Is hormesis an underestimated factor in the development of herbicide resistance?

Ist Hormesis ein unterschätzter Faktor bei der Entwicklung von Herbizidresistenz?

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Abstract

The growing impact of herbicide resistant weeds increasingly affects weed management and the delay of resistance evolution has become a major task of chemical weed control. Hormesis and, thus, the phenomenon that low doses of herbicides can boost weed growth could be of importance in this regard since the recommended field rate may represent a low dose for weeds that have evolved resistance to the applied herbicide and, thus, a potential hormetic dose. Applying the field rate may thus not only directly select resistant biotypes, it may also indirectly promote the success and spread of resistant biotypes via hormesis. Nevertheless, hormetic effects in resistant weeds are hitherto merely randomly observed and, thus, a clear quantitative basis to judge the significance of hormesis for resistance evolution is lacking. Therefore, this study aimed at quantifying the degree and frequency of herbicide hormesis in sensitive and resistant weed species in order to provide a first indication of whether the phenomenon deserves consideration as a potential factor contributing to the development of herbicide resistance. In germination assays complete dose-response experiments were conducted with sensitive and resistant biotypes of *Matricaria inodora* (ALS-target-site resistant; treated with iodosulfuron-methyl-sodium/mesosulfuron-methyl), *Eleusine indica* (glyphosate-resistant; treated with glyphosate), and *Chenopodium album* (triazine/triazinone-target-site resistant; treated with terbuthylazine). After 10 days of cultivation under controlled conditions plant growth was analyzed by measuring shoot/root length and mass. Results indicated that herbicide hormesis occurred on average with a total frequency of 29% in sensitive/resistant biotypes with an average growth increase of 53% occurring typically within a dose zone exceeding 350fold. Hormetic effects occurred, however, very variable and only for specific endpoints and not plant growth in general. If such a variable stimulation of specific traits will translate to resistance relevant growth promotion under more practical conditions is uncertain. None-the-less, for a full understanding of the development of herbicide resistance, hormetic effects should be considered as a potential factor in resistance evolution.

Keywords: Biphasic, growth stimulation, herbicides, target-site resistance

Zusammenfassung

Der wachsende Einfluss der Herbizidresistenz in Unkräutern erschwert zunehmend das Unkrautmanagement und das Hinauszögern der Resistenzentwicklung ist zu einem wichtigen Aspekt der chemischen Unkrautkontrolle geworden. Hormesis und damit das Phänomen, dass niedrige Dosierungen von Herbiziden das Unkrautwachstum fördern können, könnte in diesem Zusammenhang von Bedeutung sein, da die empfohlene Aufwandmenge für resistente Unkräuter eine niedrige Dosis und somit eine hormetische Dosis darstellen kann. Eine Applikation der empfohlenen Aufwandmenge könnte somit nicht nur direkt resistente Biotypen selektieren, sondern die Ausbreitung von resistenten Biotypen durch Hormesis indirekt fördern. Bisher wurden hormetische Effekte bei resistenten Unkräutern allerdings nur zufällig beobachtet, sodass die Datenbasis nicht ausreicht, um die Relevanz hormetischer Effekte für die Resistenzentwicklung abzuschätzen. Ziel dieser Studie war es deshalb, das Ausmaß und das Auftreten von Hormesis in sensitiven und resistenten Unkautbiotypen zu untersuchen, um einen Hinweis darauf zu erhalten, ob Hormesis als potentieller Mechanismus zur Resistenzentwicklung beitragen könnte. In Keimtests wurden deshalb Dosis-Wirkungsversuche durchgeführt mit sensitiven und resistenten Biotypen von *Matricaria inodora* (ALS-Target-Site Resistenz; Behandlung mit Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl), *Eleusine indica* (Glyphosat-resistent; Behandlung mit Glyphosat) und *Chenopodium album* (Triazin/Triazinon-Target-Site Resistenz; Behandlung mit Terbuthylazin). Nach 10 Tagen Versuchsdauer wurden die Länge und das Gewicht von Spross und Wurzel als Wirkungsparameter erhoben. Die Ergebnisse zeigten, dass hormetische Effekte sowohl beim sensitiven, als auch beim resistenten Biotyp im Durchschnitt mit einer Frequenz von 29% auftrat bei einer durchschnittlichen Wachstumsstimulierung von 53% und einem hormetischen Dosisbereich von durchschnittlich über 350fach. Hormetische Effekte zeigten jedoch eine hohe Variabilität und waren nur jeweils für spezifische Wirkungsparameter zu beobachten und nicht das Pflanzenwachstum im Allgemeinen. Ob die beobachtete variable und spezifische Stimulierung einzelner Wachstumsparameter unter Praxisbedingungen

zur Resistenzentwicklung beitragen kann ist fraglich. Für ein vollständiges Verständnis der Entwicklung von Herbizidresistenz sollten hormetische Effekte dennoch als ein potenzieller Faktor der Resistenzevolution in Erwägung gezogen werden.

