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Glyphosate in the rhizosphere—Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants

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ABSTRACT

Glyphosate is the most widely used non-selective, systemic herbicide. It is easily translocated from shoot to roots and released into the rhizosphere, where it is immobilized at the soil matrix or microbially degraded. However, contradictory results are reported in the literature concerning the bio-availability of glyphosate residues in soils and the potential risks for intoxication of non-target organisms. This study addresses the question whether plant residues of glyphosate-treated weeds (model plant perennial rye grass, *Lolium perenne* L.) or direct soil application of glyphosate bears an intoxication risk for subsequently cultivated sunflower (*Helianthus annuus* L.) seedlings. The experiments were conducted as greenhouse studies on two soils with contrasting properties (acidic, sandy Arenosol, calcareous loess subsoil). Also the potential role of different waiting times between glyphosate application and sunflower cultivation was considered.

On both soils, sunflower seedling growth and biomass production was strongly impaired by glyphosate pre-sowing treatments in the variants with 0 d waiting time and recovered within a waiting time of 7–21 d. Generally, the detrimental effects were more pronounced after glyphosate weed application (90% biomass reduction) compared with direct soil application (55–70% biomass reduction) at waiting time 0 d. The inhibitory effects on seedling growth were associated with a corresponding increase in shikimate accumulation in the root tissue as physiological indicator for glyphosate toxicity. Glyphosate intoxication of sunflower seedlings was also associated with an impairment of the manganese-nutritional status, which was still detectable after a waiting time of up to 21 d, particularly on the Arenosol in the variants with glyphosate weed application. These findings indicate an important and yet uninvestigated role of glyphosate in plant residues in determining the risk of non-target plant intoxication.

1. Introduction

Glyphosate (*N*-phosphonomethylglycine) is the most widely used broad-spectrum herbicide on global scale. After foliar application, it is absorbed by the foliage and translocated throughout stems, leaves and roots of the entire plant, finally accumulating preferentially in young growing tissues (Franz et al., 1997). The herbicidal effect is based on inhibition of the shikimate pathway enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), involved in the biosynthesis of aromatic amino acids and phenolic compounds (Della-Cioppa et al., 1986; Franz et al., 1997). Therefore, glyphosate application frequently induces intracellular accumulation of shikimate, which can be used as a sensitive physiological indicator for glyphosate toxicity (Henry et al., 2007).

Glyphosate can reach the soil via foliar wash-off and undirected spray drift contamination (Al-Kathib and Peterson, 1999; Ellis and Griffin, 2002) and by exudation from roots or death and decomposition of treated plant residues (von Wiren-Lehr et al., 1997; Neumann et al., 2006; Laitinen et al., 2007). However, risks of glyphosate toxicity to non-target organisms in soils are generally considered as marginal, since glyphosate is almost instantaneously inactivated by adsorption to clay minerals and cationic binding sites of the soil matrix (Piccolo et al., 1992; Dong-Mei et al., 2004), while glyphosate in the soil solution is prone to rapid microbial degradation (Giesy et al., 2000).

An additional potential pool of glyphosate accumulation and stabilization in soils is represented by the plant residues of glyphosate-treated weeds. Since in many plant species, glyphosate is not readily metabolized, considerable amounts can accumulate particularly in young tissues (Reddy et al., 2004). However, the fate of bound glyphosate in plant residues has not been widely considered in the past. Studies with soybean and wheat suggested unspecific and non-covalent binding of glyphosate to starch

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and cell wall components (Komoßa et al., 1992). The release and degradation of ^{14}C-labelled glyphosate in various agricultural soils correlated with the soil-microbial activity but only after direct soil application. No such correlation was observed after soil incorporation of lyophilized soybean tissue cultures, contaminated with glyphosate. These findings suggest different mechanisms for degradation of glyphosate adsorbed to the soil matrix and bound in plant residues in the soils, respectively. No information exists on factors determining the stabilization and release of glyphosate bound in plant residues and the potential risks for non-target organisms getting in contact with these residues.

An increasing number of yet unexplained observations of negative side effects after glyphosate application has been reported in the literature (Smiley et al., 1992; King et al., 2001; Kremer et al., 2001; Charlson et al., 2004; Fernandez et al., 2005; Huber et al., 2005; Yamada, 2006; Neumann et al., 2006), which have been related to direct toxicity of glyphosate, impairment of the micronutritional status and increased susceptibility to plant diseases. This study was initiated to investigate the influence of glyphosate residues in the root tissue of glyphosate-treated weeds on plant biomass production, intracellular shikimate accumulation as indicator for glyphosate toxicity and the micronutrient status of subsequently cultivated non-target plants in comparison with direct glyphosate soil application. The study was conducted using rye grass (*Lolium perenne* L. cv. Kelvin) as target weed and sunflower (*Helianthus annuus* L. cv. Frankasol) seedlings as non-target plants, considering also the impact of different waiting times after glyphosate application for the subsequent culture, as well as two contrasting soils with different binding properties for glyphosate. In addition, the findings of these model pot experiments were compared with observations of field experiments of local farmers.

