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Is there a Weed Shift in Roundup Ready Maize? (Poster Presentation at the 4th International Workshop on PMEM of Genetically Modified Plants, Quedlinburg, Germany, 2010)

Gibt es eine Verschiebung im Unkrautspektrum im Roundup Ready Mais-Anbau? (Poster-Beitrag beim 4. internationalen Workshop zum Anbaubegleitenden Monitoring von gentechnisch veränderten Pflanzen, Quedlinburg, Deutschland 2010)

Abstract

Weed effects of the use of glyphosate were investigated in a 6-years field study of continuous transgenic herbicide-resistant maize rotation (2003–2008). It was conducted at three sites in Germany which differed in terms of soil, climate, field history, and consequently initial weed spectrum. The studies focussed on the comparison between local herbicide standards and split applications of Roundup Ready (360 g L^{-1} glyphosate) applied at dosages of 1.5 + 1.5; 2 + 2 and 3 + 3 L ha^{-1} Roundup Ready.

Concerning the potential changes in weed communities, the study indicated no negative effect on weed infestation, communities or diversity, of the glyphosate treatments compared to the local herbicide standard. Possible shifts of the abundances of individual species were more affected by the initial and site specific weed spectrum rather than the herbicide treatment. Similar results were seen for the Shannon's diversity index and Shannon's evenness index.

In sum, and looking at annual effects, it could be concluded from this study that 1) there are no statistically significant differences between local standard herbicide treatments and the glyphosate treatments assessed in this study on the mean values of seedbank, species richness, species diversity and dominance; 2) the data collected on the different parameters showed an enormous variability within sites and years; 3) a dosage of 3 + 3 L ha^{-1} Roundup Ready avoids spread of less sensitive species like *Chenopodium album* and *Urtica urens*; 4) as far as the standard herbicides are efficiently applied, they will have the same effect as the Roundup Ready treatments.

Key words: Weeds, maize, glyphosate, Roundup Ready, biodiversity, weed seedbank, species richness, species diversity

Zusammenfassung

Auswirkungen des Einsatzes von Glyphosat auf die Unkraut-Flora wurden in 6-jährigen Feldstudien mit kontinuierlichem Anbau von transgenem Herbizid-toleranten Mais untersucht (2003–2008). Die Untersuchungen wurden an drei Standorten in Deutschland durchgeführt, die sich hinsichtlich Boden, Klima, Anbau-Historie und (infolge dessen auch) im anfänglichen Unkraut-Spektrum unterschieden. Die Studien verglichen den lokalen Einsatzstandard an Herbiziden mit verschiedenen Anwendungen von Roundup Ready (360 g L^{-1} glyphosate) bei den Einsatzmengen 1.5 + 1.5; 2 + 2 and 3 + 3 L ha^{-1} Roundup Ready.

Es konnten keine (negativen) Veränderungen der Unkraut-Gesellschaften zwischen lokal üblichem Herbizideinsatz und Glyphosat-Behandlungen hinsichtlich der Intensität der Verunkrautung, der Pflanzengesellschaft oder Diversität festgestellt werden. Etwaige Verschiebungen der Häufigkeiten einzelner Arten konnten eher

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Accepted
2 May 2011

auf die Ausgangs- und Standortbedingungen zurückgeführt werden. Betrachtungen des Shannon's Diversity Index und Shannon's Evenness Index kommen zu gleichen Ergebnissen.

Zusammenfassend lassen die Untersuchungen folgende Schlussfolgerungen zu: 1) statistisch signifikante Unterschiede zwischen ortsüblichem Herbizid-Einsatz und (den beschriebenen) Glyphosat-Anwendungen können unter Berücksichtigung der Mittelwerte für Samenbank, Artenreichtum, Diversität und Dominanz nicht gefunden werden; 2) die Variabilität der untersuchten Variablen ist zwischen den Standorten und Jahren sehr hoch; 3) die Anwendung von 3 + 3 L ha^{-1} Roundup Ready kann die Ausbreitung der weniger sensitiven Arten wie *Chenopodium album* und *Urtica urens* vermindern; 4) ein effizienter, ortsüblicher Herbizideinsatz hat vergleichbare Effekte auf die Unkraut-Flora wie der Einsatz von Roundup Ready.

