EFFECTS OF DELAYED WEED CONTROL IN GENETICALLY MODIFIED HERBICIDE-TOLERANT SUGAR BEET ON THE ABUNDANCE AND DIVERSITY OF ARTHROPODS

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ABSTRACT

The proposed introduction of genetically modified herbicide tolerant (GMHT) crops has prompted fears about possible adverse environmental impacts of their widespread adoption, particularly on arable weeds, insects and associated farmland birds. In two of four field trials with glyphosate-tolerant sugar beet, in which the first overall applications of glyphosate in a two spray programme were delayed, or where first applications were applied in a band with the second applied overall, there was no consistent effect of treatments on the cumulative numbers of carabids, staphylinids or spiders trapped in pitfall traps. This was almost certainly due to the low weed populations at these sites (*circa* 11-12 /m^2 in untreated plots), which did not alter the structure of the habitat sufficiently to influence the populations of these arthropods. At the other two sites, where weed numbers in untreated plots were three to five times greater (27 and 61/m^2 respectively), there were significant correlations between weed biomass (including dead and dying weeds) in late July and the cumulative numbers of staphylinid beetles during the sampling period June-August. The correlations were much weaker, although still significant, for carabids, but non-significant for spiders. There was no difference for any species of carabid or staphylinid, or their combined totals, between the conventional treatments and the early overall glyphosate treatment. This suggests that the response of the beetles was to the removal of weeds, and not to the chemicals used. Within any site there was no significant difference in the α index of biodiversity between any treatment on any one sampling date, or when the cumulative catch over all sampling dates was considered.

ABRÊGÊ - LES EFFETS D'UN DÉSHERBAGE TARDIF DES BETTERAVES À SUCRE MODIFIÉES GÉNÉTIQUEMENT AFIN DE RÉSISTER AUX HERBICIDES SUR LA DIVERSITÉ DES ARTHROPODES

La proposition d'introduire des cultures génétiquement modifiées afin de résister aux herbicides (genetically modified herbicide tolerant (GMHT)) a provoqué de vives craintes en ce qui concerne les possibles impacts environnementaux causés par une utilisation généralisée; en particulier sur la mauvaise herbe des terres arables, les insectes et les oiseaux dont le milieu est associé aux terres cultivées. Il n'y eut pas d'effet significatif sur le nombre cumulatif de *carbodiae*, *staphylinidae* et araignées piégées dans deux des quatre champs expérimentaux cultivés avec des betteraves à sucre génétiquement modifiées résistantes au glyphosate. Ces champs subirent deux applications retardées; la première fut réalisée en bande au dessus des cultures et la seconde, généralisée à tout le champ. Ceci fut certainement dû a la faible quantité de mauvaises herbes peuplant ces sites (de 11 a 12/m^2 dans les parcelles non traitées), ce qui n'altère donc pas suffisamment la structure des habitats pour avoir une quelconque influence sur la population en arthropodes. Dans les deux autres sites, la quantité de mauvaises herbes dans les parcelles non-traitées était 3 a 4 fois plus importante (respectivement 27 et 61/m^2), par conséquent, il y eut une corrélation significative entre la biomasse représentant les mauvaises herbes (incluant les mauvaises herbes mortes et mourantes) à la fin juillet et le nombre total de coléoptères staphyliniques pendant cette période d'échantillonnage allant de juin a août. Ces corrélations furent plus faibles mais toujours significatives en ce qui concerne les *carabidiae* et non significatives pour les araignées. Il n'y eut aucune différence entre le traitement conventionnel et le traitement généralisé retardé en glyphosate pour aucune des espèces *carbodiae* ou *staphylinidae* ou leur nombre total. Cela amène donc a penser que la réponse des coléoptères était due a

l'élimination des mauvaises herbes et non a la nature du produit chimique utilisé. Dans aucun site, il n'y eut de différence significative dans l'index α de biodiversité entre les divers traitements à aucune date d'échantillonnage ou même quand le nombre cumulatif des prises pendant toute la phase d'échantillonnage fut considéré.

