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PHYTOTOXIC ANTIBIOTIC SULFADIMETHOXINE ELICITS A COMPLEX HORMETIC RESPONSE IN THE WEED *LYTHRUM SALICARIA* L.

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□ In order to evaluate the hormetic response of the weed *Lythrum salicaria* to drug exposure we investigated the effects of the antibiotic Sulfadimethoxine by growing *Lythrum* plants for 28 days on culture media containing different drug concentrations (between 0.005 and 50 mg.L⁻¹). The antibiotic was absorbed by plants and can be found in plant tissue. The plant response was organ-dependent: roots, cotyledons and cotyledon petioles, were always affected by a toxic effect, whilst internodes and leaves length, showed a variable dose-depending response, with an increased growth at the lower drug concentrations and toxic effects at the higher ones. This variable response was probably dependant on different levels of local contamination resulting from a balance between accumulation rate and drug dilution in the increasing plant biomass. As a consequence, drug toxicity or hormetic response varied according to concentration and were different in each of the examined plant organ/tissue. Thus, even if hormesis can be considered a general plant response, each plant organ/tissue responds differently, depending on the local drug concentration and exposure time.

Keywords: Hormesis, *Lythrum salicaria*, Sulfadimethoxine, Antibiotic, Phytotoxicity, Weed

INTRODUCTION

Hormesis is an adaptive response characterized by a biphasic dose-dependent response, which has been found in different organisms/biological systems as a consequence of the exposure to a wide range of stimuli (Stebbing, 1998; Calabrese and Baldwin, 2001, 2002; Calabrese, 2008; Chapman, 2001). Hormetic response generally implies some stimulation at low stimulus doses (typically a 30-60% variation as compared to the

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control) and an inhibition at higher doses. The hormetic curve can be either U- or inverted U-shaped depending on the endpoint. A U-shaped curve is found when a detrimental effect in an organism is measured, *i.e.* an increase in death rate, an alteration of a physiological function or the incidence of a disease (cancer, heat strokes, etc.). A U-inverted curve is found when physiological functions are measured, as growth or survival rate (Calabrese and Baldwin, 2002).

Several authors have stated that hormesis, which can occur in all living organisms including plants (Calabrese & Blain, 2009), can be due to changes of physiological state or to the alteration of regulatory mechanisms induced by external agents (Stebbing, 1998). The first report on the existence of an hormetic response in plants concerned the exposure of wheat to different compounds (Jensen, 1907). Later, Wagner *et al.* (2003) showed an hormetic growth in corn seedlings treated with glyphosate. Velini *et al.* (2008) demonstrated that glyphosate, at sub-toxic doses, elicits hormesis in several plant species as maize, soybean, *Commelina benghalensis*, *Eucalyptus* and *Pinus caribea*. More recently, however, Cedergreen *et al.* (2009) have established that the ability to increase plant vegetative growth, at low doses, is not common to all the chemicals. A detailed assessment of the occurrence of hormesis in plants has been published by Calabrese and Blain (2009) who demonstrated that hormesis has been broadly observed and is highly generalized with respect to plant species, endpoints measured and chemical classes/physical stressors.

Antibiotics (mainly sulfonamides, tetracyclines, quinolones and macrolides) are widely used in intensive farming to prevent the outbreak of diseases. As a general rule, drugs administered by oral route are slowly absorbed and excreted with faeces. Thus, animal waste from intensive farming, often used for soil fertilization, still contains antibiotics and other drugs in active forms. As a consequence of this fertilization practice, drugs may contaminate both aquatic and terrestrial ecosystems (Halling-Sørensen *et al.*, 1998; Jørgensen and Halling-Sørensen, 2000; Jjemba, 2002; Kay *et al.*, 2005; Brambilla *et al.*, 2007; De Liguoro *et al.*, 2007). The amount of drug in animal faeces, coupled with the regular application of animal wastes to soil, and the rates of drug degradation and dilution in the ploughed soils, typically results in low drug-contamination levels in terrestrial ecosystems (De Liguoro *et al.*, 2003; Brambilla *et al.*, 2007). However, plants can absorb and accumulate these antibiotic contaminants resulting in phytotoxic effects (Jjemba, 2002; Brain *et al.*, 2005; Migliore *et al.*, 1995, 1996, 1997, 2000). Low concentrations of phytotoxic compounds (as antibiotics) in soil may elicit an hormetic response in plants (Migliore *et al.*, 2007). A recent study of the effects of animal wastes disposal containing low doses of tetracyclines (at concentrations ranging from 62.5 to 500 ng.g⁻¹ soil, dry weight) on the crop plant *Zea mays* has been performed in the field and in pot tests. Such a study