2. Materials and methods

2.1. Conditions for plant growth

Experiments were conducted under greenhouse conditions, using two contrasting soils with different cationic binding sites for glyphosate: a sandy acidic Ap horizon of an Arenosol with low buffering capacity (pH ($CaCl_2$) 4.5; C_{org} 0.16%; water-extractable Ca^{2+} and Mg^{2+} (Beck et al., 2000) [mg kg^{-1} soil]: 0.4 and 0.4), and with a well-buffered calcareous loess subsoil (pH ($CaCl_2$) 7.6; C_{org} < 0.3%; $CaCO_3$ 23.3%; water-extractable Ca^{2+} and Mg^{2+} [mg kg^{-1} soil]: 59.9 and 11.3). Calcium chloride–diethylenetriamine penta-acetic acid (CAT)-extractable micronutrient concentrations [mg kg^{-1} soil]: Mn = 7.4, Fe = 369.0, Zn = 0.8, B = 0.9, and Cu 0.5 (VDLUFA, 2004), exchangeable Al^{3+} (McLean, 1982) = 0.04 cmol kg^{-1} soil for the Arenosol and Mn = 15.0, Fe = 7.8, Zn = 0.6, B = 0.2 and Cu = 0.7 (VDLUFA, 2004).

Soils were sieved by passing through a 2 mm mesh size and fertilized with N as $Ca(NO_3)_2$ (100 mg N kg^{-1} soil), K as K_2SO_4 (150 mg K kg^{-1} soil), Mg as $MgSO_4$ (50 mg Mg kg^{-1} soil) and P as $Ca(H_2PO_4)_2$ (80 mg P kg^{-1} soil). In addition, the calcareous subsoil was supplied with Fe as FeEDTA (20 μmol kg^{-1} soil). Plant culture was performed in pots containing 500 g of fertilized soil and soil moisture was adjusted to 70% of the soil water-holding capacity (15%, w/w for the Arenosol and 18%, w/w for the calcareous loess subsoil). Water losses were determined gravimetrically and replaced by daily applications of de-ionized water.

2.2. Glyphosate plant application

To investigate the effects of glyphosate residues in the root tissue of target weeds on subsequently cultivated non-target plants, rye grass (*L. perenne* L. cv. Kelvin) was pre-cultivated as model-weed