Stichwörter: Unkrautflora, Mais, Glyphosat, Roundup Ready, Biodiversität, Unkraut-Samenbank, Artenreichtum, Artenvielfalt

Introduction

The cultivation of transgenic glyphosate-resistant crop varieties can simplify chemical weed control in crops such as maize, oilseed rape or sugar beets. Advantages to the use of non-selective herbicides in the glyphosate-resistant crops lie in their highly reliable efficacy and crop safety as well as in the broad flexibility of application (Schulte, 2005). On the other hand, the repeated use of broadly effective herbicides, especially in the same crop, can have a negative effect on biodiversity. Such undesirable effects are reduced by the diversified crop rotations, but in continuous maize culture with the use of glyphosate exclusively, weed shifts could be extremely accelerated and amplified. Extensive tests in Great Britain have shown that biodiversity is affected even after a short time by the cultivation of herbicide-resistant crop plantings (Heard et al., 2003).

The composition of weed flora and the weed seedbank in the soil, especially in maize, is also determined by crop rotation, soil tillage and the intensity of the herbicide applications (Barberi et al., 1998). Weeds can be extremely competitive with maize, and therefore the cultivation of maize requires consistent and effective weed control. For that purpose, the farmer currently has a number of selective and highly effective herbicides available. Consequently, even conventional chemical weed control can have a negative impact on the weed species diversity. The purpose of these tests was therefore to determine whether any such long-term effects of conventional herbicide application differ from the multi-year use of glyphosate.

Material and Methods

Trial sites and experimental design

For the comparison of the different herbicide treatments, identical tests in continuous maize cultivation were initiated in 2003 at 3 sites. The areas selected represented typical maize cultivation sites in Northern and Eastern Germany, which differed in terms of their prior field use, soil characteristics and climate (Tab. 1).

The soil was prepared before and during the test period by plowing in Vesbeck and Werne, although the Gerbitz site had been in non-conversion-tillage production for many years. The Roundup Ready maize was planted as usual between April 29 and May 18.

With a total test area of 81 m × 93 m (including a buffer of non-transgenic maize), the plot size was 18 m × 18 m. In our study we compared eight different herbicide treatments (see below), each treatment in one plot. In each of these treatment plots, 4 square sub-plots in a fixed position with an area of 9 m^2 were systematically installed for the weeds assessments. This test design with relatively large plots, but no real replicates, was designed to ensure accurate positioning and to prevent disruptive peripheral effects, e.g. by the accidental carry-over of soil.

Herbicide treatments

Eight herbicide treatments were tested in the period between 2003 and 2008. For comparison with the local standard treatment (treatment 1), Roundup Ready (360 g L^{-1} glyphosate) in splitting application was used exclusively in treatments 3–5, each with 2 split applications totaling 3, 4 and 6 L ha^{-1}.

Tab. 1. Characteristics of the trial sites

	Gerbitz	Vesbeck	Werne
Latitude (N)	51° 50′ 21″	52° 35′ 33″	51° 41′ 44″
Longitude (E)	11° 50′ 00″	09° 37′ 55″	07° 37′ 06″
Altitude (m)	64	50	82
Mean temperature (°C)	8.9	8.7	9.6
Annual rainfall (mm)	483	699	749
Soil texture	silt loam	loamy sand	sand
pH	7.5	5.5	5.8

Treatments 6–8 were treated similar to treatment No. 4, but in every 3rd year (2005 and 2008) there was a change with herbicides with different mode of action (Tab. 2).

According to the specific weed situation, the standard treatment contained the following herbicides in combinations of 2–3 herbicides in tank mixtures: Callisto (100 g L^{-1} mesotrione), Cato (250 g kg^{-1} rimsulfuron), Gardo Gold (312.4 g L^{-1} s-metolachlor and 187.5 g L^{-1} terbuthylazine) and Motivell (40 g L^{-1} nicosulfuron). The herbicides were applied between May 10 and June 16, i.e. 3–38 days after the maize was planted. The first Roundup Ready treatments were applied simultaneously with the standard treatment; the second application was made after 14 to 40 days, depending on the site and year.

