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STIMULATION VERSUS INHIBITION—BIOACTIVITY OF PARTHENIN, A PHYTOCHEMICAL FROM *PARTHENIUM HYSTEROPHORUS* L.

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□ *Parthenium hysterophorus* L. is an invasive weed that biosynthesizes several phytochemicals. The sesquiterpene lactone parthenin receives most attention regarding allelopathy of the plant or potential herbicidal properties. Since parthenin exhibits dose-dependent phytotoxicity with low dose stimulation, this study investigated the occurrence and temporal features of parthenin hormesis in *Sinapis arvensis* L. sprayed with parthenin under semi-natural conditions. Dose/response studies showed that the occurrence and the magnitude of hormesis depended on climatic conditions and the parameter measured. Within the tested dose range, stimulatory responses were only observed under less-stressful conditions and were most pronounced for leaf area growth [138 % of control; 13 days after treatment (DAT)]. Temporal assessment of leaf area development showed that doses causing a stimulatory response at the end of the experiment ($< 0.42 \pm 0.04$ kg/ha; 13 DAT) were initially inhibitory up to ED₅₀ values (2 DAT). This clearly demonstrated an overcompensatory response. Inhibition of leaf area at 13 DAT reached ED₅₀ values on average at 0.62 ± 0.12 kg/ha, and *S. arvensis* was completely inhibited at doses exceeding 1.81 ± 0.56 kg/ha (ED₉₀). Based on these findings, implications of parthenin hormesis are discussed with respect to allelopathy of *P. hysterophorus* and exploitation of growth stimulatory responses in agriculture.

Keywords: dose/response modeling, dose/time responses, hormesis, *Sinapis arvensis*, spray application

INTRODUCTION

Biphasic dose/response relationships that are characterized by a stimulatory response in the measured parameter at low doses of a stressor and inhibition at higher doses are well recognized in toxicology and pharmacology (Calabrese and Baldwin 2001b; Calabrese and Blain 2005). This dose/response phenomenon is termed hormesis and represents an evolutionarily conserved process of adaptive, potentially beneficial responses to low doses of a stressor agent/condition (Calabrese *et al.* 2007). It has been observed with many chemically diverse compounds, with a wide range of measured endpoints, and in essentially all organisms studied so far. Still, little documentation exists on hormetic dose responses in plants, whether effects of synthetic herbicides or natural phytotoxins are concerned (Duke *et al.* 2006). Especially in the field of allelopathy, defined as both harmful and beneficial biochemical interactions among plants

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mediated by the release of plant-produced phytotoxins, reports of hormesis are rare. Examples of hormesis with natural phytotoxins are reported with single compounds (e.g. gramine and hordenine, benzoxazolin-2(3*H*)-one, and allyl isothiocyanate), plant extracts (e.g. *Triticum aestivum* L.), and root exudates (e.g. *T. aestivum* and *Triticum spelta* L.) (An *et al.* 1993; Belz *et al.* 2005, 2007c). Based on these reports, it was hypothesized that hormesis may be a widespread dose/response feature of allelopathic phytotoxins, also referred to as allelochemicals (Rice 1984; An *et al.* 1993; Liu *et al.* 2003).

The sesquiterpene lactone parthenin produced by certain populations of the invasive weed *Parthenium hysterophorus* L. is another example for the stimulation/inhibition properties of some allelochemicals (Figure 1). The compound is biosynthesized during the entire life cycle of the plant, reaching maximum values during generative stages (Reinhardt *et al.* 2006). It is sequestered in capitate-sessile trichomes on leaves, stems, and the achene-complex of *P. hysterophorus* (Reinhardt *et al.* 2004). Parthenin is released from plant material by being washed from ruptured trichomes or from decomposing tissues and may contribute to the plant's interference with surrounding neighbors. Laboratory studies described parthenin's phytotoxic properties against a broad range of plant species, including weeds and crops [e.g. *Ageratum conyzoides* L., *Amaranthus viridis* L., *Avena fatua* L., *Cassia tora* L., *Chenopodium murale* L., *Phaseolus aureus* Roxb., and *T. aestivum* (Batish *et al.* 1997b, 2002a,b; Datta and Saxena 2001)]. The focus of these studies was adverse effects and, thus, recognition of parthenin hormesis was often constrained by the lack of doses below inhibition range.

Therefore, stimulatory effects were at first only reported in association with plant extracts of *P. hysterophorus* (Table 1). Although parthenin was isolated as the major extract constituent, it is just one of several phytochemicals in this extract and, thus, a causal involvement in the

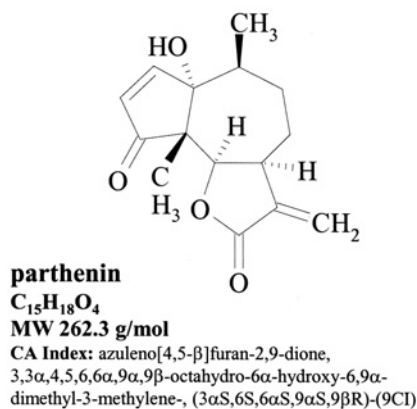


FIGURE 1. Structure of parthenin.

