Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops

L. G. Firbank^{1,*}, P. Rothery², M. J. May³,
S. J. Clark⁴, R. J. Scott¹, R. C. Stuart¹,
C. W. H. Boffey⁴, D. R. Brooks⁵, G. T. Champion³,
A. J. Haughton⁵, C. Hawes⁶, M. S. Heard²,
A. M. Dewar³, J. N. Perry⁵ and G. R. Squire⁶

¹Centre for Ecology and Hydrology, Lancaster Environment Centre,
Library Avenue, Bailrigg, Lancaster LA1 4AP, UK

²Centre for Ecology and Hydrology, Abbots Ripton, Huntingdon,
Cambridgeshire PE28 2LS, UK

³Broom's Barn Research Station, Higham, Bury St Edmunds, Suffolk
IP28 6NP, UK

⁴Centre for Ecology and Hydrology, Winfrith Technology Centre,
Dorchester, Dorset DT2 8ZD, UK

⁵Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

⁶Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

*Author for correspondence (lgf@ceh.ac.uk).

The Farm Scale Evaluations (FSEs) showed that genetically modified herbicide-tolerant (GMHT) cropping systems could influence farmland biodiversity because of their effects on weed biomass and seed production. Recently published results for winter oilseed rape showed that a switch to GMHT crops significantly affected weed seedbanks for at least 2 years after the crops were sown, potentially causing longer-term effects on other taxa. Here, we seek evidence for similar medium-term effects on weed seedbanks following spring-sown GMHT crops, using newly available data from the FSEs.

Weed seedbanks following GMHT maize were significantly higher than following conventional varieties for both the first and second years, while by contrast, seedbanks following GMHT spring 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 for reasons that are not clear. These new data provide important empirical evidence for longer-term effects of GMHT cropping on farmland biodiversity.

Keywords: Farm Scale Evaluations; arable weeds; farmland biodiversity

1. INTRODUCTION

Genetically modified herbicide-tolerant (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 Farm Scale Evaluations

The electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2005.0390> or via <http://www.journals.royalsoc.ac.uk>.

(FSEs) of GMHT winter oilseed rape provided evidence of differences in the weed seedbanks between GMHT and conventional cropping systems for 2 years after the crops had been sown (Bohan *et al.* 2005). Such differences could lead to longer-term effects on weed populations (Heard *et al.* 2005), in turn affecting animal populations higher up the arable food chain by altering the quality and quantity of forage resources (e.g. Watkinson *et al.* 2000; Hawes *et al.* 2003). The evidence from the FSEs 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). Here we revisit this issue, by analysing weed seedbank data following GMHT and conventional crops of beet, maize and spring oilseed rape to include those seedbank samples taken during 2003 and 2004.

2. METHODS

The FSE experiment was a randomized 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). Farmers managed the two treatments in each half-field to achieve the goal of cost-effective weed control (Champion *et al.* 2003). We measured a range of plant and invertebrate indicators before, during and after the crops were sown (Firbank *et al.* 2003b). There were three cohorts of sites, sown in 2000, 2001 and 2002. After a single year of contrasting GMHT and conventional varieties, the fields were sown with the non-GMHT crops of choice of the farmer. Nearly all fields were revisited in the two subsequent years for spring assessments of the soil seedbanks. Soil samples were taken, and weed seedlings were germinated and identified at a range of locations around the field, using the same methods, and the same sample locations, as in the previous seasons (see Heard *et al.* 2003a for details of methods, and of analyses using data collected during 2000–2002). We quantified any differences in weed seedbank counts before the crops were sown (year t) and between the half-fields sown with GMHT and conventional crops a year after sowing (year $t+1$) and a year later after a subsequent crop (year $t+2$). The significance of any differences between treatments was tested with a paired randomization test (Perry *et al.* 2003). The size of treatment effects on seed counts were measured as R , the multiplicative treatment ratio of the counts in the GMHT treatment divided by the conventional (i.e. where there is no difference in effect $R=1$) (Perry *et al.* 2003). Comparisons with total counts of one or zero were excluded, giving rise to different sample sizes for different tests. Tests for samples taken before the GMHT crops were sown (time t) are given, even though no significant difference was expected. Analyses are given for the total seedbank, for the monocot and dicot seedbanks, and also for the 12 individual species for which demographic data were reported by Heard *et al.* (2003b).

