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Title

Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops.

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Introduction

Glyphosate is the world´s most important non-selective, systemic herbicide. The primary reason why glyphosate is regarded as a herbicide with negligible residual activity is its strong sorption to soil minerals (Piccolo et al. 1994). Consequently, crops may be planted or seeded into treated areas directly following glyphosate application.

The globally increasing adoption of no-till or reduced tillage systems, where pre-sowing weed control is achieved with herbicides is another factor pressuring farmers towards using more glyphosate (Torresen et al. 1999). In such systems, glyphosate is applied pre-sowing for weed control, and glyphosate residues may remain in the straw. Soil disturbance usually occurs only at crop sowing, which might lead to incorporation of the glyphosate contaminated straw into the upper soil layer where seed germination occurs.

This study was initiated to investigate the potential risk of glyphosate phytotoxicity to non-target plants in reduced tillage systems, where glyphosate is employed as a means of weed control and minimal tillage is done during sowing without removing the glyphosate desiccated weed residues.

Materials and methods

For this experiment, two plant species were used as model plants: sunflower (*Helianthus annuus* L. cv Frankasol) as non-target and rye grass (*Lolium perenne* L. cv. Kelvin) as target plant. Experiments were conducted under greenhouse conditions, using two contrasting soils: a sandy acidic Ap horizon of an Arenosol with low buffering capacity and a well-buffered calcareous loess subsoil (Luvisol).

Rye grass weed was cultivated in continuously aerated nutrient solution. For glyphosate treatment, leaf surface area was calculated (7802 cm^2 per pot). After converting the recommended field application rate of glyphosate (200L of 28.4mM glyphosate solution per hectare), pots were sprayed with 15.60 ml of 28.4mM glyphosate spray solution using a hand sprayer. Twelve hours after glyphosate treatment, rye grass was harvested and shoot material was chopped into 1 cm length using scissors to be applied to the soil.

Soils were sieved to pass through a 2 mm mesh size and fertilized with essential nutrients. During fertilization, the glyphosate-treated or untreated rye grass leaves (1200 mg DM kg^{-1} soil) were mixed with the soil. Additional controls consisted of direct application of 2.36 ml of 28.4 mM glyphosate spray solution per 500 g soil and bare soil without any glyphosate application.

Soils were filled into 500 g pots, and 7 seeds of sunflower (*Helianthus annuus* L. cv Frankasol) were directly sown. Ten days after sowing, five seedlings were harvested for shikimate analysis in the roots by HPLC (Neumann 2006). The remaining two sunflower plants were grown for a total of 26 days. Youngest fully expanded leaves were selected for mineral analysis.

Results and Discussion

There was a striking difference between the two soils with respect to the inhibition of shoot and root growth by glyphosate residues. In the Arenosol, incorporation of glyphosate-treated rye grass leaves induced a strong inhibition of sunflower shoot and root growth, while on the Luvisol shoot or root growth was not visibly reduced (Fig. 1 and 2). This soil type dependent residual phytotoxicity of glyphosate is associated with a difference in the detoxification capacity between the two soils. At this level of glyphosate supply, the detoxification capacity of the highly buffered calcareous subsoil, with a high clay content as potential sorption site, mediating glyphosate immobilization and inactivation in soils (Sprankle et al. 1975) might have played a primary role

in preventing glyphosate toxicity. In addition, the expression of growth inhibition induced by direct soil application of glyphosate only on the Arenosol but not to the Luvisol confirms that the soil type dependent residual phytotoxicity is related to the detoxification capacity difference between the two soils.

Arenosol　　　　**Luvisol**

-Gly　　　**+Gly**　　**-Gly**　　　**+Gly**
Shoot residues

Fig. 1. Depression of shoot and root growth of sunflower seedlings by glyphosate-treated rye grass residues incorporated either into a highly buffered Luvisol or a less buffered Arenosol. Residues were applied at a rate of 1200 mg dry matter kg^{-1} soil.

