Effect of Lithium and Lanthanum on Herbicide Induced Hormesis in Hydroponically-Grown Cotton and Corn

William J. Allender, Geoff C. Cresswell, Jill Kaldor, and Ivan R. Kennedy

ABSTRACT

Pretreatment of hydroponically-grown cotton and corn with lithium (Li⁺) and lanthanum (La³⁺) chlorides, respectively, inhibited 2,4-dichlorophenoxyacetic acid (2,4-D) and metribuzin [4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one (MTZ)]-induced hormetic growth in both plant species. Lithium chloride proved to be a more effective inhibitor of hormetic growth. This may be due to the fact that the Li⁺ ion readily crosses cell membranes and interferes with calcium (Ca²⁺) metabolism. Lanthanum, on the other hand, has a relatively high affinity for specific sites in various types of Ca²⁺ channels and can displace Ca²⁺ from the cell wall, but cannot penetrate the cell membrane via the channel pore. Thus it acts as an inhibitor of Ca²⁺ channel activity and regulation. This work provides indirect evidence that a Ca²⁺ influx is involved in a hormetic (growth stimulation) response by cotton and corn plants when exposed to sublethal dosages of herbicide.
Reports of stimulation of plant growth from sublethal doses of potentially toxic agents have been described in the literature. Increases in growth and uptake of nutrients have occurred from applications of sublethal concentrations of 2,4-D (Wort, 1964; Miller et al., 1962; Wiedmann and Appleby, 1972; Pozuelo et al., 1989) and triazine herbicides (Pozuelo et al., 1989; Freney, 1965; Ries and Gast, 1965; Gramlich and Davis, 1967; Arvik et al., 1973). Southam and Ehrlich (1943) proposed the term “hormesis” to describe the stimulatory effect of sublethal concentrations of toxic substances on an organism. Since that time, sublethal doses of a number of pesticides have been shown to stimulate growth in a variety of plants (Wiedmann and Appleby, 1972; Pozuelo et al., 1989; Stebbing, 1982). Usually, the growth stimulation produced is small, highly variable, and biphasic in nature, ranging from 10 to 30% (Wiedmann and Appleby, 1972; Calabrese et al., 1987). The response appears to be transitory and difficult to reproduce in the field. This may be because the dosage rate is critical, depending upon plant species and sensitivity. Although the exact mechanism which produces the hormetic response is not known, it seems likely that a Ca$^{2+}$ influx in the cytosol, possibly due to increased plasma membrane (PM) permeability, is involved (Kennedy, 1971; Zocchi and Hanson, 1982; Low and Heinstein, 1986). Calcium channel blocking agents, such as La$^{3+}$, block the slow Ca$^{2+}$ channels of the cell membrane leading to decreased Ca$^{2+}$ influx (Das et al., 1988; Hurwitz, 1991; Lakshmiarayanaiah, 1991) and reduced biochemical activity. Lithium, on the other hand, interacts with peripheral Ca$^{2+}$-dependent processes by crossing cell membranes (Richelson, 1977; Reisberg and Gershon, 1979; Hinz and Fisher, 1975), and shares common physiochemical properties with Ca$^{2+}$ (Hinz and Fisher, 1975; Eisenmann, 1976; Poovaiah and Leopold, 1976). However, neither Li$^+$ nor La$^{3+}$ is perfectly selective for Ca$^{2+}$ channels, as high concentrations can depress sodium (Na$^+$) and potassium (K$^+$) channel currents in animal systems (Hille, 1992). In addition, Li$^+$ and La$^{3+}$ can produce some growth stimulation as well (Edwards, 1941; Asher et al., 1990; Harmet, 1979). Hence, it was necessary to establish the zero equivalence point (or ZEP) of each antagonist to improve selectivity. The ZEP is the inflection point where the treatment dose (or concentration) response is indistinguishable from the control (nil treatment). Doses smaller than the ZEP are stimulatory or biopositive, while larger doses are inhibitory or bionegative (Luckey, 1991). It is conceivable that Ca$^{2+}$ channel blocking agents, by decreasing the inward Ca$^{2+}$ flux may be capable of inhibiting herbicide induced hormesis.

The present work was aimed at testing this hypothesis, by producing hormetic growth with sublethal doses of two different herbicides: 2,4-D (an auxin-type herbicide) and MTZ (a photosynthetic inhibitor-type herbicide) in hydroponically-grown cotton (a C$_3$ plant) and corn (a C$_4$ plant), respectively, using La$^{3+}$ as a Ca$^{2+}$
blocker. To further elucidate a possible mechanism of action, the effect of Li$^+$ on the herbicide induced hormesis in hydroponically-grown cotton and corn was also investigated.

**MATERIALS AND METHODS**

**Plant Material**

Cotton and corn seedlings were grown using the nutrient film technique (NFT) to overcome the many complex factors which would be introduced with soil. Also, the hydroponic system permitted the controlled delivery of a known dosage of herbicide.