Stichwörter: Biphasisch, Herbizide, Wachstumsstimulierung, Wirkortresistenz

Introduction

The growing impact of herbicide resistant weeds increasingly affects chemical weed management and the delay of resistance evolution has become a major task. Several factors that trigger and accelerate the development of herbicide resistance have been discovered such as biological factors (*e.g.*, initial frequency of resistant biotypes), genetic factors (*e.g.*, mechanism of resistance), or weed management aspects (*e.g.*, type of herbicide, rotation of modes of action) (*e.g.*, RENTON *et al.*, 2011). Based on this, strategies have been deduced to reduce selection pressure on resistant biotypes and are more and more adopted thanks to the growing awareness of the resistance problem (BECKIE, 2006; NORSWORTHY *et al.*, 2012). One aspect that is hitherto not on the list of factors contributing to the development of herbicide resistance is stimulatory effects of herbicides or else hormesis. However, the phenomenon that low doses of herbicides can boost weed growth may be of particular importance for the use of herbicides for which weeds have evolved resistance (BELZ *et al.*, 2011). Since the recommended field rate may represent a low dose and, thus, a hormetic dose to the resistant biotype, the growth of herbicide-resistant weeds may be promoted by regular herbicide applications. Moreover, highly resistant individuals are believed to be especially responsive to hormesis (CALABRESE and BALDWIN, 2002). Hence, on the one hand field rates may directly select resistant biotypes from a sensitive population and on the other hand they may indirectly promote the success and spread of resistant biotypes due to hormetic growth stimulation. Hormesis could thus indirectly influence the development of resistance by making hormetically enhanced resistant weeds more competitive, more reproductive, or more resistant to a second weed control measure rather than causing direct selection pressure (BELZ *et al.*, 2011). If stimulated plants are more reproductive, hormesis may directly facilitate evolution of resistance under field conditions. In an ecosystem context, a higher competiveness than weeds that are not or are adversely affected by herbicides may lead to undesired changes in weed species composition in favour of the resistant species (CEDERGREEN, 2008). A lower sensitivity to a second weed control measure due to *e.g.* biomass gain or induction of detoxification processes may be particularly unwanted since a second herbicide application is a common reaction to weed survival in practice. Furthermore, if a lower sensitivity due to enhanced metabolic activity may be epigenetically inherited without fitness consequences, coupling of the hormetic response with detoxification gene induction may further assist in resistance development (GUEDES and CUTLER, 2013). Research addressing these issues is yet absent and reports on such a hormetic enhancement of resistant weeds are lacking, especially when it comes to field conditions. Here, however, a growth increase may not easily be detected nor explicitly attributable to herbicide hormesis. Therefore, a first support for the hypothesis that resistant weeds may be prone to herbicide hormesis stems accidentally from a few greenhouse studies conducted as part of resistance monitoring projects. For example, at doses regularly applied to combat weeds, ACCase target-site resistant biotypes of *Alopecurus myosuroides* Huds. (Leu_{1781}-Allel) showed a maximum stimulation of shoot biomass of 39% after treatment with fenoxaprop-P-ethyl and a maximum stimulation of 54% at reduced cycloxydim doses (PETERSEN *et al.*, 2008; BELZ *et al.*, 2011).

In view of this, the current study was conducted to primarily demonstrate hormetic growth stimulation in resistant weeds and to focus on the following objectives: (1) are resistant biotypes more prone to develop hormesis?; (2) does hormesis promote overall plant fitness?; (3) does the occurrence of hormesis influence the level of resistance?; and (4) is the hormetic effect frequent enough to deduce an involvement in resistance development? For this purpose, complete dose-response experiments were conducted for sensitive and resistant biotypes of three weed species, namely *Eleusine indica* (L.) Gaertn. with resistance to glyphosate, *Matricaria inodora* L. with ALS target-site resistance, and *Chenopodium album* L. with *psb*A target-site resistance. Experiments

were conducted as germination assays as an experimental design that allows capturing hormesis in a useful and promptly way. Early stage enhancement of root and shoot growth served as an indicator for competitive ability and was evaluated in six independent experiments.

Material and Methods

Biotypes. For all three weed species used, a sensitive and a resistant biotype were included in the present study (Tab. 1). While for the glyphosate-resistant biotype of E. indica the mechanism of resistance is not characterized, the other resistant biotypes are characterized as target-site resistant with resistance-endowing amino acid substitutions at position 197 on the ALS gene for M. inodora conferring resistance to sulfonylurea herbicides and 264 on the psbA gene for *C. album* conferring resistance to triazines and triazinones.

Herbicides. All herbicides used were commercially available formulated products (Tab. 1) that were mixed in demineralized water to give various test solutions. Glyphosate treatments were carried out within a concentration range of 1.0 pg/ml to 25.0 mg/ml, iodosulfuron-methyl-sodium/mesosulfuron-methyl treatments within 0.008 pg/ml to 0.2 mg/ml, and terbuthylazine treatments within 6.4 pg/ml to 20.0 mg/ml.

Experimental Design. Germination bioassays were done in form of dose-response experiments in 6-well cell culture plates (Cellstar, greiner bio-one). Each well was prepared with one layer of filter paper (Ø 34 mm, MN 615, Macherey-Nagel) and 15 seeds were transferred to each well before 1.5 ml of herbicide solution or demineralized water was added. Each experiment evaluated 12 herbicide concentrations that were replicated six times (one plate) and there were 12 control replicates with demineralized water (two plates). Plates were sealed with parafilm and cultivated according to a completely randomized design. Cultivation was done in a growth cabinet [Binder KBW 720 (E5.1)] that was attuned to a day/night cycle of 12/12 h starting at 8 am with 24/18 °C and a 12 h light period of 50-70 µmol m-2 s-1 photosynthetic active radiation. After 10 days, plates were frozen at -4 °C prior to measuring root and shoot length (≥ 1 mm), shoot fresh weight, and root dry weight of 10 seedlings per replicate. If more than 10 seeds per well had germinated, 10 seedlings were randomly selected for measurement. In all other cases, all germinated seedlings were selected and non-germinated seeds counted as zero. Effects on root elongation were evaluated for all three species in six independent experiments of which three also evaluated additional effects on shoot length and shoot/root mass.