Parallel to plant growth inhibition, nutrient concentrations (Mn and Ca) in leaves of sunflower seedlings grown on the Arenosol supplied with glyphosate-treated rye grass leaves were significantly lower compared to controls (-glyphosate). Similarly, Mn concentration in sunflower leaves declined after direct soil application of glyphosate into the Arenosol (Table 1). In contrast, on the Luvisol, there was no effect on leaf concentration of mineral nutrients associated with glyphosate phytotoxicity (Table 1).
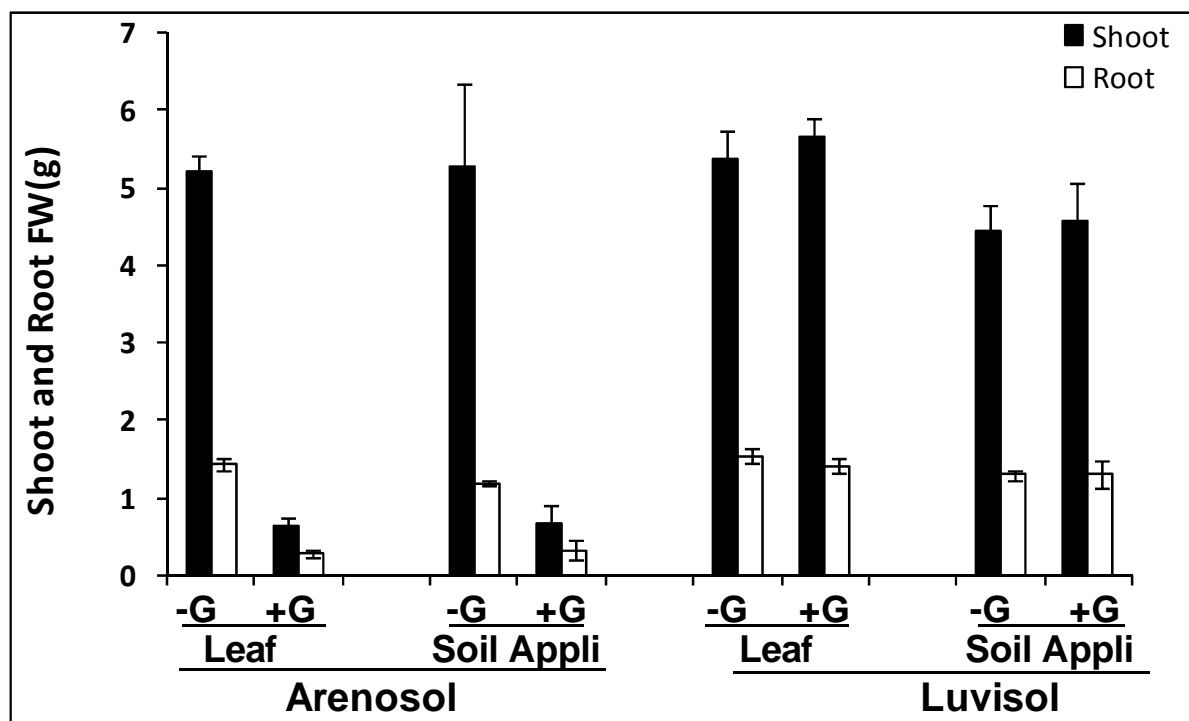
Fig.2. Shoot and root fresh weight of sunflower seedlings grown on the Arenosol or Luvisol supplied with glyphosate-treated rye grass leaves (1200 mg dry matter kg^{-1} soil) or direct soil application (4.72 ml of 28.4mM glyphosate spray solution kg^{-1} soil).

In correspondence to plant growth inhibition on the Arenosol, sunflower plants also showed strong intracellular shikimate accumulation in roots as a physiological indicator of glyphosate phytotoxicity (Table 2). In contrast, there was no intracellular shikimate accumulation in roots of sunflower seedlings grown in the Luvisol with a soil incorporation of glyphosate-treated rye grass residue (Table 2). The primary mode of action of glyphosate is the inhibition of the shikimic acid pathway resulting in the accumulation of high levels of shikimate in plant tissues (Steinrucken and Amrhein 1980).

Table 1. *Mn and Ca concentration of youngest fully expanded leaves of sunflower seedlings grown in the Arenosol or Luvisol supplied with glyphosate enriched rye grass leaves or direct soil application. Leaf material was supplied as 1200 mg dry matter kg^{-1} soil and direct soil application at 4.72 ml kg^{-1} soil.*

Treatment	Mn concentration (µg g^{-1} DM)		Ca concentration (mg g^{-1} DM)	
	Arenosol	**Luvisol**	**Arenosol**	**Luvisol**
Leaf-gly	271.4±77.0	139.2±3.1	21.5±2.2	35.6±2.1
Leaf+gly	59.1±13.2	139.3±11.1	5.7±1.0	35.6±1.2
Soil-gly	548.2±176.7	124.1±3.6	20.3±0.7	36.7±2.8
Soil+gly	74.1±28.6	124.6±7.8	10.7±3.0	35.5±1.2

Table 2. *Intracellular shikimate accumulation in roots of sunflower seedlings grown on the Arenosol and Luvisol supplied either with glyphosate enriched rye grass leaves (1200 mg dry matter kg^{-1} soil) or direct soil application (4.72 ml 28.4mM glyphosate spray solution kg-1 soil).*

Treatment	Shikimate concentration (μg g^{-1} FW)	
	Arenosol	**Luvisol**
Leaf -gly	10.0±2.2	5.0± 1.5
Leaf +gly	474.2±184.8	5.5±3.5
Soil -gly	11.0±2.6	5.0±1.3
Soil +gly	557.5±150.1	45.2±35.7

Consequently, the correlation of plant (shoot and root) growth inhibition and intracellular shikimate accumulation due to incorporation of glyphosate-treated rye grass leaves (Fig. 1 and 2; Table 2) implies glyphosate phytotoxicity as primary cause. These findings clearly demonstrate a potential risk of residual phytotoxicity of glyphosate originating from decaying organic residues of glyphosate-desiccated weeds. Special care must be given particularly in nutrient-poor light soils.

References

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