Low dose responses of different glyphosate formulations on plants

Subletale Wirkungen verschiedener Glyphosat-Formulierungen auf Pflanzen

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Summary

Although glyphosate clearly has real and potential commercial uses as a growth regulator at low doses, its main commercial significance has been as an herbicide. An important prerequisite for low dose applications gaining significance is a high efficiency and reliability of effects. This, however, seems to be a major constraint, especially regarding the approach of increasing yield by glyphosate hormesis. Glyphosate is marketed in various formulations, but potential disparities in low dose responses are unknown. Therefore, this study evaluated the expression and reliability of hormetic effects of different glyphosate formulations as a possible means for glyphosate hormesis to be more reliably and sustainably be achieved. Four commercial products sold in Germany (Glyfos, Glyfos Supreme, Glyfos Dakar, and Roundup Speed) were evaluated in germination assays with *Lactuca sativa*. Experiments were conducted as dose-response assays and evaluated for root length and shikimic acid production. In bioassays with exposure of seeds, none of the commercial formulations induced hormesis, while all formulations showed a similar hormetic effect if methanol pre-treated seedlings were exposed. Evaluating the reliability of the observed hormetic effect showed that the effect could only be reproduced in one out of three repeats independent of the formulation used. Hence, results indicated that in controlled bioassays, the induction of hormesis by glyphosate is independent of the formulation used and requires a preconditioning, although this does not ensure a hormetic effect. Therefore, the reliability of glyphosate hormesis may remain a major constraint for potential practical uses of this phenomenon despite new formulations claiming a safer response.

Keywords: Crop enhancement, dose-response, growth stimulation, hormesis, pelargonic acid

Zusammenfassung

Obwohl Glyphosat in niedrigen Dosierungen ein wachstumsregulatorisches Potenzial besitzt, ist seine kommerzielle Bedeutung weitgehend auf den Einsatz als Herbizid beschränkt. Eine wichtige Voraussetzung zur Nutzung des Potenzials niedriger Dosierungen, ist eine hohe Effizienz und Zuverlässigkeit der Wirkung. Dies scheint jedoch vor allem hinsichtlich der Nutzung hormetischer Effekte von Glyphosat zur Ertragssteigerung problematisch zu sein. Da Glyphosat in verschiedensten Formulierungen vertrieben wird und bisher keine Erkenntnisse über mögliche Unterschiede in der hormetischen Wirkung vorliegen, wurden in dieser Studie die Expression und die Zuverlässigkeit hormetischer Effekte verschiedener Glyphosat-Formulierungen untersucht. Vier kommerzielle Produkte, die in Deutschland vertrieben werden, wurden dazu in Keimtests mit *Lactuca sativa* geprüft (Glyfos, Glyfos Supreme, Glyfos Dakar und Roundup Speed). Die Versuche wurden als Dosis-Wirkungsversuche durchgeführt und Auswirkungen auf die Wurzellänge und den Shikimisäuregehalt wurden erhoben. In Versuchen mit Samen ergab sich bei allen Produkten regelmäßig keine Hormesis, während bei Behandlung von in Methanol vorgekeimten Keimlingen alle Produkte einen ähnlichen Hormesiseffekt zeigten. Dieser konnte allerdings unabhängig vom eingesetzten Produkt nur in einem von drei Versuchen reproduziert werden. Dies zeigt, dass Glyphosat Hormesis im Biotest mit *L. sativa* weitgehend unabhängig vom eingesetzten Produkt und nur nach Präkonditionierung auftritt, obgleich letzteres keine Garantie für das Auftreten von Hormesis gewährt. Die Zuverlässigkeit der hormetischen Wirkung wird deshalb trotz vielfältiger Glyphosat-Formulierungen vermutlich auch in Zukunft ein wichtiger Hemmfaktor für einen möglichen praktischen Einsatz dieses Phänomens bleiben.