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TABLE 1. Some examples of actual/presumed hormesis related to *Parthenium hysterophorus*.

| treatment | test plant | increased parameter | reference |
|--|-------------------------------|--|--|
| parthenin | <i>Echinochloa crus-galli</i> | root length* | Belz <i>et al.</i> 2007a |
| | <i>Eragrostis curvula</i> | root length* | |
| | <i>Phaseolus aureus</i> | shoot dry weight* | Batish <i>et al.</i> 1997a |
| | <i>Triticum aestivum</i> | shoot length* | |
| | | root/shoot length | Batish <i>et al.</i> 1997b |
| aqueous extracts of plant material | | | |
| leaves (fresh material) | <i>Eragrostis tef</i> | root length* | Belz <i>et al.</i> 2007a |
| leaves (dry material) | <i>Triticum aestivum</i> | shoot fresh weight | Pandey <i>et al.</i> 1993b |
| leaves, flower, stem, root (dry material) | <i>Oryza sativa</i> | carotenoid content chlorophyll content fresh weight root/shoot length | Pandey 1994b |
| leaves, flower, stem, root (dry material) | <i>Eichhornia crassipes</i> | fresh weight no. of healthy leaves | |
| | <i>Salvinia molesta</i> | fresh weight no. of healthy fronds | Pandey <i>et al.</i> 1993b Pandey 1994a,b |

* statistical significance verified

observed extract hormesis remained initially unverified. Subsequent dose/response studies with the pure compound provided a first indication for the existence of hormesis by parthenin (Batish *et al.* 1997a,b), and complete dose/response modeling finally proved that parthenin significantly stimulates growth at low doses (Belz *et al.* 2007a). Pure parthenin treatments in equimolar amounts as present in extracts could reproduce the stimulatory response, which suggests that parthenin may play a key role in eliciting observed extract hormesis (Belz *et al.* 2007a).

Taking this into account, two aspects received consideration in the present study. From a practical point of view, stimulatory effects on crop plants suggest a potential use as a growth promoter. This application might come into focus with the renewed attention on herbicide-related hormesis (Cedergreen *et al.* 2005, 2007; Duke *et al.* 2006) and alternative uses of herbicides (Duke *et al.* 2007). As parthenin proved to be fairly unstable in a soil environment (Belz *et al.* 2007b), treatments of above-ground plant parts may be more promising in this regard and hormetic effects of parthenin on shoot elongation and dry weight of *P. aureus* point to the possibility (Batish *et al.* 1997a). Therefore, this study investigated the bioactivity of parthenin applied as a conventional spray application under semi-natural conditions. Experiments used *Sinapis arvensis* L. as a model test species and evaluated bioactivity of parthenin on different growth parameters as well as temporal features of dose responses.

A second aspect related to parthenin bioactivity is the question of the ecological significance and possible impacts of hormesis. Parthenin can

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be released by leaching from living plant parts or by decomposition of plant residues (Kanchan and Jayachandra 1980a; Reinhardt *et al.* 2004). While the latter mode of release is believed to release inhibitory levels of parthenin, it is still unknown if leaching of parthenin by rain, mist or dew is of a magnitude sufficient to cause a biological effect, whether stimulatory or inhibitory. Dropping of parthenin-containing leachates on leaves of target plants growing under the canopy of *P. hysterophorus* may be one mechanism of the plant's allelopathic capacity. Literature reports from other plant species demonstrated that such natural leachates can exhibit inhibitory allelopathic effects, *e.g.* *Acacia dealbata* Link (Carballeira and Reigosa 1999). However, phytotoxin concentrations found under field conditions in leachates are often below bioactive levels, *e.g.* *Cucurbita* spp. (Fujiyoshi *et al.* 2002). Thus, investigating the bioactivity in case of a spray application also establishes effective doses as a base to assess the significance of direct effects of parthenin dropped on the leaf surface of target plants by leaching from *P. hysterophorus* plant parts.

MATERIALS AND METHODS**Isolation of parthenin**

The sesquiterpene lactone was isolated from organic solvent extracts of the leaf surface of greenhouse grown plants of a South African population of *P. hysterophorus* as described by Belz *et al.* (2007a). Extracts were obtained by dipping fresh leaf material for 10 s in tert-butyl methyl ether (250 mg/ml) followed by a filtration over anhydrous sodium sulphate. Vacuum concentrated extracts were redissolved in tert-butyl methyl ether and parthenin crystallized after heating (40 °C) by addition of small amounts of 2,2,4-trimethylpentane. After the supernatant was removed, parthenin crystals were washed twice with tert-butyl methyl ether and finally dried with nitrogen gas. Purity of isolated parthenin was checked using HPLC-DAD analysis ($\geq 95\%$) and results confirmed by HPLC-ESI-MS.

