treatment may be less than what is actually present on the site. However, it does not negate the validity of the treatment comparisons.

Two other techniques frequently used for assessment of species diversity differences were also investigated; Log series α and Berger-Parker dominance (Magurran, 1988). The latter identifies the importance of the most dominant species whilst the former assesses species number in a slightly different way to SlogN. All three of these techniques were also used in the FSE studies (Heard *et al*., 2003).

Visual assessments

A range of visual assessments were made throughout the growing season in each year in order to monitor crop and weed growth. These included assessments of percentage cover of weeds and crop, percentage control of weed species and assessment of crop damage post-herbicide treatment. The results of these assessments were not analysed and are not presented, but are used to assist with the interpretation of results at each centre. These records are kept on file at each centre and can be inspected on request.

Crop yields

Yields were recorded for both WOSR and sugar beet at all five sites. For WOSR that was not swathed, single or multiple combine cuts of defined length (generally the length of the plots) were harvested and the seed weighed and sampled. Single cuts were used when plots were small (eg Rotn 4) or where the plot was very long, as in the Year 1 rape plots. Areas harvested per plot exceeded $75m^2$ in the main rotations 1 and 3. Sub-samples were taken to estimate moisture content and yields were re-calculated at 9% moisture. Where the WOSR was swathed whole plots were harvested at NIAB and SAC (year 1) and the entire output was weighed. At SAC in year 4 two swathes (4.27m wide) / plot were harvested and weighed. In sugar beet the roots were harvested from at least 2 rows/plot (harvesting full length of plots) using a mobile tare house which washed and weighed the roots and extracted samples which were processed to determine sugar content. Yields were calculated as fresh weight per hectare, sugar content and sugar yield per hectare. The full description of the mobile tare house operation is available from the authors on request.

Additional assessments relating to volunteer rape

Rotation 1

Immediately after the applications of glyphosate and glufosinate to the WOSR in Year 4, quadrats were counted on each sub plot, once the herbicide symptoms were apparent, to assess the number of volunteer rape plants (arising from cultivars planted in year 1) killed by the two herbicides (See Table 4.7.3 for details).

Rotation 3

Prior to the first applications of glyphosate and glufosinate to the beet varieties in Year 3, volunteer oilseed rape plants were counted on each plot treated with these herbicides. A 3m (approx) strip along one edge of each subplot (using the same side on each subplot) was treated only with glufosinate or glyphosate whereas the tank mixtures were applied on the main part of the plots.

Between 10 and 25 days after application of glyphosate and glufosinate, the number of rape plants that survived treatment was counted. Newly emerged rape plants (cotyledon to early true leaves stage plants) were counted separately so that they could be distinguished from plants surviving post-emergence treatment. Doing this allowed both the total numbers of rape volunteers and the number tolerant to either glufosinate or glyphosate to be estimated in each plot.

4.5 Soil core sampling in years 1 and 4 to determine weed seedbank composition

Introduction

As part of the assessments on BRIGHT, 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, and again in the same places in the spring/summer/autumn of Year 4. The second sample was taken once all the herbicides had been applied and no further seed return from surviving weeds was possible. The soil was sieved and seeds were extracted and identified so that the weed seedbank could be analysed at these two points in time and therefore pinpoint any changes in seed numbers of individual weed species which may have occurred.

Soil sampling protocol

Each sub-plot was divided into 4 zones and the middle marked. A square 2m x 2m around each mid point was marked and 12 cores were taken at random within that area i.e. a total of 48 cores, 2.5cm diameter and 25-30cm deep from each sub plot. All the soil from each position was bulked, but the four zones within each sub-plot were kept separate. Thus for each sub-plot there were 4 samples each containing 12 cores of soil.

The soil from each bag of 12 cores was weighed and thoroughly mixed and 1 sub sample of 500g was removed for processing. Where necessary, samples were stored frozen at -20^{o}C prior to processing.