Tab. 1 Specifications of biotypes and herbicides used.

Tab. 1 *Spezifikationen der verwendeten Biotypen und Herbizide.*

species	origin (donor)	resistance status	treatment
Eleusine indica	Herbiseed	sensitive	Glyfos Supreme [450 g a.i./l glyphosate (607 g/l isopropylamine salt)]
Eleusine indica	Herbiseed	resistant	
Matricaria inodora	Herbiseed	sensitive	Atlantis OD (2 g a.i./l iodosulfuron-methyl-sodium; 10 g a.i./l mesosulfuron-methyl)
Matricaria inodora	Freiburg/Elbe, Germany (J. Petersen)	ALS[1] TSR[2] $Prolin_{197}$-Glycin	
Chenopodium album	Schriek, Belgium (J. Petersen)	sensitive	CLICK (500 g a.i./l terbuthylazine)
Chenopodium album	Outgaarden, Belgium (J. Petersen)	*psb*A TSR $Serin_{264}$-Glycin	

[1]ALS=acetolactate synthase; [2]TSR=target-site resistance

Statistical Analysis. Dose-response modeling was done with IBM® SPSS® Statistics 20. Responses per dose (y) were modeled as a nonlinear function of dose (x) to the dose-response regression model that provided the best fit for the dataset evaluated. Model comparisons for best fit were based on the ratio of residual sum of squares and residual degrees of freedom (SS/df) derived from the regression procedure so that the model with the lowest ratio was chosen. Reduced forms of three models were considered: the monophasic function of STREIBIG (1988) (Eq. 1) and the biphasic functions of BRAIN and COUSENS (1989) (Eq. 2) or CEDERGREEN *et al.* (2005) (Eq. 3).

Eqs. (1) (2) (3)

$$y = d/(1+\exp(b*\ln(x/ED_{50}))) \quad y = (d+fx)/(1+\exp(b*\ln(x/e))) \quad y = (d+(f\exp(-1/x^a))/(1+\exp(b*\ln(x/e)$$

where d denotes the mean response of the untreated control, b determines the slope of the decreasing curve part, the size of a determines the steepness of the increasing curve part, and ED50 the dose causing 50% inhibition, while parameters e and f have no straightforward biological meanings (CEDERGREEN *et al*., 2005). Response variance heterogeneity was accounted for by using the inverse variance of replicates at each dose as weight. The significance of hormesis was assessed according to SCHABENBERGER *et al*. (1999) and was given if the 95% confidence interval for the estimate of f did not cover the value zero. Besides the directly estimated parameters of the original models, further quantities describing the stimulatory effect were estimated by reparameterizations according to SCHABENBERGER *et al*. (1999) and BELZ and PIEPHO (2012, 2013a). The dose where hormesis is maximal (M), the dose where the hormetic effect disappears or the limited dose for stimulation (LDS), the distance between M and LDS doses (dist 2), and the doses causing 50% inhibition (ED50) were estimated. The corresponding maximum stimulatory response ymax at M and the relative ymax value (ymax*100/d) were estimated as prediction at x=M. Resistance factors between sensitive (S) and resistant biotypes (R) were calculated at the ED50 dose level as ED50 (R)/ED50 (S).

Results

Effects on Root Elongation. Results revealed a general low frequency of hormesis in root elongation with nine of the total 36 dose-response curves showing hormesis (25%). The frequency of hormesis in root elongation was highest for *M. inodora* (33%) followed by *E. indica* (25%), while terbuthylazine effects on *C. album* showed the lowest frequency of hormesis with 17%. Stimulation of root elongation was observed for both biotypes except for *C. album* such that resistant biotypes were stimulated within five assays and sensitive biotypes only in four (Tab. 2 and 3).

Experiments with *E. indica* showed significant hormesis or a biphasic curve as best fit for both biotypes in exp. 1 and for the sensitive biotype in exp. 2 (Fig. 1A and 1B). All other dose-response relationships were monophasic (Fig. 1C; Tab. 3). Hence, the frequency of monophasic dose-response effects on root elongation was with a total of 75% considerably higher than the occurrence of hormesis. Analyzing the quantitative features showed 11- 48% maximum stimulation of root length for the sensitive and 61% for the resistant biotype. The dose range where hormesis occurred comprised a dose distance between *M* and *LDS* (*dist* 2) of 13-252fold for the sensitive and 504fold for the resistant biotype (Tab. 2).