3. RESULTS

Weed seed numbers increased following the conventional beet crops, but changed little following GMHT beet crops, resulting in significant differences between the two treatments (table 1). Significant differences were also observed for two individual species, *Persicaria maculosa* and *Stellaria media* (table 2; details of analyses for individual species are given in the electronic supplementary material). Between the first ($t+1$) and second years ($t+2$), there was a weak tendency for seedbank numbers to decline more following conventional crops than following GMHT crops (table 3), falling back to levels observed at the start of the experiment (table 1). As a result, the differences in seedbanks between treatments were

Table 1. Weed seedbank densities (numbers m^{-2} in the top 15 cm of soil) before the conventional (C) and GMHT crops were sown (time t) and 1 and 2 years later (times $t+1$, $t+2$). (Values are geometric means for the two treatments, with the multiplicative treatment ratio, $R=10^d$ where d is the mean of the differences between the two treatments on a logarithmic scale; confidence limits for R are back-transformed from those generated for d . p levels are given, with * indicating $p<0.05$, ** $p<0.01$. Note that results for total weeds are not the sum of those for dicots and monocots because of the use of geometric means.)

		n	C	GMHT	R	upper 95%	lower 95%	p -value
<i>beet</i>								
total weeds	t	64	1996	1779	0.89	0.76	1.05	0.151
	$t+1$	61	2367	1861	0.79	0.67	0.92	0.002**
	$t+2$	63	1980	1872	0.95	0.81	1.11	0.487
dicots	t	64	926	900	0.97	0.82	1.15	0.756
	$t+1$	61	1423	1085	0.77	0.64	0.91	0.004**
	$t+2$	63	1088	996	0.92	0.76	1.11	0.359
monocots	t	63	673	566	0.85	0.68	1.06	0.128
	$t+1$	60	591	447	0.76	0.60	0.97	0.032*
	$t+2$	61	609	534	0.88	0.67	1.15	0.351
<i>maize</i>								
total weeds	t	57	2266	2519	1.11	0.97	1.28	0.142
	$t+1$	47	2386	2935	1.23	1.00	1.50	0.050*
	$t+2$	44	2029	2711	1.33	1.06	1.68	0.012*
dicots	t	57	1211	1338	1.10	0.93	1.31	0.282
	$t+1$	47	1354	1736	1.28	1.01	1.63	0.035*
	$t+2$	44	1228	1671	1.36	1.02	1.81	0.037*
monocots	t	57	752	872	1.16	0.91	1.47	0.250
	$t+1$	47	677	888	1.30	0.97	1.74	0.068
	$t+2$	44	602	725	1.20	0.94	1.52	0.130
<i>spring oilseed rape</i>								
total weeds	t	65	2065	2050	0.99	0.77	1.28	0.967
	$t+1$	64	3070	2399	0.78	0.66	0.94	0.006**
	$t+2$	64	2884	2302	0.80	0.67	0.95	0.018*
dicots	t	65	1096	1085	0.99	0.79	1.24	0.930
	$t+1$	64	2045	1419	0.70	0.56	0.86	0.003**
	$t+2$	64	1926	1392	0.73	0.60	0.88	0.003**
monocots	t	64	621	697	1.12	0.81	1.54	0.503
	$t+1$	63	514	540	1.05	0.85	1.30	0.667
	$t+2$	62	577	537	0.93	0.72	1.20	0.589

Table 2. Differences in the seedbanks of individual species between GMHT and conventional treatments before the crops were sown (time t), and 1 year ($t+1$) and 2 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. Full details of these analyses are provided in the electronic supplementary material.)

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

much reduced and were no longer significant except for *P. maculosa* (table 2).

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 (table 1). *Capsella bursa-pastoris* and, again, *P. maculosa*, showed significant effects (the latter following a much weaker, but

Table 3. Analyses of changes in seedbank between samples taken at $t+1$ and $t+2$ with respect to the GMHT/conventional treatment in year t . (A statistically significant difference implies that the rate of change of the seedbank has been influenced by the GMHT/Conventional treatment imposed in year t , e.g. by a density-dependent change in weed numbers, or differential crop management practices, in year $t+1$.)

