Title
Evidence for glyphosate damage of winter wheat depending on waiting-times after pre-crop glyphosate application and density of desiccated weed plants under field and experimental conditions

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Introduction

Due to low production costs and high efficiency, glyphosate is the most widely used non-selective herbicide on global scale. Glyphosate acts as a systemic herbicide, inhibiting the shikimate pathway and it is rapidly translocated from shoot to roots with subsequent release into the rhizosphere. Until recently, negative side effects on non-target plants have been considered to be marginal due to rapid immobilisation and microbial degradation of glyphosate in soils. Therefore, currently no waiting times for sowing after pre-crop glyphosate application are recommended.

In previous worst-case model studies, high doses of glyphosate application to weeds in combination with short waiting times before sowing of the subsequent crop resulted in much stronger and longer lasting crop damage than the same amount of glyphosate directly mixed with the soil. (Tesfamariam et al., 2009, submitted). These findings suggested that glyphosate residues in target weeds may act as a transient storage pool of active glyohosate associated with a risk of contact contamination of non-target crops subsequently sown after short waiting times.

To evaluate short waiting times (less than 20 days) and high weed density as potential risk factors for glyphosate toxicity to non-target crops under field conditions, three field trials were conducted in a no-tillage system with winter wheat. To verify the relevance of the previous model experiments also at lower levels of glyphosate application, the effects of short waiting times after pre-crop glyphosate application were tested using a track spraying device for glyphosate application, simulating application conditions in the field.

Material and methods

The experimental set-up of the field trials located in Tauberbischofsheim, Dusslingen and Starzach (all Southwest Germany) included waiting times of 20, 10 and 2 days between herbicide application and sowing of winter wheat as non-target crop. Glyphosate was applied as Roundup Ultra Max® in a concentration of 2 L ha^{-1} diluted in 200 L water. Controls included the application of a mixture of the herbicides Basta® and Agil-S® 20 days before sowing (DBS) and of Basta® at 2 DBS. At the field site in Starzach, different weed densities were achieved by pre-sowing of 100 kg wheat ha^{-1} in addition to the natural weed population.
In model experiments, 4g of wheat was sown into pots filled with 550g of field soil (pH 6.9) to achieve a high density of target-plants. Basal fertilization was performed with N (100 mg N kg^{-1} soil), P (80 mg P kg^{-1} soil), K (150 mg K kg^{-1} soil) and Mg (50 mg Mg kg^{-1} soil). After 10 days, wheat seedlings were treated with Roundup Ultra Max (4 L ha^{-1} diluted in 400L) applied with a track-spraying device. Two days after glyphosate application, shootsof the glyphosate-treated target wheat plants were re-moved by cutting and non-target wheat was sown to evaluate possible effects of glyphosate toxicity. In control treatments without glyphosate application, shoots of target-plants were removed manually by cutting at the soil level with a sharp knife.

In all experiments, visual plant damage, chlorosis scoring (SPAD-values), biomass production, the mineral nutritional status and intracellular shikimate accumulation in roots and shoots as physiological indicator of glyphosate toxicity were recorded.

Results

Field experiments:

At all field sites, visual scoring of germination rates and plant development as well as determination of shoot biomass production shortly before or during tillering revealed clear indications for damage to non-target plants in case of short waiting times after pre-crop glyphosate application (Fig. 1) and to some extent also in the Basta® treatment.

