ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on the results of a scientific study which investigated the botanical and rotational implications of genetically-modified herbicide tolerance in oilseed rape and sugar beet crops

Date: 18 May 2005

A. Introduction and background

1. The final report of BRIGHT[1], a four year research project which studied the botanical and rotational implications of growing herbicide tolerant crops with the aim to contribute information for guidelines on the management of genetically modified herbicide tolerant (GMHT) crops, was published on 29 November 2004.

2. BRIGHT studied herbicide tolerant sugar beet and oilseed rape grown in rotation with conventional cereals. The project compared different types of herbicide tolerance with each other and with conventional practice. The main focus of the BRIGHT project was on agronomic aspects and efficacy of weed and volunteer control. The study also provides information regarding the environmental impact of GMHT crops.

3. BRIGHT was a Sustainable Arable LINK project and started in autumn 1998 (i.e. one year before the Farm-Scale Evaluations (FSE)). BRIGHT was conducted by a consortium of research institutions (NIAB, Broom's Barn, Rothamsted Research, Scottish Agricultural College (Aberdeen) and Morley Research Centre (now part of The Arable Group)) and companies (Agrovista, BASF, Bayer, BBRO, HGCA and Monsanto).

4. The BRIGHT report was peer-reviewed by four anonymous referees prior to publication on the Home-Grown Cereals Authority's website.[2] Publication of the report received considerable media attention.

5. ACRE considered the BRIGHT report and the implications of this study for assessing the environmental impact of GMHT oilseed rape and beet in their meeting on 3 February 2005. The Committee also considered whether the BRIGHT report has any implications for the advice issued previously by ACRE on the Farm-Scale Evaluations[3].

6. In its advice on the implications of the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops published on 13 January 2004 ACRE concluded as follows concerning beet and spring-grown oilseed rape:

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[1] Botanical and Rotational Implications of Genetically-Modified Herbicide Tolerance (BRIGHT)

[2] http://www.hgca.com/cms_publications.output/2/2/Publications/Publication/Botanical%20and%20rotational%20implications%20of%20genetically%20modified%20herbicide%20tolerance%20in%20winter%20oilseed%20rape%20and%20sugar%20beet%20(BRIGHT%20Project).mspx?fn=show&pubcon=1805

[3] http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice44.pdf

> *46. Based on the evidence provided by the FSE results published in October 2003, if GMHT beet were to be grown and managed as in the FSEs this would result in adverse effects on arable weed populations, as defined and assessed by criteria specified in Directive 2001/18/EC, compared with conventionally managed beet. The effects of arable weeds would be likely to result in adverse effects on organisms at higher trophic levels (e.g. farmland birds), compared with conventionally managed beet.*
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> *55. Based on the evidence provided by the FSE results published in October 2003, if spring-sown GMHT oilseed rape were to be grown and managed as in the FSEs this would result in adverse effects on arable weed populations, as defined and assessed by criteria specified in Directive 2001/18/EC, compared with conventionally managed spring-grown oilseed rape. The effects of arable weeds would be likely to result in adverse effects on organisms at higher trophic levels (e.g. farmland birds), compared with conventionally managed oilseed rape.*

7. ACRE has recently considered an application for the commercialisation of the glufosinate-tolerant oilseed rape that was used in the Farm Scale Evaluations (Ms8xRf3)[4], although the scope of this application has been reduced to exclude cultivation in the EU. There is no current pending application for the cultivation of glyphosate-tolerant oilseed rape in the EU. Concerning beet; there was an application for the cultivation of GM sugar beet when the Farm Scale Evaluations were initiated, but this application has now lapsed. No new application for the cultivation of GM sugar beet in the EU has since been issued although there is a pending application for use of GM sugar beet in food and animal feed.

8. Defra is funding further research on the survival of crop volunteers and weed populations following GMHT crops, on the quantification of geneflow from GMHT crops and the factors influencing such geneflow under UK conditions. Defra has also commissioned research on the environmental impact of crop production practice beyond the FSE.