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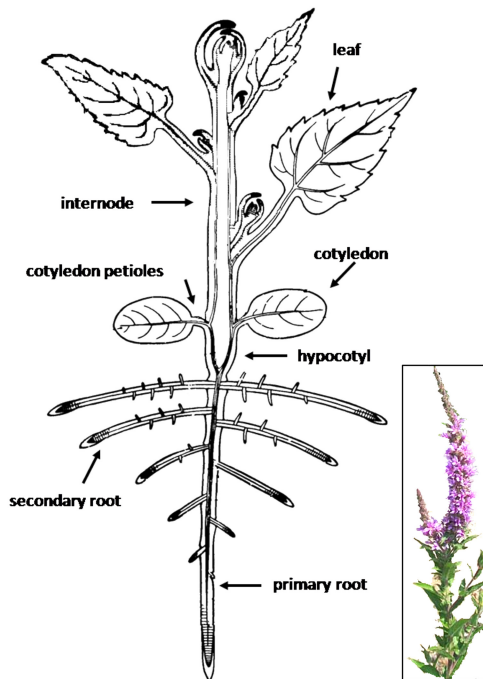


FIGURE 1. *Lythrum salicaria* L.

revealed an hormetic response mainly in terms of increased plant height and number of produced cobs (Migliore *et al.*, 2010). To disentangle the actual hormetic effect due to chemicals from other potential biotic/abiotic interactions with the fertilized soil, the cultivation of plants in a soil-free model is necessary. With this aim, we evaluated the effect of different concentrations of Sulfadimethoxine on the weed *Lythrum salicaria* L. (Figure 1), commonly present in cultivated fields, to detect the plant response in terms of both growth entity and drug uptake in axenic cultures and controlled conditions. The toxic effect of Sulfadimethoxine has already been demonstrated in laboratory models on terrestrial crop plants (Migliore *et al.*, 1995, 1996), weeds (Migliore *et al.*, 1997), and aquatic plants (Migliore *et al.*, 1998; Forni *et al.*, 2002). In the present study, we investigated the low dose interval of the hormetic response, by determining, in a 28 day long test, the growth of the target plants (roots, hypocotyls, cotyledons, cotyledons petioles, internodes and leaves elongation) and the drug content.

MATERIAL AND METHODS

Lythrum salicaria L. (Lythraceae), common name purple loosestrife, is an erect perennial herb or small shrub, 50 - 150 cm tall, with raceme

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inflorescences (Figure 1). Native of Eurasia, the species is spread in all temperate regions (mainly North-America) due to its wide tolerance of cultural conditions (for example, *L. salicaria* L. is highly tolerant to salinity) and elevated seed production. As consequence of its invasive nature, this plant occurs as weed in cultivated soils and may represent a useful bioindicator of their chemical contamination (Migliore *et al.*, 2000).

Lythrum seeds were obtained from the wild, in the Varriconi area, at the estuary of the Volturno River (near Naples, Italy). Seeds, deprived of fruit involucre, were sterilized and transferred to Petri dishes for germination at 25 °C under dark conditions. After germination (98%), seedlings (five for each jar) were transferred into transparent plastic jars (8 cm diameter; 15 cm height. PBI, Milan; Italy) containing sterile agarized Murashige and Skoog culture medium (Sigma, Milan, Italy), with or without Sulfadimethoxine (Sigma, Milan, Italy) (Migliore *et al.*, 1995, 1996, 1997, 1998). The drug was added to the medium at nominal concentration of 0.005 – 0.05 – 0.5 – 5 – 50 mg.L⁻¹. All procedures were performed in a biohazard cabinet and plants were maintained into sealed jars in sterile conditions throughout the experiment. Both control and Sulfadimethoxine treated jars were transferred into a growth room at 25 °C under continuous light (light intensity: 50 mE.m⁻²s⁻¹). After 28 days all control plants were completely developed and plant size was compatible with the jar dimension. At the end of the experiment a) the length of primary root, hypocotyls, cotyledons, cotyledon petioles, 1st – 6th internodes, b) the number of secondary roots and leaves and c) the length of leaves were measured (see Figure 1). Plants were weighed and frozen at -20 °C until chemical analysis. A total of 248 plants were tested; 40 plants for each treatment group, except the treatment group of 0.5 mg.L⁻¹ which had 48 plants. Biometric data were analyzed by using one-way ANOVA and, in one instance, using Student-*t* test.

Chemical analysis

Pooled plants from each treatment group were dried at 50 °C for 24 h and then weighted to obtain dry weight. Sulfadimethoxine in plants was extracted according to the method of Forni *et al.* (2000). The above reported procedure leads to recoveries that span from 86.1 to 88.7 % in the plant.