Three or four cotton (*Gossypium hirsutum* L. cv Siokra) and corn (*Zea mays* L. cv Terrific) seeds were sown into rockwool blocks contained in plastic pots clipped into the cover of the plastic hydroponic channels. When the seedlings were about 2 cm high, they were thinned to one per pot. Two parallel 2.5 m long channels each holding 12 plants were connected to a common 50 L reservoir. A commercial

![Graph](image-url)  
**FIGURE 1.** Effect of 2,4-D applied to roots of hydroponically-grown cotton on leaf areas and dry weight of tops. The error bars represent the standard errors of the means.
nutrient solution of the following composition (mg/L) was used in all experiments: NO$_3$-N (150); NH$_4^+$-N (40); PO$_4^{3-}$ (40); K$^+$ (260); S (46); Cl$^-$ (35); Ca$^{2+}$ (140); Mg$^{2+}$ (45); Mn (0.3); Cu (0.5); Zn (0.1), and Fe (2.2). This solution was regularly adjusted with additional nutrient salts to maintain an electrical conductivity of 2.0 dS/m$^{-1}$ and a pH of 6 (either phosphoric acid or lime was used to lower or raise the pH, respectively). Water was continually replenished to maintain a constant volume.

Hormetic Levels of 2,4-D and MTZ

A stock solution of 2,4-D (99.5%) (Sigma-Aldrich, Sydney) was prepared by dissolving 200 mg of the herbicide in 200 mL of acetone. A 10 mL aliquot was then diluted to 1 L with distilled water to provide a working 2,4-D concentration of 10 mg/L. This solution was then used to prepare six graded treatments: 0, 1, 2, 4, 8, and 16 μg/L of 2,4-D. Similarly, a stock solution of MTZ (99.8%) (Pestanal, Sydney) was prepared by dissolving 200 mg of the herbicide in 200 mL of acetone.

![Figure 2](image-url)

**FIGURE 2.** Effect of metribuzin applied to roots of hydroponically-grown corn on leaf areas and dry weight of tops. The error bars represent the standard error of the means.
and diluting to provide a working solution as above. This solution was then used to prepare six graded treatments: 0, 2, 4, 16, and 32 μg/L of MTZ. When the cotton and corn seedlings were established (first true leaf after germination) an aliquot of nutrient solution was taken out of each reservoir and replaced with the same volume containing the appropriate treatment dosages of 2,4-D and MTZ, respectively. A randomized block design of 3 replicates of the herbicide treatments was employed.

**Zero Equivalence Point of Calcium Antagonists**

Stock solutions (1.0 g/L) of Li⁺ and La³⁺ were prepared by dissolving LiCl and LaCl₃·7H₂O, respectively, in distilled water. Aliquots of these stock solutions were later added to nutrient solutions. The ZEP (zero equivalent point) concentrations of Li⁺ and La³⁺, were determined by growing plants in six treatments: 0, 5, 10, 20, 50, and 100 mg/L⁻¹.

![FIGURE 3. Effect of lanthanum (La³⁺) applied to roots of hydroponically-grown cotton on leaf areas and dry weight of tops. The error bars represent the standard error of the means.](image)

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*EFFECT OF LITHIUM AND LANTHANUM*
Effect of Lithium and Lanthanum on 2,4-D and MTZ-Induced Hormesis

Once the cotton plants were established (about a week after germination when the first true leaf had appeared) the following six treatments were carried out: control (nil treatment); Li$^+$ only; La$^{3+}$ only; La$^{3+}$ followed by 2,4-D treatment; Li$^+$ followed by 2,4-D treatment, and 2,4-D. Cotton plants were pre-incubated with the ZEP level of each Li$^+$ and La$^{3+}$ for 4 hours in separate trials. Plants were then dosed with an aliquot of 2,4-D solution to produce a nutrient solution containing 2 µg/L of 2,4-D, and grown for an additional three weeks.

The corn seedlings were treated in a similar way as above. Corn plants were also pre-incubated with the ZEP dosage of each antagonist for 4 hours. Plants were then dosed with an aliquot of MTZ solution to produce a nutrient solution containing 4 µg/L of MTZ, and grown for three more weeks. Eight plants from each plot were harvested after 4 weeks and leaf areas were determined using a leaf area meter (LAMBA Instruments Corp. LI-3000A).

FIGURE 4. Effect of lanthanum (La$^{3+}$) applied to roots of hydroponically-grown corn on leaf areas and dry weight of tops. The error bars represent the standard error of the means.
All plant samples were dried at 80°C for one week, weighed, and ground to pass through a 1 mm screen prior to analysis. Mineral analyses were carried out by inductively coupled plasma-emission spectrometry (ICP-AES) based on the 1991 Environment Protection Authority (EPA) Method 3050/ICP.