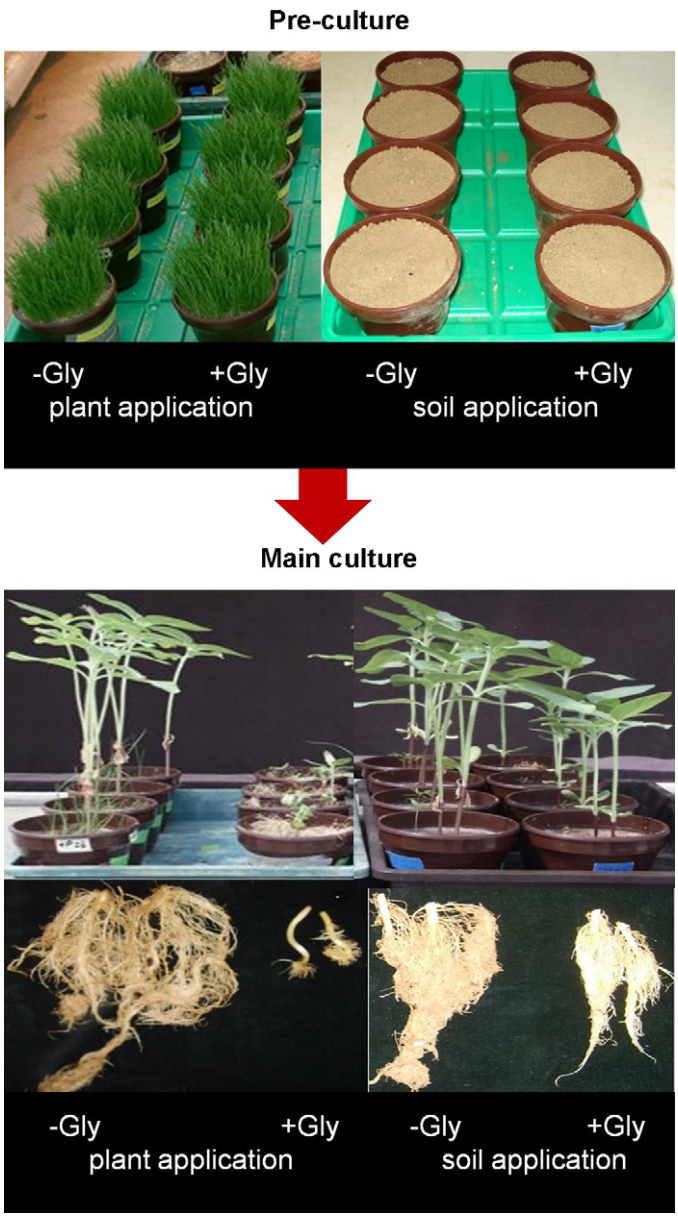

Fig. 1. Shoot and root development of sunflower seedlings grown for 25 DAS on an acidic Arenosol with (+Gly) or without (−Gly) pre-sowing glyphosate treatments on a pre-culture with *Lolium perenne* or direct glyphosate soil application.

in 500 g pots filled with the fertilized soils. A sowing density of 2.2 g rye grass seeds (germination rate 70%) per pot with a surface area of 100 cm^2 was used to simulate high weed coverage of the soil with intense root development (Fig. 1). At 10 d after sowing (DAS), the young rye grass seedlings were sprayed with the recommended dilution of Roundup Ultramax® glyphosate formulation (Monsanto Agrar, Düsseldorf, Germany), containing a glyphosate concentration of 28.4 mM in the spray solution using a hand-held sprayer. Each pot received 6.7 mL of glyphosate spray solution on the leaves, based on determination of the rye grass leaf area coverage (approximately 3300 cm^2 per pot) and the plants died within 7 d, a typical time period usually observed also under field conditions (Pilot experiments with lower doses of glyphosate failed to desiccate the rye grass plants completely even within 3–4 weeks). Subsequently, sunflower seeds (*H. annuus* L. cv. Frankasol) were sown into the same pots (7 seeds per pot) at 0, 7, 14 and 21 d after rye grass glyphosate application. After desiccation, rye grass residues were removed and no disturbance of the soil in the pots was under-

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Table 1
Shoot and root dry matter of sunflower plants (25 DAS) grown on an acidic Arenosol with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively.

Treatment	Shoot biomass (g)		Root biomass (g)	
	Plant application	Soil application	Plant application	Soil application
0 d – Gly	0.59 ± 0.05^{ab}	0.58 ± 0.03^{ab}	0.27 ± 0.03^{ab}	0.27 ± 0.03^{ab}
0 d + Gly	0.07 ± 0.03^{c}	0.26 ± 0.06^{bc}	0.04 ± 0.02^{c}	0.09 ± 0.02^{bc}
7 d – Gly	0.32 ± 0.04^{bc}	0.56 ± 0.02^{ab}	0.32 ± 0.07^{a}	0.27 ± 0.02^{ab}
7 d + Gly	0.40 ± 0.3^{abc}	0.52 ± 0.03^{ab}	0.27 ± 0.19^{ab}	0.26 ± 0.01^{ab}
14 d – Gly	0.37 ± 0.06^{bc}	0.56 ± 0.07^{ab}	0.35 ± 0.02^{a}	0.35 ± 0.05^{a}
14 d + Gly	0.57 ± 0.06^{ab}	0.55 ± 0.02^{ab}	0.33 ± 0.06^{a}	0.28 ± 0.01^{ab}
21 d – Gly	0.75 ± 0.11^{a}	0.54 ± 0.05^{ab}	0.41 ± 0.03^{a}	0.32 ± 0.04^{a}
21 d + Gly	0.46 ± 0.46^{ab}	0.56 ± 0.05^{ab}	0.24 ± 0.24^{abc}	0.31 ± 0.03^{a}

Data represent means and standard deviations (±SD) of 4 independent replicates. Significant differences between treatments within a column are indicated by different characters.

taken. This time period was defined as "waiting time". In control treatments without glyphosate application, rye grass shoots were removed by cutting at the soil level with a sharp knife. A time schedule with sequential sowing dates for the rye grass pre-culture was employed to ensure the same sowing day and thus the same external growth conditions for all sunflower seedlings, irrespective of the waiting time. All treatments were performed in 4 replicates.

2.3. Glyphosate soil application

To assess the effects of glyphosate in the soil on non-target plants, the same amount of glyphosate as applied to the target weeds (6.7 mL of a Roundup Ultramax® solution containing a glyphosate concentration of 28.4 mM) was mixed directly with 500 g of the fertilized soils. Controls received only mineral nutrients and water. After a waiting time of 0, 7, 14 and 21 d, sunflower seeds were sown (7 seeds per pot) at the same day as in the treatments with rye grass weed pre-culture.