	<i>n</i>	<i>R</i>	upper 95%	lower 95%	<i>p</i> -value
<i>beet</i>					
total weeds	61	1.22	1.03	1.46	0.025
dicots	61	1.18	0.95	1.47	0.125
monocots	61	1.15	0.86	1.54	0.357
<i>maize</i>					
total weeds	40	1.16	0.94	1.42	0.178
dicots	40	1.09	0.82	1.45	0.527
monocots	40	1.06	0.83	1.35	0.628
<i>spring oilseed rape</i>					
total weeds	62	0.98	0.80	1.21	0.884
dicots	62	0.99	0.78	1.25	0.923
monocots	62	0.91	0.72	1.15	0.460

still significant treatment difference before the crops were sown; this was one of three such cases (table 2), all considered to be Type I statistical errors) (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 $t+2$ (table 1). Again, significant effects were observed for *P. maculosa* and *C. bursa-pastoris*, along with several other species (table 2). There was no suggestion of treatment effects on seedbank change between $t+1$ and $t+2$ for maize or spring oilseed rape (table 3).

4. DISCUSSION

GMHT crops are managed using different herbicide regimes to conventional varieties, and it is these herbicide regimes that affect weed seed production (Heard *et al.* 2003a). This new evidence shows that differences in weed seed production within GMHT and conventional crops resulted in significant differences in weed seedbanks a year after the crops were sown ($t+1$) for maize in addition to beet and spring and winter oilseed rape as reported earlier (Heard *et al.* 2003a; Bohan *et al.* 2005).

The size of the treatment effects following maize and spring oilseed rape were consistent in both the first and second years after the treatments had been imposed. This finding implies that GMHT spring 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, as is the case for winter oilseed rape (Bohan *et al.* 2005), while maize GMHT cropping will raise dicot seedbank levels. 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 $\times$ year interaction for some species, notably *S. media*. The reason for this phenomenon 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).

Break crops such as those studied within the FSEs are important for maintaining weed populations, 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 spring oilseed rape are likely to increase from rotation to rotation, assuming that crop management were to remain similar to that used in the FSEs (Heard *et al.* 2005).

We therefore conclude that the differences in weed seed production between GMHT and conventional maize and spring oilseed rape (and, less certainly, beet) crops are likely to be perpetuated for at least two seasons afterwards, and that the new data provide important empirical evidence for longer-term effects of GMHT cropping on farmland biodiversity.

This work was funded by Defra. Rothamsted Research receives grant-aided support from the BBSRC. A more complete list of acknowledgements is given by Bohan *et al.* (2005).

- Bohan, D. A. *et al.* 2005 Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. *Proc. R. Soc. B* **272**, 463–474. (doi:10.1098/rspb.2004.3049)
- Champion, G. T. *et al.* 2003 Crop management and the agronomic context of the Farm Scale Evaluations. *Phil. Trans. R. Soc. B* **358**, 1801–1818. (doi:10.1098/rstb.2003.1405)
- Firbank, L. G. *et al.* 2003a The implications of spring-sown genetically modified herbicide-tolerant crops for farmland biodiversity: a commentary on the Farm Scale Evaluations of spring sown crops. See <http://www.defra.gov.uk/environment/gm/fse/results/fse-commentary.pdf>.
- Firbank, L. G. *et al.* 2003b An introduction to the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *J. Appl. Ecol.* **40**, 2–16. (doi:10.1046/j.1365-2664.2003.00787.x)
- Hawes, C. *et al.* 2003 Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Phil. Trans. R. Soc. B* **358**, 1899–1913. (doi:10.1098/rstb.2003.1406)
- Heard, M. S. *et al.* 2003a Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Effects on abundance and diversity. *Phil. Trans. R. Soc. B* **358**, 1819–1832. (doi:10.1098/rstb.2003.1402)
- Heard, M. S. *et al.* 2003b Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 2. The effects on individual species. *Phil. Trans. R. Soc. B* **358**, 1833–1846. (doi:10.1098/rstb.2003.1401)

- Heard, M. S., Rothery, P., Perry, J. N. & Firbank, L. G. 2005 Predicting longer-term changes in weed populations under GMHT crop management. *Weed Res.* **45**, 331–338. (doi:10.1111/j.1365-3180.2005.00465.x)
- Perry, J. N., Rothery, P., Clark, S. J., Heard, M. S. & Hawes, C. 2003 Design, analysis and power of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *J. Appl. Ecol.* **40**, 17–31. (doi:10.1046/j.1365-2664.2003.00786.x)
- Watkinson, A. R., Freckleton, R. P., Robinson, R. A. & Sutherland, W. J. 2000 Predictions of biodiversity responses to genetically modified herbicide-tolerant crops. *Science* **289**, 1554–1557. (doi:10.1126/science.289.5484.1554)