Report

Heard, M.; Brooks, D.. 2006 *Farm Scale Evaluations follow-up biodiversity assessments of weeds and soil seedbank: 2004-2006.* Final Report. Centre for Ecology and Hydrology, 18pp. (CEH: Project Report Number C02632)

Farm Scale Evaluations Follow-up Biodiversity Assessments of Weeds and Soil Seedbank: 2004-2006

FINAL REPORT

June 2006

Matthew Heard
CEH Monks Wood, Abbots Ripton, Huntingdon, Cambs.

David Brooks
Rothamsted Research, Harpenden, Herts

EXECUTIVE SUMMARY

1. This study was commissioned to monitor the effects of the management of genetically modified herbicide tolerant (GMHT) crops on the weed flora in subsequent crops. It was undertaken on behalf of Defra to complete the full round of monitoring for the Farm Scale Evaluations (FSEs).
2. The study completed all t+1 and t+2 measurements for spring-sown beet, maize and oilseed rape and the autumn-sown winter oilseed rape remaining at the end of the financial year 2003/04. These data have been incorporated into the FSEs publicly accessible database along with all other base-year (t), t+1 and t+2 measurements.
3. The weed seedbank and above ground counts of weeds and their biomass were monitored at the FSE sites originally sown with 13 beet, 45 winter and 24 spring oilseed rape and 27 maize crops.
4. Weed seedbanks were assessed by identifying germinating seedlings from field collected soil cores in glasshouses. Field surveys later in the season were used to assess the densities of weeds in different size classes and total weed biomass.
5. Weed seedbanks following GMHT maize were significantly higher than following conventional crops for both the first and second years. In contrast, dicot seedbanks following GMHT spring and winter oilseed rape were significantly lower over this period. Seedbanks following GMHT beet were smaller than following conventional crops in the first year after the crops had been sown, but this difference was much reduced by the second year.
6. There were few significant treatment effects on above ground vegetation in following years due mainly to efficacious herbicide management in the subsequent cereal dominated crops. Any significant effects on above-ground weed densities mirrored the significant treatment effects on weed densities in the seedbank.
7. In general densities of reproductive plants were very low in the subsequent crops. In beet and spring oilseed rape the numbers of dicot reproductive plants were much reduced relative to year *t* (2- to 4-fold reduction in beet, 9- to 18-fold reduction in rape). In maize the opposite was true with slightly increased densities. Reproductive monocot densities had generally slightly higher densities than in year *t.*
8. There were no treatment effects on weed biomass production in subsequent crops. In general mean weed biomass production was much reduced relative to production in the conventional break crops in year *t*.
9. These data provide important empirical evidence for longer-term effects of GMHT cropping on farmland biodiversity.

INTRODUCTION AND RATIONALE

The Farm Scale Evaluations (FSEs) were a four-year programme of research designed to compare the effects of weed management practices associated with four genetically modified herbicide-tolerant (GMHT) and conventional crops on farmland wildlife in the UK. They were initiated in response to concerns that the introduction of GMHT crops might further exacerbate the substantial declines in farmland wildlife that had been observed in the UK in the latter half of the twentieth century which have been linked to agricultural intensification (Robinson & Sutherland, 2002). The potential ecological effects of GMHT cropping on farmland wildlife must be considered in this context. GMHT crop management can achieve significantly more efficient weed control than conventional crop management (Buckmann *et al.*, 2000; Dewar *et al.*, 2000). There is concern that if widely adopted in the UK, these crops may exacerbate the negative impacts of farming on farmland biodiversity by reducing populations of weeds that are important resources of food and habitat for animals (Johnson, 1999; Hails, 2000; Watkinson *et al.*, 2000).

The main focus of the FSEs was to test, for a given set of indicator groups, the null hypothesis of no difference in abundance of key indicator groups between the GMHT and conventional treatments, and to estimate the sizes of treatment effects. The initial results were published in 2003 and 2005 (Brooks *et al.*, 2003; Champion *et al.*, 2003; Haughton *et al.*, 2003; Hawes *et al.*, 2003; Heard *et al.*, 2003a; Heard *et al.*, 2003b; Roy *et al.*, 2003; Bohan *et al.*, 2005). They showed that GMHT crops impact upon the richness and abundance of species in and around arable fields because of the efficacy of the herbicides applied to control weeds (Firbank *et al.*, 2003a). The paper on the effect of GMHT winter oilseed rape provided evidence of persistent differences in weed seedbanks between GMHT and conventional cropping systems for two years after the crops had been sown (Bohan *et al.*, 2005). Such differences could lead to longer-term effects on weed populations, in turn affecting animal populations higher up the arable food chain by altering the quality and quantity of forage resources (Watkinson *et al.*, 2000; Hawes *et al.*, 2003). The evidence for longer-term trends in weed seedbanks following spring-sown GM cropping systems was much less conclusive, possibly due to the small sample sizes available at the time of publication (Heard *et al.*, 2003a).

In this report we revisit this issue, by analysing weed seedbank data, and weed densities and biomass data collected prior to harvest in crops following both GMHT and conventional beet, maize and spring and winter oilseed rape. These data include all follow-up samples taken during 2003, 2004 and 2005. Effects on the seedbank and emerged vegetation are particularly relevant since these are the primary organisms at which the crop management was aimed. The study assessed whether the effects found in the year of the comparison fade or disappear in the two subsequent years, testing the hypothesis that GMHT and conventional cropping cause no subsequent difference in seedbank, vegetation or field management.