2.4. Plant harvest

At 12 d after sowing (DAS), a first set of sunflower seedlings was removed from the pots. Roots and shoots were separated, frozen in liquid nitrogen and stored at −20 °C for shikimate analysis. In each pot, two seedlings were kept and further cultivated until 25 DAS. At final harvest, the root systems were washed out from the soil, and shoot and root parts were separated for biomass determination. The youngest fully expanded leaves were selected for analysis of micronutrients.

2.5. Shikimate analysis

Shikimate in acidic tissue extracts was analyzed with modifications of the methods described by Singh and Shaner (1998) and Neumann (2006).

The frozen plant tissue was homogenized with 5% orthophosphoric acid (1 mL 100 mg^{-1} fresh weight) using mortar and pestle. Insoluble material was removed by centrifugation (5 min at 20,000 × *g*) and the supernatant was used for HPLC analysis after appropriate dilution with the HPLC mobile phase.

HPLC separation was performed by ion exclusion chromatography using an Aminex 87H column (Bio-Rad, Richmond, CA, USA) designed for organic acid analysis. A sample volume of 20 μL was injected into the isocratic flow (0.5 mL min^{-1}) of the eluent (2.5 mM H_2SO_4, 40 °C) and organic acids were detected spectrophotometrically at 210 nm. Identification and quantification of shikimate was conducted by comparing the retention times, absorption spectra and peak areas with a known standard.

2.6. Analysis of micronutrients

Shoot mineral nutrients were determined according to Gericke and Kurmies (1952). Dried leaves (70 °C) were ground and ashed in a muffle furnace at 500 °C for 5 h. After cooling, the samples were extracted twice with 2 mL of 3.4 M HNO_3 (v/v) and subsequently evaporated to dryness. The ash was dissolved in 2 mL of 4 M HCl, subsequently diluted 10-fold with hot de-ionized water, and boiled for 2 min. After addition of 0.1 mL Cs/La buffer to 4.9 mL ash solution, Fe, Mn and Zn concentrations were measured by atomic absorption spectrometry (UNICAM 939, Offenbach/Main, Germany).

2.7. Statistics

All treatments comprised 4 replicates and pots were arranged in the greenhouse in a completely randomized block design. Analysis of variance was performed with SPSS statistics software package (SPSS Inc., IL, USA).

Table 2
Shoot and root dry matter of sunflower plants (25 DAS) grown on a calcareous loess subsoil with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively.

Treatment	Shoot biomass (g)		Root biomass (g)	
	Plant application	Soil application	Plant application	Soil application
0 d – Gly	0.53 ± 0.04^{abc}	0.59 ± 0.06^{ab}	0.29 ± 0.02^{abc}	0.26 ± 0.01^{abc}
0 d + Gly	0.05 ± 0.02^{e}	0.23 ± 0.09^{de}	0.03 ± 0.02^{e}	0.07 ± 0.03^{de}
7 d – Gly	0.35 ± 0.04^{bcd}	0.54 ± 0.03^{abc}	0.28 ± 0.03^{abc}	0.26 ± 0.02^{abc}
7 d + Gly	0.38 ± 0.19^{bcd}	0.48 ± 0.11^{abc}	0.17 ± 0.12^{cd}	0.22 ± 0.05^{bc}
14 d – Gly	0.32 ± 0.04^{cd}	0.45 ± 0.03^{abcd}	0.33 ± 0.05^{ab}	0.26 ± 0.03^{abc}
14 d + Gly	0.31 ± 0.19^{cd}	0.42 ± 0.07^{abcd}	0.22 ± 0.07^{bc}	0.22 ± 0.06^{bc}
21 d – Gly	0.65 ± 0.11^{a}	0.47 ± 0.16^{abcd}	0.38 ± 0.07^{a}	0.30 ± 0.06^{abc}
21 d + Gly	0.57 ± 0.02^{ab}	0.53 ± 0.02^{abc}	0.30 ± 0.03^{abc}	0.30 ± 0.05^{abc}

Data represent means and standard deviations (±SD) of 4 independent replicates. Significant differences between treatments within a column are indicated by different characters.

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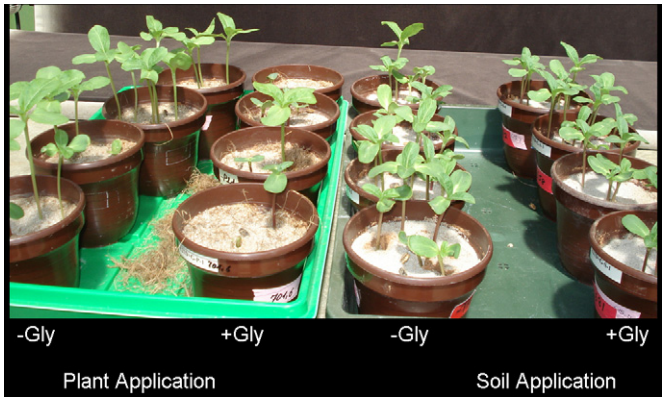

Fig. 2. Germination and seedling development of sunflower plants grown on an acidic Arenosol at 21 d after desiccation of a ryegrass pre-culture by foliar glyphosate application (plant application) and after direct soil application of the same glyphosate dose (soil application).

