

Exploring the effects of glyphosate products on weed composition

Untersuchung des Einflusses von Glyphosat auf Unkrautzusammensetzungen

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Abstract

Glyphosate is a non-selective, broad-spectrum, systematic herbicide that is the world's most widely used herbicide since its introduction in the 1970s as a pre-plant, post-directed and post-harvest herbicide application with further technological developments leading to its use within glyphosate resistant crops (GRCs) as of the 1990s. In countries around the world, weed shifts have accompanied weed management systems employing glyphosate products. The farmer actions and weed reactions that have contributed to these weed shifts will be discussed. Evidence of glyphosate-induced weed shifts has not yet been documented in Germany, but through consideration of the conditions that have led to glyphosate-induced weed shifts elsewhere the outlook for Germany will be assessed. An ongoing research project will be introduced in which weed monitoring is being employed to find out if glyphosate-induced weed shifts can be identified in northeastern German arable farm fields and in which field experiments are also being conducted to further explore the reactions of weed communities and populations under the intensive application of glyphosate products.

Keywords: Arable farming, Germany, glyphosate, weed shift

Zusammenfassung

Glyphosat ist ein nicht-selektives, breit wirksames Blattherbizid mit systemischer Wirkung, das sich, seit seiner Einführung als Vorsaats-, Voraufbau und Nach-Ernte-Herbizid in den 1970er Jahren zu dem weltweit am häufigsten eingesetzten Herbizid entwickelt hat. Weitergehende technologische Entwicklungen führten in den 1990er Jahren zum Einsatz in genetisch veränderten Kulturpflanzen. Anbauverfahren unter Verwendung von Glyphosat haben weltweit zu Veränderungen der Artenzusammensetzung von Unkräutern geführt. In der vorliegenden Arbeit werden Managementfaktoren sowie die darauffolgenden Unkrautreaktionen diskutiert. In Deutschland sind aktuell noch keine Veränderungen der Artenzusammensetzung von Unkräutern bekannt, doch durch die Betrachtung der Faktoren, die anderswo zu veränderten Artenzusammensetzungen geführt haben, soll ein Ausblick für Deutschland gegeben werden. Es wird ein laufendes Forschungsprojekt vorgestellt, in dem mögliche Veränderungen der Unkrautflora durch Bonituren ermittelt werden. Innerhalb des Projektes werden außerdem Feldexperimente durchgeführt, die die Reaktionen von Unkrautgesellschaften und Populationen auf intensiven Glyphosateinsatz eruieren.

Stichwörter: Ackerbau, Deutschland, Glyphosat, Veränderung der Artenzusammensetzung

Introduction

Glyphosate is a non-selective, broad-spectrum, systematic herbicide that has become the world's most widely used herbicide since its introduction in the 1970s as a pre-plant, post-directed and post-harvest herbicide application with further technological developments leading to its use within glyphosate resistant crops (GRCs) as of the 1990's. In countries around the world, weed shifts have accompanied weed management systems employing glyphosate products. CULPEPPER (2006) attributed the weed shifts in the United States to the combination of a rise in conservation tillage and a high reliance on glyphosate products for weed management while the use of residual herbicides declines, leading to more weeds tolerant to glyphosate and weeds that emerge after glyphosate application. The causes behind glyphosate-related weed shifts have also been attributed to evolved resistance (HEAP, 2014), the selection of species (community level) and biotypes (population level) that are naturally resistant (NANDULA et al., 2005), plants strategically avoiding glyphosate with their germination and development timing (HILGENFIELD et al., 2004) and

the growth of species that are attracted to reduced tillage farm management systems (SWANTON et al., 1999).

There is currently a heavy reliance on chemical management in European arable farming. Non-chemical options exist, but need to be improved through research and actually implemented by farmers (MELANDER et al., 2013). Weed shifts are ultimately not avoided through tillage management schemes; rather, other species are selected for, as shown by TUESCA et al. (2001) in a long-term study of different tillage systems. Weed selection is constantly taking place in arable farming, from the crop rotation (if existent) and choice of crop sowing date to weed management through either various degrees of tillage or herbicide applications (with the accompanying choice of application time, frequency, and dose), or both. The maintenance of diversity in weed management, e.g., herbicide rotations, sequences, combinations of robust rates of different modes of action and non-chemical weed control, has been proposed as the key to sustaining glyphosate as an effective weed management tool (POWLES, 2008).