LC-ESI MS/MS analyses were performed on a 1100 series system (Agilent Technologies, Italy), coupled to an Agilent Technologies MSD SL ion trap mass spectrometer equipped with an electrospray interface. The HPLC column was a Symmetry C18 reversed-phase (150 mm x 3.0 mm, 5 µm), connected to a Sentry Guard Column Symmetry C18 5 µm (3.9 x 20 mm) (Waters, Milford, MA).

The mobile phase was constituted by solvent A [Water containing glacial acetic acid 1 % (v/v)] and solvent B (Methanol). The gradient program was:

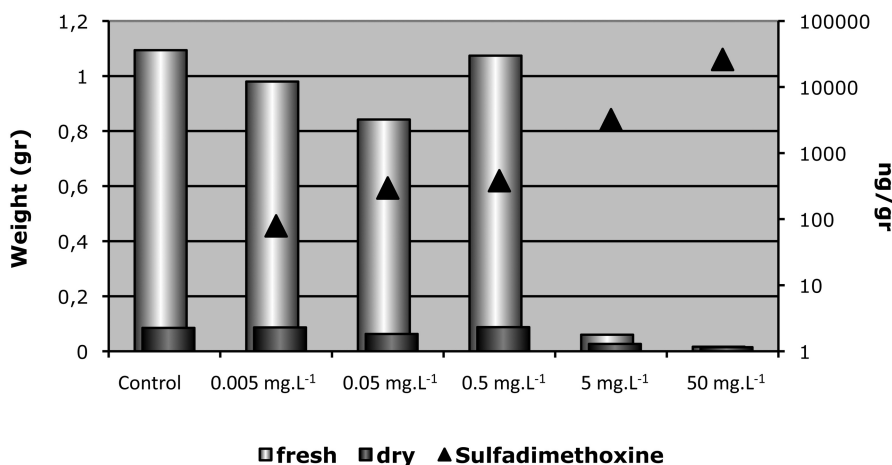
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FIGURE 2. Final fresh (light bars) and dry (dark bars) weight of control and Sulfadimethoxine treated *L. salicaria* pooled plants and drug uptake (black triangles) (ng.gr⁻¹) per plant dry weight.

from 5 % to 100 % B in 15 minutes. The flow rate was 0.350 mL.min⁻¹ and 10 µL of the extract was injected onto the column. Sulfadimethoxine was detected using electrospray ionization in the positive mode.

Nitrogen was used as dry gas at flow rate of 8 L.min⁻¹. The nebulizer pressure was set at 30 psi. The desolvation temperature was 350 °C. Acquisition parameters were optimised by direct infusion of 10 µg.mL⁻¹ standard solution in the mass spectrometer at a flow rate of 600 µL.h⁻¹. In a positive ionisation mode, protonated molecule 311 m.z⁻¹ (M+1)⁺ was obtained. The fragmentation of this precursor ion, with an energy collision of 1.2 eV, gave the following product ions: 156, 245, 218, 108 m.z⁻¹.

RESULTS

Final weight and drug uptake

L. salicaria plants, cultivated in axenic and controlled conditions, absorbed Sulfadimethoxine from the culture medium. Figure 2 shows Sulfadimethoxine uptake and final plant weight (fresh and dry) in the different experimental treatments after 28 days of drug exposure. Wet weight of the 0.5 mg.L⁻¹ treatment group was comparable to control, while wet weights in all the other treatment groups were lower. Dry weight of the 0.005 – 0.05 – 0.5 mg.L⁻¹ treatment groups were comparable to control while the other treatment groups (5 – 50 mg.L⁻¹) showed dramatically reduced dry weight. The ratio of dry to wet weight, *i.e.* water content, in control was 7.76% and in 0.005 – 0.05 – 0.5 mg.L⁻¹ treatment groups was 8.84, 7.14 and 8.16 %, respectively. This ratio was much higher in the 5 – 50 mg.L⁻¹ treatment groups (44.35 and 89.69% respectively), because of a dramatic reduction in plant water content. After 28 days, drug con-