Experiments with *M. inodora* showed significant hormesis or a biphasic curve as best fit for the sensitive biotype in exp. 1 (Fig. 1D) and 6 (Tab. 2) and the resistant biotype in exp. 2 (Tab. 2) and 5 (Fig. 1F). All other dose-response relationships were monophasic (Fig. 1E; Tab. 3). The occurrence of monopasic dose-responses was thus prevailing with a frequency of 67%. Quantifying the hormetic response of iodosulfuron-methyl-sodium/mesosulfuron-methyl on root elongation showed 25-42% stimulation for the sensitive biotype and 14-43% stimulation for the resistant biotype. The hormetic dose zone (*dist* 2) amounted at 3-12fold for the sensitive and 3-45fold for the resistant biotype (Tab. 2).

Experiments with *C. album* showed significant hormesis in root elongation in exp. 1 and 5, but only for the resistant biotype (Fig. 1G and 1I). All other dose-response relationships for the resistant biotype and all dose responses for the sensitive biotype were monophasic (Fig. 1H; Tab. 3). The observed increase in root elongation of the resistant biotype ranged between 37-57% and showed a *dist* 2 of 13-708fold (Tab. 2).

Tab. 2 Regression parameters for the biphasic modelling of dose responses of sensitive (S) and resistant (R) biotypes of Eleusine indica treated with glyphosate, Matricaria inodora treated with iodosulfuron-methyl-sodium/mesosulfuron-methyl, and *Chenopodium album* treated with terbuthylazine.

Tab. 2 *Regressionsparameter der biphasischen Modellierung dosisabhängiger Wirkungen in sensitiven (S) und resistenten (R) Biotypen von Glyphosat-behandelter Eleusine indica, Iodosulfuron-methyl-Natrium/ Mesosulfuron-methyl-behandelter Matricaria inodora und Terbuthylazin-behandeltem Chenopodium album.*

species	biotype	exp.	parameter	*d*	*b*	*f*	*a*	*M* [µg/ml]	*LDS* [µg/ml]	ED_{50} [µg/ml]	*dist 2 LDS/M*	y_{max} [%]
Eleusine indica	S	1	root length	15.2 mm	0.43	38.7	0.06	0.0041	1.045	19.100	252	148
		2	root length	27.0 mm	0.61	25.1	0.14	0.0226	0.296	6.604	13	111
		5	shoot length	3.7 mm	0.29	60.9	0.10	0.0012	0.854	50.095	685	177
		6	root weight	0.346 mg	0.37	1.1	0.05	0.0009	0.871	20.109	1025	160
	R	1	root length	11.6 mm	0.42	33.6	0.05	0.0026	1.333	22.042	504	161
		5	shoot length	4.0 mm	0.22	139.2	0.10	0.0007	0.311	63.942	446	138
		6	root weight	0.182 mg	0.50	1.7	0.08	0.0091	3.644	22.999	399	270
Matricaria inodora	S	1	root length	20.7 mm	0.72	262.7	0.15	0.0005	0.006	0.042	12	125
		4	root weight	0.359 mg	0.61	1.3	0.06	0.0001	0.009	0.066	77	156
		5	shoot length	2.9 mm	0.50	13.2	0.14	0.0044	0.033	0.806	7	112
			shoot weight	6.2 mg	0.38	0.7	0.12	0.0002	0.036	0.749	208	179
		6	root length	23.7 mm	1.74	24003	--	0.0004	0.001	0.003	3	142
			root weight	0.396 mg	1.37	2904	--	0.0002	0.001	0.006	5	133
	R	2	root length	27.3 mm	0.50	31.7	0.08	0.0005	0.020	0.707	45	114
		4	root weight	0.900 mg	0.76	1.1	0.12	0.0457	0.761	5.857	17	131
		5	root length	26.4 mm	1.75	76.5	--	0.3465	1.178	3.185	3	143
			shoot length	4.3 mm	1.05	2.4	0.09	0.1828	2.390	14.806	13	116
		6	root weight	0.404 mg	1.35	2814	--	0.0005	0.008	0.056	15	190
			shoot weight	7.3 mg	2.55	11.6	0.08	1.2385	6.034	9.971	5	158
Chenopodium album	S	4	root weight	0.309 mg	0.48	1.1	0.04	0.0488	69.998	499.820	3832	218
	R	1	root length	27.2 mm	0.38	55.0	0.05	0.0135	9.555	266.027	708	157
		5	root length	30.3 mm	1.19	275.4	--	0.2509	3.203	119.498	13	137
		6	root weight	0.598 mg	0.33	3.5	0.14	0.0822	12.606	330.095	153	155
			shoot weight	14.4 mg	2.25	9.6	0.17	535.717	3353.942	6185.991	6	147

Effects on Other Endpoints. The frequency of hormetic effects on other endpoints was with 14 out of a total of 46 dose-response relationships (30%) somewhat higher as the observed frequency of hormesis in root elongation. Most frequent were hormetic effects on root dry weight (50%) followed by shoot length (22%) and shoot fresh weight (21%). Hormetic effects occurred for both biotypes in all three species tested, however, most often with *M. inodora* (44%) followed by *E. indica* (29%) and *C. album* (21%) (Tab. 2 and 3).

Experiments with *E. indica* revealed significant hormesis or a biphasic modelling as best fit for both biotypes in exp. 5 for shoot length (Fig. 2B) and in exp. 6 for root dry weight (Fig. 2A), while exp. 4 showed no hormetic effect in any endpoint measured (Fig. 2C; Tab. 3). Hence, hormesis occurred for all four endpoints measured with a total frequency of 27%, but in each case only for one of the several endpoints measured. Quantifying the expression of hormesis revealed a maximum increase of 38-170% and a hormetic dose zone (*dist* 2) of 399-1025fold. Thus, compared to effects on root elongation, hormesis of glyphosate tended to be more pronounced in other endpoints, especially in root dry weight (Tab. 2).

