Herbicide hormesis to segregate a weed population? – A case study with Tripleurospermum perforatum (Mérat) Lainz

Kann Herbizid-Hormesis eine Unkrautpopulation spalten? – Eine Fallstudie mit Tripleurospermum perforatum (Mérat) Lainz

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Abstract

Weed populations feature within-population genetic differences. Thus, evaluating mean responses in herbicide treated populations may miss ecologically significant individual responses. Since hormesis can likewise vary between individuals, this study investigated the hypothesis that herbicide hormesis within a high-density weed population is different among slowly-growing individuals, as compared to fast-growing individuals. In a dose-response experiment, Tripleurospermum perforatum (Mérat) Lainz was exposed to 12 doses of Atlantis WG (mesosulfuron + iodosulfuron) in 24 replicates (50 plants/replicate). Root/shoot growth responses were evaluated as dose-response relationships for the population mean, the 90-97th percentile of the population (fast-growing individuals), and the 5-10th percentile (slow-growing individuals). Growth responses were generally biphasic. Slow-growing individuals had more pronounced hormesis that occurred partially at lower doses as compared to the population mean. With fast-growing individuals, hormesis was instead less pronounced and partially shifted to higher doses. Hence, hormesis was primarily associated with a stimulation of slow-growing individuals, while fast-growing individuals contributed to a lesser extent to the hormetic population response in a dense stand in vitro. This discrepancy may have the potential to segregate an herbicide exposed population and alter its sensitivity in the long-run.

Keywords: Biphasic, dose-response, growth stimulation, selective toxicity, sulfonylurea herbicide

Zusammenfassung


Stichwörter: Dosis-Wirkungsbeziehung, selektive Toxizität, Sulfonylharstoff-Herbizide, Wachstumsstimulierung, zweiphasisch

Introduction

A weed population, defined as all the individuals of the same plant species within a geographic area, is composed of individual genotypes contributing gene variants (alleles) to the gene pool of a population. This allelic diversity within the gene pool causes a distinct phenotypic plasticity and substantiates the ability of a population to adapt to local environmental conditions, including
weed control measures (ZIMDAHL, 2013). The adaptation of a weed population to chemical control is most obvious when it comes to the selection of individuals with specific alleles endowing resistance to the herbicide at action. As compared to this high-dose selection of resistance, putative adaptations of weed populations to subletal low-dose exposures of herbicides as in case of e.g. drift deposition, errors in application, or gaps in effectiveness are rarely being taken into account. Recently, however, low-dose toxicant exposures were observed to influence plant gene expression and plant size distribution of a population in dense stands (AINA et al., 2006; QUAGGIOTTI et al., 2007; SINKKONEN et al., 2008, 2009, 2011). Hence, low toxicant concentrations can result directly or indirectly via gene expression in phenotypic differentiation which may have severe effects on plant populations in the long-run. Therefore, this study investigated if low herbicide doses are also capable of directly differentiating the within-population size distribution in high-density stands. When it comes to low-dose toxicant responses in plant biology, two aspects are to consider, namely ‘selective low-dose toxicity’ and low-dose stimulatory effects, known as hormesis. Selective toxicity on subpopulations at doses lacking an effect on most of the population has been observed in dense stands for the most vigorous individuals in plant populations exposed in vitro to environmental toxicants (SINKKONEN et al., 2008, 2009, 2011). Deterioration of exceptionally fast-growing individuals at doses that do not affect mean plant size is considered to apply to many toxicants and species, but has not yet been proven for herbicides and their target weeds. However, low toxicant doses do not necessarily leave the mean plant size unchanged, but are also known to stimulate plant growth. Since hormesis studies usually focus on mean responses, it is unknown if and how the within-population size distribution is changed under low-dose exposure to a hormetic compound. Therefore, this study investigated the interplay of both low-dose phenomena in high-density stands, i.e. whether they occur simulatenously, successively, or independent of each other and to what extent hormesis alters the size distribution of the treated population. The study used Tripleurospermum perforatum (Mérat) Lainz exposed to an inhibitor of acetolactate synthase (ALS) since this combination has shown stimulatory responses in a previous study (BELZ, 2014). The experiment was conducted as in vitro dose-response germination bioassay processed at a high replication rate and a high plant density. Growth responses were evaluated for the population mean in comparison to the most long-grown (≥ 90% percentile) and the most short-grown subpopulation (≤ 10% percentile).