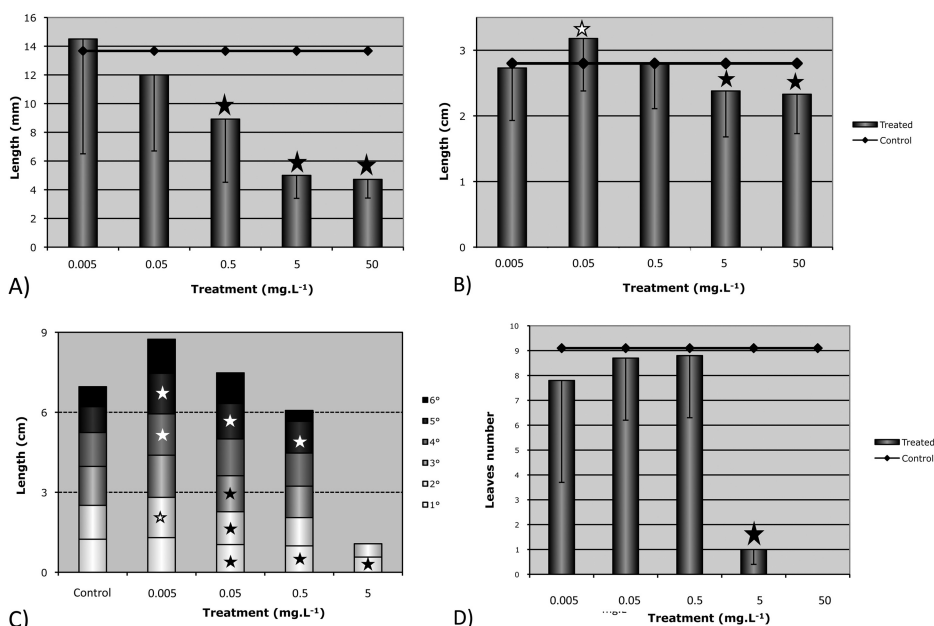
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FIGURE 3. Effect of Sulfadimethoxine on different plant features, reported as mean values: A) length of primary roots; B) length of hypocotyls; C) length of internodes and D) total number of leaves. Significant differences with control (ANOVA; $p < 0.05$) are indicated with stars: white for significant increase and black for significant decrease.

tents were 79 - 296 - 382 - 3,205 and 26,140 ng.g⁻¹ dry weight (ppm) in the 0.005 – 0.05 – 0.5 – 5 – 50 mg.L⁻¹ treatment groups, respectively.

Growth test

Figure 3A shows the mean length of primary root growth of *L. salicaria* over the test concentrations of Sulfadimethoxine (from 0.005 to 50 mg.L⁻¹) after 28 days. The drug concentration of 0.005 mg.L⁻¹ did not alter primary root length, while drug concentrations higher than 0.05 mg.L⁻¹ progressively and significantly (ANOVA, 0.5 mg.L⁻¹: $F = 29.41$, $p < 0.05$; 5 mg.L⁻¹: $F = 183.79$, $p < 0.001$; 50 mg.L⁻¹: $F = 208.01$, $p < 0.01$) reduced roots elongation from the control value (13.67 ± 3.7 mm). At all tested doses, Sulfadimethoxine progressively affected the mean number of secondary roots, which was reduced to 3.45 ± 1.6 in the 0.005 mg.L⁻¹ treatment group and to 3.71 ± 1.7 in the 0.5 mg.L⁻¹ treatment group (control group: 4.80 ± 1.8). Secondary roots were not developed in both 5 and 50 mg.L⁻¹ treatment groups. All differences among control and treatment groups were statistically significant (ANOVA, 0.005 mg.L⁻¹: $F = 8.21$, $p < 0.05$; 0.05 mg.L⁻¹: $F = 11.68$, $p < 0.05$; 0.5 mg.L⁻¹: $F = 8.40$, $p < 0.05$).

Figure 3B shows the effect of Sulfadimethoxine on the mean lengths of hypocotyls of *L. salicaria* over the test concentrations of

Sulfadimethoxine (from 0.005 to 50 mg.L⁻¹) after 28 days. A biphasic response (U-shaped dose/response effect) was found in hypocotyls: unaffected length in the 0.005 mg.L⁻¹ treatment group, a significant length increase in the 0.05 mg.L⁻¹ treatment group (ANOVA, $F = 5.75$; $p < 0.05$), unaffected length in the 0.5 mg.L⁻¹ treatment group and, finally, a significant length reduction in the 5 and 50 mg.L⁻¹ treatment groups (ANOVA, 5 mg.L⁻¹: $F = 9.46$, $p < 0.05$; 50 mg.L⁻¹: $F = 12.06$, $p < 0.05$) from the control value (2.8 ± 0.6 mm). In contrast, a phytotoxic effect was found on cotyledons and cotyledon petioles length at all drug tested concentrations. Cotyledon length was progressively reduced from the mean value 2.56 ± 0.6 mm in the 0.005 mg.L⁻¹ treatment group to the mean value 1.52 ± 0.4 mm in the 50 mg.L⁻¹ treatment group (control: 3.3 ± 0.7 mm); all differences among control and treatment groups were significant (ANOVA, 0.005 mg.L⁻¹: $F = 25.33$, $p < 0.05$; 0.05 mg.L⁻¹: $F = 23.25$, $p < 0.05$; 0.5 mg.L⁻¹: $F = 86.04$, $p < 0.05$; 5 mg.L⁻¹: $F = 188.12$, $p < 0.005$; 50 mg.L⁻¹: $F = 186.35$, $p < 0.01$). Similarly, cotyledon petioles length was reduced from the mean value of 0.83 ± 0.4 mm in the 0.005 mg.L⁻¹ treatment group to the mean value of 0.5 ± 0.2 mm the 5 mg.L⁻¹ treatment group (control: 0.97 ± 0.5 mm); differences with control, however, were not always significant (ANOVA, 0.05 mg.L⁻¹: $F = 35.09$, $p < 0.05$; 5 mg.L⁻¹: $F = 7.76$, $p < 0.05$). Cotyledon petioles were not developed at all in the 50 mg.L⁻¹ treatment group.