KURZFASSUNG - AUSWIRKUNGEN VON VERZÖGERTER UNKRAUTBEKÄMPFUNG BEI GENETISCH MODIFIZIERTEN HERBIZIDTOLERANTEN ZUCKERRÜBEN AUF DIE ABUNDANZ UND DIVERSITÄT VON ARTHROPODEN

Die vorgeschlagene Einführung von genetisch modifizierten herbizidtoleranten (GMHT) Kulturpflanzen hat Ängste über mögliche negative, durch deren weitverbreitete Einführung verursachte Umweltauswirkungen, insbesondere auf kulturfähige Unkräuter, Insekten und assoziierte Vögel, hervorgerufen. Bei zwei von vier Freilandversuchen mit glyphosattoleranten Zuckerrüben, bei denen die

erste Anwendung von Glyphosat in einem zweistufigen Spritzprogramm verzögert wurde, oder die ersten Anwendungen in Streifen ausgebracht wurden, waren keine konsistenten Auswirkungen auf die kumulative Anzahl von Carabiden, Staphyliniden oder Spinnen feststellbar. Dies erklärt sich mit großer Wahrscheinlichkeit aus den niedrigen Unkrautpopulationen an diesen Standorten (circa 11 – 12 /m^2 auf unbehandelten Parzellen), die die Struktur des Habitats nicht ausreichend änderten, um sich auf die Populationen dieser Arthropoden auszuwirken. An den anderen beiden Standorten, bei denen die Unkrautmenge auf den unbehandelten Parzellen drei- bis fünfmal größer war (27 bzw. 61/m^2), gab es signifikante Korrelationen zwischen der Unkrautbiomasse (einschließlich toter und absterbender Unkräuter) Ende Juli und der kumulativen Anzahl von Staphyliniden vonJuni bis August. Die Korrelationen für Carabiden waren viel schwächer, aber noch signifikant, für Spinnen jedoch nicht signifikant. Für keine der Carabiden- oder Staphylinidenarten oder ihrer kombinierten Gesamtanzahl war ein Unterschied zwischen den konventionellen Behandlungen und der frühen Glyphosat-Gesamtbehandlung feststellbar. Dies deutet an, dass die Käfer auf die Beseitigung der Unkräuter reagierten und nicht auf die verwendeten Chemikalien. Innerhalb eines Standortes gab es keine signifikanten Unterschiede im α-Index für Biodiversität zwischen den verschiedene Behandlungen , weder für individuelle Probenzeitpunkte noch when when alle Probenzeitpunkte kombiniert wurden.

1.- INTRODUCTION

Genetically modified herbicide-tolerant (GMHT) sugar beet offers farmers a more flexible, and more cost-effective way of controlling the wide range of weeds, both monocotyledonous and dicotyledonous species, that occur in sugar beet fields (May, 2003). However, the better efficacy of the broad-spectrum herbicides such as glyphosate and glufosinate ammonium, to which the tolerance has been developed in GM sugar beet (Moll, 1997; Brandts and Harms 1998; Read and Bush 1998; Wilson 2002), has prompted concerns that the introduction of GMHT varieties will lead to even greater intensification of agriculture and exacerbate the general decline in farmland birds (Chamberlain *et al.* 2000), especially those that feed on weed seeds in the autumn (Watkinson *et al.* 2000). These concerns have been voiced by English Nature (1998, 2000) and other environmental scientists (Krebs 1999,Hails 2000).

However, the use of broad spectrum herbicides in GMHT beet can offer the potential of managing weeds for the benefit of the environment by allowing later weed control than is possible by conventional herbicides, which have to be applied when weed seedlings are small to achieve reasonable kill. In this paper we describe the effects of leaving weeds, either by delaying applications of herbicides, or by using band sprays early followed by overall sprays later, on the number and diversity of three groups of arthropods that commonly occur in beet fields – the Carabidae, Staphylinidae and Araneae. Carabid beetles are regarded as good indicators of biodiversity, comprising predators, and herbivores (Luff and Woiwod 1995), and both staphylinid beetles (Powell *et al.* 1985) and spiders (Haughton *et al.* 1999) contain species that are known to respond to herbicide regimes.