BRIGHT results

(a) Agronomic aspects

9. The consortium reported that the weed control regimes based on glyphosate or glufosinate-tolerant sugar beet and oilseed rape were effective and flexible and often achieved similar or greater levels of weed and volunteer control as conventional practice, depending on site, season and other factors. Glyphosate in combination with the glyphosate-varieties tended to be the most effective weed control programme, although less so in sugar beet than in oilseed rape.

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[4] http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice48.pdf

10. The consortium concluded that herbicide tolerance in oilseed rape and sugar beet would provide farmers with greater flexibility of herbicide timing and management.

11. Weed management in crops following GMHT crops was not affected except when GMHT sugar beet followed GMHT oilseed rape with the same type of herbicide tolerance. In the latter case additional herbicides were needed to control volunteer rape. Weed control was often more effective in the cereals than in GM or conventional oilseed rape and sugar beet.

12. The use of GMHT crops reduced the amount of herbicide active ingredient applied to sugar beet. In both crops the costs of glufosinate and glyphosate were lower than those of conventional broad-leaved weed and grass weed herbicide treatments.

13. Another advantage of the use of the GMHT crops was the greater control of weeds closely related to the crops (such as weed beet in sugar beet), which are difficult or expensive to control in conventional crops.

14. BRIGHT also studied outcrossing between oilseed rape crops and confirmed results of earlier studies. Cross pollination declined with distance from the source and levels of outcrossing were higher to a varietal associations with a high proportion of male sterile plants due to reduced competition from self-pollen.

15. In BRIGHT, oilseed rape varieties with different types of herbicide tolerance were grown in close proximity to study if volunteer rape resulting from gene stacking of more than one tolerance trait would present a weed control problem. As expected, gene stacking occurred but the commonly used weed control programmes applied in the cereal crops gave good control of all GMHT volunteers.

16. An average of 1000 oilseed rape seeds per m² survived over the 4 year crop rotation, supplying a potential source of volunteers in future crops. The scientists state that this could pose a serious problem with respect to the coexistence of GM and non GM crops grown in sequence in the same rotation. They predict that oilseed rape volunteers derived from a seed bank of GMHT oilseed rape at a level similar to that recorded in BRIGHT would result in the subsequent rape crop breaching the present EU threshold for GM material in non GM crops. Monitoring of BRIGHT sites is continuing as part of an EU funded project. The scientists recommend measures for reducing the volunteer rape seedbank.

(b) Environmental impact

17. The BRIGHT consortium concluded that there was no evidence of any direct environmental effects of the GM plants since differences observed between treatments were explained by the herbicide programmes.

18. GMHT crops required fewer herbicide applications than conventional crops. One application of glyphosate or glufosinate was used in most GM oilseed rape plots. In GM beet, a mean of 1.3 applications of glyphosate and 1.7 applications of glufosinate were used to give effective weed control. In

comparison, one or two herbicide products were normally used in conventional oilseed rape and 2.7 applications (each including several products) were required in conventional beet.

19. Numbers of weed seeds in the soil increased in all rotations and all treatments between the beginning and the end of the BRIGHT study (see Annex A to this paper for further details), a result that differs from the results obtained in the FSE. The increase observed in BRIGHT took place predominantly in the oilseed rape and the beet crops rather than the cereal crops. According to the authors of the BRIGHT report the data of the two studies are not easily compared since BRIGHT assessed seedbank levels over a longer period of time, used different methodology to measure the numbers of seeds in the soil and observed higher initial levels of weed seeds in the soil than the FSE.

20. The impact of the different conventional and GM herbicide treatments on weed density and weed biomass varied greatly between sites, partly due to the differences in herbicide management and the presence of different dominant weed species at individual sites (Annex A). Glyphosate-treated plots had most frequently the lowest weed densities but this was not a consistent effect. Glufosinate-treated plots sometimes had the lowest and sometimes the highest weed densities but more often results from glufosinate-plots were intermediate between the conventional and glyphosate treatments. Although treatments had significant effects on number of plant species present, the direction of these effects varied greatly between sites and years, with the GMHT plots sometimes having more and sometimes fewer weed species compared to conventional plots (Annex A). Carry-over effects of herbicide treatments into subsequent years were observed in some cases but no general pattern emerged. BRIGHT also showed that weed biomass was often much lower in the cereal crops than in any of the oilseed rape and sugar beet treatments.