Figure 3C shows the effect of Sulfadimethoxine on the mean length of the 1st to 6th internodes (piled as in plants stem) of *L. salicaria* over the test concentrations of Sulfadimethoxine (from 0.005 to 50 mg.L⁻¹) after 28 days. A biphasic response (U-shaped dose/response effect) was found. Plants of the 0.005 mg.L⁻¹ treatment group were the tallest due to a generalized increased length of all the internodes; differences with control were significant for the 2nd, 4th and 5th internodes (ANOVA, $F = 4.44$, $p < 0.05$; $F = 7.85$, $p < 0.05$ and $F = 29.37$, $p < 0.05$, respectively). Plants of the 0.05 mg.L⁻¹ treatment group were also taller than control, due to the significant increased length of 5th internode, although significant reductions were found in the 1st, 2nd and 3rd internodes ($F = 9.84$, $p < 0.05$; $F = 4.81$, $p < 0.05$ and $F = 8.10$, $p < 0.05$, respectively). A slight reduction in plant growth was observed in the 0.5 mg.L⁻¹ treatment group; the length reduction was significant for the 1st internode ($F = 5.41$, $p < 0.05$), but a significant length increase was found in the 5th internode ($F = 10.69$, $p < 0.05$). In plants of the 5 mg.L⁻¹ treatment group only 1st and 2nd internodes were produced with the former being significantly reduced in length ($F = 16.50$, $p < 0.05$). In the 50 mg.L⁻¹ treatment group no internodes were produced.

The fraction of plants producing internodes (Table 1A) also showed a U-shaped biphasic response. In the 0.005 and 0.05 mg.L⁻¹ treatment groups, the percentage of plants producing from 1st to 5th internodes was lower than controls. This percentage was comparable or slightly lower than controls for the 0.5 mg.L⁻¹ treatment group, but clearly lower for the 5 and

*Hormesis in Lythrum salicaria exposed to sulfadimethoxine***TABLE 1.** Percentage of plants producing internodes (A.) and leaves (B).

A.

Internodes	1	2	3	4	5	6
<i>Control</i>	100	100	90	87.5	72.5	5.0
<i>0.005 mg.L⁻¹</i>	87.5	80	75	72.5	52.5	22.5
<i>0.05 mg.L⁻¹</i>	97.5	97.5	87.5	82.5	57.5	20
<i>0.5 mg.L⁻¹</i>	100	91.7	89.6	81.2	62.5	27.1
<i>5 mg.L⁻¹</i>	17.5	2.5	0	0	0	0
<i>50 mg.L⁻¹</i>	0	0	0	0	0	0

B.

Leaves	I	II	III	IV	V	VI
<i>Control</i>	100	97.5	92.5	90	70	5
<i>0.005 mg.L⁻¹</i>	87.5	80	75	72.5	52.5	22.5
<i>0.05 mg.L⁻¹</i>	100	97.5	87.5	82.5	52.5	17.5
<i>0.5 mg.L⁻¹</i>	100	97.9	91.7	83.3	62.5	27.1
<i>5 mg.L⁻¹</i>	40	10	0	0	0	0
<i>50 mg.L⁻¹</i>	0	0	0	0	0	0

50 mg.L⁻¹ treatment groups. However, an high percentage of plants in the 0.005 – 0.05 and 0.5 mg.L⁻¹ treatment groups produced the 6th internode.

Figure 3D shows the effect of Sulfadimethoxine on the mean number of leaves produced *per* plant over the test concentrations of Sulfadimethoxine (from 0.005 to 50 mg.L⁻¹) after 28 days. For leaves production a U-shaped biphasic response was found, as for internodes (the two datasets being slightly different because in some plants the internodes growth was not followed by a corresponding leaves production). In detail, in the 0.005 and 0.05 mg.L⁻¹ treatment groups, leaves number was slightly reduced; in the 0.5 mg.L⁻¹ treatment group leaves number was comparable to the controls, while in the 5 – 50 mg.L⁻¹ treatment groups, a dramatic reduction or absence of leaves was detected (Table 1B). A decreased number of leaves was found both at the highest (no leaves production) and at the lowest concentrations (reduced I-V leaves production). The VI leaf was produced by a higher number of treated plants (9 - 7 - 13 plants, respectively) than control (only 2 plants).