Materials and Methods

Dose-Response Bioassay

The assay was conducted as a complete dose-response germination experiment with T. perforatum (Herbiseed, England) as the test species and root and shoot elongation as the response parameter for effects of Atlantis® WG (30 g/kg mesosulfuron-methyl, 6 g/kg iodosulfuron-methyl-natrium; Bayer CropScience). The assay was done in 6-well cell culture plates (Cellstar, greiner bio-one). Each well was prepared with one layer of filter paper (MN 615, Macherey-Nagel) and 60 T. perforatum seeds before 1.5 ml of 12 concentrations of Atlantis WG (0.8 pg a.i./ml to 4.0 µg a.i./ml) were added. A control with demineralized water was prepared similarly. All treatments were replicated 24 times. Plates were sealed with parafilm and cultivated according to a completely randomized design in a growth cabinet (day/night cycle of 12/12 h with 24/18 °C and 50-70/0 µmol m⁻² s⁻¹ photosynthetic active radiation). After 48 h, seedling number was harmonized to 50 per replicate by removing all potentially ungerminated seeds. After ten days, plates were frozen at -4 °C prior to measuring root and shoot length (≥ 1 mm) of all seedlings per well using Fitomed (CASTELLANO et al., 2001). Seedlings with a root or shoot length < 1 mm were counted as zero.
Statistical Analysis

Selective Low-Dose Toxicity. An univariate analysis of variance was performed with Tukey test ($\alpha=0.05$) using IBM SPSS to sort out treatments with significantly different values in mean root/shoot length from non-exposed control treatments. If the Tukey test indicated no difference in treatment means, we compared seedlings at the right (97, 95 and 90%ile) and left (10, 8, and 5%ile) tails of the size distribution diagrams of control and exposed treatments with Mann-Whitney U tests ($\alpha=0.05$) (SINKKONEN et al., 2009, 2011).

Dose-Response Modelling. In order to compare mean dose responses with selective effects on large and short individuals of a population, dose-response relationships were modelled and compared for mean values of the 24 replicates per dose and for the 10 and 95%ile. The NLMIXED procedure of SAS was used to fit root/shoot length values per dose ($y$) as a nonlinear function of dose ($x$) to the hormetic dose-response models of BRAIN and COUSENS (1989) or CEDERGREEN et al. (2005). The significance of hormesis was assessed according to SCHABENBERGER et al. (1999) by means of the model parameter $f$ requiring a 95% confidence interval that does not cover the value zero. Besides the directly estimated parameters of the original dose-response functions, further hormetic features were deduced using reparameterizations of the original models (BELZ and PIEPHO, 2012, 2013): the maximum stimulatory response $y_{\text{max}}$ at the dose where hormesis is maximal ($M$), the LDS dose as the upper dose limit for stimulatory responses, and the $ED_{50}$ or $ED_{90}$ as the dose levels of 50 or 90% inhibition. Response variance heterogeneity was accounted for by using the inverse variance of replicates at each dose as weight. Model comparisons for significant differences between dose-response curves were done by pairwise comparisons of regression parameters using the CONTRAST statement within the NLMIXED procedure.

Results

Selective Low-Dose Toxicity

Mean root elongation was not significantly different from the untreated control at eight of the 12 doses (Tab. 1). The average root length of these treatments was $19.3 \pm 0.8$ mm (mean ± stdev.). Regarding long-rooted seedlings at the right tail of the size distribution diagram (90, 95, 97%ile), only one treatment (0.002 µg a.i./ml) at the 90%ile significantly differed from the control, whereas root length was significantly enhanced. Regarding short-rooted seedlings at the left tail of the size distribution diagram (5, 8, 10%ile), differences between controls and exposed treatments occurred at all three percentiles evaluated such that a dose of 0.0003 µg a.i./ml significantly enhanced root elongation. Thus, results provided no indication for selective low-dose toxicity against the most long-rooted seedlings.