Seed extraction from soil samples

This method of seedbank determination involved extraction and identification of seeds. Direct counting of the extracted seeds determined the total seed numbers in the soil. The technique was based on that described by Roberts and Ricketts (1979). Soil samples were processed using a wet sieving technique. A Fritsch Analysette vibratory shaker was set up with a stack of sieves (4mm, 1mm and 0.5mm, 0.25mm) and set to vibrate/sieve for approximately 20-25 mins depending on soil type. The process was completed once the outflow water was clear, signifying that all the soil had been washed though.

The sieve stack was then dismantled and the top sieve checked for any large seeds. The sediment was washed from the other sieves into a large Petri dish by using a water jet and a brush and the washings inspected for seeds. The sediment was checked for seeds by adding 50-100ml of a saturated solution of calcium chloride ($CaCl_2$) which caused the seeds to float. The solution was stirred and inspected for seeds under a large lens.

Seeds were either identified and recorded immediately or stored in sample bottles for later identification. To determine apparent viability, seeds were inspected and gently squeezed between forceps, firm seeds were assumed to be viable (Ball and Miller, 1990).

4.6 Studies of cross pollination between herbicide tolerant and conventional varieties of winter oilseed rape

Experimental Sites

NIAB, Cambridge

Two blocks of three herbicide tolerant and two conventional winter oilseed rape varieties were established in adjacent areas of 92m x 92m in a 10 hectare field in autumn 1998 (Rotation 1a) at NIAB experimental farm, Cambridge, UK.

Rothamsted Research, Harpenden

Four blocks of three herbicide tolerant and a conventional winter oilseed rape variety (24m x 120m) were established in autumn 1998 (Rotation 1a and 1b) at Rothamsted Research experimental farm, Harpenden UK. (Figure 6.8.5 and 6.8.6).

Scottish Agricultural College, Aberdeen

Four blocks of three herbicide tolerant and a conventional winter oilseed rape variety (24m x 120m) were established in autumn 1998 (Rotation 1a and 1b) at Scottish Agricultural College, Aberdeen, UK. (Figure 6.8.7).

Seed sampling and testing procedure

Location of sampling points at NIAB

Three linear transects across each block were sampled at 1.5m, 6.5m, 11.5m, 16.5m, 21.5m, 41.5m, 61.5m and 81.5m from the adjacent oilseed rape variety. Conventional plots, which contained two winter rape varieties in smaller but equal areas, were sampled at more frequent distances; 1.5m, 6.5m, 11.5m, 16.5m, 21.5m, 26.5m, 31.5m, 41.5m, 51.5m, 61.5m, 71.5m, 81.5m, 91.5m.

Location of sampling points at Rothamsted Research

A similar layout of sampling points was used as described above, although in the smaller plots only 5 distances across each transect were sampled (0m, 5m, 10m, 15m, 20m away from the ‘test’ herbicide tolerant variety).

Location of sampling points at SAC

Samples were taken at the same distances described for Rothamsted Research. Seed samples were only taken from the conventional variety plots and were tested for glufosinate and glyphosate tolerance.

Seed harvesting

The main raceme was removed from 20 plants within a $1m^2$ quadrat at each sample point. Racemes were collected in large cloth bags and dried in ambient conditions for approximately 14 days. Seeds were removed from pods by crushing the racemes in cloth bags and hand sieving to remove debris.

Seed testing for herbicide tolerance

NIAB

Seeds were randomly sub-sampled in order to test two replicates of 1000 seeds per sample using a digital automated seed counter. Seed samples of GM herbicide tolerant and conventional winter oilseed rape varieties were grown in seed trays containing a multi-purpose peat based potting compost under glasshouse conditions. A herbicide susceptible control (a conventional non-tolerant winter oilseed rape variety; Falcon or Apex) was grown with each herbicide tolerance test. Trays were arranged on glasshouse benches using a randomised block design. Plants were sprayed at growth stage 1.2 with either glufosinate-ammonium (200g/l) in a 1% solution, glyphosate (360 g/l) in a 0.5% solution or with imazamox (40g/l + wetter) in a 1% solution, applied using a hand sprayer. The numbers of surviving plants were assessed approximately 10-15 days after treatment for glufosinate, and after 15 -25 days for glyphosate and imazamox. Survivors from each replicate of 1000 plants were re-treated with herbicide at growth stage 1.3. Surviving plants were counted after the same intervals as the first treatment.