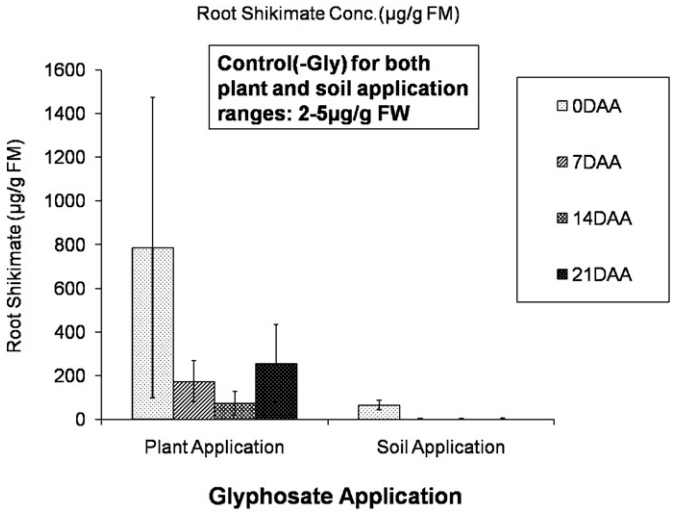

Fig. 3. Intracellular shikimate accumulation in the root tissue of sunflower seedlings (12 DAS) grown on an acidic Arenosol with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively. Data represent means and standard deviations of 4 independent replicates. The background levels of shikimate concentrations are shown as numeric values.

3. Results

Biomass production of sunflower seedlings was not influenced by the two contrasting soils (acidic Arenosol, calcareous loess subsoil) used for plant culture. However, glyphosate pre-sowing treatments substantially reduced seedling dry matter, particularly in the variant with a waiting time of 0 d after glyphosate application for sowing of sunflower (Tables 1 and 2). The inhibitory effect was more strongly expressed when glyphosate was applied on a pre-culture of rye grass, associated with a reduction of root and shoot biomass by approximately 90%, compared with direct soil application, leading to a reduction of shoot biomass by 55–57% and of root biomass by 67–73% (Fig. 1; Tables 1 and 2). The inhibitory effects declined with increasing waiting times, but still remained detectable even at 21 d after glyphosate application, although the differences were not significant in all cases.

The detrimental effects of glyphosate pre-sowing treatments on plant growth were reflected in a corresponding increase in shikimate concentrations in the root tissue as a physiological indicator for glyphosate toxicity (Figs. 3 and 4). In this case, the differences between the two glyphosate application modes already observed for inhibition of seedling growth (Tables 1 and 2) were even more expressed, and intracellular shikimate accumulation was increased by 10–100-fold in the treatment with glyphosate applied to pre-cultured rye grass seedlings, compared with direct soil application (Figs. 3 and 4).

In contrast to direct soil application of glyphosate, the treatments with glyphosate application to the *Lolium* pre-culture were characterized by non-homogeneous germination and large differences in seedling development of sunflower (Fig. 2). This was reflected in a high variability of biomass data (Tables 1 and 2) and intracellular shikimate accumulation in the respective treatments (Figs. 3 and 4).

The pre-culture of rye grass without glyphosate application obviously increased Mn acquisition of sunflower on the Arenosol but not on the calcareous loess subsoil (Fig. 5). On both soils, glyphosate pre-sowing treatments affected Mn concentrations in the youngest fully expanded leaves in treatments with 0 d waiting time (Figs. 5 and 6). Manganese concentrations recovered with increasing waiting times in all variants with exception of the rye grass glyphosate pre-sowing treatment on the Arenosol. In this case, glyphosate application induced a decline of Mn leaf concentrations even after a waiting time of three weeks and in some cases Mn concentrations dropped close to the critical level of Mn deficiency (Bergmann, 1992) (Fig. 5).

In contrast to the Mn-nutritional status, Fe and Zn nutrition of the sunflower seedlings were not affected by glyphosate pre-sowing treatments and Fe and Zn concentrations even increased in the glyphosate-treated variants with rye grass pre-culture and 0 d waiting time (data not shown).

As a general feature of all measured parameters, data obtained from the treatments with glyphosate application to the rye grass pre-culture exhibited a much higher variation compared with those from the treatments with direct soil application of glyphosate (Tables 1 and 2, Figs. 3–6).