In contrast to the monoculture wheat and corn belts of North America, it is still common in German arable farming to employ a crop rotation, even if only containing two crops. The main crops in Germany are winter wheat, winter barley, winter oilseed rape, sugar beet and legumes, with the portion of corn on the rise.

Glyphosate resistant weeds around the world tend to be found on fields where glyphosate products have a history of being applied with great frequency (e.g., 2-3 times per year) (COLLAVO and SATTIN, 2014). As of November 2015, Germany does not have any recorded cases of glyphosate resistant weeds (HEAP, 2015). In crops equipped with glyphosate resistance it is possible to apply glyphosate products even after crop growth has begun. In Germany, glyphosate application on agricultural land is restricted to stubble, pre-sowing and pre-harvest time periods since glyphosate resistant crops are not permitted to be grown (BMEL, 2015). While not allowed on agricultural lands, in-crop application in Germany does take place between the rows of perennial crops, such as in orchards, in vineyards and on grasslands. German regulations for the use of glyphosate products are strict. Current regulations, updated with tightened measures in May 2014, only permit two applications a year separated by a time span of 90 days. A maximum of 3.6 kg a.i. per hectare per year may be used. Siccation is only permitted on partial field areas when a harvest would otherwise be hindered by weeds; it may not be employed to steer the harvest date or optimize threshing (BVL, 2014).

Two recent farmer questionnaires conducted by a research group based in Göttingen, Germany (STEINMANN et al., 2012; KONING et al., 2015) both show that glyphosate products are applied on the greatest percent of crop area to the stubble, followed by at pre-sowing time, and finally at pre-harvest time (68.1, 20.7 and 11.2% and 22.7, 12.8, and 2.4%, respectively). The effect of the stronger siccation regulations recently enacted are evidenced in the farmer responses as it appears that an immense reduction in stubble applications took place between 2009 and 2014 (reference years of STEINMANN et al., 2012; KONING et al., 2015).

Although weed shifts in general are an unavoidable companion to agriculture, weed shifts based on the use of glyphosate products may be postponed for the foreseeable future in Germany due to the implementation of crop rotations, lack of GRCs, and strict glyphosate application regulations that keep German farmers from applying amounts that have proven elsewhere to lead to glyphosate-induced weed shifts.

Many recent publications have focused on detecting how glyphosate affects plants on a molecular level and how application leads to resistance (VILA-AIUB et al., 2013), while other studies highlight glyphosate-induced weed shifts occurring in agricultural systems where glyphosate resistant crops are grown (CERDEIRA et al., 2011). Our study contributes to glyphosate research by taking an ecological approach to assessing the effect of glyphosate on plant communities and populations, as well as looking for signs of glyphosate-induced weed shifts in Germany's non-GRC agricultural

landscape. The methods of two experiments and a monitoring are presented including preliminary results from the first and second year of experimenting.

Materials and Methods

Weed monitoring

Weeds are monitored in commercial winter wheat fields of northeastern Germany with the goal of determining if certain weed species have greater presences in fields where no herbicides are used, any herbicide active ingredient other than glyphosate is used, or glyphosate products are used at low, medium, or high intensities. Glyphosate product use intensity classes are created for the fields sampled in the monitoring relative to the field histories that date back 5 to 10 years. Weeds are monitored twice a year with the same fields being visited in the second period as were monitored in the first period: in the month leading up to the winter wheat harvest (i.e. July) and while the field is being prepared for the next crop, preferably after a round of tillage has instigated fresh germination (i.e. late August until late September). Each of three profiles per field is 2-by-50 meters in size and weed densities at the species level are scored in classes. Monitoring takes place on around 50 unique fields per year for three consecutive years. Data acquisition is in progress.

Weed community experiment

An experiment in Latin Square Design on a research field with a naturally occurring weed population and no crop has been set up to study the weed community composition under the influence of four different management tools employed twice a year (June and August):

- 100% of the manufacturer's recommended glyphosate product dose (1080 g a.i./ha for fields without *Elymus repens*, Glyfos Supreme, Cheminova Deutschland GmbH),
- 50% of the recommended dose,
- chisel ploughing and
- mouldboard ploughing.