METHODS

Overall design of the Farm Scale Evaluations

The FSEs were a randomised block experiment comparing GMHT and conventional cropping systems, in which the two treatments were allocated to half-fields at random. Each crop (beet, maize, spring oilseed rape and winter oilseed rape) was considered as a separate experiment (Perry *et al.*, 2003). For each crop, about 60 fields were selected from a pool on the basis that they satisfied a number of criteria relating to environmental and farm management regimes and agricultural intensity. This provided a sample of sites throughout the lowlands of Britain, which was broadly representative of current agriculture (Firbank *et al.*, 2003b).

Crop management

Details of crop management, including the timing and type of pesticide applications, used in the experimental year are given by (Champion *et al.*, 2003). All management decisions for the conventional crops were made by the farmers, who were asked to apply 'cost effective' weed control using their normal practices. Advice on herbicide applications to the GMHT crops was provided by simulated manufacturer labels and SCIMAC (Supply Chain Initiative for Modified Agricultural Crops) advisers where necessary. In general the GMHT crops received less herbicide active ingredient per crop with later and fewer applications than the conventional varieties. Inputs for each site were audited by agronomists qualified under the British Agrochemical Supply Industry Scheme (BASIS). They confirmed that overall the management was appropriate and reflected current conventional practice.

In subsequent years (t+1, t+2) growers followed their normal crop rotations and grew crops of their choice in the fields Crop management was recorded for these crops using a farmer questionnaire. The following was recorded: crop type and drilling date, basic categorisations of crop (cereal vs. non-cereal; winter or spring crop), primary cultivation type and date (also basic categorisation of inversion or non-inversion tillage), herbicide use (pre-emergence herbicides types, application rates and dates; post-emergence herbicide types, application rates and dates). All records were audited by BASIS qualified agronomists.

Vegetation response

The vegetation was sampled systematically from 12 transects around the edge of each half-field (Heard *et al.*, 2003a). Transects ran from the field margin out into the crop, with sampling points located at 2 m, 4 m, 8 m, 16 m and 32 m from the field margin. Previous work has shown that species richness and abundance decline rapidly with distance from field boundaries (Marshall, 1989; Wilson & Aebischer, 1995) and that there is typically little difference between values at 32 m and those in the middle of a field (Critchley & Fowbert, 2000).

The seedbank was sampled to compare the effect of treatments on seed densities across a wide range of arable sites. For the follow-up measurements soil samples were taken in spring (for beet, maize and spring oilseed rape) and autumn (for winter oilseed rape) at the same sample locations at approximately the same time of year as initial FSE samples. Samples were taken at a subset of the loci sampled for vegetation in each half of the split field, at 2 and 32 m on four out of the twelve transects (to capture potential differences between edge and field centre; figure1). About 1.5 kg of soil was sampled to

a depth of 0.15 m at each locus using a soil auger or spade, and then weighed and passed through a sieve of mesh size 10 mm. Stones exceeding 10 mm in diameter were removed and weighed. About 1.2 litres of the sieved sample was weighed and placed in a plastic tray to a depth of 40 mm. The trays were arranged in an unheated glasshouse on benches fitted with capillary matting, which was kept moist. Emerged seedlings were removed and identified. Typically, 80% of the seedbank emerges in the first flush of seedlings using this technique, but additional seedlings can still appear up to two or three years later. In this study, the number of seedlings of each species emerging during the first flush, up to 18 weeks after sample preparation, was taken as the standard measure of seedbank composition. The number of seedlings emerging from a tray was expressed per unit field area to the sampling depth of 0.15 m.

Above-ground vegetation was sampled in following crops (t+1, t+2) at all sites between May 27th and July 11th for each year. Counts of individual plants, identified to species, were made on a subset of six transects at the same locations as previous counts (reduced from 12 used in year t). Weeds were counted in quadrats of size 0.25 m × 0.5 m with the longest side centered on sampling points 2 m, 4 m, 8 m, 16 m and 32 m along each of 6 of the transects. Exceptionally, when densities of some species were very high (e.g. >100 per quadrat, equivalent to 800 plants m^{-2}), counts were made for these species in a half or quarter of the quadrat selected at random and multiplied as applicable to achieve standardized estimates of density. Plants were recorded in three development classes: plants with fewer than four leaves (excluding cotyledons), plants with four or more leaves but not flowering, and reproductive individuals either flowering or seeding. At all stages, moribund plants were ignored unless they were reproductive individuals dying back after having shed seed. From 2003 onwards weed biomass was sampled at the same time. Samples were taken at 2 m and 32 m from the field edge along each transect using a 0.25 m × 0.5 m quadrat (the same quadrat used for individual counts). All weeds rooted within the boundary of the quadrat were cut at ground level, sorted to species, dried for 24 hours at 80°C and weighed.

Crops

For the spring sown crops, the follow-on measurements were made in 64 fields originally sown in 2002 (13 beet, 27 maize and 24 spring rape). In these fields t+2 seedbank samples were taken in spring 2002 and t+2 vegetation measurements were made in summer 2004. For winter oilseed rape the situation was a little more complicated, since crops mature the calendar year after they are sown. In this case t+2 vegetation samples were made in June 2004 in 29 fields originally sown in autumn 2001. In the 16 fields originally sown in autumn 2002 t+1 vegetation measurements were made in summer 2004, t+2 seedbank samples were made in autumn 2004 and t+2 vegetation samples were made in summer 2005.