B. Advice

21. ACRE considered the BRIGHT project a helpful additional set of studies and particularly welcomed its approach of investigating the impact of GMHT crops over several years within the context of agricultural rotations. The Committee concluded that the results of the BRIGHT project are not unexpected based on what is already known about weed germination patterns as well as the known activity range and lack of residual effects of certain herbicides. The Committee noted that both the BRIGHT project and the FSE concluded that the impacts observed were due to the herbicide management regime, not the genetic modification itself. The results of the FSE and BRIGHT project are not directly comparable as they differed in approach and methodology. The FSE was a highly replicated study using large field-scale plots managed by farmers. BRIGHT was a longer term study carried out at research institutes, allowed more flexibility in weed control and used a different experimental design, involving smaller plots and more limited replication than the FSE.

22. The BRIGHT results suggest that it may be possible to manage weeds using GMHT beet or oilseed rape such that the impact on arable weeds would be less or comparable to that of conventionally managed beet or oilseed rape, respectively. ACRE noted the finding of BRIGHT that GMHT crops required fewer herbicide applications than conventional crops.

23. ACRE welcomed that the BRIGHT project attempted to compare conventionally-bred herbicide-tolerant oilseed rape with GMHT oilseed rape. It is unfortunate that no full comparison was possible to a level that good data on this comparison could be obtained.

24. ACRE welcomed the additional information the BRIGHT project provided on levels and survival of GMHT oilseed rape seeds in soil but emphasised that it is important to also establish how the seed bank subsequently translates into seedling and plant density. The Committee noted that Defra is already funding further research on this issue[5].

25. ACRE reinforced its earlier advice that the environmental impact of GMHT crops should be viewed from a wider perspective. In the BRIGHT study conventional winter cereal crops often had a greater impact on weed survival than GMHT oilseed rape and sugar beet crops. The study also demonstrated again the importance of oilseed rape as a crop that gives weeds a chance to replenish their seed bank. ACRE supports the research recommendation of the BRIGHT consortium that there is a need to look at the subject holistically across crop rotations and to address the potential impacts of all crops, not just those potentially including GMHT systems. The findings of BRIGHT will form part of the evidence to be considered in the report of the ACRE Subgroup on the Wider Issues Raised by the Farm-Scale Evaluations of GMHT crops.

C. Annex A

Overview of weed biomass, density, diversity and seed bank data from the BRIGHT project (by the ACRE secretariat)

26. The main focus of the BRIGHT project was on agronomic aspects of genetically modified herbicide tolerant (GMHT) crop management but the data collected by BRIGHT on weed populations were also intended to provide information on the environmental impact of GMHT crops.

27. BRIGHT was conceived and started before the FSE. Although a large field study conducted across several sites, BRIGHT was a much smaller project than the FSE, both in financial and scale terms.

Materials and methods used in BRIGHT

28. In BRIGHT, oilseed rape and sugar beet were grown in rotation with winter cereals (barley or wheat) over a four year period. Data were collected from the cereal crops as well as from the oilseed rape and sugar beet crops. BRIGHT studied the following five four-year rotational scenarios:

- *Rotation 1:* rape – cereal – cereal - rape
- *Rotation 2:* beet – cereal – cereal - beet
- *Rotation 3:* rape – cereal – beet - cereal
- *Rotation 4:* (augmented OSR seed bank)[6] - cereal – rape – cereal - cereal
- *Rotation 5:* (augmented beet seed bank) - cereal – cereal – beet – cereal

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[5] http://www.defra.gov.uk/environment/gm/research/reports.htm#Current

[6] Sown in the first year and ploughed under before main crop was sown to simulate seed shed from a previous crop.

Rotations 1 and 2 were chosen to represent conventional practice for oilseed rape and sugar beet, respectively, as far as the constraints of a 4 year programme allowed. Rotation 3 was chosen as a scenario expected to cause management problems (particularly where glufosinate tolerant beet followed glufosinate tolerant oilseed rape and where glyphosate tolerant beet followed glyphosate tolerant oilseed rape. The main objective of choosing rotations 4 and 5 was to study how the different herbicide systems coped with volunteer oilseed rape and annual weed beet, respectively.