Experiments with *M. inodora* revealed significant hormesis or a biphasic modelling as best fit for both biotypes in exp. 4 (Fig. 2D) and exp. 6 for root dry weight and in exp. 5 for shoot length (Fig. 2E). The shoot fresh weight was significantly stimulated in exp. 5 for the sensitive biotype (Fig. 2F) and in exp. 6 for the resistant biotype. Hence, two experiments showed significant hormesis in two endpoints, namely root/shoot length (exp. 5) and root/shoot weight (exp. 6) in case of the resistant biotype and shoot length/weight (exp. 5) and root length/weight (exp. 6) for the sensitive biotype (Tab. 2). Overall, hormesis occurred with a total frequency of 40% for all four endpoints measured. Quantifying the expression of hormesis revealed a maximum increase of 12-90% and a *dist* 2 of 5-208fold. Hence, as observed for glyphosate hormesis, the observed stimulation by iodosulfuron-methyl-sodium/mesosulfuron-methyl tended to be more pronounced for other endpoints than root elongation, especially regarding shoot weight (Tab. 2).

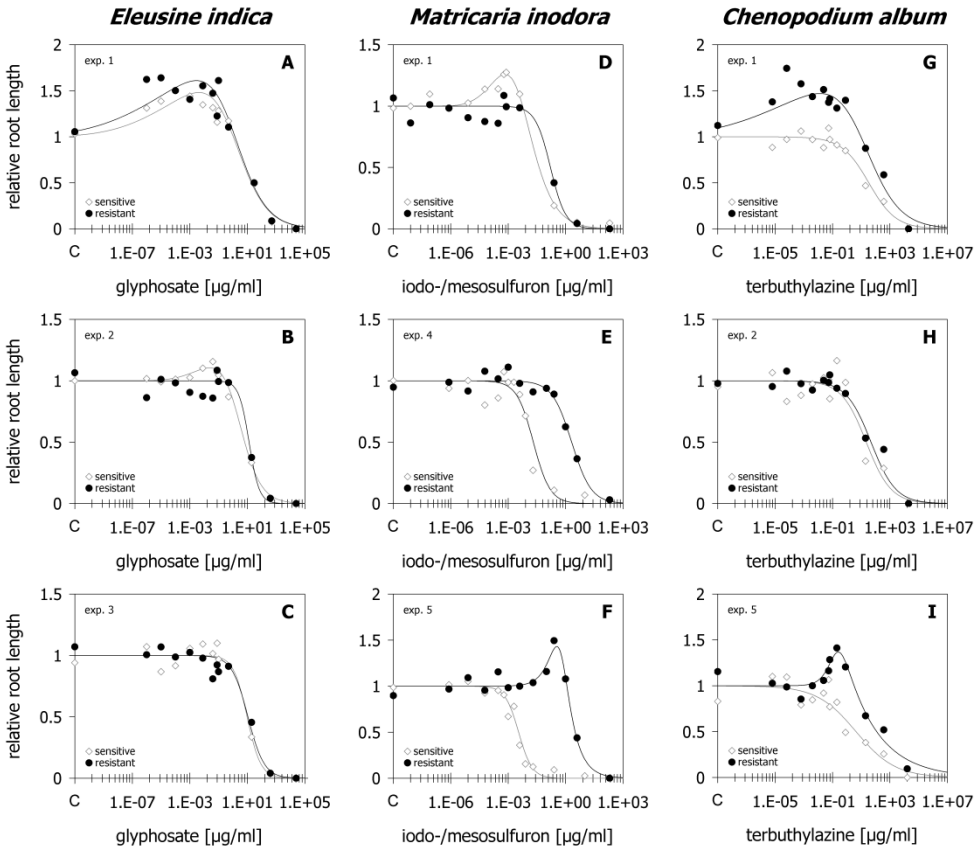

Fig. 1 Ein- und zweiphasige Dosis-Wirkungszusammenhänge für die Reaktion des Wurzellängenwachstums von sensitiven und resistenten Unkrautbiotypen in drei von sechs unabhängigen Versuchswiederholungen. (A-C) Wirkung von Glyphosat auf Biotypen von Eleusine indica; (D-F) Wirkung von Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl auf Biotypen von *Matricaria inodora*; (G-I) Wirkung von Terbuthylazin auf Biotypen von *Chenopodium album*.

Abb. 1 *Mono- and biphasic dose-response relationships for the response of root elongation of sensitive and resistant weed biotypes in three out of six independent experiments. (A-C) Effects of glyphosate on biotypes of Eleusine indica; (D-F) effects of iodosulfuron-methyl-sodium/mesosulfuron-methyl on biotypes of* Matricaria inodora*; (G-I) effects of terbuthylazine on biotypes of* Chenopodium album*.*

Experiments with *C. album* revealed a significant hormetic effect on root dry weight of the sensitive biotype in exp. 4 (Fig. 2G) and in exp. 6 for the resistant biotype. Shoot response parameters measured were only stimulated once in the resistant biotype (Fig. 2H and 2I). Hence, terbuthylazine-treated *C. album* showed hormesis primarily for root response parameters albeit with a low total frequency of 19%. With a maximum increase of 47-118% and a *dist* 2 of 6-3832fold, the stimulation also tended to be more pronounced in other endpoints than in root length (Tab. 2).