Statistical Analysis

The statistical models and analyses developed for the FSE have been set out in detail elsewhere (Perry *et al.*, 2003). The main objective was to determine whether the total density of weeds (all species lumped together) differed between the GMHT and conventional treatments in subsequent crops. Separately for each crop, the number of individuals in each half-field was analysed by a standard randomized block ANOVA.

The field was the blocking factor, with the treatments (conventional or GMHT) replicated once in each field. Data were log-transformed prior to analysis with the total count, c_{ij}, per half-field, for treatment i at site j, transformed to $l_{ij} = \log(c_{ij}+1)$. Sites for which the whole-field total count was zero or one were excluded. Let n be the number of sites remaining to be analysed. The null hypothesis was tested with a paired randomization test using the test statistic $d = \Sigma_j [l_{2j} - l_{1j}] / n$. Further analyses separated plants into two groups (monocots and dicots) and three development classes. These categories were analysed similarly.

The second objective was to determine the effects of the treatments on weed biomass. In this analysis the total mass, in grams, w_{ij}, of weeds collected in each half-field was log-transformed to $m_{ij} = \log (w_{ij} + 0.005)$ (the added constant being half of 0.01 g, the minimum measurable mass per sample). Sites for which the whole-field total biomass was zero were removed from the analysis.

Treatment effects were estimated by R, the multiplicative treatment ratio (GMHT/conventional), calculated as $R = 10^d$. Confidence limits about R were obtained by back-transformation of the confidence interval of d on the logarithmic scale, derived from the standard error of d and $t_{(0.05)}$. For each treatment, average counts across sites were calculated as the geometric mean, defined as the antilog of the mean log-transformed counts minus one.

RESULTS

Seedbank

Weed seed densities increased following the conventional beet crops but were the same before and after GMHT beet crops, generating significant differences in total (R=0.79), monocot (R=0.76) and dicot (R=0.77) seedbanks for year t + 1 (Tables 1, 2 & 3). In general for this crop monocots decreased and dicots increased from their original densities in both halves of the field. In the second year (*t+2*), the differences between treatments were much reduced as seedbank densities in conventional crops fell back to their approximate year t levels (Tables 1, 2 & 3). Significant differences after beet crops were also observed for two individual species, *Persicaria maculosa* and *Stellaria media* (Table 4). In the second year (*t+2*), the differences between treatments were much reduced as seedbank numbers in conventional crops fell back to t levels (Table 1), and were no longer significant except for *P. maculosa* (Table 4).

Dicot and total weed seedbanks were significantly higher following GMHT maize than conventional maize in both the first (t+1) and second years (t +2) after the crops had been sown (Tables 1 & 2). Again, *P. maculosa* was one of the species showing a significant effect, the other being *Capsella bursa-pastoris* (Table 2).

Dicot seedbanks increased following both GMHT and conventional spring oilseed rape crops, but at a faster rate following conventional crops, resulting in significant treatment effects for both dicot and total weed seedbanks in year t +1, that persisted at the same levels in year t +2 (Tables 1 & 2). Again, significant effects were observed for *P. maculosa* and *Capsella bursa-pastoris*, along with several other species (Table 4).

In winter oilseed rape the treatment effects on dicot seedbanks were similar to those observed in spring oilseed rape (R=0.7) with increases (approximately 1.6-fold) observed in both treatments. This treatment effect and approximate seedbank densities

were maintained in year *t*+2. In contrast there was no significant treatment effect on monocot seedbanks in any year, although they increased 1.3- fold from *t*+ 1 to *t*+2.

Plant density, size and biomass

Plant densities in the following crops averaged 28.37m^{-2} in year *t*+1, and 27.5m^{-2} in year *t*+2. About 81% of the *t*+1 following crops were cereals or other monocot crops, 15% broad-leaved crops and 4% set-aside. In the following year (*t*+2) about 64% were cereals, 31% broad-leaved crops and 5% set-aside.

There were few significant treatment effects in the following years although weed densities in spring oilseed rape were significantly lower on previous GMHT half-fields for both *t*+1 (*R*=0.75) and *t*+2 (*R*=0.75) years reflecting similar treatment effects forseedbanks (Table 1). In winter oilseed rape dicot weed densities were significantly reduced in both follow-up years on previous GMHT half-fields. Again, these effects were of a similar magnitude (*R* = 0.66 and 0.77 in each year respectively) to the treatment effects observed in the seedbank (Table 2). Monocot densities were reduced in the GMHT treatment in the first beet follow-up crop (*R*=0.72) but greater in the crops following winter oilseed rape (*R*=1.57) (Table 3).

In contrast to year *t*, there were also relatively few significant treatment effects on plant size classes (Tables 5 & 6). In general densities of reproductive plants were very low in following years (mean dicot density = 1.6 m^{-2} and 2.1 m^{-2}, mean monocot density = 6.7 m^{-2} and 3.7 m^{-2}). In beet and spring oilseed rape the numbers of dicot reproductive plants were much reduced in subsequent crops relative to year *t* (2- to 4-fold reduction in beet, 9- to 18-fold reduction in rape). In maize the opposite was true with slightly increased densities. Reproductive monocot densities had generally slightly higher densities than in year *t.*

There were no treatment effects on weed biomass production in subsequent crops (Table 1). In general mean weed biomass production was much reduced in subsequent crops relative to the conventional break crops in year *t*. In beet mean biomass was 2.9-fold lower in *t*+1 and 1.6-fold lower in *t*+2; in maize only slightly reduced in *t*+1, and 5-fold lower in *t*+2; in spring and winter oilseed rape it was 11-fold lower in the first year and 18-fold lower in the second year.