Stichwörter: Dosis-Wirkungsbeziehung, Hormesis, Kulturpflanzenförderung, Pelargonsäure, Wachstumsstimulation

1. Introduction

Glyphosate is currently the most important active ingredient for controlling weeds. However, glyphosate shows useful effects in addition to killing weeds at high doses. Several non-lethal, low dose applications offer real and potential commercial uses of glyphosate as a growth regulator such as the commercially significant application to increase the levels of extractable sugar from sugar cane or the use of growth stimulating, hormetic doses to increase crop yield (CEDERGREEN et al., 2009; DALLEY and RICHARD, 2010; EL-SHAHAWY and SHARARA, 2011ab). Although hormetic approaches are not yet developed for practical usage, greenhouse and field studies showed that glyphosate applied at a range of 2-143 g a.i./ha can increase yield by 12-175 % in several plant species and response parameters (VELINI et al., 2008; CEDERGREEN et al., 2009; EL-SHAHAWY and SHARARA, 2011ab). An important prerequisite to transfer this effect into harvestable yield under field conditions is a high efficiency and reliability of the hormetic phenomenon. This, however, seems to be one of the major constraints of hormetic approaches including glyphosate hormesis (APPLEBY, 1998; CEDERGREEN, 2008; BELZ et al., 2011). Although glyphosate is marketed in several formulations, studies on glyphosate hormesis mainly used Roundup or Glyphonova (*e.g.* VELINI et al., 2008; CEDERGREEN et al., 2009; EL-SHAHAWY and SHARARA, 2011ab) and potential impacts of alternative formulations on low dose responses are widely unknown, especially when it comes down to new formulations free of toxic polyethoxylated tallow amine (POEA). As BRANTS and GRAHAM (2000) observed a tendency towards an improved hormetic performance of specific glyphosate formulations, alternative formulations may offer a means for glyphosate hormesis to be more reliably and sustainably be achieved. Therefore, this study evaluated the expression and reliability of hormetic effects of Glyfos with those of three claimed faster and safer formulations: the POEA-free formulation Glyfos Supreme, the newly developed, granulated formulation Glyfos Dakar and the mixed formulation Roundup Speed (pelargonic acid & glyphosate). In addition, the influence of pelargonic acid on the dose-response performance of glyphosate was further evaluated in joint action experiments and by analysis of shikimic acid levels in treated plants. A possible link between the occurrence and expression of hormesis and the primary mode of inhibitory action of glyphosate was evaluated.

2. Materials and methods

2.1 Herbicide bioassay

Herbicides. All herbicides used were commercially available formulated products (Tab. 1) that were mixed in demineralized water to give various test solutions for bioassay application.

Bioassay design. The efficacy of the different herbicides was evaluated in dose-response germination assays using *Lactuca sativa* var. *capitata* cv. Maikönig (lettuce) as test species. Assays were conducted in 6-well cell culture plates (Cellstar, greiner bio-one). Each well was prepared with one layer of filter paper (MN 615) and six lettuce seeds before 1.5 ml of herbicide solution was added per well. Controls were performed with demineralized water only. Plates were sealed with nescofilm and cultivated in a randomized design in a growth cabinet with a day/night cycle of 12/12 h, 24/18 °C and 50-70/0 µmol/m^2/s photosynthetic active radiation (PAR). After five days, root length (≥ 1 mm) of five seedlings per well was measured using Fitomed (CASTELLANO et al., 2001). Alterations from this standard design conditions (experiment 1 and 4) comprised a three day pregermination of lettuce seedlings in 4 % methanol (experiment 2 and 3).

Tab. 1 Specifications of herbicides used.

Tab. 1 *Spezifikationen der verwendeten Herbizide.*

Trade name (abbreviation)	Active ingredient	Source
Glyfos (GLY)	360 g a.i./l glyphosate (HRAC G)	Stähler Deutschland
Glyfos Supreme (SUP)	450 g a.i./l glyphosate (HRAC G)	Stähler Deutschland
Glyfos Dakar (DAK)	680 g a.i./kg glyphosate (HRAC G)	Stähler Deutschland
Roundup Speed (SPEED)	7.20 g a.i./l glyphosate (HRAC G) 9.55 g a.i./l pelargonic acid (HRAC Z)	Scotts Celaflor
Bayer Garten 3 Stunden Bio-Unkrautfrei (PA)	186.7 g a.i./l pelargonic acid (HRAC Z)	Bayer CropScience

a.i. = active ingredient; HRAC = herbicide resistance action committee

Dose-response design. Experiments 1-3 were conducted with 13 doses per herbicide ranging from 0-148 µmol a.i./ml for Glyfos formulations and from 0-88 µmol glyphosate/ml for SPEED. Treatment solutions were prepared in demineralized water from stock solutions of technical herbicides. Each treatment was triplicated and there were six common controls.