Mean shoot elongation was not significantly different from the untreated control at nine of the 12 doses (Tab. 1). The average shoot length of these treatments was $3.4 \pm 0.2$ mm. Regarding short-stemmed seedlings (5, 8, 10%ile), a treatment of 0.0003 µg a.i./ml lead to significantly enhanced shoot elongation at all three percentiles evaluated. Thus, results provided no indication for selective low-dose toxicity against the most short-stemmed seedlings. Regarding the most long-stemmed seedlings (90, 95, 97%ile), three doses at the 90%ile showed significantly shorter shoots as compared to the control. Thus, selective low-dose toxicity in shoot elongation appeared among the most long-stemmed seedlings.
Tab. 1 | Statistical significance of effects of Atlantis WG (iodosulfuron-methyl-sodium/mesosulfuron-methyl) on root and shoot length of *Tripleurospermum perforatum* at the population mean and the 5, 8, 10, 90, 95 or 97%ile. Data given as mean ± standard deviation.

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<th>Parameter</th>
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\(^a\)doses for which the mean root/shoot length was not significantly different from control according to Tukey test \((a=0.05)\); \(^b\)significant different from control according to Mann-Whitney-U test \((a=0.05)\).

Dose-Response Modelling

*Population Mean.* Root and shoot growth responses to Atlantis WG showed significant hormesis, although the hormetic effect was with a maximum stimulation \(y_{\text{max}}\) of 106% of control for root length and 110% of control for shoot length not very pronounced (Fig. 1).

Root growth showed a maximum stimulation at a dose \(M\) of 1.058 ng ai./ml and the upper dose limit \(LDS\) for stimulatory responses amounted at 2.826 ng ai./ml. A 50% inhibition of root length was given at an \(ED_{50}\) of 0.020 µg ai./ml. As compared to this, shoot growth was significantly less sensitive. The dose-response curve for shoot length was shifted to higher doses by a factor of 13-fold at the \(M\) dose level, by 21-fold at the \(LDS\), and by 158-fold at the \(ED_{50}\).

*Subpopulations.* Modelling and comparison of dose-response curves at the 10%ile (slow-growing individuals) and the 95%ile (fast-growing individuals) showed that root and shoot responses were significantly hormetic at both percentiles. However, hormesis occurred at lower doses and was significantly more pronounced with the slow-growing individuals as compared to the fast-growing individuals (Fig. 2).
Fig. 1 Biphasic dose-response curves for effects of Atlantis WG (iodosulfuron-methyl-sodium/mesosulfuron-methyl) on root and shoot length of *Tripleurospermum perforatum*; data given as means of 24 replicates per dose.

Abb. 1 Zweiphasische Dosis-Wirkungskurven für die Wirkung von Atlantis WG (Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl) auf das Wurzel- und Sprosswachstum von *Tripleurospermum perforatum*; dargestellt sind Mittelwerte aus 24 Wiederholungen pro Dosierung.

Fig. 2 Biphasic dose-response curves for effects of Atlantis WG (iodosulfuron-methyl-sodium/mesosulfuron-methyl) on (A) root and (B) shoot length of *Tripleurospermum perforatum* at the 10% or 95%ile of the tested population.

Abb. 2 Zweiphasische Dosis-Wirkungskurven für die Wirkung von Atlantis WG (Iodosulfuron-methyl-Natrium/Mesosulfuron-methyl) auf das Wurzel- (A) und Sprosswachstum (B) der 10%ilen und 95%ilen Teilpopulation eines Biotyps von *Tripleurospermum perforatum*.