Experimental design*Cultivation of plants*

Seeds of *S. arvensis* (Herbiseed, Great Britain) were pregerminated in vermiculite for seven days and transplanted to 8 x 8 cm Jiffy-strips (BayWa, Germany) filled with compost soil at a plant density of two plants/cavity. Plants were cultivated for another eight days under semi-field conditions in a vegetation hall at the University of Hohenheim, Stuttgart, Germany, prior to parthenin treatment. Plants were located in the glass roofed part of the vegetation hall that simulates outdoor conditions under rain protection. Ambient climatic conditions during the trial period (July/August 2006) are given in Table 2 (data represent outdoor conditions). Watering of plants was done with tap water as required.

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TABLE 2. Climate data of Stuttgart-Hohenheim (lon. 9.21, lat. 48.7, 407 m NN).

| weather condition | | July 2006 | August 2006 |
|--|---------------------------------------|---------------------|--------------------|
| average air temperature (min./max.) [°C] | | 22.9 (14.0/34.0) | 15.5 (7.7/26.6) |
| average max. temp. | [°C] | 29.0 | 20.1 |
| average min. temp | [°C] | 17.1 | 12.0 |
| days > 25 °C | [no.] | 29 | 2 |
| days > 30 °C | [no.] | 13 | 0 |
| sunshine duration | [hrs] | 323.4 | 129.4 |
| sum of global radiation | [J cm ⁻² d ⁻¹] | 75628.0 | 44828.0 |
| average relative humidity | [%] | 64 | 79 |

Spray application

Fifteen days after germination, plants at the 2-leaf stage were treated with parthenin using a laboratory sprayer equipped with a flat-fan nozzle (type 8004, TeeJet-Spraying Systems, USA; 2.5 bar pressure; 400 L/ha spray volume). Plants were treated with up to 11 doses of parthenin (0-3 kg/ha) prepared with deionized water (incl. 0.2 % Tween[®]20, Roth) from stock solutions of parthenin in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide at each treatment was 10 %. Controls were performed with solvent only and replicated 12 times. Parthenin treatments used one Jiffy-stripe each with six replications and were cultivated in a completely randomized design as described above.

Evaluation

The leaf area of plants was estimated non-destructively at 2, 4, 6, 8, 11, and 13 days after treatment (DAT) using a bi-spectral camera (IR spectrum > 680 nm; VIS spectrum 620-660 nm). As the distance between leaves and camera varied with time, concentration, and insertion on the stem, overarm images merely give an estimate of the real leaf area. Grey scale images were binarised using an image processing procedure with manually chosen thresholds and the relative coverage of leaves was determined from thresholded images (1024 x 766 pixel) as ratio of white to black pixels. Shoot length was recorded at 14 DAT as well as dry weight of aboveground plant parts after drying at 80 °C for three days.

Statistical analysis

The relationship between the average response (y) of dry weight, shoot length, or relative leaf area and parthenin concentration (x) was modeled by the modified four parameter logistic regression model of Cedergreen *et al.* (2005):

$$y = c + [(d - c) + f \cdot \exp(-1/x^\alpha)] / \{1 + \exp[b \cdot \ln(x/e)]\} \quad (1)$$

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where c denotes the lower asymptote at infinite doses, d denotes the mean response of the untreated control, and f denotes the theoretical upper bound of the hormetic effect. The remaining parameters α , b , and e have no straightforward biological meaning, nevertheless, the size of b determines the steepness of the curve at inhibitory doses, e gives a lower bound on the ED_{50} (dose causing 50 % inhibition), and α is proportional to the rate of increase in responses at subinhibitory doses (Cedergreen *et al.* 2005). The model was fitted to data by nonlinear regression analysis using *R* (*R* Development Core Team 2006) with the add-on package *drc* (Ritz and Streibig 2005). Variance of responses was stabilized by an optimal box-cox transformation and the quality of curve fitting was assessed by *F* test for lack-of-fit based on an analysis of variance ($P=0.05$). The value of α was fixed to 0.5 according to the smallest residual sum of squares resulting for the three built-in hormesis functions of the *drc* package (Cedergreen *et al.* 2005, 2007). Significance of hormesis was assessed by the 95 % confidence intervals (CI_{95}) for the estimates of f and was given for $f > 0$. In such cases, where the 95 % confidence interval for f included zero, f was set to zero and equation 1 reduced to a monotonic decreasing four parameter logistic function. In case of hormesis, the dose M giving maximum response, the maximal response y_{max} , and the dose where the hormetic effect has disappeared or the limited dose for stimulation LDS [corresponds to ED_1 and is also called ED/EC_0 or no-observable-adverse-effect-level (NOAEL) (Cedergreen *et al.* 2007)] were computed using the *drc* package (Ritz and Streibig 2005). Comparison of dose/response curves was done by horizontal assessment (*F* test, $P=0.05$) for similarity in upper and lower limits, b parameters and e values. Relative potencies of curves were assessed at the ED_{10} and ED_{50} levels.