Weed assessment and data analysis

Within the sub-plots, a visual assessment of the percentage of weed coverage was made at a maximum of 6 times a year. At the same time we reported the growth stage (BBCH), ground cover and possible phytotoxic symptoms of the maize crop (results not reported here).

The seedbank, e.g. the number of weed seeds per species, was determined by germination tests in the glasshouse: Each year, shortly after the maize was planted, soil samples were taken from the sides of the above referenced sub-plots. For this purpose, undercuts from a depth of 30 cm respectively 590 cm^3 soil volume were combined into a mixed sample. These soil samples were spread in a 2–3 cm thick layer in flat pans over an area of approximately 550 cm^2. In order to assess as many viable seeds as possible by breaking the dormancy, the soil samples were mixed and spread out multiple times, cooled and dried in between germination timings.

On account of the extremely high spatial variability, the weed dynamic was calculated primarily on the basis of individual annual differences within the same sub-plot. No standard distribution of the data and no homogeneity of the variances were found, so if possible non-parametric statistical tests like the Kruskal-Wallis test were used to analyse treatment effects. Also descriptive methods like box-whisker-plots and frequency distributions were applied. Statistical calculations and graphic presentations were done using the Statgraphics Plus program (Version 5.1). We put the main focus on the comparison of the local standard (LOCSTD) with the Roundup Ready treatments (1.5 + 1.5, 2 + 2, 3 + 3).

Furthermore treatment effects were assessed by plotting the relative abundance of the weed species against the species list, ranked in order of descending abundance (relative species abundance). This descriptive approach is especially applicable to detect possible shift of rare weed species (Preston, 1948).

Due to the different site conditions mostly all evaluations were done site by site. The evaluation of weed diversity was based on the following indicators:

- Species richness: (S)
- Abundance per species (n) and total (N)
- Dominance: (D) = n_{max} N^{-1}
- Shannon's index of diversity: (H) = (N ln N − Σ n ln n) N^{-1}
- Shannon's index of evenness: (E) = H $(\ln S)^{-1}$
- Relative species abundance (log plot diagram)

Results

General weed situation

Weed density and composition differed significantly between the sites which was likely an effect of the different situation in terms of climate, soil and field history (Tab. 1 and 3). Before starting the treatments in 2003, the weed diversity at the site Werne was much higher than at Gerbitz. The same was true for the weed seed density in the soil.

Tab. 2. Herbicide treatments

No.	Herbicide	Rate	2003	2004	2005	2006	2007	2008
1	Local Standard	a.r.	•	•	•	•	•	•
2	MON 69447[a] + Roundup Ready	2.0 + 3.0	•	•	•	•	•	•
3	Roundup Ready	1.5 + 1.5	•	•	•	•	•	•
4	Roundup Ready	2.0 + 2.0	•	•	•	•	•	•
5	Roundup Ready	3.0 + 3.0	•	•	•	•	•	•
6	Roundup Ready	2.0 + 2.0	•	•		•	•	
	Lentagran WP[b] + Bromotril 250 SC[c]	2.0 + 0.5			•			•
7	Roundup Ready	2.0 + 2.0	•	•		•	•	
	MaisTer [d]	0.15			•			•
8	Roundup Ready	2.0 + 2.0	•	•		•	•	
	Terano[e]	1.0			•			•

a) 360 g L^{-1} acetochlor, b) 450 g kg^{-1} pyridate, c) 250 g L^{-1} bromoxynil, d) 9.6 g kg^{-1} iodosulfuron + 300 g kg^{-1} foramsulfuron, e) 25 g kg^{-1} metosulam + 600 g kg^{-1} flufenacet

Tab. 3. Initial weed situation at the 3 sites in 2003

Parameter	Code	Unit	Gerbitz	Vesbeck	Werne
Seedbank	N	n m^{-2}	3810	28388	15633
Species richness of seedbank	S_{sb}	n	8	19	17
Species richness of flora	S_f	n	5	12	10
Shannon's diversity index	H	–	1.10	1.77	2.03
Shannon's evenness index	E	–	0.52	0.59	0.72