In the 0.005 mg.L⁻¹ treatment group the number of plants producing leaves was significantly reduced (as Student-*t* test comparison between control/expected and treated/observed batches) except for those producing the V leaf (*I leaf*: $t = 2.36$, $p < 0.05$; *II leaf*: $t = 2.54$, $p < 0.05$; *III leaf*: $t = 2.16$, $p < 0.05$; *IV leaf*: $t = 2.03$, $p < 0.05$; *V leaf*: $t = 1.61$; n.s.; *VI leaf*: $t = -2.32$, $p < 0.05$). In the 0.05 and 0.5 mg.L⁻¹ treatment groups the number of plants producing leaves was significantly increased only for those pro-

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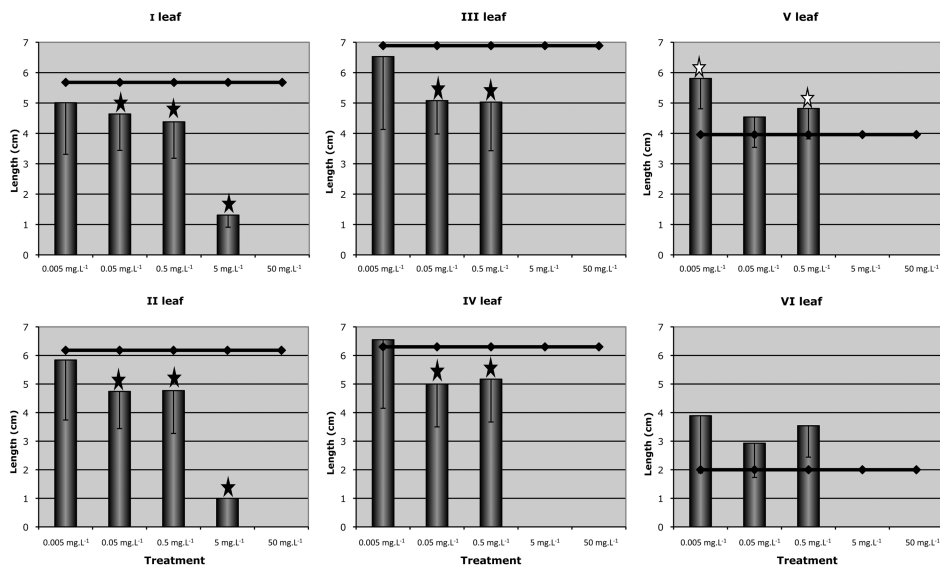


FIGURE 4. Effect of Sulfadimethoxine on the mean length of leaves. Significant difference with control are indicated with stars: white for significant increase and black for significant decrease.

ducing the VI leaf ($t = -1.78$, $p < 0.05$ and $t = -2.836$, $p < 0.05$, respectively), while in the 5 mg.L^{-1} treatment group the number of plants producing leaves was significantly reduced except for those producing the V leaf (*I leaf*: $t = 7.65$, $p < 0.05$; *II leaf*: $t = 16.16$, $p < 0.05$; *III leaf*: $t = 21.93$, $p < 0.05$; *IV leaf*: $t = 18.73$, $p < 0.05$; *V leaf*: $t = 9.54$; n.s.; *VI leaf*: $t = 1.43$, $p < 0.05$).

Figure 4 shows the effect of Sulfadimethoxine on the mean lengths of I to VI leaf produced *per* plant over the test concentrations of Sulfadimethoxine (from 0.005 to 50 mg.L^{-1}) after 28 days. A biphasic response from the first to the last produced leaf was found between the 0.005 and 0.5 mg.L^{-1} treatment group: I, II and III leaves have a reduced mean length; the IV leaf was slightly longer in the 0.005 mg.L^{-1} treatment group but significantly reduced in the $0.05 - 0.5 \text{ mg.L}^{-1}$ treatment groups. In contrast, V and VI leaves were always longer than in the controls; at the highest concentrations, in the $5 - 50 \text{ mg.L}^{-1}$ treatment groups, leaves were absent; in the 5 mg.L^{-1} treatment group only a few plants produced very small I and II leaves. Differences with control were significant in the following cases: I leaf (0.05 mg.L^{-1} : $F = 11.29$, $p < 0.05$; 0.5 mg.L^{-1} : $F = 20.13$, $p < 0.05$; 5 mg.L^{-1} : $F = 125.3$, $p < 0.001$); II leaf (0.05 mg.L^{-1} : $F = 13.69$, $p < 0.05$; 0.5 mg.L^{-1} : $F = 13.72$, $p < 0.05$; 5 mg.L^{-1} : $F = 25.30$, $p < 0.05$); III leaf (0.05 mg.L^{-1} : $F = 22.05$, $p < 0.05$; 0.5 mg.L^{-1} : $F = 21.55$, $p < 0.05$); IV leaf (0.05 mg.L^{-1} : $F = 9.56$, $p < 0.05$; 0.5 mg.L^{-1} : $F = 8.17$, $p < 0.05$); V leaf (0.005 mg.L^{-1} : $F = 8.55$, $p < 0.05$; 0.5 mg.L^{-1} : $F = 4.53$, $p < 0.05$).