RESULTS AND DISCUSSION**Bioactivity of parthenin**

Dose responses of *S. arvensis* to leaf-applied parthenin showed a biphasic relationship (Figure 2). However, the magnitude and, thus, the significance of hormesis at the end of the experiment (13/14 DAT) varied depending on the parameter studied (Figure 3). Significance of hormetic effects could be statistically assessed only for the stimulation in relative leaf area [38 % maximum stimulation compared to control (CI_{95} 28/49); $f=33.1$ (CI_{95} 2.2/63.9)]. f Values for dose responses in shoot dry weight [$f=1.0$ (CI_{95} -1.8/3.8)] and shoot length [$f=47.8$ (CI_{95} -33.8/129.4)] were not significantly different from zero, although both endpoints showed a stimulatory trend [13 % stimulation in dry weight (CI_{95} -5/31); 21 % stimulation in shoot length (CI_{95} 11/30)]. These results strengthen the importance of the response parameter for interpretation of hormesis, as hormetic dose responses are endpoint-specific.

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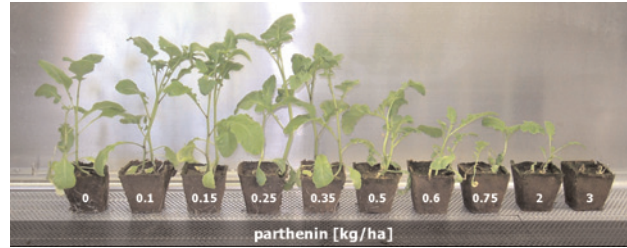


FIGURE 2. Dose responses of *Sinapis arvensis* on parthenin at 14 days after treatment (exp. 2, August 2006).

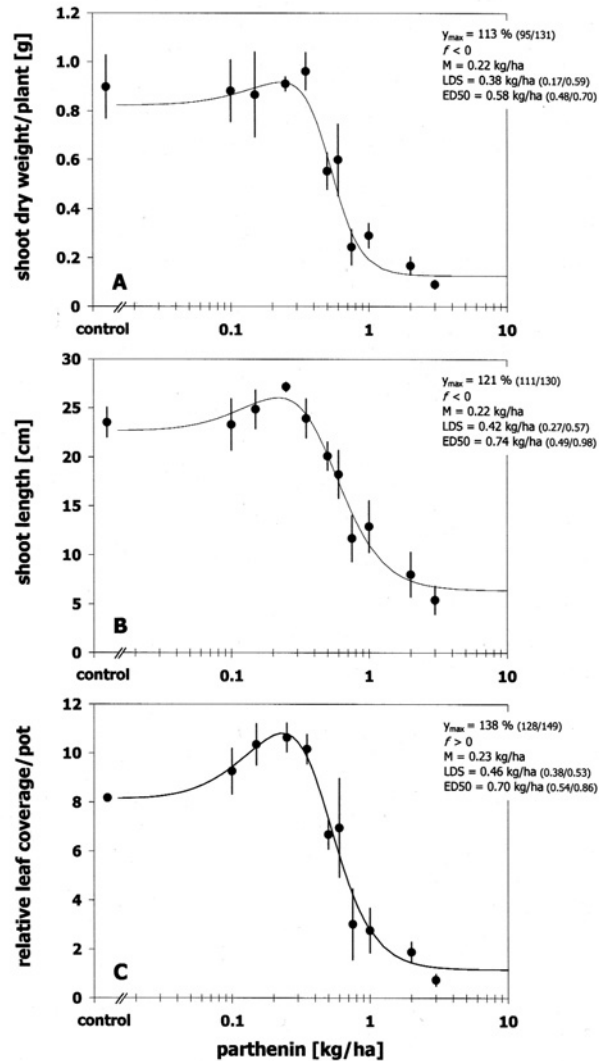


FIGURE 3. Effect of parthenin on growth of *Sinapis arvensis* at 13/14 days after spray application (exp. 2, August 2006). (A) shoot dry weight, (B) shoot length, (C) relative leaf area. Bars indicate \pm standard error of means. Y_{max} = maximal response; f = theoretical upper bound of the hormetic effect; M = dose giving maximum stimulation; LDS = limited dose for stimulation or ED₁ (dose causing 1 % inhibition); 95 % confidence interval for estimates in parentheses.

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Owing to a supposed differential binding to stimulatory and/or inhibitory receptors or resource trade-offs for optimized growth under stressful conditions, dose responses in different traits do not necessarily correlate (Calabrese and Baldwin 2001a, 2002a; Cedergreen *et al.* 2007; Duke *et al.* 2006). For example, the herbicide metsulfuron-methyl stimulated leaf length of *Hordeum vulgare* L. at low doses, while total dry weight remained unaffected (Cedergreen *et al.* 2005). In this study, a stimulatory trend was observed for all endpoints measured and, thus, a trade-off between traits or a differential binding is not indicated. Batish *et al.* (1997a) correspondingly found stimulatory responses in seedling length and dry weight of *P. aureus* exposed to parthenin. Whether the stimulation of several plant traits by parthenin will result in an overall improvement of plant fitness over the long term is yet to be proven. Besides the endpoint measured, hormetic dose responses can vary depending on other factors of the study design, including doses used and species tested. For example, Batish *et al.* (2002b) also investigated the bioactivity of parthenin in a spray application, but did not observe hormetic effects, as doses used were inhibitory. The magnitude of hormesis on *S. arvensis* in this study was in the range of average hormesis responses observed for various herbicides in plants and algae (20-30 %; Cedergreen *et al.* 2005, 2007). However, maximum hormesis effects by parthenin on root length in germination assays reached higher values of up to 82 % stimulation using *Eragrostis curvula* (Schrader) Nees and *Echinochloa crus-galli* (L.) P. Beauv. as test species (Belz *et al.* 2007a).