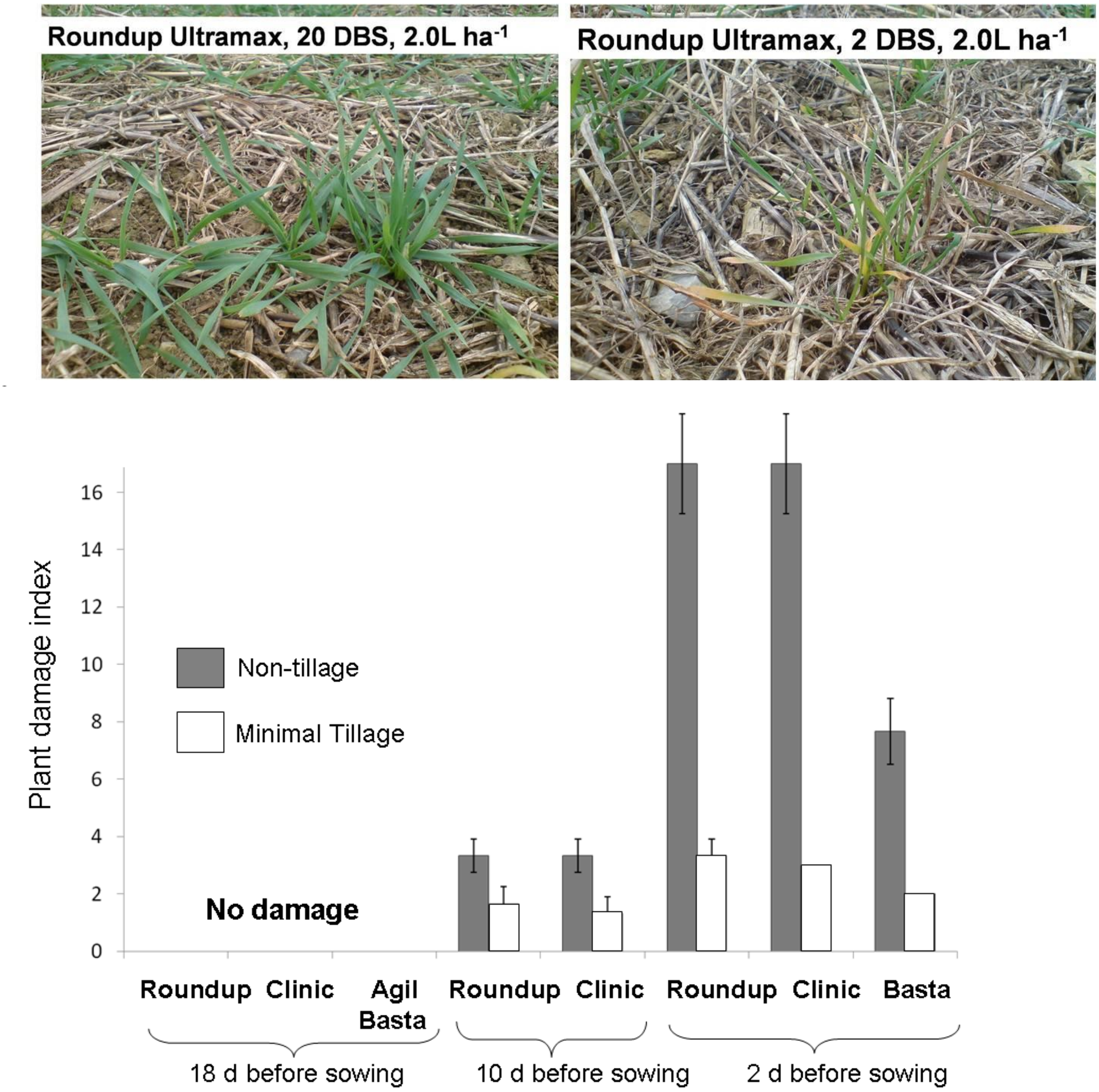

Fig. 1: Visual damage of winter wheat on a field site in Tauberbischofsheim after pre-crop glyphosate application with waiting times of 20, 10 and 2 days before sowing (DBS). Plants exhibit delayed and weak development in case of short waiting times (2 d) after pre-crop glyphosate application.

Additionally, results of the field trial in Starzach revealed a stronger expression of damage to non-target plants in treatments with high weed density (data not shown). At least at the field sites in Tauberbischofsheim and Dusslingen, short waiting times after pre-crop glyphosate application revealed induction of chlorosis (decline in SPAD-value), associated with significantly reduced concentrations of Zn and Mn in the shoots of winter wheat plants (Tab. 1).