29. The BRIGHT project studied three types of herbicide tolerant winter oilseed rape and two types of herbicide tolerant sugar beet. Herbicide tolerant oilseed rape varieties were either tolerant to glyphosate, glufosinate[7] or imidazolinone herbicides. The first two varieties were genetically modified but the imidazolinone-tolerant variety was developed through conventional plant breeding. The imidazolinone-tolerant variety did not perform as well as the other varieties and was not available beyond the second year of the study. The two herbicide-tolerant sugar beet varieties used in BRIGHT were both genetically modified and were tolerant to either glyphosate or glufosinate. In comparison, the FSE studied glufosinate GMHT oilseed rape (winter and spring varieties) and glyphosate tolerant beet. BRIGHT therefore provides information on two GMHT crops (glyphosate tolerant winter oilseed rape and glufosinate tolerant sugar beet) not studied by the FSE.

30. BRIGHT used a different experimental design than the FSE. In BRIGHT, treatments were replicated within a site and each rotation on each site was analysed separately. The experimental design of BRIGHT involved larger treatment plots in the first year, which were split into sub-plots for the planting of the next HT crop. Deliberate release approvals at the start of the project restricted the area that could be planted, so treatments had only between two and six replicates per site in the first year. Plot sizes and replication varied with site and rotation. BRIGHT plot sizes for the main studies with winter oilseed rape were 0.2 ha in the first year. These plots were subdivided for the second HT crop in year 4 and each sub-plots measured 0.05 ha. For the rotation that included sugar beet and no oilseed rape, initial plots were 0.3-0.6 ha, subdivided for the second HT crop into sub-plots measuring between 0.08 and 0.23 ha. Plots were smaller in the other rotations. BRIGHT used sites in Cambridgeshire, Hertfordshire, Suffolk, Norfolk and Aberdeenshire. Each rotation was tested on two or three sites. In contrast, the FSE used a split-field design and sampled c. 60 fields per crop spread throughout England and Scotland. Fields used by the FSE measured up to 15 ha (half of which would have been planted to HT oilseed rape or beet).

31. Application of herbicide to the herbicide-tolerant plots followed the recommended rates and timings given by the agrochemical companies supplying the herbicides. Decisions on herbicide products and timing for conventional crops were made by managers at each site based on weed infestations present at individual sites.

32. Weed density and weed biomass were assessed for each treatment in each season, for total weeds as well as for individual weed species. Although all plots received the same treatment during years when cereals were grown,

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[7] = glufosinate ammonium

weed density and weed biomass were also assessed during these years to study carry-over effects of treatments applied during previous GMHT oilseed rape or sugar beet crops. Weed seeds in the soil (seed bank) were assessed at the beginning of the project and at the end of the project.

Results

33. A high level of variability was encountered and although statistically significant treatment effects were observed, results from different sites (2-3 sites per rotation) often contradicted each other. Different weed species dominated the weed flora at different sites and this tended to influence the effects of treatments on overall weed numbers. The herbicides used differed in their efficacy against particular weeds and thus resulted in shifts in the weed flora in subsequent years.

34. Results in the report are presented for each rotation and each site separately. Data for abundant weed species are presented in the BRIGHT report for each species individually as well as for total weeds but weed species were not grouped into broad-leaved weeds and grass weeds for analysis.

A. Oilseed rape rotated with cereals

(i) Rotation 1

35. Rotation 1 was the main rotation in BRIGHT, which investigated the effect of herbicide tolerant oilseed rape on weed populations. In this rotation oilseed rape was grown in the first and final years with winter cereal crops grown in the two intervening years. This rotation was tested at three sites. In most cases GMHT oilseed rape plots received one spray of glufosinate or glyphosate.