Although the amplitude of parthenin hormesis varied with endpoint in this study, the stimulatory dose range corresponded. Maximum stimulation appeared on average at parthenin doses of 0.22 ± 0.01 kg/ha ($M \pm$ standard deviation) and disappeared at doses exceeding 0.42 ± 0.04 kg/ha (LDS). Thus, the mean distance between M and LDS doses comprised a 1.87 ± 0.12 fold increase in concentration, which is rather small compared to literature reports of an average 5 fold dose difference (Calabrese and Baldwin 2002a,b). An equally narrow stimulatory dose range for parthenin was observed in germination assays for root growth of *E. curvula* and *E. crus-galli* with an average 2.10 ± 0.02 fold increase in concentration (Belz *et al.* 2007a). In contrast to this, wider distances were observed in herbicidal studies [6 to 26 fold (Cedergreen *et al.* 2005, 2007)]. The limited quantitative features of parthenin hormesis observed in different biological models indicate that stimulation by parthenin may rather represent an overcompensation stimulation hormesis than a direct stimulation. In the latter case, a broader hormetic dose range (up to $\geq 10^3$ fold) and a higher amplitude of the stimulatory response (up to 2 fold of control) would be expected (Calabrese 1999, 2001; Calabrese and Baldwin 2002b). Furthermore, a narrow stimulatory dose zone may indicate that only few mechanisms underlie the hormetic response (Calabrese and Baldwin

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2002b). Both hypotheses still lack sufficient biological understanding of parthenin hormesis to allow for a definite conclusion.

ED₅₀ values ranged between 0.58 kg/ha (CI₉₅ 0.48/0.70) for dry weight to 0.70 kg/ha (CI₉₅ 0.54/0.86) for relative leaf area and 0.74 kg/ha (CI₉₅ 0.49/0.98) for shoot length. Thus, the dose range of apparent stimulation was at all endpoints at concentrations between zero and 65 ± 5 % of the ED₅₀ which is somewhat higher than observed in herbicidal studies (average upper limit of 20-25 %; Cedergreen *et al.* 2005, 2007), but equals observations in laboratory studies with parthenin (average upper limit of 68 %; Belz *et al.* 2007a). This may result from the narrow stimulatory dose range observed for parthenin in contrast to herbicidal studies and indicates that the span between stimulation and inhibition for parthenin is small. With average ED₉₀ doses of 1.34 ± 0.33 kg/ha for all endpoints, parthenin proved to be a moderately effective herbicidal compound for the control of *S. arvensis*. This relatively weak activity, along with the observed hormesis effect, may account for the fact that parthenin has not been considered as a natural pesticide for weed management since its discovery in 1959 (Herz and Watanabe 1959). Furthermore, considering the limited solubility of parthenin in water and the observed effective doses, it seems unlikely that leaching of parthenin from intact leaves may be of a magnitude sufficient to have a direct lethal effect if dropped on plants growing under the canopy of *P. hysterophorus*.

Temporal features of parthenin bioactivity

Evaluating multiple time points, Pandey *et al.* (1993a) observed that low concentrated leaf extracts of *P. hysterophorus* initially reduced biomass development of the aquatic weed *Eichornia crassipes* (Mart) Solms, followed by a recovery of plants and increase in biomass compared to the untreated control. Pandey *et al.* (1993a) suggested a nutritional effect, but in consideration of apparent hormesis, it may have been an over-compensation response following initial injury by low levels of inhibitors in the extract. If parthenin plays in fact a decisive role for hormesis of leaf extracts of *P. hysterophorus*, bioactivity of the single compound should exhibit a comparable temporal feature.