CONCLUSIONS

The complete follow-up data set shows that differences in weed (and dicot) seed production within GMHT and conventional crops resulted in significant differences in weed seedbanks at the start of the following springs (*t*+1, *t*+2) for spring and winter oilseed rape and maize. This extends earlier reported findings (Heard *et al.*, 2003a; Bohan *et al.*, 2005) by showing that these effects persist for longer and in many cases the results are more robust. The results also demonstrate that the treatment effects on seedbanks can also affect plant recruitment in subsequent crops.

The size of the treatment effects on dicot seedbanks following maize, spring and winter oilseed rape were consistent in both the first and second years after the treatments had been imposed. This finding implies that GMHT spring and winter oilseed rape cropping, if managed in the same way as in the FSE, will depress dicot seedbanks for at least several seasons under current commercial agriculture, while maize GMHT cropping may raise dicot seedbank levels. The low densities and low biomass of reproductive dicot

plants in the mainly cereal crops means that there were few opportunities for plants to produce seed and replenish their seedbanks thus maintaining the treatment effects. In maize the slight increase in the density of reproductive dicots resulted from the mainly cereal weed management regime being slightly less effective than that predominantly observed for maize (Perry *et al.*, 2004). The herbicide management strategies in cereal crops are mainly targeted at dicot control, through the use of selective herbicides. In general, the relatively few treatment effects observed for above ground vegetation are likely due to the effectiveness of this subsequent control. .

The situation appears more complex for beet, because the apparent reduction in treatment effect among the dicots in year t+2 is influenced by a significant treatment x year interaction for some species, notably *S. media*. The reason for this observation is not clear. It may only be a chance effect, given that the confidence limits for $R_{(t+1)}$ and $R_{(t+2)}$ overlap. However, it may be explained by density dependent changes in numbers of dicots, that are consistent with longer-term treatment effects in rotations dominated by cereal crops (Heard *et al.*, 2005). The situation is more complex for beet because the apparent reduction in treatment effect for dicots in year t+2 disguises a significant treatment x year interaction for some species, notably *S. media*. The reason for this is not clear, and it may be a chance effect restricted to certain crops, as analyses focusing on only those sites that were followed by winter cereal crops gave results more similar to those from t+1 (Heard *et al.*, 2005).

Break crops such as those studied within the FSEs are important for maintaining weed populations, especially dicots, as seed production tends to be much higher than during the rest of the rotation, especially if it includes mostly cereals. Therefore, the treatment differences in seedbank levels reported here for maize and the oilseed rapes are likely to increase from rotation to rotation, assuming that crop management remained similar to that used in the FSEs (Heard *et al.*, 2005). We therefore conclude that the differences in dicot seed production between GMHT and conventional maize and the oilseed rape crops (and, less certainly, beet) crops are likely be perpetuated for at least two seasons afterwards, and that these data provide important empirical evidence for longer-term effects of GMHT cropping on farmland biodiversity.

ACKNOWLEDGEMENTS

We would like to thank all those at CEH, Rothamsted Research and SCRI involved in sample collection and data processing and the farmers involved for allowing access to their land.

REFERENCES

Bohan, D., Boffey, C., Brooks, D., Clark, S., Dewar, A., Firbank, L., Haughton, A., Hawes, C., Heard, M., & May, M. (2005) Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. *Proceedings: Biological Sciences*, **272**, 463-474.

Brooks, D.R., Bohan, D.A., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S., Clark, S.J., Dewar, A.M., Firbank, L.G., Perry, J.N., Rothery, P., Scott, R.J., Woiwod, I.P., Birchall, C., Skellern, M.P., Walker, J.H., Baker, P., Bell, D., Browne, E.L., Dewar, A.J.G., Fairfax, C.M., Garner, B.H., Haylock, L.A., Horne, S.L., Hulmes, S.E., Mason, N.S., Norton, L.R., Nuttall, P., Randle, Z., Rossall, M.J., Sands, R.J.N., Singer, E.J., & Walker, M.J. (2003) Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1847 - 1862.

Buckmann, H., Petersen, J., Schlinker, G., & Marlander, B. (2000) Weed control in genetically modified sugar beet - two year experiences of a field trial series in Germany. *Journal of Plant Diseases and Protection*, 353-362.

Champion, G.T., May, M.J., Bennett, S., Brooks, D.R., Clark, S.J., Daniels, R.E., Firbank, L.G., Haughton, A.J., Hawes, C., Heard, M.S., Perry, J.N., Randle, Z., Rossall, M.J., Rothery, P., Skellern, M.P., Scott, R.J., Squire, G.R., & Thomas, M.R. (2003) Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1801 - 1818.

Critchley, C.N.R. & Fowbert, J.A. (2000) Development of vegetation on set-aside land for up to nine years from a national perspective. *Agriculture, Ecosystems and Environment*, **79**, 159-174.

Dewar, A.M., Haylock, L.A., Bean, K.M., & May, M.J. (2000) Delayed control of weeds in glyphosate-tolerant sugar beet and the consequences on aphid infestation and yield. *Pest Management Science*, **56**, 345-350.