This paper complements that of May *et al.* (2003) also published in this issue, which describes the effects of similar treatments on weeds and yield of beet.

2.- MATERIALS AND METHODS

2.1.- TRIAL DESIGN

In four trials carried out in 1999 and 2000, a subset of the herbicide treatments included in adjacent yield trials (described by May *et. al* 2003 in this issue), was set up to investigate the effects of weeds on number and diversity of field-resident arthropods. Plot sizes were larger than the yield trials (12 x 6 m (12 rows) in trial B, and 12 x 12 m (24 rows) in the other three) to allow the collection of invertebrates with minimal interference from neighbouring plots.

A glyphosate-tolerant variety of sugar beet (Line No 77 from Monsanto) was sown in late April in 1999 and early May in 2000. These relatively late sowing dates were due to poor weather in April in both years, and this may have affected the number of weeds that subsequently emerged at each site.

The experiments were carried out on a range of soil types typical of those on which sugar beet is grown in the UK. Between 12 and 22 different weed species were present on each experiment, many of them important components of farmland bird diets (Krebs *et al.* 1999). The most abundant species recorded at most sites were *Chenopodium album*, *Fallopia convolvulus*, *Sonchus asper*, *Veronica persica*, *Cirsium arvense*, *Tripleurospermum inodorum*, *Gallium aparine* and *Stellaria media*,. Total population densities in untreated plots ranged from 11 to 60 /m^2.

2.2.- TREATMENTS

Herbicide treatments were compared to an untreated control. The conventional herbicide programmes varied at each site depending on the weed species present. The number of active ingredients ranged from 4 to 7, the simplest including phenmedipham, metamitron, lenacil and ethofumesate (Site 1, 1999), and the most complex including paraquat, diquat, phenmedipham, desmedipham, ethofumesate, triflusulfuron-methyl and clopyralid (Site 4, 2000). The number of applications ranged from 2 to 4 applied pre-emergence (in one trial, site 4), but mostly post-emergence starting between 79 and 222°Cd (above 3°C) after sowing. In 1999, applications of some treatments, particularly in the two conventional programmes, were delayed by adverse weather conditions.

Treatments of glyphosate (at 1080 g a.i./sprayed ha) were applied either overall at three timings - 207 (4-6 leaf stage of beet), xxx (10 leaf stage) and 864 (14+ leaf stage) day degrees above 3°C (°Cd) after sowing, or over the sugar beet rows only at xxx°Cd (2000 only). The overall treatments were followed by a second application at xxx, 698 (12 leaf stage) and 1022°Cd respectively, and the band spray by an overall application at 811°Cd after sowing.

2.3- OBSERVATIONS

Weed numbers and species were recorded on two or three occasions in June July and August from 10 x 0.25 m^2 quadrats, and weed biomass, including that of living and dead or dying weeds, was recorded from 2 x 0.25 m^2 quadrats on two or three occasions. The last sample was collected two weeks after application of the last herbicide, in late July or early August.

Ground active arthropods were sampled using standard pitfall traps (Baars 1979), 6.5 cm in diameter, 8 cm deep containing ethyl alcohol, glycerol and water in 1999 and 50% ethylene glycol in 2000. Three traps were placed in the centre and 3 m from each end between the central two rows of each plot. In 1999 traps were changed weekly from early June to mid- August, and in 2000 they were changed fortnightly over the same period. The change in preservative from alcohol to ethylene glycol allowed longer exposure before changes needed to be made. Not all samples dates of the total collected were sorted and identified to species. Those identified were chosen to represent the effects of treatments over the whole sampling period i.e. from June, July and August.

3.- RESULTS

3.1.- EFFECTS OF TREATMENTS ON WEEDS

The density of weeds at each site varied considerably from 11 – 61 /m^2. Glyphosate applied early overall on the first occasion consistently had the lowest weed biomass by late July/early August, two weeks after the last treatments had been applied. Later treatments left greater biomass. Only the latest overall first spray left substantial weed biomass at site 1, but both the mid-timng and late applications of glyphosate did so at sites 3 and 4. Weed biomass at site 2 was never high, even in the untreated plots, and that at site 3 was dominated by *Chenopodium alba*.