DISCUSSION

Sulfadimethoxine, in a concentrations range from an high (50 mg.L⁻¹) to a low amount (0.005 mg.L⁻¹), altered post-germination development of *Lythrum salicaria* plants. Growth alterations showed biphasic responses, a *continuum* between the toxic effect and the hormetic response (*sensu* Stebbing (1998): increased growth). Toxic and hormetic alterations were contemporary found in different plant organs/tissues. Both types of alteration were related to the drug uptake in the plants. The dynamic of the plant response to drug exposure was complex and the final effect not clear-cut as it was due to the combination of increasing/decreasing growth effect at each developmental step: in each plant organ/tissue, the final amount of Sulfadimethoxine will depend on both the test concentration in the culture medium and on the time length of drug exposure (i.e. at the same concentration, VI leaf will be less exposed than primary root).

The highest Sulfadimethoxine test concentrations (i.e. in the 50 – 5 mg.L⁻¹ treatment groups) significantly depressed development of all plant organs. Roots, hypocotyls, cotyledons, cotyledon petioles, internodes and leaves length and number showed a general decrease, up to their absence in some plants. At these high test concentrations Sulfadimethoxine content in *Lythrum* plants has been found in the order of magnitude of µg.g⁻¹ dry weight, and these concentrations were responsible for the clear toxic effect. A toxic effect of Sulfadimethoxine has been already demonstrated in several crop plants and weeds under similar experimental conditions (Migliore *et al.*, 1995, 1996, 1997, 1998; Forni *et al.*, 2002) and it could depend on competition between the drug and the folic acid (Woods, 1962) which is involved in the synthesis of purines, thymidylate, panthonetate, and methionine (Neuburger *et al.*, 1996). The highest drug content induced plant death. This was confirmed by the low water content of exposed plants (i.e., an elevated ratio of dry/wet weight when compared to control plants).

The lower Sulfadimethoxine test concentrations (i.e. in the 0.5 – 0.05 – 0.005 mg.L⁻¹ treatment groups) caused a decrease, no effect, or an increase in organs size and/or number. At these test concentrations the drug content in plants was in the order of magnitude of ng.g⁻¹ dry weight (largely lower than the above cited higher test concentrations) and all treated plants appeared fully viable, as confirmed by their water content (dry/wet weight ratio) comparable to control plants.

At the lower Sulfadimethoxine test concentrations, drug content in plants increases with drug concentration in the culture medium in all treatment groups but at 0.5 and 0.05 mg.L⁻¹. In these latter two treatment groups, a comparable drug content (few hundred ng.gr⁻¹ dry weight) was found. This could indicate that the drug was not passively compartmentalised in vacuoles or other cell compartments (a passive compartmentalization would more likely produce a linear accumulation). Moreover,

in *Lythrum* plants, roots are probably the main site of drug accumulation, as it was already demonstrated for other plant species (Migliore *et al.*, 1995, 1996). This local accumulation likely explains the toxic effect always found in roots elongation (even secondary roots were dramatically affected).

A further issue complicates the observed complex response dynamic, *i.e.* plant size could affect the local drug content in the different plant organs/tissues. The 0.5 mg.L⁻¹ treatment group had higher weight than the 0.05 mg.L⁻¹ treatment group. As a consequence, the same amount of drug seems more ‘diluted’ in those plants with larger biomass and higher water content. Thus, the comparable drug content in plants grown in one order of magnitude different test concentration (0.5 *vs* 0.05 mg.L⁻¹) can either be cause or effect of hormesis.

Although at the end of the experiment the drug content in *Lythrum* plants increased with test concentration, we ignore the dynamics of the drug uptake depending on exposure time. Thus, we can not exclude possible consequences of different uptake times. For instance, the significant opposite growth response found in different internodes at the same experimental test concentration (Figure 3C) may depend on a different amount of drug reaching the different plant organs/tissues at different times.