Firbank, L.G., Bennett, S., Bohan, D.A., Brooks, D.R., Champion, G., Clark, S.J., Daniels, R.E., Dewar, A., Fairfax, C., Haughton, A.J., Hawes, C., Haylock, L., Heard, M.S., Hill, M.O., Hulmes, S., Lovell, L., May, M., Nuttall, P., Osborne, J.L., Parish, A., Perry, J.N., Randall, Z., Rossall, M.J., Rothery, P., Roy, D.B., Scott, R.J., Singer, E., Skellern, M.P., Squire, G., Todd, A.D., Woiwod, I.P., & Young, M. (2003a) The potential implications of spring-sown GMHT crops on farmland biodiversity. *Philosophical Transactions B.*

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., & Perry, J.N. (2003b) An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology*, **40**, 2-16.

Hails, R.S. (2000) Genetically modified plants - the debate continues. *Trends in Ecology and Evolution*, **15**, 14-18.

Haughton, A.J., Champion, G.T., Hawes, C., Heard, M.S., Brooks, D.R., Bohan, D.A., Clark, S.J., Dewar, A.M., Firbank, L.G., Osborne, J.L., Perry, J.N., Rothery, P., Roy, D.B., Scott, R.J., Woiwod, I.P., Birchall, C., Skellern, M.P., Walker, J.H., Baker, P., Browne, E.L., Dewar, A.J.G., Garner, B.H., Haylock, L.A., Horne, S.L., Mason, N.S., Sands, R.J.N., & Walker, M.J. (2003) Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1863 - 1877.

Hawes, C., Haughton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.R., Champion, G.T., Dewar, A.M., Heard, M.S., Woiwod, I.P., Daniels, R.E., Young, M.W., Parish, A.M., Scott, R.J., Firbank, L.G., & Squire, G.R. (2003) Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1899 - 1913.

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J., Parish, A.M., Perry, J.N., Rothery, P., Scott, R.J., Skellern, M.P., Squire, G.R., & Hill, M.O. (2003a) Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1819 - 1832.

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J., Parish, A.M., Perry, J.N., Rothery, P., Roy, D.B., Scott, R.J., Skellern, M.P., Squire, G.R., & Hill, M.O. (2003b) Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. II. Effects on individual species. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1833 - 1846.

Heard, M.S., Rothery, P., Perry , J.N., & Firbank, L.G. (2005) Predicting longer-term changes in weed populations under GMHT crop management. *Weed Research*, **45**, 1-8.

Johnson, B. (1999) Go slow for GMOs. *Plantlife*, **Spring 1999**, 6-7.

Marshall, E.J.P. (1989) Distribution patterns of plants associated with arable field edges. *Journal of Applied Ecology*, **26**, 247-257.

Perry , J.N., Firbank, L., Champion, G., Clark, S., Heard, M., May, M., Hawes, C., Squire, G., Rothery, P., Woiwod, I., & Pidgeon, J. (2004) Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. *Nature*, **428**, 313-316.

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

Robinson, R.A. & Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, **39**, 157-176.

Roy, D.B., Bohan, D.A., Haughton, A.J., Hill, M.O., Osborne, J.L., Clark, S.J., Perry, J.N., Rothery, P., Scott, R.J., Brooks, D.R., Champion, G.T., Hawes, C., Heard, M.S., & Firbank, L.G. (2003) Invertebrates and vegetation of field margins

adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **358**, 1879 - 1898.

Watkinson, A.R., Freckleton, R.P., Robinson, R.A., & Sutherland, W.J. (2000) Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science*, **289**, 1554-1557.

Wilson, P.J. & Aebischer, N.J. (1995) The distribution of dicotyledonous arable weeds in relation to distance from the field edge. *Journal of Applied Ecology*, **32**, 295-310.

Table 1. Weed seedbank densities (numbers m^{-2} in top 15 cm), plant densities (numbers m^{-2}) and biomass (g m^{-2}) per half-field in relation to crop, sampling occasion and treatment. Values are geometric means for GMHT and conventional (C) treatments. Multiplicative treatment ratio, $R = 10^d$, where d is the mean of the differences between GMHT and C treatments on the logarithmic scale; confidence limits for R are back-transformed from those for d. CI, confidence interval.