Joint action design. The quality of interaction between pelargonic acid (PA) and glyphosate was evaluated under standard design conditions based on the multiplicative survival model (MSM) as reference (STREIBIG and JENSEN, 2000) (experiment 4). Therefore, the dose-response curves for PA applied alone or in mixture with glyphosate were designed. There were 13 doses of PA ranging from 0-63 µmol a.i./ml applied alone or in mixture with a Glyfos formulation at the ED_{50} (dose causing 50 % inhibition; here 0.130 µmol a.i./ml). Each treatment was triplicated and there were six common controls performed with water only (PA) or with 0.130 µmol/ml glyphosate (mixture).

2.2 Shikimic acid extraction and analysis

The shikimic acid concentration in lettuce roots at the end of the bioassays was evaluated for selected dose-response relationships according to VELINI et al. (2008) and ZELAYA et al. (2011). The fresh root biomass (root length ≥ 5 mm) of all replicates per treatment was pooled in a 2 ml tube and stored at -20 °C until analyzed. The frozen root material was ground using a Retsch MM 400 (2 min at 30 Hz; 5 mm steel ball) before 1 ml/100 mg fresh root biomass of 0.25 M hydrochloric acid was added. The extracts were shaken (2 min at 30 Hz) and then centrifuged at 13000 rpm for 15 min. The supernatant (50 µl) reacted with 0.5 ml of a 1 % solution of periodic acid. After 3 h at room temperature, 0.5 ml of 1 M sodium hydroxide and 0.3 ml of 0.1 M glycine were added per sample, samples were centrifuged again and absorbance measured at 380 nm. The shikimic acid concentration was quantified using external standard solutions (0-20 µmol/ml; Sigma-Aldrich) that were similarly prepared.

2.3 Statistical analysis

Dose-response analysis. Root length (y) as a function of herbicide dose (x) was modeled by logistic regression models using IBM SPSS® Statistics. Response variance was stabilized at each dose by using the inverse standard deviation of replicates as weight. A monotonic model (STREIBIG, 1988) was used when no significant hormesis was observed (Dunnett-test, α = 0.05) or its expansion in case of significant hormesis (Eq. 1) (CEDERGREEN et al., 2005).

$$\text{(Eq. 1)} \quad y = [d+f^*\exp(-1/x^a)]/(1+\exp\{b^*[\ln(x/e)]\})$$

where d denotes the mean response of the untreated control, and f denotes the theoretical upper bound of the hormetic effect ($f > 0$ as a necessary condition for the presence of hormesis). Parameters a, b, and e have no straightforward biological meaning. The values of a were freely estimated or fixed between 0.06 and 0.16 according to the smallest residual sum of squares (CEDERGREEN et al., 2005). The dose giving maximum response (M), the corresponding response (y_{max}), and the dose where the hormetic effect is no longer present (limited dose for stimulation (LDS)) were estimated by reparameterizations of equation 1. Dose-response curves of different treatments were finally compared by horizontal assessment (F-test, α = 0.05).

Joint action analysis. The response P to mixtures satisfying MSM was predicted based on the response P_a observed for the dose z_a = 0.130 µmol/ml glyphosate and the modeled dose responses P_b for PA applied alone at various doses z_b after STREIBIG and JENSEN (2000) (responses expressed as percent of untreated control):

$$\text{(Eq. 2)} \quad P = (100-P_a)+(100-P_b)-[(100-P_a)^*(100-P_b)]/100.$$

The dose-response curves modeled from predicted dose responses P were subsequently compared to the observed dose-response curves for the binary mixtures (F-test, α = 0.05).

3. Results

3.1 Experiment 1: Standard bioassay conditions

Under standard conditions with exposure of seeds, none of the four tested glyphosate formulations significantly increased the root growth of *L. sativa* at low doses (Fig. 1). Comparing the resulting dose-response relations showed no significant differences between the three Glyfos formulations with an average ED_{50} of 0.165±0.026 µmol a.i./ml (Tab. 2). Thus, compared to GLY, 20 % less product of SUP is needed to achieve the same effect and 47 % less product of DAK due to a higher product content of glyphosate. In contrast to the Glyfos formulations, the dose-response curve for the effect of SPEED was significantly steeper and 4-fold less pronounced than the average response to Glyfos formulations at ED_{10}, while 2-fold more pronounced at ED_{50} and 12-fold at ED_{90} (Fig. 2, Tab. 2). Thus, the efficacy of glyphosate in mixture with PA seems to be impaired at lower effective doses, while the mixture seems advantageous at high response levels.