Rothamsted Research

The herbicide tolerance test at Rothamsted used a similar method as described for NIAB except 100 seeds were tested per replicate and a 1% solution of both herbicides was applied. All plots were sampled and tested for their tolerance to either glufosinate or glyphosate. No imazamox tolerance testing was carried out at Rothamsted.

Scottish Agricultural College

The same method was used as described above for Rothamsted. No imazamox tolerance testing was carried out at SAC.

4.7 The behaviour of seeds of oilseed rape after crop harvest and in subsequent years

Seed losses at harvest

Estimates of the number of seeds remaining in the field after harvest were obtained by counting quadrats in each plot the day after harvest (NIAB, Rothamsted, Scottish Agricultural College (SAC)), or, by collection of seeds in gutters at harvest (Brooms Barn, Morley). Quadrat and gutter number and size varied according to each site and each year (Table 4.7.1). Numbers of quadrats counted in Year 4 tended to be lower than in the previous years, as the data were intended simply to confirm earlier trends and were not to form the basis of calculations of seed decline in subsequent years.

Table 4.7.1 The number and size of quadrats or gutters used to count oilseed rape seeds for each site and year

Site	Year	Rotation	Seed loss collection method
Rothamsted	1	1	5 quadrats (10x10cm) per plot
	2	4	6 quadrats (10x10cm) per plot
	4	1	3 quadrats (10x10cm) per plot
NIAB	1	1 and 3	10 quadrats (15x15cm) per plot
	2	4	5 quadrats (10x10cm) per plot
	4	1 and 3	3 quadrats (20x20cm) per plot
SAC	1	1	15 quadrats (10x10cm) per plot. (10 quadrats under swathe, 5 outside swathe)
	2	4	15 quadrats (10x10cm) per plot (10 quadrats under swathe, 5 outside swathe)
	4	1	3 quadrats (20x20cm) per plot (2 quadrats under swathe, 1 outside swathe)
Brooms Barn	1	3	4 gutters ($0.22m^2$) per plot
Morley	1	3	3 gutters ($0.22m^2$) per plot

Post-harvest seed counts and seed germination

Rape seeds, or seedlings, post-harvest were counted on Rotation 1a/b (Year 1 and 4) and Rotation 4 (Year 2) at SAC and Rothamsted only. In year 1 and 2 SAC counted two quadrats per plot from under the swathe 19 days post harvest. At Rothamsted in Year 1 all quadrats were re-counted twice, at 10 and 22 days post harvest, whilst in Year 2 all quadrats were counted 3 times at 8, 16 and 22 days after harvest. In Year 4 all quadrats were re-counted 13 days after harvest. The quadrats were the same size as those used to count seeds lost at harvest (Table 4.7.1)

Estimation of the numbers of oilseed rape seeds in the seedbank

Each winter after an oilseed rape crop soil cores were taken at all sites to assess the decline in the rape seedbank (Table 4.7.2). At all sites the cores taken were 2.5cm diameter by 25-30cm depth, to ensure that the soil was sampled down to below the plough layer. The number of cores increased after the first winter as there were fewer rape seeds present. Numbers of cores and month of sampling are given in Table 4.7.2. When possible the soil sample was washed out immediately using a 4mm and a 1mm sieve. Otherwise, the sample was frozen and washed out as above at a later date. Whole seeds were then removed from the contents of the 1mm sieve. The seeds were squeezed to test their viability and viable seed numbers were recorded.

Table 4.7.2 Date and number of cores taken to assess rape seed decline in the seedbank.