Present dose/time evaluations showed that *S. arvensis* plants were visually damaged from parthenin treatments starting from the first day after application. Visual symptoms mainly involved local necrosis on leaf surfaces, most likely caused by a direct contact effect. At 2 DAT this resulted in a strictly decreasing dose/response relationship with inhibitory effects on relative leaf area at doses exceeding 0.20 kg/ha (CI₉₅ 0.10/0.30; ED₁₀) and a 50 % inhibition at 0.50 kg/ha (CI₉₅ 0.45/0.58) (Figure 4). A significant direct stimulatory effect on leaf area development was thus neither visible at the first day after treatment nor measur-

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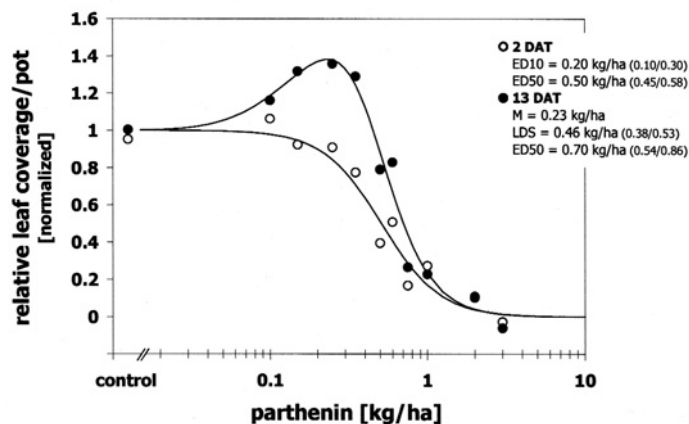


FIGURE 4. Effect of parthenin on leaf area of *Sinapis arvensis* at two (2 DAT) and 13 days after spray application (13 DAT; exp. 2, August 2006). Responses are normalized to controls. M = dose giving maximum stimulation; LDS = limited dose for stimulation or ED_1 (dose causing 1 % inhibition); ED_K = dose causing K % inhibition; 95 % confidence interval for estimates in parentheses.

able at 2 DAT. Dose/response relationships at 4 and 6 DAT did not display a hormetic trend as well, in contrast to dose responses at 8, 11 and 13 DAT, although hormesis was significant only at 13 DAT. This demonstrates that plants recovered from the initial injury and overcompensated for inhibitory effects within a certain dose range. Comparing dose/response relationships at 2 and 13 DAT clearly showed that doses inducing stimulatory responses at 13 DAT (0.23-0.46 kg/ha and lower), decreased leaf area initially between 10 to 50 % (Figure 4). An initial 10 % inhibition caused a maximum stimulation at the end of the experiment and plants showing an initial 50 % inhibition recovered to response levels of the untreated control (LDS). Other test systems were able to overcompensate after an initial 100 % inhibition, which shows that overcompensatory responses can occur across a broad range of initial inhibitory dose levels (Calabrese 1999, 2001). This may suggest that the observed maximum of 50 % initial inhibition for overcompensatory responses might have been higher if the experiment had been extended. On the other hand, current findings correspond with overcompensated inhibition levels observed for the treatment of *Mentha x piperita* L. with the synthetic herbicide fosfon (2,4-dichlorobenzyl tributylphosphonium chloride), where an initial growth inhibition of more than 50 % could not be compensated until five weeks after treatment (Calabrese 1999).

Observed temporal features of parthenin hormesis along with the limited quantitative features confirm the hypothesis that hormesis by parthenin may represent the overcompensation stimulation type of hormesis. This supports the assumption of Calabrese (1999, 2001) that growth hormesis in general represents an overcompensation to previous injury and, thus, a disruption in homeostasis. The molecular and physio-

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logical mechanism underlying this phenomenon in case of parthenin are however unknown. Based on literature reports and their own findings Batish *et al.* (1997a) assumed that parthenin may act as plant growth regulator comparable to plant auxins. Synthetic auxins have been shown to elicit hormesis (Morré 2000; Allender *et al.* 1997). Furthermore, Batish *et al.* (1997a, 2002b) assumed that parthenin may inactivate enzymes containing a thiol group by non-reversible alkylation. The same mechanism is supposed to underlie phytotoxicity of natural isothiocyanates (Drobnica *et al.* 1977) and for one compound of this group of phytochemicals, allyl isothiocyanate, marked hormesis was observed as well (Belz *et al.* 2007c). Thus, hormetic responses were observed in association with both hypothetical modes of action, but especially a potential growth regulatory action may open to exploit parthenin as a stimulant for crop seedling growth. This type of application was also proposed by Pandey (1994b) for plant extracts of *P. hysterophorus*. An essential prerequisite for a successful application as growth promoter is however a high reproducibility of hormetic responses.

Reproducibility of parthenin bioactivity

In order to evaluate reproducibility of dose responses by parthenin, experiments were carried out twice during July and August 2006 applying the same experimental protocol. Results showed that at 2 DAT, the two dose/response curves for relative leaf area were parallel, but shifted towards lower doses in the second experiment conducted in August (exp. 2). In comparison with the first evaluation in July (exp. 1), relative potencies were not significantly different at the ED₁₀ dose level (0.22 ± 0.02 kg/ha), while the observed 1.7 fold shift to lower ED₅₀ doses at exp. 2 was significant. Thus, the initial efficacy of parthenin was higher in exp. 2. Nevertheless, in both experiments the inhibitory dose range started at doses exceeding 0.22 ± 0.02 kg/ha, and dose/response curves were strictly decreasing (Figure 5; 2 DAT). The equivalent was observed for evaluations at 4 and 6 DAT, which indicated a roughly similar initial inhibition in both experiments. Despite this, dose/response curves developed to the contrary starting from 8 DAT. In exp. 2 plants began to overcompensate the initial inhibition at low doses, while no compensation occurred in exp. 1 (Figure 5; 8 DAT). By the end of the experiments dose responses were biphasic in exp. 2 and strictly decreasing in exp. 1 (Figure 5; 13 DAT). The dose M giving maximum stimulation in exp. 2 equaled the ED₂₀ in exp. 1. Furthermore, ED₅₀ values in exp. 2 increased from 0.50 kg/ha (CI₉₅ 0.45/0.58; 2 DAT) to 0.70 kg/ha (CI₉₅ 0.54/0.86; 13 DAT) and decreased in exp. 1 from 0.86 kg/ha (CI₉₅ 0.70/1.03) to 0.53 kg/ha (CI₉₅ 0.09/0.98). This shows that plants were not able to compensate inhibitory effects in exp. 1 and the efficacy of parthenin increased over time in contrast to plants in exp. 2.