Tab. 2 Effective doses (*ED* in µmol glyphosate/ml) for the effect of glyphosate formulations on root length of *Lactuca sativa* in a germination assay (5 days after treatment; mean ± standard error).

Tab. 2 *Effektive Dosierungen (ED in µmol Glyphosat/ml) der Wirkung von Glyphosat Formulierungen auf das Wurzelwachstum von Lactuca sativa im Keimtest (5 Tage nach Applikation; Mittelwert ± Standardfehler).*

Response level	Glyfos	Glyfos Supreme	Glyfos Dakar	Roundup Speed
ED_{10}	0.00074 ± 0.00054	0.00036 ± 0.00023	0.00041 ± 0.00029	0.00157 ± 0.00103
ED_{50}	0.143 ± 0.048	0.140 ± 0.036	0.215 ± 0.062	0.091 ± 0.022
ED_{90}	27.7 ± 17.6	54.6 ± 29.7	111.3 ± 60.7	5.3 ± 3.4

3.2 Experiment 2: Methanol pretreatment

Exposing methanol pre-treated seedlings showed significant growth stimulation at low doses for all tested formulations (Fig. 1). The statistical analysis showed no significant differences between the biphasic dose-response relations of the three Glyfos formulations. The maximum stimulation was on average induced by $M = 0.019 \pm 0.006$ nmol a.i./ml (0.007 % of ED_{50}) and accounted for 134 %. Hormesis was observed up to a dose of $LDS = 3.5 \pm 1.9$ nmol a.i./ml (1.3 % of ED_{50}) confirming a broad hormetic dose range with a 186-fold distance between *M* and *LDS* doses. Due to a significantly steeper slope, the biphasic curve for SPEED showed a narrower hormetic dose range characterized by a 39-fold distance between *M* (0.070±0.034 nmol glyphosate/ml; 0.09 % of ED_{50}) and *LDS* (2.7±1.9 nmol glyphosate/ml; 3.6 % of ED_{50}). However, with a maximum stimulation of 137 %, SPEED was as effective in inducing hormesis as the Glyfos formulations. Although not significant, the observed y_{max} values increased from 133-141 % in the order GLY < SUP < SPEED < DAK.

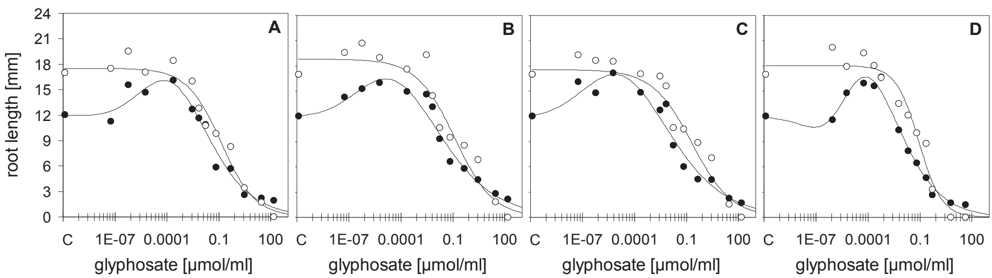

Fig. 1 Effect of Glyfos (A), Glyfos Supreme (B), Glyfos Dakar (C) and Roundup Speed (D) on root growth of *Lactuca sativa* with (●) or without (○) pregermination in methanol; C = control.

Abb. 1 *Wirkung von Glyfos (A), Glyfos Supreme (B), Glyfos Dakar (C) und Roundup Speed (D) auf das Wurzelwachstum von Lactuca sativa mit (●) oder ohne (○) Vorkeimen in Methanol; C = Kontrolle.*

3.3 Experiment 3: Reliability of glyphosate hormesis

Investigating the reliability of the hormetic response under the condition of a methanol preconditioning showed that the hormetic effect could only be reproduced in one out of three repeats whereas the occurrence of hormesis did not differ between the tested formulations (Fig. 2).