Regarding root elongation, the short-rooted seedlings at the 10%ile showed an $y_{\text{max}}$ of 172% of control and an 11.0-fold dose-distance between $M$ and $LDS$ as an indicator for the hormetic dose zone. The relative $y_{\text{max}}$ was thus 1.7-fold higher and the dose-distance $LDS/M$ was 4.5-fold broader as compared to the 95%ile. Dose $M$ giving maximum stimulation did, however, not significantly differ between percentiles (0.634±0.089 ng a.i./ml on average) (Fig. 2A). As compared to the population mean, relative $y_{\text{max}}$ was 1.6-fold higher and the dose-distance $LDS/M$ 4.1-fold broader at the 10%ile, while both features were 0.9-fold lower at the 95%ile. Dose $M$ for mean root length was with 1.058 ng a.i./ml significantly higher than the average of both percentiles. Hence, slow-growing individuals proved significantly more responsive to low-dose effects than the average population. Fast-growing individuals were also more sensitive, but less stimulated than the average of the population. Regarding the inhibition of root length at higher doses, the $ED_{50}$ values did not significantly differ between the population mean and both percentiles (0.022±0.002 µg
a.i./ml on average). However, the $ED_{90}$ dose level significantly increased from the 10%ile with 0.176 µg a.i./ml by 4.2-fold at the population mean and by 6.5-fold at the 95%ile.

A similar trend was observed for shoot elongation. The short-stemmed seedlings at the 10%ile showed a relative $y_{max}$ of 129% of control at $M=0.116$ ng a.i./ml and a 10.0-fold $LDS/M$ dose-distance. The relative $y_{max}$ was thus 1.2-fold higher and at an 8.9-fold lower dose as compared to the 95%ile. The $LDS/M$ dose-distance was, however, 0.6-fold narrower at the 10%ile (Fig. 2B). As compared to the population mean, relative $y_{max}$ was 1.2-fold higher at the 10%ile and the $LDS/M$ dose-distance 2.2-fold broader. At the 95%ile shoot stimulation was 0.9-fold less pronounced as at the population mean despite a 3.5-fold broader $LDS/M$ dose-distance. Dose $M$ for mean shoot length equalled those of the 95%ile, but was significantly higher as compared to the 10%ile. Hence, slow-growing individuals also proved significantly more responsive in shoot elongation to low-dose stimulation than the average population and fast-growing individuals. Regarding shoot growth inhibition at higher doses, the $ED_{50}$ values significantly increased from the 10%ile with 0.097 µg a.i./ml by 32.7-fold at the population mean and by 115.3-fold at the 95%ile.

**Discussion**

The study confirmed previous reports of low-dose stimulatory effects of sulfonylurea herbicides on plants (CEDERGREEN, 2008; BELZ, 2014). The observed hormetic quantities for mean responses with an $y_{max}$ between 106-111% of control and a dose distance $LDS/M$ between 2.7-4.5-fold indicated a modest stimulatory response as compared to general quantitative features reported in the literature (CALABRESE and BALDWIN, 2002; CALABRESE, 2008). Proving the significance of a stimulatory response $\leq 10\%$ requires excellent statistical power and sufficient replicates (CALABRESE, 2008). This was obviously granted by the current experimental design that could even model significant hormesis for a maximum stimulation of 101% of control for root elongation at the 95%ile. For such exceptionally low examples of hormesis, the hormetic dose range cannot be modelled as usual as the dose distance between the dose giving 10% stimulation and the $LDS$. Taking the dose giving 1% stimulation as a basis for the lower dose limit for hormesis, stimulation of mean root length occurred between 0.063 ng a.i./ml and 0.003 µg a.i./ml and of mean shoot length between 0.306 ng a.i./ml and 0.060 µg a.i./ml.