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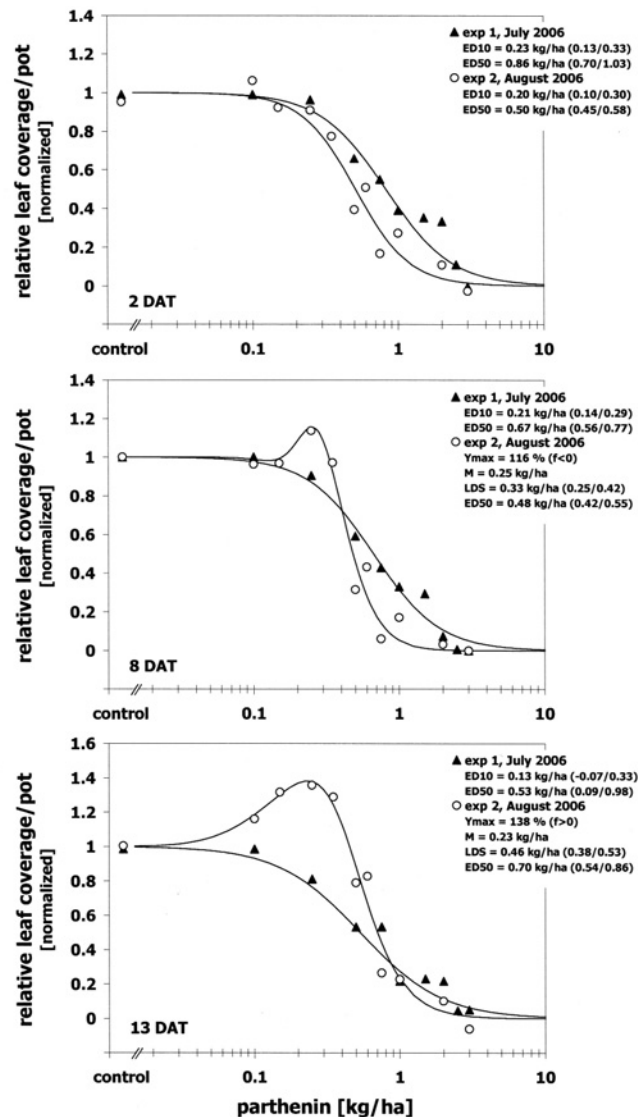


Figure 5. Effect of a spray application of parthenin on leaf area of *Sinapis arvensis* cultivated for two (2 DAT), eight (8 DAT), and 13 days after treatment (13 DAT) under semi-field conditions in July 2006 (exp. 1) and August 2006 (exp. 2) at Stuttgart-Hohenheim, Germany. Responses are normalized to controls. Y_{max} = maximal response; f = theoretical upper bound of the hormetic effect; M = dose giving maximum stimulation; LDS = limited dose for stimulation or ED_1 (dose causing 1 % inhibition); ED_K = dose causing K % inhibition; 95 % confidence interval for estimates in parentheses.

As the experimental protocol was equal in both experiments, it may be speculated that climatic conditions during the test period accounted for the observed disparity of results. With 29 days over 25 °C in exp. 1, it was much warmer than in exp. 2 where temperatures over 25 °C were only reached during two days (Table 2). Conditions in exp. 1 were further on characterized by a 2.5 fold longer photoperiod and a 1.7 fold higher

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sum of global radiation. Higher temperatures along with a more intense radiation in exp. 1 may have been responsible for the higher efficacy of parthenin at 13 DAT, a phenomenon that is especially known for temperature from studies with herbicides (*e.g.* Madafiglio *et al.* 2000; Fauser and Renner 2001). The higher efficacy may have shifted the stimulatory dose zone towards lower doses. Such a shift has been observed previously owing to experimental conditions (Calabrese and Baldwin 2002a,b), but it would not have been captured by the dose range tested.