During the complete test period we found 94 weed species in the seedbank at the three sites, but 15 species occurred only once. The most frequent weed species were *Chenopodium album*, *Solanum nigrum* and *Stellaria media*, but only *Chenopodium album* showed reasonably similar densities at all 3 sites (Tab. 4). Compared to the extensive weed survey by MEHRTENS et al. (2005), the weed communities can be described as typical for maize fields in Germany. However, compared to this study *Echinocloa crus-galli* and *Matricaria chamomilla* were found less whereas others species like *Gnaphalium uliginosum* and *Digitaria ischaemum* appeared more frequently.

Starting from the beginning in 2003 the number of weed species increased from 37 to 53 at the end of the study in 2009. Of those, 33 species occurred with a frequency of less than 25% and 18 of those were found only on one site in 1–2 years. Alternatively, 14 weed species were found in at least 75% of the study.

Tab. 4. Frequency (%) of weed species (extract of seedbank data, 2003–2009)

Weed species	Gerbitz	Vesbeck	Werne	All sites	Germany°
CHEAL	35.0	25.8	11.9	72.7	79.7 *
SOLNI	4.8	35.3	29.8	70.0	36.3
STEME	8.0	29.4	29.8	67.2	61.0
POAAN	0.8	30.6	20.5	51.9	27.7 *
AMARE	25.6	0.2	18.0	43.8	15.6 *
VIOAR	0.2	12.3	27.0	39.5	47.8
CAPBP	1.7	27.5	2.0	31.3	33.8
GNAUL	6.6	10.5	12.2	29.2	0.4 *
URTUR	24.4	0.0	0.3	24.7	4.3 *
RUMAA	0.0	21.3	0.2	21.4	12.7 *
DIGIS	0.0	2.3	17.0	19.4	7.0 *
ECHCG	6.9	2.3	9.7	18.9	53.0
POLPE	0.0	17.7	1.1	18.8	24.8
URTDI	2.7	13.4	2.2	18.3	4.3 *
CHEPO	0.0	0.9	16.1	17.0	79.7 *
VERAG	0.0	0.0	15.0	15.0	31.1 *
MATCH	0.8	2.2	11.6	14.5	50.3 *
GASCI	0.2	0.6	13.6	14.4	11.4 *
LOLPE	0.0	13.8	0.0	13.8	2.3 *
SONOL	5.0	1.4	7.2	13.6	19.9 *
SENVU	6.4	3.6	1.4	11.4	2.2
TRFRE	0.0	1.1	10.3	11.4	2.7 *
SOLTU	10.8	0.0	0.2	10.9	2.2
VERPE	0.5	0.0	10.3	10.8	31.1 *
VERAR	0.9	0.0	9.7	10.6	31.1 *
ERPVE	0.2	9.8	0.0	10.0	‹0.1
RUMOB	0.6	9.4	0.0	10.0	12.7 *

°) acc. to MEHRTENS et al. (2005), * reported for genus

Both the spectrum of weed species and the seedbank size were highly location-dependent. Averaged across the sites and treatments, the relative frequency of the following weeds increased within the six years: *Amaranthus retroflexus, Solanum nigrum* and *Chenopodium album*. In some years the soil seedbank increased by a factor of up to 5. There was a significant decrease in the seed number of *Capsella bursa-pastoris, Juncus bufonius* and *Solanum nigrum.* Other weeds such as *Echinocloa crus-galli, Gnaphalium uliginosum* and *Galinsoga ciliata* reacted weakly and indifferently.

Looking at the complete data set, there was a clear interaction between site and treatment effects: Especially at Gerbitz, the site with the lowest initial weed diversity both seedbank and species richness increased independently from the herbicide treatment. In contrast to Gerbitz, the weed diversity at Vesbeck declined during the study period (Fig. 1).