36. Different weeds dominated at each of the three sites and treatment effects on weed densities (plants/m^2) differed between the sites. In the first oilseed rape year, there was evidence that glyphosate-treated oilseed rape plots had lower total weed densities on two sites compared to conventional and glufosinate-treated plots but not on the third site. Weed density on glufosinate-treated oilseed rape plots did not differ from conventional oilseed rape plots on the first two sites but glufosinate-treated oilseed rape plots had the lowest weed density of all treatments on the third site. The conventionally-bred oilseed rape crop tolerant to imidazolinone had significantly higher levels of total weeds on one site compared to the conventional and GMHT plots but the weed infestation was so high that it resulted in lower yields. No differences in weed density between conventional and GMHT treatments appeared to have carried over into the following cereal crop. Plots treated with glyphosate in the previous year had similar weed densities as conventional plots. Weed densities in the next cereal crop (year 3) showed significant differences due to treatments applied in year 1 on two of three sites. Imidazolinone-treated plots had significantly higher numbers of grass weeds as a carry-over effect of year 1. Conventional plots had significantly higher numbers of *Viola arvensis* (field pansy) and *Myosotis arvensis* than glyphosate or gluphosinate plots on one site but not at the other two sites. During the two cereal years all plots were treated the same but in the fourth year different herbicide regimes were again applied to the oilseed rape crop. The imidazolinone-tolerant variety was not available anymore and was replaced with a second conventional treatment. Effects of herbicide treatments on weed densities varied between sites in year

4. Carry-over effects of year 1 treatments were observed on one site where *Senecio vulgaris* (groundsel) and *Stellaria media* (chickweed) were more numerous in conventional plots than in both types of GMHT plots. Year 4 treatments had significant effects on total weeds or on individual weed species at all sites. Trends were, however, very different between sites, partly due to the presence of different weed species. On one site the conventional plots had the lowest total weed density and glyphosate-treated plots the highest, a trend that seemed to be mainly due to numbers of *Alopecurus myosuroides* (blackgrass), a weed that was not present at the other two sites. *Viola arvensis* was significantly more common on conventional plots than glyphosate-treated plots on two sites but the effect of glufosinate on this species was more variable. This variation may have been due to differences in timing of herbicide applications. *Papaver rhoeas* (field poppy) was also more common on the conventional plots than on glyphosate-treated plots on one site.

37. Treatment effects on weed biomass (g dry weight/m²) followed similar trends as weed density in year 1 but there were fewer statistically significant differences between herbicide treatments. The imidazolinone-treated plots had the highest levels of weed biomass, particularly due to grass weed species. Glyphosate-treated oilseed rape plots had significantly a lower biomass for some weed species on two of the three sites. Weed biomass in the following cereal year was not significantly affected by year 1 treatments on two sites. On the third site a carry-over effect of the less effective weed control of imidazolinone and glufosinate could be detected in the weed biomass data in year 2. In the following cereal year (year 3) *Stellaria media* was more common on conventional plots compared to GMHT plots on one site and *Viola arvensis* on another. Weed biomass results in the oilseed rape crop in the last year (year 4) were again inconsistent. Glyphosate-treated plots had the lowest weed biomass on one site but had similar or higher weed biomass as conventional plots on the two other sites. No carry-over effects from the first oilseed rape crop (year 1) to the second oilseed rape crop (year 4) were observed in the weed biomass data.

38. The assessment of plant species number, as an indicator of plant diversity, showed significant treatment effects, particularly of the treatments applied in year 4, but there were no clear overall trend as to which of the treatments resulted in the most or least species. At two sites fewest species were found in glyphosate-treated plots but this was not case on the third site, where fewest species were found in the glufosinate-treated plots and in one of the conventional treatments. On one site the treatments causing the minimum and maximum number of species were completely opposed in years 1 and 4.