Site	Rotation	Number of cores per plot	Dates
Rothamsted	1	40	Jan - Feb 2000 (Yr 2)
		80	Jan - Feb 2001 (Yr 3); Jan 2002 (Yr 4)
	4	80	Feb 2001 (Yr 3); Feb 2002 (Yr 4)
NIAB	1	50	Nov 1999 (Yr 2)
		80	Nov 2000 (Yr 3); Nov 2001 (Yr 4)
	3	50	Jan 2000 (Yr 2)
		80	Jan 2001 (Yr 3); Dec 2001 (Yr 4)
	4	80	Mar 2001 (Yr 3); Mar 2002 (Yr 4)
SAC	1	40	Mar 2000 (Yr 2)
		80	April 2001 (Yr 3); Feb 2002 (Yr 4)
	4	40	April 2001 (Yr 3)
		80	Feb 2002 (Yr 4)
Broom's	3	40	Nov 1999 (Yr 2)
Barn		60	Dec 2000 (Yr 3); Jan 2002 (Yr 4)
Morley	3	24	Mar 2000 (Yr 2)
		30	Feb 2001 (Yr 3)
		60	Feb 2002 (Yr 4)

Intrinsic dormancy of the cultivars of oilseed rape used in the BRIGHT project (Rothamsted test).

Seeds were harvested from the four cultivars grown on the BRIGHT trials in July 1999 at Rothamsted Research (RES) and Brooms Barn (BB). After harvest the seeds were stored in seed store until ready to use (20ºC ± 2ºC). In October 1999 two tests were done. In the first, 50 seeds of the four cultivars, replicated 6 times, were placed in Petri-dishes with 7ml of water, in the light in an incubator at 20^{o}C. These were left for a week and germinated seeds were counted. In the second experiment the same numbers of seeds were placed in Petri dishes containing Polyethylene glycol (PE.G. 6000) at a concentrating aimed to generate a water potential of –1.5Mpa (for details see Pekrun *et al.,* 1997). There were again six replicates. These seeds were placed in the dark in an incubator at a constant temperature of 20ºC for 4 weeks. After 4 weeks seeds were examined under a green safe light and any germinated or mouldy seeds were removed. The seeds were then transferred to clean Petri-dishes containing 8ml of clean water and were returned to the dark incubator for a further 2 weeks. The number of germinated seeds was recorded. This experiment was repeated in January 2000. The data on numbers of germinated seeds were transformed using logit transformations, the results of the two tests were amalgamated and means and standard errors calculated.

Estimates of the proportion of the rape seedbank that emerges and produces new plants.

Following the application of glyphosate and glufosinate to the rape grown in year 4 in Rotation 1, volunteers of the other cultivars arising from the crops sown in year 1, showed symptoms of phytotoxicity and eventually died. These dying plants were counted on random quadrats placed on the affected plots (Table 4.7.3). These assessments were done at NIAB, Rothamsted and the Scottish Agricultural College.

Table 4.7.3 Details of dates and assessment methods used to record the number of dying rape plants present on Rotation 1 in year 4

	NIAB	Rothamsted	Scottish Agricultural College
Date of herbicide application	25 September 01	1 November 01	26 November 01
Date of assessment	1 October 01	19 November 01	31 January 02
Number of quadrats/plot	20	12	10
Size of quadrats	0.25 m^2	1m^2	0.25m^2

At Brooms Barn in year 3 it was possible to record the number of volunteer rape plants in the sugar beet (Rotation 3) prior to the application of herbicides to control it. Counts were done on 25 June and 14 July and the highest value was assumed to represent the maximum number of plants. Twelve $0.5m^2$ quadrats were counted on each plot. It is possible that some of the plants present in May might have died before June and so the later count would have been an under-estimate. However, it was concluded that this would only have affected a minority of plants.

The number of dying plants was compared to the nearest soil seedbank assessment; winter 2001/02 for the rape and winter 2000/01 in the sugar beet crops. Details of the methods used to measure the soil seedbank are given earlier in this Section.