sampling occasion, year	*n*	C	GMHT	*R* (95% CI)	*p*-value
beet					
seedbank, *t* +1	61	2367.34	1861.03	0.79 (0.67-0.92)	0.002**
follow-up, *t* +1	61	35.46	29.65	0.84 (0.67-1.05)	0.12
biomass, *t* +1	13	7.09	8.66	1.22 (0.48-3.11)	0.65
seedbank, *t* + 2	63	1979.74	1872.45	0.95 (0.81-1.11)	0.49
follow-up *t* +2	61	24.59	24.42	0.99 (0.81-1.21)	0.93
biomass, *t* +2	35	4.47	3.96	0.89 (0.35-2.22)	0.78
maize					
seedbank, *t* +1	47	2386.01	2934.77	1.23 (1.00-1.50)	0.03*
follow-up, *t* +1	38	34.29	36.79	1.07 (0.79-1.46)	0.64
biomass, *t* +1	23	8.96	8.29	0.93 (0.5-1.72)	0.81
seedbank, *t* + 2	44	2386.01	2934.77	1.33 (1.06-1.68)	0.012*
follow-up *t* +2	40	22.69	22.04	0.97 (0.74-1.28)	0.84
biomass, *t* +2	29	1.30	1.36	1.04 (0.49-2.22)	0.91
spring oilseed rape					
seedbank, *t* +1	64	3069.60	2398.78	0.78 (0.66-0.94)	0.006**
follow-up, *t* +1	62	25.37	19.08	0.75 (0.59-0.97)	0.028*
biomass, *t* +1	24	3.13	5.23	1.67 (0.72-3.86)	0.22
seedbank, *t* + 2	64	2884.31	2302.28	0.8 (0.67-0.95)	0.018*
follow-up *t* +2	61	38.04	30.41	0.8 (0.66-0.98)	0.034*
biomass, *t* +2	47	2.98	2.27	0.76 (0.31-1.85)	0.55
winter oilseed rape					
seedbank, *t* +1	65	2799.36	2624.91	0.94 (0.76-1.16)	0.56
follow-up, *t* +1	65	21.21	25.14	1.18 (0.96-1.45)	0.079
biomass, *t* +1	24	2.34	3.84	1.67 (0.72-3.86)	0.22
seedbank, *t* + 2	49	2941.82	2941.39	1.00 (0.84-1.2)	1.00
follow-up *t* +2	61	27.37	30.36	1.11 (0.88-1.4)	0.39
biomass, *t* +2	56	1.48	2.53	1.7 (0.82-3.55)	0.16

Table 2. Dicotyledon seedbank densities (numbers m^{-2} in top 15 cm), plant densities (numbers m^{-2}) and biomass (g m^{-2}) per half-field in relation to crop, sampling occasion and treatment. Values are geometric means for GMHT and conventional (C) treatments. Multiplicative treatment ratio, $R = 10^d$, where d is the mean of the differences between GMHT and C treatments on the logarithmic scale; confidence limits for R are back-transformed from those for d. CI, confidence interval.

sampling occasion, year	*n*	C	GMHT	*R* (95% CI)	*p*-value
beet					
seedbank, *t* +1	61	1423.05	1084.48	0.77 (0.64-0.91)	0.004**
follow-up, *t* +1	61	15.86	12.51	0.79 (0.57-1.1)	0.16
biomass, *t* +1	13	2.14	0.95	0.45 (0.12-1.72)	0.23
seedbank, *t* + 2	63	1089.32	996.09	0.92 (0.76-1.11)	0.36
follow-up *t* +2	59	10.11	7.70	0.77 (0.56-1.05)	0.095
biomass, *t* +2	33	2.38	1.92	0.81 (0.39-1.67)	0.55
maize					
seedbank, *t* +1	47	1353.96	1735.50	1.28 (1.01-1.63)	0.035*
follow-up, *t* +1	38	16.89	18.53	1.1 (0.77-1.56)	0.59
biomass, *t* +1	23	3.61	2.77	0.77 (0.27-2.21)	0.62
seedbank, *t* + 2	44	1227.75	1671.66	1.36 (1.02-1.81)	0.037*
follow-up *t* +2	38	10.96	10.93	1 (0.75-1.33)	0.99
biomass, *t* +2	29	0.73	0.52	0.72 (0.3-1.74)	0.50
spring oilseed rape					
seedbank, *t* +1	64	2045.49	1418.64	0.7 (0.56-0.86)	0.003**
follow-up, *t* +1	62	8.45	6.78	0.81 (0.56-1.15)	0.26
biomass, *t* +1	24	0.43	0.54	1.26 (0.33-4.82)	0.73
seedbank, *t* + 2	64	1926.0	1391.63	0.73 (0.6-0.88)	0.003**
follow-up *t* +2	59	18.32	14.05	0.77 (0.57-1.03)	0.079
biomass, *t* +2	46	0.81	0.78	0.97 (0.37-2.56)	0.95
winter oilseed rape					
seedbank, *t* +1	64	1543.65	1087.65	0.7 (0.56-0.86)	0.002**
follow-up, *t* +1	64	7.47	4.89	0.66 (0.5-0.89)	0.006**
biomass, *t* +1	43	0.70	0.36	0.52 (0.21-1.27)	0.14
seedbank, *t* + 2	49	1385.29	1074.11	0.78 (0.63-0.97)	0.026*
follow-up *t* +2	60	9.12	6.95	0.77 (0.61-0.96)	0.019*
biomass, *t* +2	51	0.30	0.39	1.3 (0.59-2.84)	0.50

Table 3. Monocotyledon seedbank densities (numbers m^{-2} in top 15 cm), plant densities (numbers m^{-2}) and biomass (g m^{-2}) per half-field in relation to crop, sampling occasion and treatment. Values are geometric means for GMHT and conventional (C) treatments. Multiplicative treatment ratio, $R = 10^d$, where d is the mean of the differences between GMHT and C treatments on the logarithmic scale; confidence limits for R are back-transformed from those for d. CI, confidence interval.