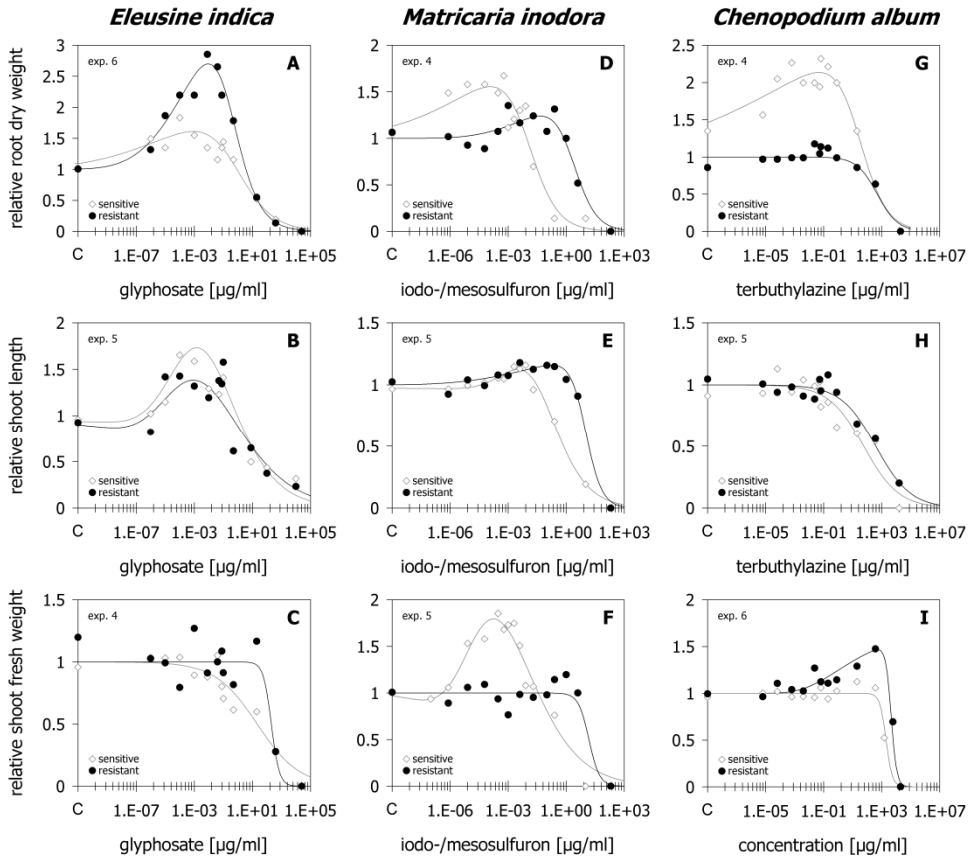

Fig. 2 Ein- und zweiphasige Dosis-Wirkungszusammenhänge für verschiedene Wirkungsparameter bei sensitiven und resistenten Unkrautbiotypen. (A-C) Wirkung von Glyphosat auf Eleusine indica; (D-F) Wirkung von Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl auf *Matricaria inodora*; (G-I) Wirkung von Terbuthylazin auf *Chenopodium album*.

Abb. 2 *Mono- and biphasic dose-response relationships for different response parameters measured in sensitive and resistant weed biotypes. (A-C) Effects of glyphosate on Eleusine indica; (D-F) effects of iodosulfuron-methyl-sodium/mesosulfuron-methyl on* Matricaria inodora*; (G-I) effects of terbuthylazine on* Chenopodium album*.*

Resistance Factors (RF). The RFs varied in all three species tested depending on the response parameter measured and the experiment conducted (Tab. 3). Overall, RF values increased in the order *E. indica* < *C. album* < *M. inodora* from a total mean value of 3.5 in *E. indica* to 7.2 in *C. album* to 131.6 in *M. inodora*.

Experiments with *E. indica* revealed RF values between 0.9-11.6. Root response parameters showed lower RF values with a mean of 1.9 compared to 6.4 for shoot response parameters. The occurrence of hormesis in one of the two biotypes or both was not obviously related to the degree of resistance and the five highest RFs observed were achieved when hormesis was absent in the response parameter evaluated (Tab. 3).

Experiments with *M. inodora* revealed RF values between 2.7 - 907.5. Here, root response parameters showed considerably higher RF values than shoot response parameters with a mean of 206.7 for root length/mass compared to 18.9 for shoot length/mass. Relating the RFs to the occurrence of hormesis did not indicate a clear relation, however, two of the three lowest RFs were observed when only the sensitive biotype was stimulated (Tab. 3).

Experiments with *C. album* revealed RF values between 1.0-27.5. Root and shoot parameters measured showed on average fairly equal RF values with a mean of 7.3 for root length/mass and 7.0 for shoot length/mass. A clear relation between the occurrence of hormesis and the level of resistance was indeterminable here as well, however, the highest RF was observed when only the resistant biotype was stimulated and the lowest RF when only the sensitive biotype was stimulated (Tab. 3).