Plots measure 5 x 6 meters. Weed richness, density and cover (after Braun-Blanquet with a scale of 1 to 5 but excluding r and +) are recorded at the outset of the growing season (June) and approx. 9 weeks after each treatment (August and October) with ten 0.1 m² random sample sub-plots per plot. The field was grassland up until four years prior the current experiment when it became the location of various arable farming experiments that were managed mechanically and chemically. In the fall of 2013 the field was tilled with a mouldboard plough and in the spring of 2014 harrowing broke up and smoothed the soil surface as preparation for the current experiment. In March of 2015, after one experimental season, the field was re-set for the second season by mulching the biomass in the glyphosate-treated plots and managing all of the experimental plots with a rotary harrow. The experiment runs for three consecutive years, with results currently available for the first two years.

Weed population experiment

A field experiment under semi-natural conditions in a randomized block design is being conducted to evaluate the effect of applying glyphosate product doses reduced from the manufacturer's recommended dose (1080 g a.i./ha for fields without *Elymus repens*, Glyfos Supreme, Cheminova) at which a portion of a sown weed population survives to produce seeds. Two appropriate doses for each weed species were determined in greenhouse bioassays prior to outdoor application. The reproductive success of four weed species, *Arabidopsis thaliana* (thale cress), *Lolium multiflorum westerwoldicum* (westerwold ryegrass), *Papaver rhoeas* (field poppy) and *Senecio vulgaris* (common groundsel), are being followed over two to three generations. The parent seeds do not have a history of being exposed to glyphosate herbicides. Sowing takes place in 1 m² plots at densities of 10000 seeds/m² for *A. thaliana* and *P. rhoeas* while *L. multiflorum* and *S.*

vulgaris are sown at 1000 seeds/m². The presence of additional, naturally growing vegetation in the plots is not prohibited, but is kept to a moderate level through mechanical weeding so as to encourage strong growth of the target plants while at the same time not inhibiting their success. The glyphosate product application occurs once a year for each species: *A. thaliana* at the rosette stage in early spring, *L. multiflorum* just prior to tillering in early summer, *P. rhoeas* at 1-6 true leaves in early summer and *S. vulgaris* at 1-6 true leaves in late summer. The number of plants growing prior to the herbicide treatment, the number of seed-bearing plant components, e.g., inflorescences, capsules and pods that develop after treatment, in addition to the number of seeds produced are recorded as measures of reproductive success. This paper will only evaluate the germination rates of the parent and F1 generations. Germination rates were calculated by dividing the number of seedlings before herbicide application by the number of seeds initially sown. Additionally, a difference in germination rate was determined by subtracting the germination rate of the F1 generation from the germination rate of the parent generation.

Results

Weed community experiment

After two seasons, the results of the weed community experiment have been evaluated for weed richness, density, taxonomic representation (dicotyledon and monocotyledon), and Raunkiaer lifeform representation. An overview of the 2014 and 2015 survey data with the species presence/absence data from all four treatments compiled under the respective survey session shows high similarities between the two seasons (Tab. 1). Total richness saw a greater decrease from the first to second and third surveys in 2015 than in 2014, but the 2015 average contained only three species less the 2014 average. The share of monocot and dicots recorded at each session show a similar trend in both years with the dicot portion increasing steadily over the course of the season. Raunkiaer lifeforms presented a much greater contrast in therophyte dominance over hemicryptophytes in 2014 compared to 2015, but the two years showed agreement in the low contributions of geophytes and chamaephytes to the weed communities.

Tab. 1 Overview of 2014 and 2015 survey data with all four treatments data compiled into survey sessions.

Tab. 1 Übersicht der Vegetationsaufnahmedaten aus 2014 und 2015 mit allen vier Behandlungen nach Vegetationsaufnahmetermin zusammengestellt.

Season	Total Richness	Monocot Species	Dicot Species	Raunkiaer Lifeforms* T-H-G-C (%)
2014				
June	52	8 (15%)	44 (85%)	63-27-8-2
August	51	7 (14%)	44 (86%)	61-31-6-2
October	51	5 (10%)	45 (90%)	52-38-8-2
2015				
June	54	9 (17%)	45 (83%)	48-44-6-2
August	45	7 (16%)	38 (84%)	44-44-9-2
October	46	6 (13%)	40 (87%)	48-41-9-2

*Raunkiaer Lifeforms: T-Therophyte H-Hemicryptophyte G-Geophyte C-Chamaephyte

The cover classes of the living vegetation recorded in the 2015 season show a common trend over all the treatments for a mid-summer low in cover (Fig. 1). Management with the chisel plough lead to the highest cover at the first and second surveys but the 100% glyphosate treatment had a slightly higher cover at the time point of the third survey. The plots managed with the mouldboard plough consistently had the lowest cover at each survey session.