Effects of Roundup Ready on the weed seedbank

Based on the year-to-year comparisons on sub-plot level at the three sites there were no differences between the local standard and the full Roundup Ready treatment (Fig. 1a). However, lower dosages of glyphosate resulted in a higher weed seedbank at all sites. Although there were no clear trends during the first three years, at the end of the study the seedbank has increased to 52% at the 1.5 + 1.5 treatment (mean value of the three sites). For example at Gerbitz the seedbank ratios (relation between two subsequent years) ranged between 0.42 and 5.08 ($\bar{x}$ = 2.02). The seedbanks at Vesbeck and Werne responded to a lower extend: 0.64–1.54 ($\bar{x}$ = 1.03) and 0.61–2.29 ($\bar{x}$ = 1.53) respectively. Looking to the reasons for the strong increase especially *Chenopodium album* and *Urtica urens* could be identified for a high seed rain. This was not the case at the full glyphosate treatment and also not at the local standard where the seedbank ratios achieved 1.12 and 1.23 respectively.

The data reveal that reducing the glyphosate dosage from 2 × 3 L/ha^{-1} by 50% will result in a heavy weed buildup. Also the full glyphosate dosage as well as the standard treatments could not avoid additional weed seed production in each year, but finally these treatments obtained the least weed seed densities.

Effects of Roundup Ready on the weed species richness and weed diversity

Similar to the seedbank data we also could expect major site effects on weed species richness and weed diversity. Averaged across all sites, subplots, and years 9 weed species were found. Whereas the species number declined during the study period at Vesbeck ($\bar{x}$ = 11) from 14 to 9, the number remained at a similar level at Gerbitz ($\bar{x}$ = 6) and Werne ($\bar{x}$ = 12). Fig. 4b shows slight differences between the sites, but it also shows that weed species richness was not affected by the different herbicide treatments. According to these findings there was also no significant treatment impact on the Shannon's diversity index (Fig. 4c). Since it is strongly affected by the density