4. Discussion

In contrast to the common and recommended practice of glyphosate pre-sowing treatments, which frequently allows herbicide application even until the first days after sowing (Monsanto, Roundup Ultramax® product information), the results of this study

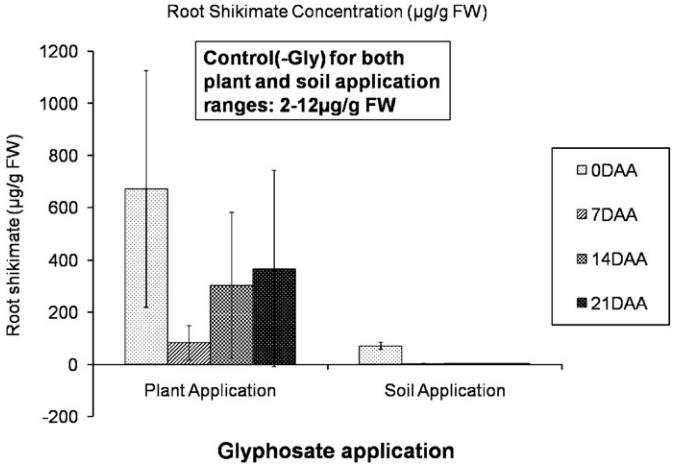

Fig. 4. Intracellular shikimate accumulation in the root tissue of sunflower seedlings (12 DAS) grown on a calcareous loess subsoil with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively. Data represent means and standard deviations of 4 independent replicates. The background levels of shikimate concentrations are shown as numeric values.

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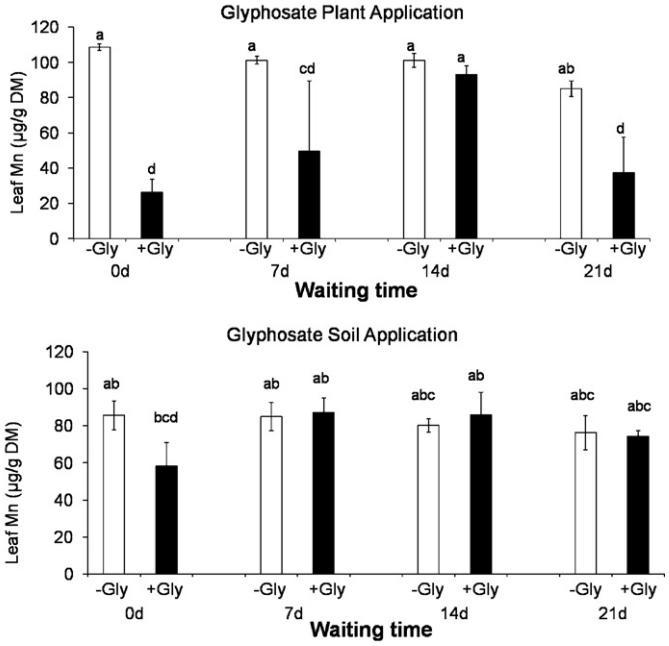

Fig. 5. Manganese concentration in the youngest fully expanded leaves of sunflower plants (25 DAS) grown on an acidic Arenosol with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively. Data represent means and standard deviations of 4 independent replicates. Significant differences between treatments are indicated by different characters.

underline the importance of waiting times, to avoid or at least minimize detrimental effects on the following culture. The analysis of physiological parameters, such as intracellular shikimate accumulation as metabolic indicator for glyphosate toxicity or the micronutrient status revealed, that the risk of toxic effects, induced by glyphosate pre-sowing treatments, increases with declining waiting time and can persist up to three weeks (Fig. 5), even when clearly visible effects on seedling growth and development are no more detectable by the first view (Fig. 1, Table 1). Similarly, Cornish (1992) reported detrimental effects of glyphosate pre-transplanting treatments on tomato in field and pot experiments on sandy loam soils, which were still detectable after waiting times of 3–4 weeks. However, this study used young tomato plants and no seeds which increases the risk of plant damage by glyphosate application.