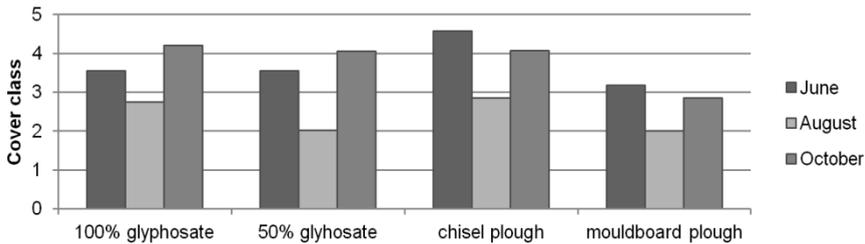


Fig. 1 Cover classes of the living vegetation for the 2015 season (surveys in June, August and October) sorted in the bar graph by treatment.

Abb. 1 Deckungsgrad der vitalen Vegetation für die Saison 2015 (Vegetationsaufnahme in Juni, August und Oktober), sortiert nach den Behandlungen in dem Balkendiagramm.

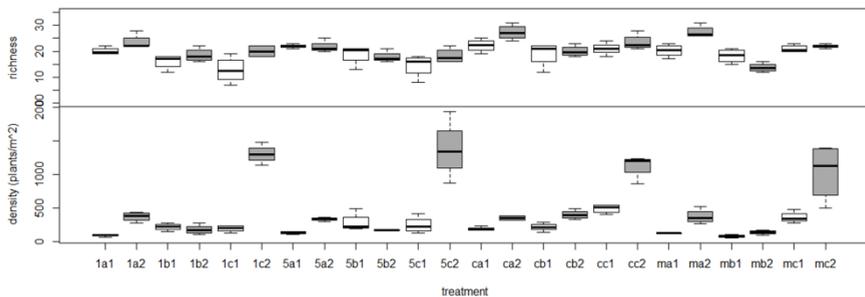


Fig. 2 Box-and-whisker plots for the 2014 (white boxes) and 2015 (dark grey boxes) field seasons showing the species richness of the treatments (1 = 100% recommended glyphosate dose, 5 = 50% recommended glyphosate dose, c = chisel plough and m = mouldboard plough) and the species density (plants/m²) at the time of the three vegetation surveys (a, b and c conducted in June, August and October of each year, respectively).

Abb. 2 Box-Whisker-Plots der Feldsaisons 2014 (weiße Boxen) und 2015 (dunkelgraue Boxen) sortiert nach Behandlung (1 = 100 % der empfohlenen Glyphosataufwand, 5 = 50 % der empfohlenen Glyphosataufwand, c = Grubber und m = Pflug) Artenreichtum und Gesamtdichte der Arten (Pflanzen/m²) aus drei Vegetationsaufnahmen (a, b und c durchgeführt jeweils in Juni, August und Oktober in jedem Jahr).

Species richness had a total range of 7 to 31 species, but each treatment average over the course of the season was around 20 species. Species density remained within a 0-500 plants/m² range until the third survey in the second season when the density jumped to a maximum of nearly 1500-2000 plants/m² in each treatment. An evaluation of the species richness and density with the aid of box-and-whisker plots shows a tendency for richness and density to decrease in the middle of the summer (i.e. August) and increase again in the fall (i.e. October) (Fig. 2, season 1 is white and season 2 is dark grey). Exceptions to this tendency in the case of richness were the 100% and 50% glyphosate treatments in season one in which the richness constantly declined over the course of the three surveys. As for density, in the first season only the mouldboard plough treatment followed the above mentioned trend while the other treatments lead to constant rises in density over the course of the three surveys. In the second season only the chisel plough treatment diverged from the above mentioned trend and constantly increased in density.