The efficacy of conventional treatments varied between sites; at site 1 control of weeds was as good as the early and mid-timing sprays of glyphosate; at site 2, weed control was relatively ineffective; at sites 3 and 4 weed biomass in the conventional plots was as great as in the plots sprayed late with glyphosate (Table 1).

Although ground cover was related to the density of weeds it was not always correlated with the weed biomass. For example, maximum weed biomass in untreated plots at sites 1 and 3 was very similar in late July / early August, but the number of weeds was three times higher at site 1, and the ground cover was double (Table 1). This was due to the disproportionate contribution of *C. alba* to the total biomass (75%) at site 3. However, *C. alba* is a tall weed and does not affect the ground cover as much as lower growing weeds with lateral growth that were more prevalent at site 1, hence the discrepancy.

Table 1 Effect of herbicides on the biomass of weeds (g/m²) 14 days after the last treatment had been applied in GMHT trials

*e = early; m = mid-timing; l = late; ll= later; b = band spray; * = significantly less than untreated at P<0.05*

Treatment	Site 1: 20 July 1999	Site 2: 23 July 1999	Site 3: 9 Aug 2000	Site 4: 27 July 2000
No. of weeds/m^2 at start	**27**	**12**	**11**	**61**
Max ground cover (%) in July	**35**	**23**	**16**	**96**
Conventional	2*	31.4	24.9*	245*
Glyphosate e + l	1*	1.6*	0*	133*
Glyphosate m + l	2*	0.2*	160.5	143*
Glyphosate l + ll	145*	0.7*	34.2*	222*
Glyphosate eb + l	-	-	3.6*	749*
Untreated	427	70.4	349.4	3299
SED (21-x D F)	88.9	20.11	90.28	687.1
LSD (5%)	182.5	41.95	186.33	1418.2

3.2.- EFFECTS OF TREATMENTS ON ARTHROPODS

In 1999, samples were sorted and identified from four sample weeks, but this was increased in 2000 to 9 and 7 sample weeks at sites 3 and 4 respectively, when samples were collected over two weeks instead of one, except the last sample which was only one week prior to harvest. In our study sites the number of species of carabids, staphylinids and spiders was typical of arable fields (Kromp 1999). Carabids were numerically greater than the other two groups at all sites, comprising at least 44% of the total collected (site 4), but as high as 84% at site 2, which was situated next to a beetle bank. Staphylinids comprised between 5% (sites 2 and 3) and 30% (site 4), while spiders comprised between 10% (site 2) and 31% (site 1) of the total. Site 3 was the most diverse (particularly in spiders), and caught the largest number of specimens over the sampling period (31911) even taking account of the longer period of collection (Table 2). Site 1 had moderate populations, while site 4 had the fewest individuals.

Table 2. Number of species in each indicator group caught in all pitfall traps

	Site							
	Trial 1: 1999		Trial 2: 1999		Trial 3: 2000		Trial 4: 2000	
Soil type	Sandy loam		Loamy sand		Sandy loam		Peaty loamL	
Sampling weeks	4		4		9		7	
Taxonomic group	N	No. of species	N	No. of species	N	No. of species	N	No. of species
Carabids	6710	21	10852	19	20913	23	4324	23
Staphylinids	2043	24	660	12	1550	26	2996	25
Spiders	3979	25	1340	25	9448	37	2569	20
Total	**12732**	**70**	**12852**	**56**	**31911**	**86**	**9889**	**68**
Biodiversity index (alpha ± SE)	**9.80 ± 1.26**		**7.52 ± 1.08**		**10.80 ± 1.24**		**9.83 ± 1.29**	

Among the carabids, the dominant species at all sites was *Pterostichus melanarius*, which comprised at least 70% of the carabids caught. *Agonum dorsale*, *Bembidion lampros*, *Calathus fuscipes*, *Harpalus rufipes* and *Trechus quadristriatus* were among the most common of the rest, and together the top five species represented over 90% of the carabid population at any site.