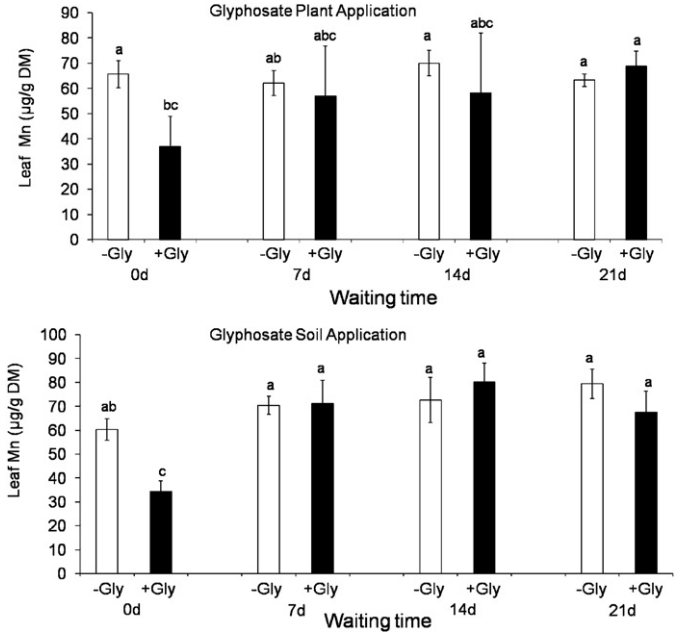

Fig. 6. Manganese concentration in the youngest fully expanded leaves of sunflower plants (25 DAS) grown on a calcareous loess subsoil with glyphosate application at 0, 7, 14 and 21 d before sowing to a pre-culture of rye grass or directly incorporated into the soil, respectively. Data represent means and standard deviations of 4 independent replicates. Significant differences between treatments are indicated by different characters.

Glyphosate-induced impairment of Mn nutrition was more strongly expressed on the sandy Arenosol with low buffering capacity compared with the well-buffered calcareous subsoil (Figs. 5 and 6), indicating a role of different soil types in determining the expression of glyphosate toxicity. This was not associated with corresponding differences of intracellular shikimate accumulation or plant biomass production (Tables 1 and 2; Figs. 3 and 4), suggesting rather soil-specific differences in Mn availability than differential expression of glyphosate toxicity on the two investigated soils as possible causes. Accordingly, soil analysis by CAT extraction (VDLUFA, 2004) revealed lower levels of available Mn in the Arenosol [7.4 mg kg^{-1} soil] as compared with the calcareous loess subsoil [15.0 mg kg^{-1} soil]. Glyphosate can form poorly soluble complexes with Mn (Sprankle et al., 1975) and may thereby reduce the already low level of available Mn in the Arenosol. Also glyphosate-induced inhibition of root growth (Fig. 1, Tables 1 and 2) may counteract Mn acquisition with the strongest consequences for Mn uptake on the Arenosol with low levels of plant-available Mn. Detrimental effects of glyphosate applications on the micronutrient status and particularly on Mn nutrition have been previously reported when glyphosate reached non-target plants as drift contamination in sub-lethal dosage (Eker et al., 2006), via rhizosphere transfer from target weeds (Neumann et al., 2006), or even in glyphosate resistant soybean (Jolley and Hansen, 2004). Since micronutrients, such as Mn and Zn are important physiological cofactors for mechanisms of plant disease resistance (Cakmak, 2000; Thompson and Huber, 2007), glyphosate-induced impairment of the micronutrient status may be linked with the observations of a higher susceptibility to plant diseases (e.g. *Fusarium, Corynespora, Rhizoctonia, Gaeumannomyces* and pathogenic nematodes) in response to glyphosate treatments (Smiley et al., 1992; King et al., 2001; Kremer et al., 2001; Charlson et al., 2004; Jolley and Hansen, 2004; Fernandez et al., 2005; Huber et al., 2005).

In contrast to the Mn-nutritional status in this study, Fe and Zn concentrations in the youngest fully developed leaves were not affected by glyphosate application, except of the treatments with rye grass pre-culture and 0 d waiting time. In these cases, Fe and Zn concentrations even increased in the leaves of glyphosate-treated variants (data not shown). Most probably, this represents a concentration effect of Fe and Zn seed reserves due to the extreme growth depression of the seedlings in these treatments.

Also calcium and magnesium are discussed as potential ligands, mediating glyphosate immobilization and inactivation in soils (Sprankle et al., 1975) and in plants (Duke et al., 1985). However, despite of much higher levels of $CaCO_3$ and of free water-extractable Ca^{2+} [59.9 mg kg^{-1} soil] and Mg^{2+} [11.3 mg kg^{-1} soil] in the calcareous subsoil compared with the Arenosol [Ca^{2+}: 0.4 mg kg^{-1} soil; Mg^{2+}: 0.4 mg kg^{-1} soil], glyphosate-induced inhibition of plant growth (Tables 1 and 2) and intracellular shikimate accumulation (Figs. 3 and 4) were similarly expressed on both soils. This finding suggests that on both soils, the plants were exposed to similar levels of free glyphosate, which induced similar effects of toxicity. The lack of Ca^{2+} and Mg^{2+} in the Arenosol may be compensated by much higher concentrations of available Fe^{3+} [369 mg kg^{-1} soil] and exchangeable Al^{3+} [0.04 cmol kg^{-1}] compared with the calcareous loess subsoil Fe^{3+} [7.8 mg kg^{-1} soil] and negligible exchangeable Al^{3+} as ligands for binding and complexation of glyphosate.