sampling occasion, year	*n*	C	GMHT	*R* (95% CI)	*p*-value
beet					
seedbank, *t* +1	60	590.91	447.04	0.76 (0.60-0.97)	0.032*
follow-up, *t* +1	60	10.49	7.55	0.72 (0.54-0.96)	0.019*
biomass, *t* +1	13	2.36	4.82	2.04 (0.76-5.51)	0.17
seedbank, *t* + 2	61	608.83	533.55	0.88 (0.67-1.15)	0.35
follow-up *t* +2	57	11.17	10.85	0.97 (0.78-1.22)	0.79
biomass, *t* +2	24	6.46	6.92	1.06 (0.56-2.04)	0.84
maize					
seedbank, *t* +1	47	677.38	888.45	1.30 (0.97-1.74)	0.068
follow-up, *t* +1	34	12.01	11.44	0.95 (0.68-1.34)	0.78
biomass, *t* +1	20	1.60	0.91	0.57 (0.17-1.92)	0.37
seedbank, *t* + 2	44	601.80	724.63	1.20 (0.94-1.52)	0.13
follow-up *t* +2	35	7.30	8.68	1.18 (0.80-1.75)	0.39
biomass, *t* +2	12	2.87	3.92	1.30 (0.73-2.32)	0.31
spring oilseed rape					
seedbank, *t* +1	63	513.79	539.59	1.05 (0.85-1.3)	0.67
follow-up, *t* +1	59	9.24	9.34	1.01 (0.76-1.34)	0.94
biomass, *t* +1	23	0.89	2.87	3.21 (0.86-11.97)	0.09
seedbank, *t* + 2	62	577.14	536.83	0.93 (0.72-1.2)	0.59
follow-up *t* +2	61	7.59	6.56	0.87 (0.64-1.17)	0.36
biomass, *t* +2	32	7.06	5.39	0.79 (0.46-1.35)	0.38
winter oilseed rape					
seedbank, *t* +1	65	791.10	994.93	1.25 (0.91-1.71)	0.15
follow-up, *t* +1	64	9.42	14.87	1.57 (1.19-2.06)	<0.001***
biomass, *t* +1	41	0.67	2.08	3.11 (0.92-10.47)	0.077
seedbank, *t* + 2	49	1038.86	1311.53	1.26 (0.99-1.6)	0.06
follow-up *t* +2	59	11.02	13.39	1.21 (0.88-1.66)	0.24
biomass, *t* +2	38	5.89	10.16	1.65 (0.94-2.90)	0.076

Table 4. Differences in the seedbanks of individual species between GMHT and conventional treatments before the crops are sown (time t), and one year (t+1) and two years (t+2) later, presented as R values. R > 1 means that the seedbank was larger in the GMHT treatment, while R < 1 means it was larger in the conventional treatment. Statistically significant differences from R =1 (at $p < 0.05$) are indicated by bold.

	Beet			Maize			Spring oilseed rape		
	t	*t*+1	*t*+2	*t*	*t*+1	*t*+2	*t*	*t*+1	*t*+2
Capsella bursa-pastoris	0.94	0.93	1.45	1.24	**1.62**	1.32	1.40	0.78	**0.64**
Chenopodium album	1.07	0.72	0.78	0.96	1.01	0.89	1.07	0.69	**0.38**
Fallopia convolvulus	0.62	0.86	0.65	0.64	0.68	2.76	0.61	0.80	1.10
Lamium purpureum	1.38	0.96	1.20	1.26	2.26	1.89	1.12	1.54	1.15
Persicaria maculosa	0.85	**0.37**	**0.50**	**1.74**	**2.64**	1.29	1.13	**0.48**	0.55
Poa annua	0.84	0.86	1.02	1.15	1.21	1.26	1.02	1.08	0.92
Polygonum aviculare	1.07	0.72	0.82	0.92	1.18	1.31	0.81	0.86	**0.64**
Senecio vulgaris	0.93	1.09	1.19	1.10	0.90	0.71	0.92	**0.64**	1.01
Sonchus spp	1.17	1.18	0.84	0.90	1.03	1.38	0.97	**0.50**	0.71
Stellaria media	1.08	**0.69**	1.31	**1.69**	1.44	1.55	0.98	0.70	0.91
Veronica persica	1.08	1.27	0.98	1.15	1.58	1.98	1.17	0.90	0.74
Viola arvensis	0.94	1.58	1.22	1.06	1.06	2.11	**1.59**	1.22	1.30

Table 5. Dicotyledon densities (individuals m^{-2}) per half-field at follow-up counts in each of three development classes, in relation to crop, sampling occasion and treatment. Values are geometric means for GMHT and conventional (C) treatments. Multiplicative treatment ratio, $R = 10^d$, where d is the mean of the differences between GMHT and C treatments on the logarithmic scale; confidence limits for R are back-transformed from those for d. CI, confidence interval.