Tab. 3 Resistance factors (RF) and occurrence of hormesis depending on the response parameter measured and the experiment conducted in sensitive (S) and resistant (R) biotypes of *Eleusine indica* treated with glyphosate, *Matricaria inodora* treated with iodosulfuron-methyl-sodium/mesosulfuron-methyl, and *Chenopodium album* treated with terbuthylazine.

Tab. 3 *Resistenzfaktoren (RF) und Auftreten von Hormesis in Abhängigkeit des gemessenen Wirkungsparameters und der Versuchswiederholung in sensitiven (S) und resistenten (R) Biotypen von Glyphosat-behandelter* Eleusine indica*, Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl-behandelter* Matricaria inodora *und Terbuthylazin-behandeltem* Chenopodium album.

parameter	exp.	*Eleusine indica*			*Matricaria inodora*			*Chenopodium album*		
		RF	hormesis[1]		RF	hormesis		RF	hormesis	
			S	R		S	R		S	R
root length	1	1.2	✓ (48%)[2]	✓ (61%)	3.9	✓ (25%)	–	7.9	–	✓ (57%)
	2	1.5	✓ (11%)	–	74.7	–	✓ (14%)	2.2	–	–
	3	1.2	–	–	20.4	–	–	2.7	–	–
	4	3.4	–	–	100.4	–	–	3.3	–	–
	5	3.1	–	–	907.5	–	✓ (43%)	27.5	–	✓ (37%)
	6	0.9	–	–	5.4	✓ (42%)	–	9.4	–	–
root dry weight	4	—			88.8	✓ (55%)	✓ (31%)	1.0	✓ (118%)	–
	5	2.4	–	–	649.5	–	–	—		
	6	1.1	✓ (60%)	✓ (170%)	9.6	✓ (33%)	✓ (90%)	4.7	–	✓ (55%)
shoot length	4	11.6	–	–	35.5	–	–	6.3	–	–
	5	1.3	✓ (77%)	✓ (38%)	18.4	✓ (12%)	✓ (16%)	6.8	–	–
	6	1.8	–	–	12.7	–	–	6.7	–	–
shoot fresh weight	4	9.4	–	–	2.7	–	–	11.9	–	–
	5	—			20.1	✓ (79%)	–	—		
	6	6.1	–	–	24.1	–	✓ (58%)	3.1	–	✓ (47%)

[1]✓ = significant hormesis or biphasic modelling as best fit, – = no significant hormesis or monophasic modelling as best fit; [2]relative stimulation

Discussion

Effects on Growth Parameters. The general hormetic increase observed in all fields of sciences, and for different toxicants, organisms, and endpoints, ranges between 30-60% stimulation above control although this average range can be considerably exceeded to up to 200% stimulation (CALABRESE and BLAIN, 2005; CALABRESE, 2008). With a total average of 51% increase in the sensitive biotypes and 55% in the resistant biotypes and a maximum of 170% stimulation, current quantitative features are in line with the general hormetic increase reported in the literature. With a total dose distance between *M* and *LDS* (*dist* 2) of 352fold and 42% of the hormetic curves showing a *dist* 2 of > 100fold, the current dose zones of hormesis are somewhat broader than the reported mean hormetic dose zone of less than 100fold occurring in about 95% of reported hormetic results (CALABRESE, 2008; CALABRESE and BALDWIN, 2003). However, such considerable deviation from general quantitative features is known and presumed to be attributable to the type of endpoint measured and/or the biological model used (CALABRESE and BLAIN, 2011). Nevertheless, the observed average increase in early growth traits in the resistant biotypes of 55% appears to be striking provided that it is translated into an increased competitive ability under more practical conditions.

Hormesis is usually observed for a single endpoint and does not require or imply a stimulatory response for any other endpoint at any time (DUKE *et al*., 2006; KENDIG *et al*., 2010; MUSHAK, 2013). Therefore, stimulatory responses probably seldom lead to an over-all improvement of plant fitness (PARSON, 2003; FORBES, 2000). This also widely applies to current results as mainly only one of the four response parameters measured showed significant hormesis within one experiment and, furthermore, not always the same endpoint. The only exceptions to this were two experiments

with *M. inodora* where in each experiment and biotype two of the four parameters measured showed stimulation by iodosulfuron-methyl-sodium/mesosulfuron-methyl. Thus, in some cases of herbicide hormesis more than one endpoint may be stimulated or maybe even overall plant fitness. However, the number of endpoints or traits measured to illustrate hormesis is usually low and most studies have measured only a single endpoint. Therefore, it is yet uncertain if single endpoint increases or rather an improvement of several traits may prevail in herbicide hormesis. Since the observed frequency of hormesis increased in the order shoot weight (21%) < shoot length (22%) < root length (25%) < root weight (50%) and root weight stimulation was most pronounced, this endpoint may be most eligible to capture herbicide hormesis in germination assays. Regarding the observed interassay variability of stimulated endpoints, it is however indicated to measure several endpoints in order to capture hormesis. Nevertheless, if such an early stage boost in one trait will ultimately translate into a higher competitiveness, a higher reproductiveness, or lower herbicide sensitivity is yet questionable.