The Aleocharinae were the predominant group of staphylinids at two of the four sites (sites 2 and 3), constituting 79 and 50 % of the population there respectively. *Philonthus cognatus* was the most important species at the other two sites making up 43 % at site 1 and 53% at site 4. The only other species to make up more than 5% of the population at any site were *Aleochara bipustulata*, *Anotylus rugosus*, *A. sculpuratus*, *Ocypus olens*, *Sepedophilus marshami*, *Tachinus signatus*, *Tachyporus hypnorum* and *Xantholinus jarrigei*. The top five species again comprised at least 87% of the staphylinid population.

Spider communities were also dominated by a single species at three of the four sites. *Oedothorax apicatus* was the most common species at site 1 (61%), site 2 (49%), and site 3 (69%), but *Erigone atra* was the dominant species at site 4 (36%). *Bathyphantes gracilis*, *Diplostyla concolor*, *Erigone dentipalpis*, *Lepthyphantes tenuis*, *Oedothorax fuscus* and *Robertus lividus* were the other commoner species. The top five species made up at least 88% of the total at any site.

The impact of herbicide treatments on the diversity of these three groups depended on the density and diversity of weeds present and the timing and efficiency of their removal. At sites 2 and 3 there were no consistent effects of treatments on the numbers of carabids, staphylinids or spiders at any time during the growth of the crop. The only consistent transitory effect of treatments was seen in samples collected on 3 August at site 3, when significant reductions in the number of the carabid beetle, *H. rufipes*, a seed eater, and the total number of staphylinids, were recorded in plots with fewest weeds. This general lack of effect was almost certainly due to the low weed populations (circa 11-12 / m^2 in untreated plots) (Table 1), and the lower contribution of those species to the ground cover at both these sites, which did not alter the structure of the habitat sufficiently to influence the behaviour of these arthropods. Indeed the maximum ground cover afforded by those weeds in late July, was only 23 and 16% respectively in the untreated plots at these two sites, compared to 35% at site 1 and 96% at site 4 (Table 1).

At these latter two sites, weed numbers were three to five times greater. Associated with this greater biomass of vegetation there were significant differences between treatments in numbers of some beetle and spider species on some occasions, particularly after the later treatments had had some effect on the survival of the weeds. At both sites 1 and 4 there were significant correlations between the cumulative number of carabids and staphylinid beetles collected from samples taken in June, July and August, and the weed biomass (both living and dead) at the end of July/ early August (Fig. 1). The correlations were very strong for staphylinid beetles, less so for carabids, but they represent considerable increase in both groups. In contrast, spiders did not respond to differences in weed biomass in any trial.

There was no difference for any species of carabid or staphylinid, or their combined totals, on any sampling occasion or when considering cumulative totals, between the conventional treatments and the early overall glyphosate treatment. This suggests that the response of the beetles was to the presence of weeds, and not to the chemicals used.

3.2.- EFFECTS OF TREATMENTS ON BIODIVERSITY

In comparison to relatively undisturbed environments such as woodland or permanent grass leys, arable fields are not very diverse with arthropods. The catastrophic effects of cultivations, particularly deep ploughing, on small organisms has been well documented (Wilson, *et al* 1999), and correlated with declines in farmland birds which feed on such invertebrates.

The measures of biodiversity used here (the log-series alpha indices (Taylor, 1976)), produced by consideration of these three groups, took account of both the number of individuals and the number of species at each site, and was relatively independent of sample size. Thus the most diverse site was site 3 and the least, site 2, which was significantly less diverse than the former (Table 1). However there were no significant differences between any of the other sites. In this study the composition and structure of the arthropod communities, at least as they were represented by carabids, staphylinids and spiders, were relatively similar despite the large differences in soil type.

Within any site there were no significant differences between any treatment in the alpha index on any one sampling date, or when the cumulative catch over all sampling dates was considered (Table 3), even at the two sites which showed significant effects of treatments on the number of carabid and staphylinid beetles. Untreated plots were the most diverse at site 3, but not at the other three sites; plots treated with glyphosate overall early and later, which were the least weedy in all these trials, had the highest alpha index at site 4 and the lowest at site 2. Later treated plots were not necessarily more diverse at any site.