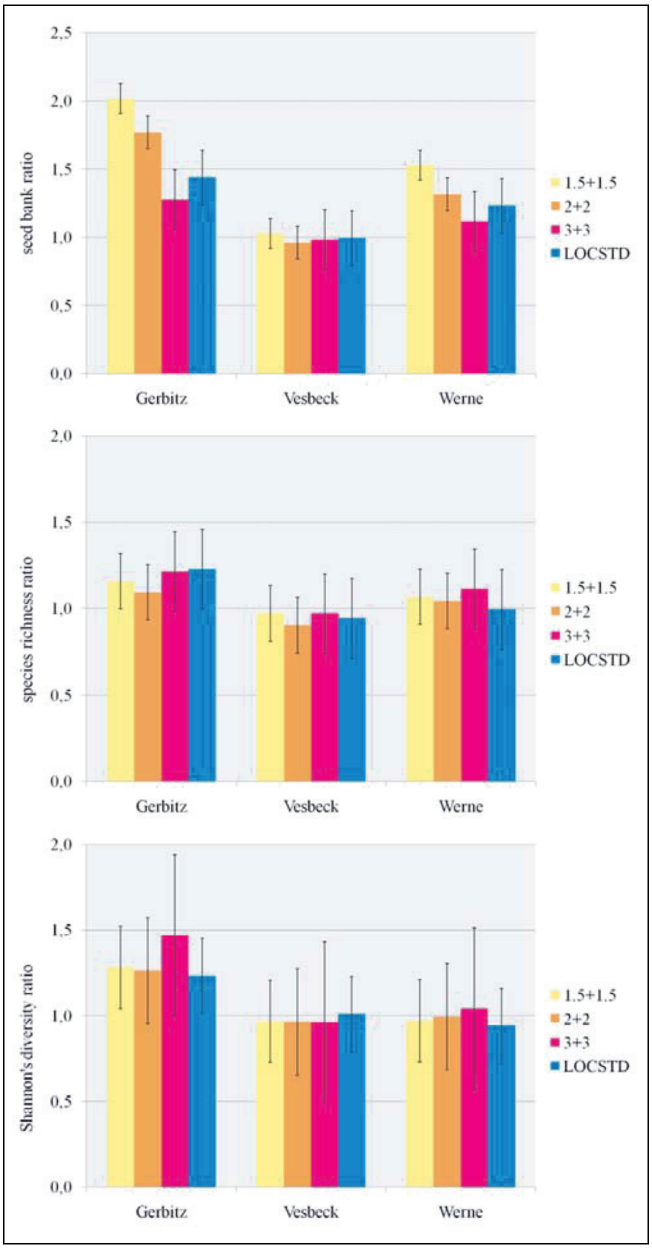

Fig. 1. Effects of herbicide treatments on the ratio of (a) seedbank, (b) species richness, (c) Shannon's diversity (a ratio of > 1 indicates an increase of the parameter from 2003 to 2009), mean values and SEM.

of predominant weed species, the Shannon index varied at a wide range at Gerbitz (0.56–1.19). However, a trend during the study period was not visible, but the index declined at Vesbeck (1.73 to 1.40) and Werne (1.89 to 1.58). The Shannon's index of evenness (data not shown) responded in the same way as the Shannon's diversity index.

Based on the above mentioned general indicators, we could not find clear evidence that the Roundup Ready system resulted in a stronger weed shift than the tested standard herbicide treatments did.

In a next step the ranking of weed species according to their relative abundance was analysed. Especially we are interested in a possible response of very rare as well as very dominant weed species. Fig. 2 shows the relative

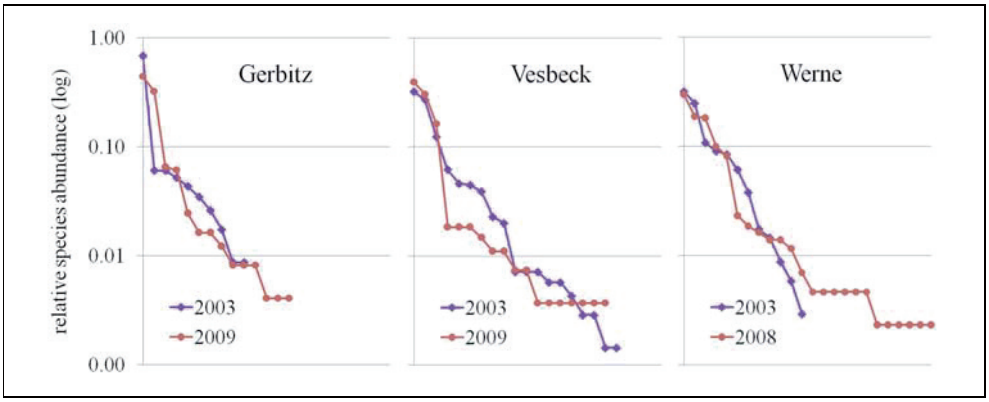

Fig. 2. Effect of the 3 + 3 L ha^{-1} Roundup Ready application on the relative species abundance.

species abundance for the 3 + 3 L ha^{-1} Roundup Ready treatments: By comparing initial and final year there is no evidence that the 3 + 3 treatment resulted in a loss of less frequent species or in a stronger dominance of other weed species.

From an agronomical point of view it is of interest if the continuous application of glyphosate will create situations with less but more dominant weed species. Fig. 3 shows the frequency distribution of the dominance indicator (D) across all sites and years (except 2003). Based on this parameter, there was no shift towards to dominant weed species in the 3 + 3 treatment. We actually found that by the full glyphosate dosage the frequency of dominant weed species (D > 0.6) was lower than in all other treatments.

Response of specific weed species and functional groups of weeds

Because of the heterogeneous nature of the data, it was difficult to detect herbicide treatment effects on certain weed species, especially for the rare and spontaneous species. Consequently the following evaluation bases upon classes of weed species according to specific characteristics:

a) Weed classes according to their sensitivity to glyphosate
b) Weed classes according to their initial frequency
c) Weed classes according to their resistance risk

Regarding the class of weed species characterised by a low sensivity against glyphosate, there was no shift until 2008, but a different situation in 2009 (Fig. 4). Since the reduced data set in the final year (no data at Werne) this result should not be overestimated. However, at Gerbitz reduced glyphosate rates resulted clearly in a serious build-up of *Chenopodium album* and *Urtica urens*. Also the seedbank of *Polygonum convolvulus* increased to some extend (80 seeds m^{-2} in 2009). Other less sensitive species like *Menthis arvensis*, *Convolvulus arvensis* and *Trifolium repens* occurred in low densities without showing any trend.