sampling occasion, year	*n*	C	GMHT	*R* (95% CI)	*p*-value
beet					
t +1, less than four leaves	55	3.97	3.34	0.85 (0.58-1.23)	0.36
t +1, greater than four leaves	61	10.50	7.70	0.74 (0.54-1.02)	0.06
t +1, reproductive	11	2.08	2.01	0.97 (0.34-2.78)	0.96
t +2, less than four leaves	49	2.06	1.81	0.89 (0.55-1.42)	0.63
t +2, greater than four leaves	58	4.65	3.42	0.74 (0.56-0.99)	0.027*
t +2, reproductive	28	3.77	2.24	0.61 (0.34-1.12)	0.10
maize					
t +1, less than four leaves	40	4.02	4.25	1.05 (0.75-1.47)	0.76
t +1, greater than four leaves	40	10.34	9.94	0.96 (0.61-1.52)	0.85
t +1, reproductive	18	2.08	2.51	1.19 (0.60-2.34)	0.59
t +2, less than four leaves	37	3.34	3.11	0.94 (0.61-1.43)	0.76
t +2, greater than four leaves	37	7.48	5.79	0.78 (0.53-1.15)	0.23
t +2, reproductive	18	1.73	2.97	1.64 (0.93-2.89)	0.09
spring oilseed rape					
t +1, less than four leaves	57	2.55	1.50	0.61 (0.42-0.9)	0.012*
t +1, greater than four leaves	63	4.21	4.02	0.96 (0.66-1.38)	0.78
t +1, reproductive	15	1.11	1.04	0.95 (0.46-1.93)	0.88
t +2, less than four leaves	55	3.42	3.56	0.94 (0.61-1.43)	0.76
t +2, greater than four leaves	60	8.96	5.81	0.65 (0.46-0.93)	0.019*
t +2, reproductive	38	2.20	1.06	0.52 (0.33-0.82)	0.013*
winter oilseed rape					
t +1, less than four leaves	52	1.65	1.18	0.74 (0.55-1.00)	0.054
t +1, greater than four leaves	63	3.32	2.23	0.69 (0.50-0.94)	0.025*
t +1, reproductive	32	0.91	0.72	0.82 (0.48-1.41)	0.46
t +2, less than four leaves	55	1.60	1.28	0.82 (0.62-1.10)	0.19
t +2, greater than four leaves	55	4.46	3.55	0.80 (0.63-1.03)	0.083
t +2, reproductive	40	1.60	1.30	0.83 (0.55-1.24)	0.32

Table 6. Monocotyledon densities (individuals m^{-2}) per half-field at follow-up counts in each of three development classes, in relation to crop, sampling occasion and treatment. Values are geometric means for GMHT and conventional (C) treatments. Multiplicative treatment ratio, $R = 10^d$, where d is the mean of the differences between GMHT and C treatments on the logarithmic scale; confidence limits for R are back-transformed from those for d. CI, confidence interval.

sampling occasion, year	*n*	C	GMHT	*R* (95% CI)	*p*-value
beet					
t +1, less than four leaves	40	1.34	0.79	0.63 (0.37-1.08)	0.095
t +1, greater than four leaves	60	8.31	5.57	0.68 (0.5-0.92)	0.01*
t +1, reproductive	10	6.01	14.82	2.4 (1.01-5.72)	0.077*
t +2, less than four leaves	32	1.37	1.11	0.83 (0.56-1.23)	0.35
t +2, greater than four leaves	55	5.12	4.78	0.94 (0.72-1.21)	0.60
t +2, reproductive	33	3.21	3.60	1.12 (0.79-1.57)	0.53
maize					
t +1, less than four leaves	29	1.68	1.30	0.79 (0.54-1.16)	0.22
t +1, greater than four leaves	34	6.47	5.61	0.87 (0.54-1.39)	0.56
t +1, reproductive	16	5.50	6.48	1.17 (0.84-1.64)	0.30
t +2, less than four leaves	25	1.56	1.55	1 (0.56-1.77)	1.00
t +2, greater than four leaves	55	4.24	3.78	0.94 (0.72-1.21)	0.60
t +2, reproductive	17	3.68	5.45	1.46 (0.73-2.9)	0.28
spring oilseed rape					
t +1, less than four leaves	33	1.35	0.91	0.71 (0.35-1.42)	0.30
t +1, greater than four leaves	60	5.23	5.43	1.04 (0.71-1.52)	0.86
t +1, reproductive	17	6.78	7.40	1.09 (0.55-2.16)	0.78
t +2, less than four leaves	37	0.92	0.70	0.79 (0.52-1.21)	0.25
t +2, greater than four leaves	57	3.79	3.14	0.84 (0.61-1.14)	0.24
t +2, reproductive	38	3.35	3.44	1.03 (0.65-1.63)	0.91
winter oilseed rape					
t +1, less than four leaves	32	0.90	0.92	1.02 (0.61-1.69)	0.95
t +1, greater than four leaves	60	3.99	5.53	1.37 (1.01-1.87)	0.038*
t +1, reproductive	35	1.99	4.39	2.11 (1.44-3.1)	0.002**
t +2, less than four leaves	31	0.75	0.53	0.75 (0.41-1.38)	0.37
t +2, greater than four leaves	54	3.81	3.82	0.84 (0.61-1.14)	0.24
t +2, reproductive	44	2.90	3.68	1.26 (0.8-1.99)	0.31

ADDITIONAL PAPERS RESULTING FROM THIS PROJECT

L. G. Firbank, P. Rothery, M. J. May, S. J. Clark, R.J. Scott, R.C. Stuart, C.W.H. Boffey, D.R. Brooks, G.T. Champion, A.J. Haughton, C. Hawes, M.S. Heard, A.M. Dewar, J.N. Perry and G.R. Squire. (2006) Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. *Biology Letters* 2 140-143.

D. R. Brooks, S. J. Clark, J. N. Perry, D. A. Bohan, G. T. Champion, L. G. Firbank, A. J. Haughton, C. Hawes, M. S. Heard and I. P. Woiwod. Invertebrate biodiversity in maize following withdrawal of triazine herbicides (2005). *Proceedings of the Royal Society (series B)* **272** 1497-1502.

Heard, M.S., Rothery, P, Perry, J. (2005) Modelling the impacts of genetically modified herbicide-tolerant crops on weed populations. *Weed Research* 45 (5) 331-338

Heard, M.S. *et al*. (2006) Effects of successive seasons of genetically modified herbicide-tolerant maize cropping on weeds and invertebrates. *Accepted to Annals of Applied Biology*