Tab. 1: Micronutrient concentrations in shoots of winter wheat grown at a field site located in South Germany (Tauberbischofsheim) after pre-crop herbicide application wth different waiting times before sowing. Statistically significant differences (P<0,05) are indicated with letters

micronutrient concentrations	herbicide treatments and waiting times of pre-crop application				
	Agil+Basta 20 DBS	Roundup 20DBS	Roundup 10DBS	Roundup 2DBS	Basta 2 DBS
Zn (µg g^{-1})	39,8^{A}	45,3^{A}	38,3^{AB}	28,7^{B}	37,7^{AB}
Mn (µg g^{-1})	47,0^{A}	46,6^{A}	37,3^{AB}	31,2^{B}	36,4^{AB}

In Tauberbischofsheim, a significant accumulation of shikimate as an indicator for glyphosate toxicity was detected in shoots of emerging wheat seedlings in case of short waiting time (2dafter pre-crop glyphosate application (data not shown).

Model experiments:

Compared with the untreated control, short waiting times (2d) after pre-crop glyphosate application .caused a significant ddecline of shoot and root biomass, expression of chlorosis, an impaired plant nutritional status of plants and needle-shaped deformations of leaf growth (Fig. 2), which have been similarly observed also under field conditions (Fig. 1). Similar results were obtained, comparing glyphosate application with the track spraying device and with a hand sprayer (Fig. 2)

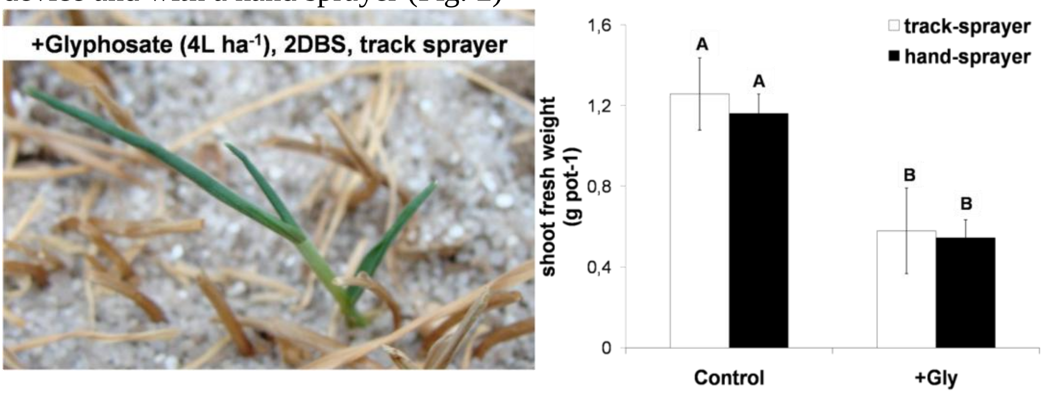

Fig. 2: Visual damage of winter wheat (left) and biomass production at final harvest (16 days after sowing, right) after pre-crop application of glyphosate (+Gly) with a waiting time of 2 d before sowing. Glyphosate was applied with a track- or a hand-held sprayer at an application level of 4L ha^{-1}. In control treatments (Control) without glyphosate application, shoots of target-plants were removed by cutting at the soil level with a sharp knife. Significant differences (P<0,05) are indicated by different letters.

Conclusions

The results of the field trials confirmed the assumption that a risk of negative side effects by pre-crop glyphosate application for non-target plants cannot be generally ruled out. Short waiting times after pre-crop glyphosate treatmrets and high weed density were identified as risk factors for glyphosate toxicity to non-target plants both, in model experiments and under farmers practice conditions in the field. The similarity of results in three independent field trials on sites differing in soil characteristics and climatic conditions was unexpected and is alarming. The results suggest that non-tillage systems are particulary sensitive to phytotoxic effects of glyphosate, which may be transiently stabilized in the root tissue of target weeds and exerting detrimental effects on the subsequent crop by contact contamination. A similar contact contamination by other herbicides seems to be also possible. The consideration of a waiting time of about 20 days could represent a practicable strategy for farmers to avoid yield losses due to glyphosate contact contamination and could additionally act as a helpful strategy to avoid green bridge effects for pathogen transfer from target weeds.