Based on the initial occurrence (Fig. 5) weed species which where less frequent at the beginning of the study

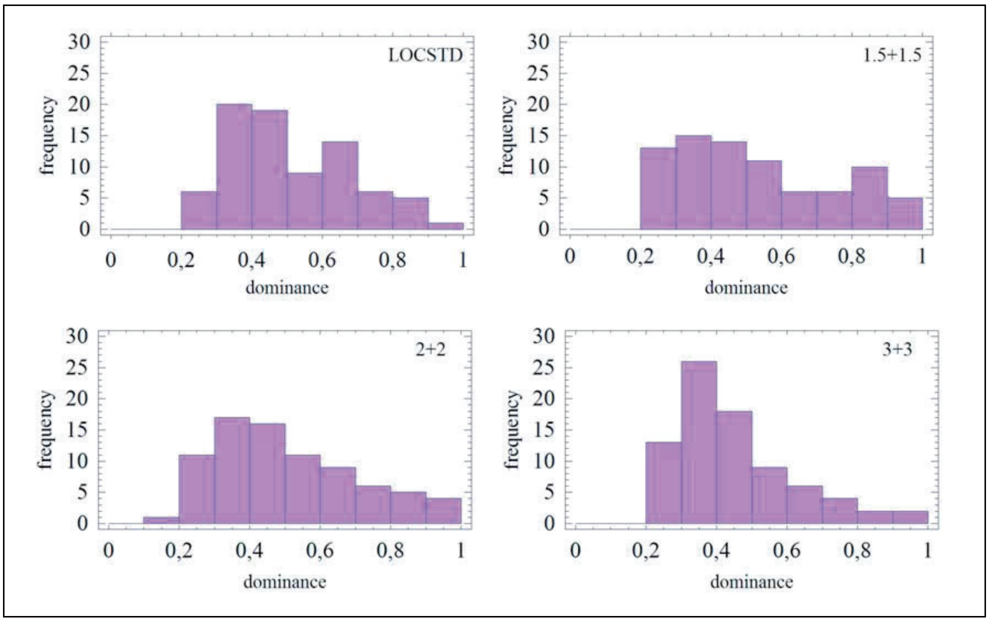

Fig. 3. Frequency of the most predominant weed species (dominance (D), data of all sites, 2004–2009).

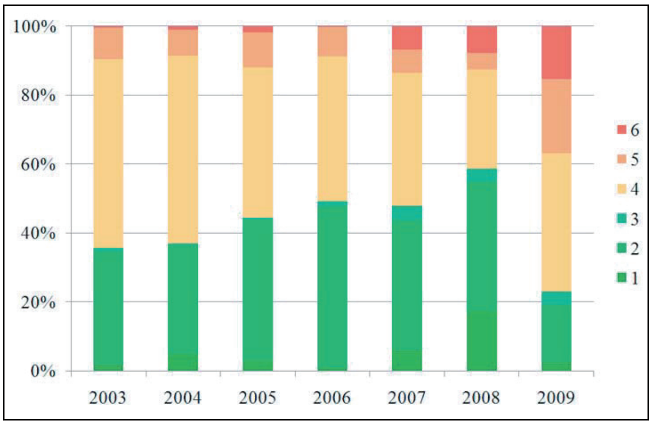

Fig. 4. Proportion of weed species according to their sensivity class (1 = high sensitivity to glyphosate, 6 = tolerant to glyphosate).