Table 2. Biodiversity indices after considering the cumulative number of carabids, staphylinids and spiders caught in pitfall traps at each site

N = number of carabids + staphylinids + spiders per treatment (all traps); e = early, m = mid-timing, l= late, ll = later, b=band

Site	Parameter	Treatments					
		Untr	Conv	Glyph e+l	Glyph m+l	Glyph l+ll	Glyph eb+l
1.	N	3346	2459	2353	2536	3131	-
	Species	48	46	47	48	50	-
	Alpha	**7.94**	**8.03**	**8.32**	**8.40**	**8.45**	**-**
	SE	1.25	1.30	1.34	1.33	1.31	-
2.	N	2752	2493	2402	2525	2646	-
	Species	37	37	33	35	40	-
	Alpha	**6.04**	**6.16**	**5.41**	**5.75**	**6.69**	**-**
	SE	1.09	1.11	1.03	1.06	1.16	-
3.	N	3796	3403	3113	3690	3207	3528
	Species	63	50	50	52	53	58
	Alpha	**10.73**	**8.31**	**8.46**	**8.57**	**9.02**	**9.86**
	SE	*1.48*	*1.29*	*1.31*	*1.3*	*1.36*	*1.42*
4.	N	1694	894	755	796	943	781
	Species	48	41	42	34	38	36
	Alpha	**9.19**	**8.87**	**9.59**	**7.22**	**7.94**	**7.8**
	SE	1.47	1.56	1.68	1.39	1.45	1.47

4.- DISCUSSION AND CONCLUSIONS

These results show that, where weed populations are high enough to affect the level of ground cover within sugar beet canopies, the number of staphylinid beetles is significantly enhanced by leaving weeds for longer, either by delaying application of glyphosate, or by applying this herbicide as a band spray at first application followed by a later overall spray when weeds become competitive. Carabid beetles were also increased but not by as great a margin. Spiders were not responsive to changes in canopy structure in this crop.

These results were similar to those reported in cereal crops (Powell *et al.* 1985; Moreby *et al.* 1999). In another study carried out in a row crop, Purvis and Curry, (1994) reported that carabids were rarely affected by weediness in sugar beet fields but staphylinid beetles were substantially increased, while spiders were unaffected. Spiders are known to be affected by herbicide regimes but the response is more apparent in some families than others (Haughton *et al.* 1999), and their lack of response here may be due to the continuing presence of the crop after removal of the weeds, which still allows the most common species (Lyniphyiids) to construct webs.

The lack of effects of treatments on biodiversity for individual sample dates may have been partly due to the relatively small plots within each trial, which allowed some larger species (e.g. *Pterostichus melanarius*) to migrate easily from one plot to another, thus diminishing the differences. It may also be that the species studied are adapted to exploiting transitory environments such as those in arable fields with annual or biennual crops, and thus were equally at home in weedy and non-weedy plots, albeit at different densities. The consequences of this on their population enhancement in the longer term, e.g. through improved breeding possibilities, could not be examined in these trials as the sites were destroyed within four months of sowing, and most of the species studied have only one generation per year.

The results presented above show that, although biodiversity is unaffected by the use of herbicides, either conventional or glyphosate, the number of some species of beetles were reduced by removal of weeds, or conversely, increased by retention of weeds. The yield trials (May *et al.* 2003; Dewar *et al.* 2003) demonstrated that it is possible to retain weeds within sugar beet crops without yield loss, by employing a band spray early in the growth of the crop, and an overall spray later when weed competition begins to effect yield. Further work is required to fine–tune this technique for fields with differing weed populations, and with different weed species than those that were prevalent in our trials. We have also shown that there is potential for improving the habitat for some species of nesting birds and at the same time encouraging sources of proteinaceous food for their chicks. However, it will not be possible to test this hypothesis on bird populations until these crops are approved and grown more widely.

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