39. Seed bank data are usually highly variable, a fact also observed in BRIGHT. The most striking observation was that the seed bank significantly increased between the start and the end of the experiment on all three sites and in all treatments. The scale of this increase (2 to 11-fold) varied between sites and was not solely due to the presence of oilseed rape seeds. Numbers of *Papaver rhoeas, Stellaria media, Poa annua* and *Alopecurus myosuroides* seeds in the soil also increased considerably, amongst other species. Significant carry-over effects of herbicide treatments in year 1 could be detected in the seed bank on two of the three sites at the end of the experiment. For example, on one site *Alopecurus myosuroides* seeds were lowest on plots that received the conventional treatment in year 1. There were also significant effects arising from the treatments in year 4. For

example, *Stellaria media* seeds tended to be more abundant in plots treated with glyphosate in both year 1 and 4. There was also a tendency for *Poa annua* seeds to be more abundant in glyphosate-treated plots compared to conventional and glufosinate-treated plots.

(ii) Rotation 4

40. Rotation 4 provided some additional information on the impact of GMHT oilseed rape on weed populations. The original purpose of this rotation was to study control of volunteer oilseed rape and the rotation consisted of cereals in year 1, oilseed rape in year 2 followed by cereals in year 3 and 4. The sites for this rotation were undersown with oilseed rape at the start of the experiment to create a seed bank of volunteer oilseed rape. However, unfavourable weather conditions prevented the establishment of a significant oilseed rape seed bank. Consequently, this rotation was treated as an extra set of data to investigate the relative performance of the four treatments and any continued effect on weed levels in the subsequent two cereal crops. This rotation was tested at three sites. GMHT oilseed rape plots received one spray of glufosinate or glyphosate in year 2.

41. Significant treatment effects of herbicides applied to the oilseed rape plots in year 2 were observed in year 2 with all common weed species and also with total weeds, both for weed density and for weed biomass. Imidazolinone-treated plots had in most cases the highest weed densities and biomass. Results for the other treatments differed between sites. On one site conventional plots had significantly lower total weed densities than GMHT plots while on a second site conventional plots had more total weeds than both GMHT treatments while on the third site conventional plots had similar weed densities as glyphosate-treated plots but lower weed densities than glufosinate-treated plots. Of the broad-leaved species that occurred on more than one site, *Galium aparine* (cleavers) and *Veronica persica* (common field speedwell) were consistently least abundant on conventional plots and most abundant on imidazolinone-treated plots. *Veronica persica* was generally more common on glyphosate-treated plots than on glufosinate-treated plots but this was not the case for *Galium aparine*. Results for *Viola arvensis* (field pansy) were very variable between sites. Apart from barley, the only common grass weed species present was *Poa annua* (annual meadow grass) and only on one site. *Poa annua* occurred at the highest density on imidazolinone-treated plots. Conventional plots had significantly higher numbers of *Poa annua* compared to both GMHT crops. Weed biomass data followed a similar trend as the weed density data.

42. Sites also differed in whether effects of the herbicides applied to the oilseed rape crops in year 2 carried over into the subsequent cereal crops. In the cereal crop in year 3 no significant differences arising from the treatments applied in year 2 were recorded on two of the sites (weed densities on one site were below one plant per square metre). Results were different on the third site, where conventional plots had significantly higher total weed densities as well as higher *Poa annua* and *Myosotis arvensis* densities than glufosinate-treated plots on this site in the cereal crop in year 3. Glyphosate-treated plots were intermediate and imidazolinone-treated plots had the highest total weed densities. On this site the same trend was observed in year 4. Significant treatment effects were also observed for the other two sites in year 4. For one site there was evidence that plots treated with imidazolinone had the highest and glyphosate-treated plots the lowest density

of weeds in year 4. In contrast, the second site had significantly higher levels of *Galium aparine* in year 4 on plots previously treated with glyphosate.

43. In rotation 4, plant species number was significantly affected by herbicide treatments in year 2 but much less so in years 3 and 4. However, the treatments with the fewest and highest number of weed species varied across the three sites. On one site the conventional treatment had the lowest species number compared to the GMHT crops while on another site it had more species. The two GMHT oilseed rape crops had similar effects of species number. The imidazolinone treatment had the most variable effect on species number.

B. Sugar beet rotated with cereals

44. Rotation 2 was the main sugar beet rotation. In this rotation two GMHT and a conventional variety of sugar beet were grown in the first and final years with winter cereal crops grown in the two intervening years. This rotation was tested at two sites. However, for one of the two sites weed data are only available for year 4. Glyphosate-tolerant sugar beet received one or two glyphosate sprays in the first year but only one glyphosate spray in the last year. Glufosinate-tolerant sugar beet plots received two glufosinate sprays in the first year and one or two glufosinate sprays in the last year. Rotation 5 concentrated on weed beet and volunteer beet control only and is therefore not included here.