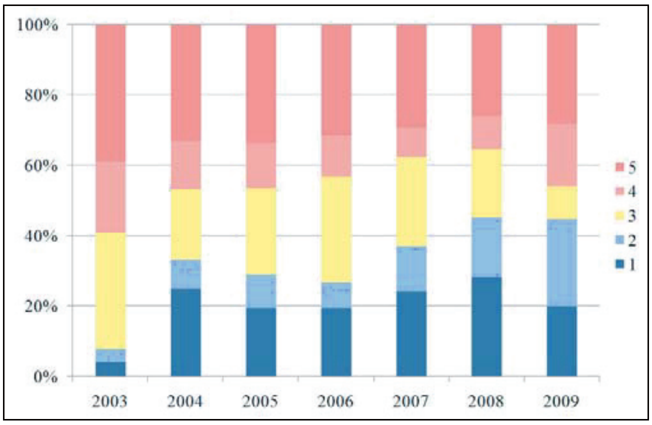

Fig. 5. Proportion of weed species according to their initial frequency in 2003 (1 = 0–1.9%, 2 = 2–9.9%, 3 = 10–79%, 4 = 80–89%, 5 = 90–100%).

increased by trend in all Roundup Ready plots. Depending on site and year we found 10–23 weed species which were not abundant in 2003 and 2004 (single plant samples not considered). Some of these species achieved rather high seedbank densities at the end, e.g. *Echinocloa crus-galli* (333 seeds m^{-2}), *Erophila verna* (1403 seeds m^{-2}), *Lamium* spp. (489 seeds m^{-2}) and *Veronica* spp. (223 seeds m^{-2}). However, due to the high spatial variation it was not possible to distinguish treatment effects on any of these weed species.

In a next step we analysed possible treatment effects on those weed species for which herbicide resistance is known. All species were considered with at least three general records by HEAP (2010) or at least one record for the HRAC group G (Glycine, including glyphosate). Out of these 24 relevant weed species, *Chenopodium album* was the only one which increased extra-proportionally. Averaged across the 3 different Roundup Ready treatments and all sites, the density of *Chenopodium album* increased from 206 to 920 seeds m^{-2} during the study period. Even the high rate of 3 + 3 L ha^{-1} Roundup Ready could not avoid spreading out of this weed.

Discussion

In comparison with the standard herbicide use and based on the analysed indicators, the continuous application of glyphosate did not result in a significant weed shift during the 6 years study. Regarding to the size and composition of the weed seedbank the variation caused by the different sites and years was considerably higher than treatment effects.

Altogether the results of this study are in line with recent studies which have been conducted in North America where glyphosate-tolerant maize is widely grown since more than 10 years. According to a survey by CULPEPPER (2006) weed shifts have not been observed in maize, but in other GMHT crops in the United States. However, similar to our findings it is also reported that low glyphosate dosages of less than 0.8 kg ha^{-1} may cause serious weed problems, especially for *Chenopodium album* (WILSON et al., 2007). Furthermore, especially in fully glyphosate-tolerant crop rotations spreading of volunteer crops like oilseed rape, soybean or winter wheat have to be considered. Because of the missing residual effect of glyphosate, a higher seed production of late emerging weeds is more likely in GMHT maize rather than in conventional cultivars (HEARD et al., 2003). For these reasons American long term experiences have clearly demonstrated that the exclusive use of glyphosate raises the agronomical risks in terms of weed shifts.

Focussing on the ecological aspect we could not find a clear proof, that the Roundup Ready system reduces the weed diversity in maize. There are similar findings of the Field Scale Evaluation in UK. According to DEWAR (2009) this is mainly due to the fact that growing of maize itself as well as any measure of weed removal are of higher relevance than the type of herbicide. As far as selective herbicides are applied at optimal conditions, an almost complete weed kill can be achieved. Because of a very low competitiveness of maize and thus a high need for an efficient weed control, there are only very few options for enhancing the biodiversity in this crop.

Acknowledgements

The author would like to thank Martina KRACHT, Jens BEHRENS and Werner LÖHR for their excellent technical assistance with the tests on the seedbank.

The planting and conducting of the field tests were performed by employees of the Landwirtschaftskammer Nordrhein-Westfalen, the Landwirtschaftskammer Niedersachsen and the Landesanstalt für Landwirtschaft und Gartenbau in Sachsen-Anhalt.

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