CALABRESE and BALDWIN (2002) hypothesized that highly resistant individuals may be especially responsive to hormesis. In the present study, hormesis occurred nearly as often with the sensitive biotypes as with the resistant biotypes. Furthermore, the most pronounced quantitative features (maximum increase and hormetic dose zone) were primarily obtained with the sensitive biotypes of the three weed species tested. Therefore, the present study is not in line with the assumption that resistant biotypes are more prone to develop hormesis. Moreover, the occurrence of hormesis in both target-site resistant biotypes may indicate that the hormetic effect is not related to the herbicide target endowing adverse effects for iodosulfuron-methyl-sodium/mesosulfuron-methyl and terbuthylazine. However, in some cases of target-site resistance, the target-site is still affected by the herbicide, but at higher doses. Thus, whether the hormetic mode of action really differs from the one at higher doses needs further evidence. In case of glyphosate, hormesis was absent in crops made resistant with a glyphosate-resistant EPSP synthase indicating that in case of glyphosate the hormetic effect is dependent on the same herbicide-sensitive target-site responsible for phytotoxicity (VELINI *et al*., 2008). Since in this study the glyphosate-resistant biotype of *E. indica* showed glyphosate-hormesis, it is to assume that the EPSP synthase of that biotype is still affected by glyphosate or a mechanism endowing resistance other than a target-site resistance exists.

Hormesis is not a universally distributed biological phenomenon and there is no evidence for a high and consistent frequency of occurrence close to 100% in any case of hormesis reported (MUSHAK, 2013). A database study of CEDERGREEN *et al*. (2007) evaluating 687 dose-response curves revealed a frequency of herbicide hormesis between < 20 to > 70% depending on the species and the compound tested. Thus, the current frequency of 32% for the resistant biotype is in line with previous findings, although at the lower end. The question is however, if the observed 'lower end' frequency of hormesis would be sufficient to figure in resistance development. On the other hand, the frequency of hormesis was shown to dramatically vary with the biological species and the herbicide (CEDERGREEN *et al*., 2007; MUSHAK, 2013) and, therefore, an involvement may primarily be indicated for those species/herbicide combinations showing a high frequency of hormesis. Considerable research is yet needed to prove this hypothesis.

Resistance Factors. In dose-response studies evaluating interassay and interspecies variability of hormesis, there appeared to be a positive correlation between the magnitude of hormesis and the ED_{50} such that the more pronounced the hormetic effect, the higher the ED_{50} dose level (BELZ and PIEPHO, 2013a,b). Thus, theoretically, the occurrence of hormesis should impact the level of resistance so that a solo stimulation of the sensitive reference should lower RF values, while a solo stimulation of the resistant biotype should enhance RF values. Correlating the occurrence of hormesis with observed RF values in this study did not provide clear evidence to support this hypothesis. However, the observed incidences of hormesis in only one of the two biotypes may have been too sparse to depict a general trend.

A further aspect to consider for the involvement of hormesis in resistance development is the fact that a sufficient difference in sensitivity between the sensitive and the resistant biotype is needed so that the resistant biotype is promoted at doses efficiently controlling the sensitive biotype. In this study, this was only the case for iodosulfuron-methyl-sodium/mesosulfuron-methyl-treated *M. inodora* in exp. 5 showing an RF of 907.5 for root length responses (Fig. 1F). Here, the dose giving 43% maximum stimulation (*M*) in the resistant biotype exceeded the ED_{90} dose level of the sensitive reference. On the contrary, RF values as low as currently especially observed for glyphosate-treated *E. indica* will never facilitate a promotion of the resistant biotype at doses used to control the species (*e.g.*, Fig. 1A, 2A, and 2B). Based on this, it may be speculated that primarily weeds with high resistance factors hold a risk for the involvement of hormesis in resistance development. Due to the observed highest frequency of hormesis, the stimulation of more than one plant trait and the high RF values, the actual used ALS-target-site resistant biotype of *M. inodora* may hold such a risk. Nevertheless, two further important issues are to consider in this context. *First*, the field rate does not necessarily represent the dose causing maximum stimulation or a hormetic dose. *Second*, growth stimulation may have no long-term impact if boosted plants are not more reproductive (BELZ *et al*., 2011).

Getting back to the initial question if hormesis may be an underestimated factor in the development of herbicide resistance, an answer is still pending. This study explored herbicide hormesis in sensitive/resistant weed biotypes using an experimental design that allowed capturing the phenomenon in a useful and promptly way. However, the findings relate to early stage growth enhancement as a measure for competitive ability under controlled conditions in an artificial bioassay system. It is thus uncertain if current findings can be transferred to more complex biological systems. Therefore, the addressed potential implications of hormesis boosting resistant weeds under more practical conditions are tentative and clearly need to be verified under more practical conditions. Nevertheless, this study showed that herbicide hormesis has the potential to considerably boost early stage growth traits and, thus, the competitive ability of resistant weeds. On the other hand, results showed that rather single plant traits of resistant weed biotypes were stimulated by herbicide hormesis than overall plant fitness. Furthermore, the observed frequency of hormesis was moderate and high levels of resistance may be required for hormesis to assist in resistance development. Therefore, it is indicated that hormesis may not assist per se in resistance development, but only certain species/herbicide/mode of resistance combinations may be at risk. Considerable research will be required to elucidate the interplay of hormesis and herbicide resistance in weeds, however, for a full understanding of combating weeds with herbicides, hormesis should be considered.

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