45. Half or more of the weeds present in the beet crop in year 1 were *Chenopodium album* (fat hen). Although significantly lower densities of this species were found on conventional plots in the pre-treatment counts of year 1 (partly due to different herbicide timings), there were no significant treatment effects after herbicide application. In the cereal crop in year 2 significant differences in weed density between treatments were only found with *Matricaria* spp (scentless mayweed, pine apple weed), numbers of which were significantly higher in the conventional treatment. Plots treated with glyphosate in year 1 had slightly higher densities of this genus than glufosinate-treated plots. No significant carry-over effects of treatments were found in the cereal crop of year 3. Total weed densities were almost identical in the three treatments in the beet crop in year 4 on this site. However, there were significant treatment effects in the density of most individual weed species. On this site, conventional plots had higher *Poa annua* and *Capsella bursa-pastoris* (shepherd's purse) numbers but lower *Fallopia convolvulus* (black bindweed), *Senecio vulgaris* (groundsel), *Veronica persica* (common speedwell), *Cirsium arvense* (creeping thistle) and *Chenopodium album* numbers compared to GMHT plots. Densities of individual weed species on glufosinate-treated plots were mostly intermediate between conventional and glyphosate-treated plots. No carry-over effects of year 1 treatment on weed densities in year 4 were observed. Year 4 results were different for the second site. Although no significant differences between individual species were found on this site, there were strong treatment effects on total weed density. Conventional plots had the lowest total weed densities and glufosinate-treated plots had the highest weed density.

46. Weed biomass assessments in the beet crop in year 1 did not show any significant treatment effects for any weed species nor for total weeds. There was, however, a trend for greater total weed biomass (mainly due to *Chenopodium album*) on conventional plots compared to GMHT plots.

Glyphosate-treated plots tended to have the lowest total weed biomass and glufosinate-treated plots were intermediate. Weed biomass was an order of magnitude lower in year 2 when cereals were grown. There were no significant carry-over effects of treatments applied in the beet year, apart from *Viola arvensis* (field pansy), which produced more biomass on conventional plots. There was a trend for higher total weed biomass in the glufosinate-treated plots compared to conventional and glyphosate-treated plots. No significant differences in weed biomass were found between treatments in the cereal crop in year 3 although there was a trend for higher weed biomass on conventional plots compared to GMHT plots, a trend which was mainly due to *Cirsium vulgare* (spear thistle) and *Poa annua*. Glyphosate-treated plots tended to have the lowest weed biomass of the three treatments. In year 4, total weed biomass did not differ significantly between treatments on this site although a trend was apparent for a higher total biomass in the conventional beet plots and for glyphosate-treated plots to have the lowest weed biomass. Statistically significant differences were only observed with one weed species: *Chenopodium album* biomass was dramatically higher in conventional beet compared to both GMHT beet varieties. Some of the differences were caused by poor crop competition and a lower crop density due to damage by wildlife early in the season when the conventional plots were weed-free and the crop plants were the only vegetation present. No year 1 or interaction effects were observed. On the second site, significant treatment effects on total weed biomass were observed in year 4. In contrast to the first site, glufosinate-treated plots had a significantly higher total weed biomass compared to conventional and glyphosate-treated plots. The first site had received two and the second site only one spray of glufosinate.

47. Seed bank data were only available for the first site. *Chenopodium album* dominated the seed bank on this site. An increase in the numbers of seeds in the soil was observed in all treatments, mainly due to changes in the level of *Chenopodium album*. The seed bank increase appears to have been linked to the seed production in the two sugar beet years, as few weed seeds were produced in the cereal years. There was a trend for conventional plots to have the highest numbers of *Chenopodium album* seeds as an effect of both year 1 and year 4 treatments. Numbers of *Chenopodium album* seeds in the soil were slightly lower in glyphosate-treated plots compared to glufosinate-treated plots. Sugar beet seeds did not contribute to the seed bank increase, since reproductive shoots (bolters) of sugar beet were removed as a requirement of the release consent.