An evaluation of the survey data for the three species with the highest frequency for each treatment at each survey in the first two seasons of the experiment produces the following species list in alphabetical order: *Arabidopsis thaliana*, *Capsella bursa-pastoris*, *Cerastium fontanum*,

Crepis/Lactuca spp., *Elymus repens*, *Fallopia convolvulus*, *Matricaria chamomilla*, *Poa annua*, *Stellaria media*, *Trifolium spp.* (predominantly the perennial *Trifolium hybridum*), and *Viola arvensis*. In a visual inspection of the frequency bar charts (Fig. 3), it is evident that high frequencies are maintained throughout both seasons for *Trifolium spp.* in the 100% and 50% glyphosate treatments. While the 100% glyphosate treatment has *Trifolium spp.* in the top three highest frequencies in six of six surveys and the 50% glyphosate treatment and the mouldboard plough management both have it in four of six surveys, the chisel plough management only had *Trifolium spp.* in the top three highest frequencies in two of six surveys. Unique to the plots under chisel plough management was the high frequency of the perennial *C. fontanum* with it being the second most frequent in four of six surveys.

Weed population experiment

Due to the complexity of each species' response to the conditions of the experiment, this paper will only look at the germination rates of the parent and F1 generations in the experimental field (Tab. 2). Germination rates for *A. thaliana* and *S. vulgaris* are only yet available for the parent generation, but these rates serve as a vital foundation for comparisons with the following generations.

Tab. 2 Germination rates in the weed population experiment.

Tab. 2 Keimraten aus den Unkrautpopulationsversuch.

	Germination Rate (%)		Difference in germination rate*
	Parent Generation	F1 Generation	
<i>Arabidopsis thaliana</i>			
Control	1.19	NA	NA
1/16 dose	0.79	NA	NA
1/8 dose	0.78	NA	NA
<i>Lolium multiflorum</i>			
Control	28.58	24.85	3.73
¼ dose	31.38	14.93	16.45
½ dose	25.28	5.23	20.05
<i>Papaver rhoeas</i>			
Control	4.73	2.74	1.99
1/16	2.48	1.96	0.52
1/8	2.58	0.52	2.06
<i>Senecio vulgaris</i>			
Control	36.78	NA	NA
1/16	39.60	NA	NA
1/8	41.15	NA	NA

*Difference in germination rate = Parent generation – F1 generation

The untreated parent generations of *L. multiflorum* and *S. vulgaris* germinated at relatively stable rates with ranges of 25.28 to 31.38% and 36.78 to 41.15%, respectively. Much greater ranges occurred in the *A. thaliana* and *P. rhoeas* untreated parent generations with 0.78 to 1.19% and 2.48 to 4.73%, although really only the control groups set themselves apart from the other two treated groups in the case of both species. Germination rates of the F1 generation are only available at this time for *L. multiflorum* and *P. rhoeas*, but even in this one aspect of reproductive success, the species do not follow the same trend. As expected, the germination rate notably decreased between the parent and F1 generation for *L. multiflorum* as the glyphosate dose increased. In contrast, the control and higher glyphosate dose (1/8 dose) of *P. rhoeas* show a similar difference in germination rate (1.99 and 2.06, respectively) while the lower glyphosate dose (1/16) had a

much greater loss in germination rate (0.52). Germination rates of the F2 generation of both species will demonstrate whether these species-specific trends continue.

Discussion

Within the framework of Germany's flora, environmental conditions, pesticide use regulations, crop regulations (e.g. non-GRC), and farmer management choices, we are investigating the possible existence and likelihood of a weed shift in northern German arable farming through weed monitoring and field experiments.