Another hypothesis related to observed disparity of results is based on the fact that overcompensation hormesis requires resource allocation for adaptive responses. However, plants allocate their resources to maintain homeostasis (Calabrese and Baldwin 2002a,b; Cedergreen *et al.* 2007) and, thus, under stressful conditions as in exp. 1, resources for vegetative growth may have been limited due to trade-offs within the plant for other physiological stress responses. This hypothesis would be strengthened by the fact that absolute values of measured parameters were lower in exp. 1, *e.g.* controls in exp. 1 achieved merely 72 % of the relative leaf area of controls in exp. 2. However, studies investigating the influence of environmental conditions on the expression and temporal features of hormesis in plants are yet absent.

Failure to reproduce bioactivity features under contrasting experimental conditions shows the importance of controlled conditions to reproduce hormesis results and the need to investigate the impact of environmental conditions on the dose/time expression of hormesis in plants.

Practical and ecological implications of parthenin bioactivity

Practical aspects. Although bioactivity of parthenin considerably varied depending on growing conditions, this study demonstrates the potential of exploiting hormetic effects for desired agronomic effects if conventionally applied. Applications of hormetic compounds may produce beneficial effects on quantitative and qualitative plant traits, *e.g.* glyphosate-mediated increase in sucrose content in sugarcane (McDonald *et al.* 2001), or plant fitness, *e.g.* elicitation of defenses against pathogens (Nelson *et al.* 2002). Although several of these applications have been proposed in the past, beneficial hormetic effects have not been used to any large extent (Duke *et al.* 2006). The reasons for this may be complex, but include variability of hormetic responses. At high doses, plants are mostly killed, leaving no great variability in responses. At stimulatory doses, the system is more variable, as here resources are allocated as needed to reestablish or maintain homeostasis (Calabrese and Baldwin 2002a,b). Furthermore, a desirable hormetic change in a certain plant trait may not necessarily conserve homeostasis under all growing conditions and, thus, may not always serve as a sink for resource allocation.

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Unpredictable long-term effects may place further constraints, as over time a hormetic change in one trait can be at the expense of another or at the expense of its own (Duke *et al.* 2006).

Ecological aspects. Considering ecological implications, this study may allow some insight into the significance of leaching of parthenin from intact leaves of *P. hysterophorus* by rain, mist or dew. One mode of biochemical interaction that is relevant in this context is dropping of leachates on leaves of target plants growing under the canopy of *P. hysterophorus*.

Despite the fact that parthenin could be identified in leaf washings (Kanchan and Jayachandra 1980a), reports on parthenin concentrations in leaf leachates that would allow for a comparison with observed effective doses are lacking. Any conclusion on the ecological impacts of parthenin leaching is thus speculative. Nevertheless, if the effective doses observed at the ends of both experiments are taken into account, an average dropping of more than 0.31 ± 0.26 kg/ha (ED_{10}) would be necessary to inhibit the leaf area growth of *S. arvensis* and 1.81 ± 0.56 kg/ha (ED_{90}) would be necessary to cause lethal effects. Based on literature reports, leaf mass of a dense stand of *P. hysterophorus* may theoretically bound 7-17 g parthenin/m² (Rodriguez *et al.* 1976, Kanchan and Jayachandra 1980b, Reinhardt *et al.* 2006, Belz *et al.* 2007a). Thus, if more than 0.1-0.7 % of the parthenin stored in leaves at dense stands of *P. hysterophorus* may be released at once, adverse effects ($> ED_{10}$) on sensitive plant species by dropping might be possible. Lethal effects ($> ED_{90}$) would require up to 3.2 %. Aqueous extraction to simulate the natural release of parthenin during decay, removed approximately 10 % of the total amount present in the leaves within 24 h (Reinhardt *et al.* 2004). A release of up to one third of this amount by rain, mist or dew seems remote and, thus, a direct lethal effect by leaching may be doubtful. Whether up to one tenth can be removed and, thus, an inhibitory amount needs to be evaluated. Lower rates of release or sparse stands of *P. hysterophorus* may in contrast lead to stimulatory effects.

Several studies proved parthenin to elicit hormesis and, thus, it is possible that hormetic effects may occur in a natural setting if doses released are low. Moreover, the fate of allelochemicals in the environment may lead to stimulatory dose levels even if inhibitory doses are initially released (An *et al.* 2002, 2003). Therefore, hormesis should be regarded as a potential low dose component of plant/plant interference and in particular in case of parthenin-mediated interactions by *P. hysterophorus*.

CONCLUSIONS

The example of parthenin demonstrated how significant hormesis can be for bioactivity features of phytotoxins. Furthermore, the observed temporal features and sensitivity of parthenin hormesis to climatic conditions showed that this phenomenon is not just 'trivial' low dose stimu-

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lation. Despite a supposed high relevance of hormesis for natural phytotoxins and allelopathic interactions, stimulatory effects of phytotoxins at low doses are rarely considered. Lack of consideration of hormesis hampers the assessment of its ecological significance and consequences in a natural environment as well as its exploitation for agricultural use. Considerable research will be needed in order to understand the ecological conditions necessary for hormesis and potential impacts, benefits, or risks of low dose stimulation by phytotoxins. The findings should be beneficial to allelopathy and agriculture.

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