Internodes length is due both to the number of stem cells derived from meristem apex cell division and to their final size, due to cell differentiation and expansion (Leyser and Day, 2002). Both cell division and differentiation/expansion can be differently affected by the local drug concentration and exposure time. For instance, the meristematic activity producing the first (and lower) internode experienced a different drug exposure if compared to the same activity producing the last (and upper) internode. These differences are related to plant biomass, both in terms of exposure time and drug concentration, and could positively or negatively affect either the number of cells produced by the plant apex for each internode and/or the cells ability to elongate by turgor-driven water uptake. The combination of different drug effects can thus explain the opposite responses (increased and decreased mean length) of consecutively produced internodes in plants exposed to the same experimental test concentration.

The hormetic response found in *Lythrum* plants exposed to different concentrations of Sulfadimethoxine can be interpreted as both a ‘running total’ of the summation of the opposite responses, decreased/increased growth, fitting the model described by Klonowski (2007) and reported in Figure 5 or as a modulated responses, as modelled for enzymatic activity (Malarczyk, 2008).

An hormetic effect of antibiotics has already been demonstrated in our previous studies on both *Lythrum* and crop plants growing at low doses of two Quinolone antibiotics (Migliore *et al.*, 2000, 2003) and on

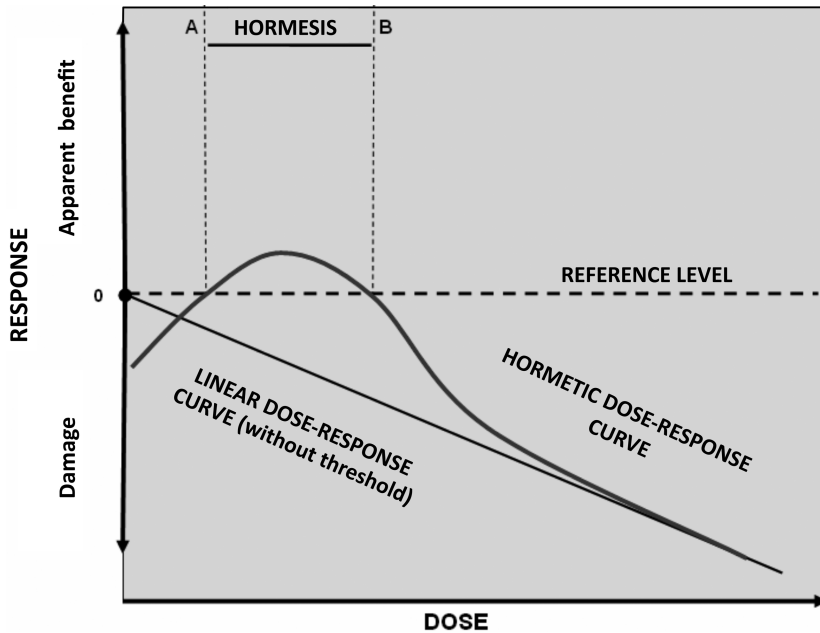
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FIGURE 5. Model of non-linear response in the hormetic process, according to Klonowsky (2007, modified).

corn plants exposed to Tetracyclines both in the lab and in the field (Migliore *et al.*, 2010). However, in the present study we highlight that the toxic threshold is different for each plant organ/tissue such as its response of apparent benefit. Thus, even if it is clear that hormesis represents a general response of the whole organism, we highlighted that each plant organ responds independently and differently, depending on local drug uptake and exposure time.

This work further supports previous statements that antibiotic-storage pattern is a common phenomenon in plants (Migliore *et al.*, 1995, 1996, 1997, 1998, 2000, 2003; Forni *et al.*, 2002; Cascone *et al.*, 2004). As a consequence, when drugs are (top) soil pollutants (Halling-Sørensen *et al.*, 1998; Kay *et al.*, 2005) they can actively/passively enter crops, being introduced by this way into the human/animal food web (Boxall *et al.*, 2006; Kumar *et al.*, 2005).

CONCLUSIONS

In conclusion, the exposure of *Lythrum salicaria* plants to Sulfadimethoxine generates an hormetic response in some traits, *i.e.* both toxic effect and increased growth, related to plant drug uptake. In this paper, a new possible toxicological outcome of sulphonamides environmental contamination at low dose has been demonstrated. Data from our experiments do not allow formulating any hypothesis about the mode of

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action of Sulfadimethoxine on plant growth at low environmental concentrations, but they indicate that attention on this hitherto little explored phenomenon must be paid. Last but not least, hormetic response in plants should be thoroughly investigated because the apparent beneficial increased growth effect can be consequence of toxicity that could otherwise affect the development, growth and biological cycle of a plant.

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