Evaluating responses for selective low-dose toxicity confirmed its occurrence for shoot growth at the right tail of the size distribution diagram, i.e. the most long-stemmed seedlings of the population ($\geq 90\%$ile). This confirms previous findings of SINKKONEN et al. (2008, 2009, 2011) and supports the hypothesis that this low-dose phenomenon may be quite common for the most vital seedlings of a population in high-density stands. Moreover, results showed that selective low-dose toxicity and hormesis do not exclude each other and should thus be considered in parallel. However, comparing the observed dose ranges of both low-dose phenomena showed that selective toxicity in shoot growth occurred between 0.008-0.600 ng a.i./ml and, thus, mainly at doses preceding the hormetic dose range for mean shoot growth. This suggests that both low-dose phenomena can occur in parallel, but presumably in a dose-dependent manner low-dose stimulation after low-dose toxicity. SINKKONEN et al. (2008, 2009, 2011) supposed density-dependent phytotoxicity as one possible reason for selective low-dose toxicity, such that fast-growing individuals take up more toxicant than slow-growing ones and are, thus, more prone to impairment. Conversely, slow-growing individuals may be more prone to stimulation which would explain the observed stimulation at the left tail of the size distribution diagram ($\leq 10\%$ile) despite negligible effects on most individuals in the population. Further evidence is, however, needed to support this hypothesis.

The question if hormesis alters the size distribution of an herbicide treated population was evaluated by comparing individual dose-response curves for the slow- (10%ile) and fast-growing (95%ile) part of that population. Results showed that the amplitude of hormesis decreased from the left to the right tail of the size distribution diagrams and that the dose-response curves were partly shifted to higher doses with the fast-growing subpopulation. This indicated that the slow-
growing individuals tended to be more sensitive to Atlantis WG and more responsive in low-dose stimulation than the fast-growing subpopulation. This shows that herbicide-hormesis can alter the size distribution in a dose-dependent manner such that the abundance of the shortest individuals decreases at low doses, while at higher but still hormetic doses the abundance of large individuals increases. Therefore, a mean hormetic response may happen due to a decrease in the frequency of the most short-grown individuals followed by an increase in the abundance of the most long-grown individuals. Moreover, the mean hormetic response of the *T. perforatum* population was widely intermediate between its slow- and fast-growing subpopulations. However, the amplitude of hormesis was always closer to the less pronounced hormetic response of the fast-growing subpopulation. This suggests that in high-density stands hormesis may be primarily associated with a stimulation of slow-growing individuals, but the degree of stimulation at the right tail of the size distribution diagram may primarily govern the effect on most of the population. Therefore, a fairly significant hormetic effect on the slow-growing subpopulation may be even missed if the effects are masked by a lack of hormesis on the normally faster growing part of the population.

The reason why hormetic effects are differentially expressed depending on individual growth rates is unsettled. However, the expression of hormesis was shown to depend on plant growth conditions such that the amplitude is impaired under conditions preventing plants from enhanced growth, *i.e.* retarded/no growth or optimal/maximum growth (Belz and Cedergreen, 2010). It is thus possible that fast-growing individuals have a lower capacity for enhanced growth. Further, within population genetic differences could account for the observed discrepancy in sensitivity between subpopulations.

**Conclusions**

The study showed that low herbicide doses are also capable of directly differentiating the within-population size distribution in dense stands. The phenomenon may be of ecological significance in wild plant populations exposed to low herbicide doses (*e.g.*, drift deposition or run-off) but also weed populations on treated fields due to errors in application, leaf contact of treated and untreated plants, protection by taller plants or mulch, herbicide resistance or gaps in effectiveness, or absorption of low doses from soil, especially after soil degradation or immobilization (Belz and Duke, 2014). Such a phenotypic segregation may directly affect plant performance and alter its survival under extreme environmental conditions. For example, lowering the frequency of short-rooted individuals may enhance a population’s resistance to drought since rooting depth is a key factor to drought resistance (Sinkkonen et al., 2009). In the longer term, such a phenotypic differentiation can lead to a genotypic adaptation and thus the formation of ecotypes with partly pronounced differences in growth and reproductive behaviour (Zwerger, 1993). Therefore, if the observed phenomena can be confirmed for other herbicides, other species and for natural plant populations, low herbicide levels may have drastic effects on the structuring of populations leading to low-dose driven changes in herbicide sensitivity, ecosystem services or biodiversity in the long-run.

**Acknowledgement**

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