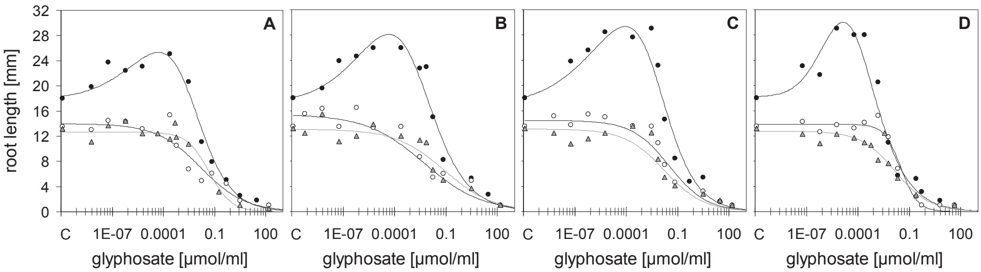

Fig. 2 Reliability of the hormetic effect of Glyfos (A), Glyfos Supreme (B), Glyfos Dakar (C) and Roundup Speed (D) on root growth of *Lactuca sativa* (pregerminated in methanol) in three independent experiments; (●) replicate 1, (○) rep. 2, (▲) rep. 3; C = control.

Abb. 2 *Zuverlässigkeit der hormetischen Wirkung von Glyfos (A), Glyfos Supreme (B), Glyfos Dakar (C) und Roundup Speed (D) auf das Wurzelwachstum von Lactuca sativa (vorgekeimt in Methanol) in drei unabhängigen Experimenten; (●) Replikation 1, (○) Rep. 2, (▲) Rep. 3; C = Kontrolle.*

Although rep. 1 revealed a more pronounced hormetic effect and a higher inhibitory efficacy for all formulations tested as exp. 2 (Fig. 1), the previously observed trends could be confirmed. The maximum stimulation increased from 141-171 % in the order GLY < SUP < SPEED < DAK, whereas the dose-response curves of the three Glyfos formulations were again not significantly different. Due to a steeper progression of the Glyfos curves in rep. 1 along with lower ED_{50} but higher *M*- and *LDS*-doses, the hormetic dose range (0-9.2±2.9 nmol a.i./ml) was narrower with a 108-fold distance between *M* and *LDS*. In contrast, all effective doses decreased in case of SPEED, while the *M*/*LDS* distance increased to 70-fold. Thus, SPEED was again most efficient and displayed a narrower hormetic dose range. Despite these similarities in overall trends, most quantitative features of the biphasic dose-response curves showed significant differences to those of exp. 2. The same was, however, true comparing the non-hormetic dose-response relations of rep. 2 and 3 (Fig. 2).

3.4 Experiment 4: Joint action analysis

Investigating the quality of interaction between the two mixture partners in SPEED, PA and glyphosate, revealed significant differences from the estimated MSM responses for all combinations tested. The observed dose-response curves for the three mixtures were significantly steeper and right shifted, indicating a primarily antagonistic response over a wide range of response levels. The antagonism was most pronounced at low response levels with an average 2.7±0.0-fold difference between the additive and the observed response at ED_{10} decreasing to 1.8±0.3-fold at ED_{50} and 1.2±0.4-fold at ED_{90}. Thus, at high response levels, the joint action shifted towards additivism and in case of GLY and DAK even slightly towards synergism (Fig. 3a-c). Equal joint action claims were observed testing fixed-ratio mixtures where, however, individual ratios showed a significant hormetic effect although both mixture partners applied alone did not induce hormesis (Fig. 3d). This indicated that the quality of interaction for the binary mixture of PA and glyphosate may depend mainly on the effective dose level, while the mixture ratio seems to bias the occurrence of hormesis.

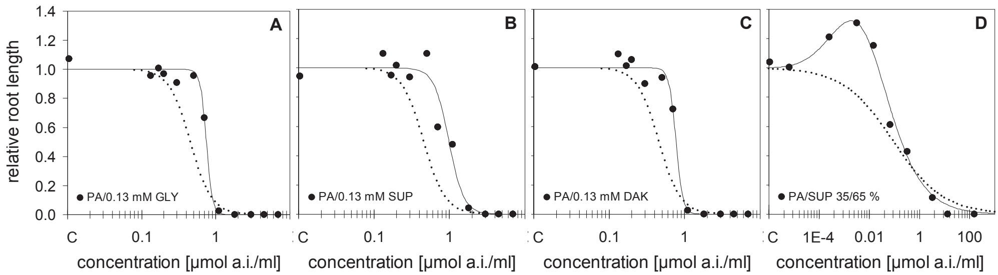

Fig. 3 Observed (—) and predicted additive response (••••) of *Lactuca sativa* to mixtures (●) of pelargonic acid (PA) with (A) Glyfos (GLY), (B/D) Glyfos Supreme (SUP), or (C) Glyfos Dakar (DAK); C = control.