C) Oilseed rape rotated with sugar beet and cereals (rotation 3)

48. Rotation 3 was established to explore the impact of planting two different herbicide tolerant GMHT crops in quite close succession in the same rotation. Oilseed rape was grown in the first year, cereals in the second, sugar beet in the third and cereals in the fourth and final year. This rotation was tested on three sites. GMHT oilseed rape plots received one spray and GMHT sugar beet plots one or two sprays of either glyphosate or glufosinate.

49. Weed density data produced contradicting treatment effects across sites in the oilseed rape crop grown in year 1. On one site conventional plots supported significantly more weeds than either of the GMHT crops, while on another site conventional plots supported significantly fewer weeds and on the third site there was no statistically significant difference between treatments. Glufosinate and glyphosate treatments had similar effects on

weed densities. In the pre-treatment count of year 2 significantly higher levels of total weeds, Stellaria media and Veronica spp. were observed in the conventional plots on one site while plots of the two GMHT treatments did not differ significantly from each other. After herbicide treatment no statistically significant differences in total weed density between plots were observed in year 2. Individual weed species were also not significantly influenced by treatments with the exception of wheat and oilseed rape volunteers. In the sugar beet crop grown in year 3 results varied again across sites. Significant carry-over effects of treatments applied to the oilseed rape crop in year 1 on total weed density and on some individual weed species in year 3 were observed in pre-treatment counts on two sites. However, while conventional plots carried most weeds on one site, glufosinate had the highest total weed density on another. Differences between sites were partly attributable to the timing of herbicides. Post-treatment weed counts in year 3 showed significant effects of treatments in year 1, year 3 as well as interactions between these years on all three sites but no general trend could be detected. On one site conventional plots had significantly higher total weed density as a carry-over effect of year 1 treatments. Effects of year 3 treatments differed between sites. Plots with glufosinate-tolerant beet had significantly higher total weed densities compared to conventional and glyphosate-tolerant beet on one site but on another site conventional beet had the highest weed densities. Significant effects of treatments applied to the oilseed rape crop in year 1 could still be observed in the cereal crop in year 4 at the pre-treatment count in year 4 on one site. There were, however, no significant differences in weed densities between plots as a result of treatments applied to the sugar beet crop in year 3. After herbicide application, the only significant treatment effects found in the spring count were with oilseed rape volunteers on one site, which continued to show an effect of treatments applied to the oilseed rape crop in year 1. On one site weed densities in year 4 were too low for analysis.

50. Total weed biomass in the oilseed rape crop in year 1 was significantly higher in conventional plots compared to one or both of the GMHT oilseed rape crops on two sites. However, on the third site the conventional plots had a lower weed biomass than both GMHT oilseed rape crops. Total weed biomass in the sugar beet crop in year 3 was significantly higher in conventional plots compared to the glyphosate plots on two sites. Total weed biomass on these two sites was similar on conventional and glufosinate plots. However, on the third site there was a strong trend for lower weed biomass on the conventional plots compared to both GMHT oilseed rape crops although the differences were not statistically significant. Total weed biomass in the cereal crops grown in years 2 and 4 was not significantly affected by treatments applied in year 1 or year 3 on any of the three sites.

51. Seed bank data were only available for two sites. Because of the high variability in seed bank data, no overall pattern emerged for treatment effects. Herbicides applied to the oilseed rape plots in year 1 or sugar beet plots in year 3 had no significant effects on the total final seed bank. At one site, *Poa annua* seeds were significantly more common on glufosinate-treated plots than on conventional plots at the end of the experiment, a cumulative effect of less effective *Poa annua* control by glufosinate both in years 1 and 3. *Poa annua* seed numbers in the glyphosate treatment were intermediate. On the second site, *Veronica* spp. were significantly less common on GMHT plots than on plots treated with conventional herbicides in year 1. The total number

of weed seeds in the soil increased on both sites between year 1 and year 4 (between 1.3- and 2.5-fold).