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Toxicity of glyphosate pre-sowing treatments on sunflower seedlings was also strongly dependent on the mode of glyphosate application: When glyphosate was sprayed on pre-cultured rye grass seedlings, detrimental effects on plant growth and the Mn-nutritional status, as well as increased intracellular shikimate accumulation in the root tissue were more strongly expressed than after direct soil application of the same amount of glyphosate. The lower expression of glyphosate toxicity after soil application is in line with the concept of rapid inactivation and detoxification of glyphosate in soils by adsorption to phosphate binding sites, such as Fe/Al-oxides and hydroxides, precipitation as calcium salts, and rapid microbial degradation of free glyphosate in the soil solution (Sprankle et al., 1975; Giesy, 2000; Monsanto, 2005a; Yamada, 2006). Accordingly, Cornish (1992) reported increased toxicity of glyphosate soil pre-treatments on tomato after simultaneous application of P fertilizers, which obviously increased the solubility and thus the bio-availability of glyphosate by competition for soil-binding sites. It remains to be established, whether also the intense expression of root-induced mechanisms for phosphorus or iron mobilization in the rhizosphere, reported for various plant species and cultivars (Neumann and Römheld, 2002), can similarly induce toxic effects by co-mobilization of glyphosate adsorbed to P sorption sites. However, in the present short-term study, no relevance of these adaptive responses to nutrient limitation is expected, since only young seedlings were investigated, relying mainly on P and Fe seed reserves in this early developmental stage.

The increased expression of toxicity effects after glyphosate pre-sowing application to the rye grass pre-culture compared with direct soil application suggests, that also the root tissue of glyphosate-treated weeds represents a storage pool for glyphosate in the investigated soils. In this experiment, the bio-availability of glyphosate in plant residues to subsequently cultivated sunflower seedlings was obviously much higher than the bio-availability of glyphosate bound at the soil matrix. In most plant species, glyphosate is not readily metabolized and is preferentially translocated to young growing tissues of roots and shoots, where it can accumulate in millimolar concentrations (Reddy et al., 2004; Monsanto, personal communication). In soil-grown target plants, this non-homogeneous distribution of glyphosate within the root tissues may lead to the formation of hot spots of root residues in soils, containing high levels of glyphosate, which is subsequently released during microbial degradation of the plant material. Without a fast immobilization of glyphosate by adsorption on the soil matrix, glyphosate toxicity to non-target plants may be induced by root contact with these hot spots. The non-homogeneous distribution of glyphosate-contaminated plant material in the soil could also explain the much higher variation of the data on sunflower biomass production, shikimate accumulation and Mn-nutritional status after glyphosate application to the rye grass pre-culture as compared to direct soil application (Fig. 2 but also Tables 1 and 2, Figs. 3–5). Since toxic effects can be expected only after direct root contact of the non-target plants with one of the hot spots of glyphosate-contaminated plant residues, sunflower seedlings without contact to the hot spots remained unaffected. In contrast, direct soil application of glyphosate resulted in a homogeneous distribution and lower bio-availability due to adsorption of the herbicide over the investigated soil profile.

The potential role of plant residues as a pool for glyphosate stabilization in soils has not been widely considered in the past. Most of the available information originates from studies of glyphosate residues in foliage (Newton et al., 1984; Feng and Thompson, 1990; Thompson et al., 1994; Reddy et al., 2004) and not in roots. In a model study with different agricultural soils, von Wiren-Lehr et al. (1997) investigated the degradation of bound ^{14}C-glyphosate residues in lyophilized cell cultures of soybean but only the water-insoluble fraction was taken into account. Komoßa et al. (1992) characterized the binding forms of glyphosate in wheat and soybean. However, in contrast to the fate of the herbicide applied to soils in a free state, systematic investigations on the bio-availability of glyphosate in real plant residues incorporated into soils are rare. The present study suggests a considerable contribution of this glyphosate pool in determining the risk of phytotoxicity to non-target organisms. The findings of this study are in line with recent field observations of plant damage in winter wheat after glyphosate pre-crop applications and waiting times shorter than two weeks in no-tillage systems (Römheld et al., 2008). To improve bio-safety in face of the global increase in agricultural use of glyphosate, open questions to be considered for the future comprise the expression of these effects under a range of different field conditions, the impact of external factors, such as soil properties, soil moisture levels, temperature, period of season, soil-organic matter and biological activity and thus speed of microbial degradation of glyphosate containing crop residues, as well as the role of plant species, rooting densities and fertilization management. The variability of these factors in agricultural practice may contribute to the explanation of contradictory results frequently reported in the literature and in field observations concerning the risks of negative side effects of glyphosate application on non-target organisms (for reviews see Monsanto, 2005a,b and Yamada, 2006 and references cited therein).

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