Abb. 3 *Beobachtetet (—) und erwartete additive Wirkung (••••) von Mischungen (●) aus Pelargonsäure (PA) und (A) Glyfos (GLY), (B/D) Glyfos Supreme (SUP) oder (C) Glyfos Dakar (DAK) auf Lactuca sativa; C = Kontrolle.*

3.5 Shikimic acid analysis

The production of shikimic acid in glyphosate exposed roots of *L. sativa* was investigated using plants from dose-response relations showing either no hormesis (SUP), a maximum growth stimulation of 141 % (GLY), or 171 % (DAK) (Fig. 4a). In each case, the level of shikimic acid increased with increasing glyphosate doses, however, the increase appeared to be unrelated to the observed growth stimulation. The increase was most pronounced in absence of hormesis with a maximum of 500 % of control and observable already at doses below ED_1. The increase was least pronounced with a maximum of 182 % at the most distinct incidence of hormesis and observable just at doses above *LDS* (Fig. 4b). Thus, significant hormesis in root growth also occurred without a measurable inhibition of the primary inhibitory target at hormetic doses.

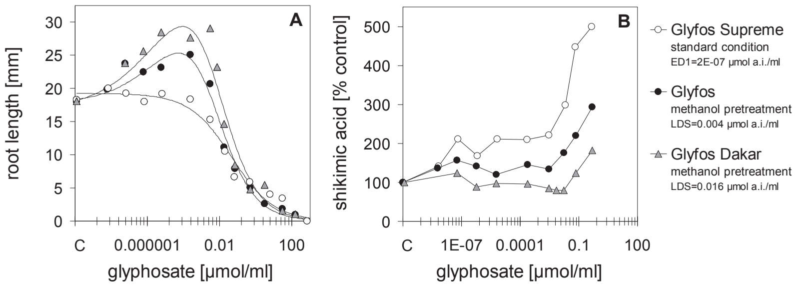

Fig. 4 Effect of Glyfos, Glyfos Supreme, or Glyfos Dakar on root growth (A) and shikimic acid content in roots (B) of *Lactuca sativa*; C = control.

Abb. 4 *Wirkung von Glyfos, Glyfos Supreme und Glyfos Dakar auf das Wurzelwachstum und den Shikimisäuregehalt in Wurzeln von Lactuca sativa; C = Kontrolle.*

4. Discussion

Low dose responses. Under the conditions of the present bioassay, all tested glyphosate formulations showed significant hormesis only after a methanol pretreatment of lettuce seedlings confirming earlier reports (BELZ et al., 2011). Despite the narrower hormetic dose range for SPEED, the quantitative features *f* and *a* characterizing the hormetic effect did not significantly differ between the tested formulations. Even so, there was a tendency towards an improved hormetic performance in the order GLY < SUP < SPEED < DAK. Regarding the reproducibility of glyphosate hormesis, none of the formulations showed a reliable performance whereas the appearance of hormesis was consistent for all formulations. This indicates that the formulation is of minor importance with regard to the expression and induction of glyphosate hormesis as well as glyphosate toxicity speaking in favor of environmentally more benign, POEA-free formulations. Furthermore, as hormesis occurred consistently and not randomly within the three repetitions, there seem to be essential, systematic factors governing the occurrence of glyphosate hormesis. Preconditioning seems to be just one of these essential factors. In greenhouse and field studies, where glyphosate hormesis appears independent of such a purposeful preconditioning (*e.g.*, VELINI et al., 2008; CEDERGREEN et al., 2009; EL-SHAHAWY and SHARARA, 2011ab), the level of light and CO_2 could be distinguished as further factors governing this phenomenon (CEDERGREEN and OLESEN, 2010). Some variability of dose-response features also occurred in the inhibitory dose range, however, here the variation comprised merely a more or less inhibitory effect that was clearly of less importance than the absence of a 30-70 % stimulation. Nonetheless, results also showed that doses $\leq M$ may under no circumstances turn inhibitory. Thus, practical approaches trying to use glyphosate hormesis at doses $\leq M$ may only bear the risk of not achieving a desired increase and whether this risk is low enough to justify treatment costs in the long-run needs to be verified. Field studies achieving a considerable yield increase by low glyphosate doses in two consecutive years point to the possibility (CEDERGREEN et al., 2009; EL-SHAHAWY and SHARARA, 2011ab).