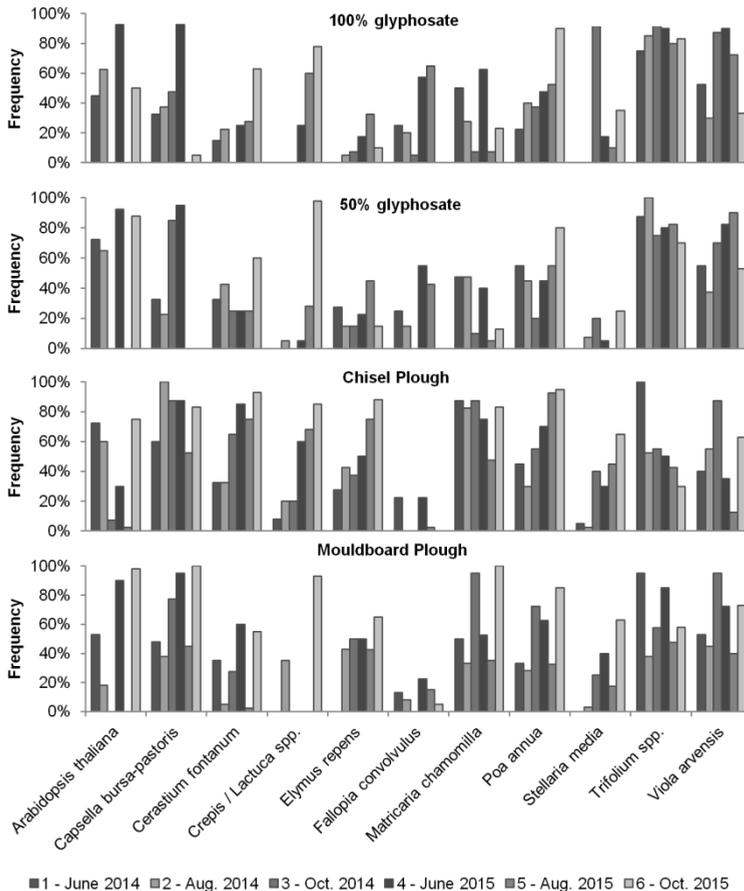


Fig. 3 Frequencies of the top three species recorded in each treatment at the six surveys taken over two seasons in 2014 and 2015.

Abb. 3 Häufigkeiten der drei häufigsten Arten je Behandlung in sechs Vegetationsaufnahmen, 2014 und 2015.

Although the effects of weed management only really become evident after long periods of time, such as from a 30-year field trial (PALLUTT, 2010), with our weed community experiment we are attempting to intensify management conditions with two treatments each year in order to compact the time period in which we see the results of our management choices. We may then be able to compare trends in the weed community composition results from the experiment with the results of the monitoring conducted on real agricultural land in the same geographical region.

Results from the first two seasons of the weed community experiment indicate that species presence is foremost driven by the time of year. Richness was the highest at the first survey of all the treatments in both years. The greater mid-summer decrease and early-fall increase of the richness in the tillage treatments compared to the glyphosate treatments can be explained by tillage exposing the soil bare in mid-summer and bringing new seeds up to the surface in the late-summer period whereas the glyphosate plots remained mechanically undisturbed with an approximately 25 cm high treated, non-compacted biomass cover. Density was the highest at the third survey of all treatments in both years. Even without the soil disturbance of tillage that brought new seeds to the surface, the glyphosate plots profited from the seed rain of their biomass covers.

The majority of the species occurring with the top three frequencies in the treatments were annuals. The perennials *C. fontanum*, *E. repens* and *Trifolium spp.* (as predominantly the perennial *T. hybridum*) were each associated with a certain growing environment. *C. fontanum* only reached high frequency in the chisel plough plots where, as demonstrated in the chisel plow experiments conducted by LOGSDON (2013), only the soil surface experienced disturbance and many plants were left simply relocated. *E. repens* only made it into the top three of species frequency in the tillage treatments where its creeping rhizomes were spread throughout the plots by the ploughs. The high frequency of *Trifolium spp.* in the glyphosate plots and mouldboard plough plots can be attributed to the lower species surface cover compared to the plots managed with the chisel plough. Although a vigorous grower in monoculture, *Trifolium spp.* grows poorly in the company of aggressive species (FRAME, 2015).

With an initial assessment of the reproductive success of *L. multiflorum* and *P. rhoeas* based only on the germination rate, this study has shown that the reproductive success of the F1 generation was reduced. Analysis of the seed-bearing plant components as well as seed production will contribute to a more thorough understanding of each species' reproductive success. It is a possibility that not all of the reproductive success factors react consistently. A generation with a high germination rate could nonetheless produce a reduced number of plant components while still turning out a high number of seeds by the end of the growth season. Research on hormesis, the stimulatory effect of a low dose of a toxicant, has demonstrated that herbicides do not influence all the characteristics of a plant consistently (DUKE et al., 2006). Unique changes in characteristics as a result of herbicide treatment may also vary from species to species, thereby selectively affecting the number of tillers produced (COUPLAND and CASELEY, 1975) or root length (BELZ, 2014).

The weed monitoring in winter wheat and field experiments with weed communities and populations continues for a third season in which further results contributing to our knowledge base of weed behavior in response to chemical management with glyphosate products in the northern German environment will be garnered.

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