4.8 Statistical Analysis of data

1. All experiments were of a factorial design with variable levels of treatments and replication. Rotation 1 at Rothamsted and SAC had four replicates, whilst the smaller Rotation 1a at NIAB had two replications. Rotations 2 and 3, all had two replications. Rotations 4 and 5 had 3 replicates. Despite the limited replication the smallest trials (Rotation 2) had 18 plots in the key final year and thus 8 df in the error, adequate for assessment of the treatment effects. Plot layouts and arrangements are described in Appendix 10.3.3.
2. Raw data, from the various rotations of the project, were collated and converted to counts per m^2 for all weed assessments, g/m^2 for biomass assessments. Weed seedbank from soil core data, counts/500g soil converted to seeds $/m^2$. Analyses were based at a site within a rotation and decisions were taken at a very early stage not to attempt any over site analyses within any rotation as the distribution of initial (and subsequent) weeds were known to be different at the different sites.
3. Genstat statistical software was used to undertake all statistical analyses. This system was flexible enough to deal with the complexity of the temporal design structure (where second GM events were utilised these were applied perpendicular to the initial primary GM event) and cope with missing values (zero or very few in most main rotations but they did exist within the full compass of the BRIGHT project). Further analytical complexities occurred due to the withdrawl of the 'IMI' treatment in the middle of the project, which GenStat dealt with in a practically workable manner.
4. To focus analytical and interpretative effort on the weeds of agronomic or biological importance, a threshold was initially applied to the data. While this action was likely to aid focus on the major weeds; it also immediately introduced bias in the overall analyses and in particular estimates of flux in species diversity. Thresh-holding was abandoned and all non-zero data retained, analysed and summarised.
5. Concerns about the spatial variability of the weeds within sites led us to explore the potential of applying covariates to the data. The original aim was to apply a suitable covariate, based on 'initial' pre-treatment assessments, to other treatments to standardise the effects to a common density. Both the initial seed

core/weed seedbank data and the autumn (Year 1) pre-treatment were available but both suffered from the same set of limitations. Firstly, there were cases where suitable weeds in the seed cores or autumn count (Year 1) existed but these weeds were not observed in the rest of the experiment (specific rotation and site). Secondly there were cases where the potential covariate had no records for weeds that subsequently were observed. There were also cases where the analysis without covariates showed statistical differences between treatments which failed to achieve significance when covariates were included. While the latter point is a desirable one (reducing the risk of a 'false positive' through application of an appropriate analyses/covariate) it was necessary to note such cases for consistency of interpretation – in many scientific studies, a non-significant result can be just as meaningful as a statistically significant one.

6. Where possible in the main R1 and R3 rotations, analysis using both potential covariates was conducted, but the adjusted treatment means, adjusted for covariates, were only used where the covariate had statistically significant effects.
7. After considerable evaluation of the quality of the data and results of preliminary analysis, all of the weed count data was analysed both as **x** raw counts / m^2 and transformed $\log_{10}(\mathbf{x}+1)$ [catering for zero counts]. The former as an aid to interpretation. Transformation was applied to the lowest strata of experimental unit - the subplot.
8. Data for weed seedbanks were subjected to both raw and $\log_{10}$ transformation but were generally reported in raw counts.
9. For temporal analyses post the primary GM event, the analyses took into account the hierarchical structure of rotation, replicate, plot and sub-plot. Where a second GM event occurred analyses were undertaken based on the initial GM event (carry over effect); the secondary GM event and a 'crossed' analysis where the treatment interaction between 'primary' and 'secondary' GM events were assessed and tested statistically.
10. In all analyses the treatment means and appropriate SED's and LSD's are summarised.
11. Appropriate cross site analysis was discussed, but because of the variability between sites it was decided that this should not be included as part of these initial analyses. More detailed analysis, including variation in 4 year weed trajectories in response to the treatments and multi site comparisons were beyond the scope and resources of this project but are planned for the future using new resources.