Joint action of glyphosate & pelargonic acid. The contact-type herbicide PA is an often used mixture partner for the slower acting systemic glyphosate in ready-to-use weed control products as it provides immediate visual injury (WEHTJE et al., 2009). Studies investigating the effect of PA on the performance of glyphosate claim that PA has no or an antagonistic effect if applied as an adjuvant but also in ratios equalling those of ready-to-use products (*e.g.* CHACHALIS and REDDY, 2004; WEHTJE et al., 2009). Evaluating the joint action based on the MSM reference model clearly confirmed a strong antagonistic interaction being most pronounced at low response levels but diminishing at high response levels up to marginal synergistic effects. This corresponds to the observed impaired effect of SPEED at low response levels and the higher efficacy at higher response levels as compared to the Glyfos formulations. Hence, for ready-to-use products the apparent antagonism seems to play a role only at low response levels, while at higher response levels the mixture seems to overcome antagonistic effects on the performance of glyphosate by the additional effect provided by PA toxicity. With sensitive species as lettuce, the practical significance of the observed antagonism is further questioned comparing the observed 1.2-fold worsening of glyphosate performance at ED_{90} with the 8-fold distance between the application rate of SPEED (102.9 µmol a.i./ml) and the rate necessary for a ED_{90} response (12.7 µmol a.i./ml). With less sensitive target species, however, the PA-based antagonism seems relevant for ready-to-use products at doses used for weed control (WEHTJE et al., 2009).

As both mixture partners are non-hormetic under standard conditions in the lettuce assay, a non-hormetic mixture response was expected and properly confirmed by the dose-response relations for SPEED (59/41 % PA/glyphosate) and the MSM approach. However, a fixed ratio mixture of 35/65 % PA/SUP significantly deviated from this non-hormetic expectation under standard conditions and is thus in contrast to previous mixture predictions of hormetic responses (BELZ et al., 2008). Although the reasons for this are unknown, it may be speculated that at certain mixture ratios the faster acting PA may provide a preconditioning effect similar to that of methanol.

Shikimic acid accumulation. Reports on the underlying molecular mechanisms of the hormetic action of glyphosate are rare. The observation of shikimate accumulation in the hormetic dose range along with the absence of hormesis in glyphosate resistant soybean lead to the assumption of a similar molecular target as the inhibitory effect, the inhibition of EPSP synthase (VELINI et al., 2008). However, the observation of growth stimulation in other glyphosate resistant crops by glyphosate in spite of an insensitive target and the patenting of this effect in 2000 are in contrast to this (BRANTS and GRAHAM, 2000). This study indicates that shikimate accumulates to an even greater extent at sublethal doses in the absence of glyphosate hormesis while when hormesis occurs, shikimate may not necessarily accumulate in the hormetic dose range. Moreover, a considerable increase in shikimate occurs just at doses above the limited dose for stimulation (*LDS*). Hence, current results confirm that shikimate may accumulate in the hormetic dose range, however, the repression of the primary inhibitory mode of action seems to promote the expression of glyphosate hormesis and, thus, the findings of BRANTS and GRAHAM (2000). More studies are certainly needed to confirm current findings, especially in consideration of different target species, treatment methods (*e.g.*, spraying) or hormetic response parameters, as results may differ under varying experimental conditions. Nevertheless, in search of the underlying molecular mechanisms of glyphosate hormesis a mode of action differing from the primal inhibitory action should also be considered.

The current state of research in the field of glyphosate hormesis is incomplete, but relevant studies show that the phenomenon exists and that we can and do make use of it. Although current results might be different with a different species or with a different treatment method, they indicate that the formulation of glyphosate plays a minor role for low dose responses as long as no active mixture partner is involved. Apart from that, results reflect that the reliability of this phenomenon remains a major challenge and, thus, elucidating the underlying molecular mechanisms and prerequisites for its occurrence is essential to assure a predictable low dose effect of glyphosate.

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