RESULTS AND DISCUSSION

Hormetic Levels of 2,4-D and MTZ

Cotton plants treated with 2 µg/L of 2,4-D exhibited a significant growth stimulation (p<0.01) compared with the controls with increases in leaf area and dry weights of plant tops of 28% and 36%, respectively, were recorded. The dose-response curve is shown in Figure 1.

Growth stimulation was also evident in corn plants after treatment with 4 µg/L of MTZ. A comparison of the treatments using ANOVA showed treatments significantly different (p<0.001). Significant quartic response (p<0.05) for leaf

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** Effect of lithium (Li⁺) applied to roots of hydroponically-grown cotton on leaf areas and dry weights of tops. The error bars represent the standard error of the means.
area initially increased as treatment level increased, then decreased as treatment level further increased. A similar response was observed for the dry weights, i.e., an increase in dry weight, followed by a decrease as treatment level increased (pr<0.05). Increases in leaf area and dry weights were observed in hormetically affected plants, averaging 40.9% and 46.6%, respectively. A dose-response curve for the corn treatments is shown in Figure 2. Both cotton and corn produced dose-response curves similar to a 'hormetic-curve' described by Stebbing (1982).

**Zero Equivalence Point of Calcium Antagonists**

The ZEP for La³⁺ occurred at a dosage rate of 20 mg/L for cotton and 64 mg/L for corn; and although much greater dosage rates than this reduced growth relative to the stimulated growth (Figures 3 and 4), no symptoms of toxicity were apparent even at the highest rates. Härmet (1979) found that rapid growth responses of oat coleoptile segments were produced when treated with 50 mM down to 20 μM concentrations of La³⁺ after an initial depression of growth (Asher et al., 1990).

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**FIGURE 6.** Effect of lithium (Li⁺) applied to roots of hydroponically-grown corn on leaf areas and dry weight of tops. The error bars represent the standard error of the means.
The ZEP for Li$^+$ was found to be at a dosage rate of 5 mg/L for cotton and 32 mg/L for corn (Figures 5 and 6). Dosage rates greater than 10 mg/L of Li$^+$ resulted in growth retardation and toxic effects. Chlorosis and necrotic spots were clearly evident at higher concentrations than 10 mg/L in the cotton plant leaves. Bingham (1966) produced Li$^+$ toxicity symptoms in avocado seedlings treated with 16 mg/L of Li$^+$ as a soil drench. The plants developed small, circular, necrotic spots with alternate light and dark-brown rings in the interveinal region of the leaf blade. This was followed by browning and curling of leaf margins (Bingham, 1966). Corn proved to be more resistant to Li$^+$ with only slight growth reduction at 16 mg/L, and showed a marked stimulation at a dosage level of 8 mg/L (Figure 6).

**Mineral Analyses**

The macronutrient content of the cotton plant tops was not significantly affected by the treatments. A moderate depression of 14% of phosphorus (P) content was observed in the 2,4-D stimulated cotton (0.72%) versus the control plants (0.84%). A similar effect was also reported with diuron-stimulated cotton (Van Rijn, 1972).

![Graph showing the effect of Li and La on 2,4-D induced hormesis in cotton on leaf area and dry weights of tops. The error bars represent the standard error of the means.](image-url)
Treatment effects were significant only for iron (Fe) \((p<0.01)\) with notable increases in Fe uptake occurring in \(\text{La}^{3+}\) treated plants \([106.3 \text{ ug/g}, \text{standard error (s.e.) } 12.7]\) compared with the control plants \([60.7 \text{ ug/g}, \text{s.e. } 7.2]\) with slightly improved uptake (average 110.3 \text{ ug/g}, s.e. 6.6) being observed in plants treated with 2,4-D, in addition to \(\text{La}^{3+}\).

Uptake of \(\text{Li}^+\) was increased in plants receiving hormetic levels of 2,4-D from 135.3 \text{ ug/g} (s.e. 37.2) to 245.7 \text{ ug/g} (s.e. 36.0) in \(\text{Li}^+\) plus 2,4-D treated plants - an increase of 82%. Smaller amounts of \(\text{La}^{3+}\) (3 \text{ ug/g}) were accumulated by \(\text{La}^{3+}\) plus 2,4-D treated plants. Uptake was not influenced significantly by hormetic levels of 2,4-D.

Overall, only small differences were observed in the macronutrient content of the plants. A moderate depression (16.3\%) of P content was also found in the MTZ- stimulated corn \([1130 \text{ ug/g}, \text{s.e. } 122]\) and \(\text{La}^{3+}\) treated plants \([1080 \text{ ug/g}, \text{s.e. } 107]\), versus the control plants \([1330 \text{ ug/g}, \text{s.e. } 98]\). Earlier work by Leonard et al. (1975) showed that \(\text{La}^{3+}\) inhibits \(\text{K}^+\) absorption in corn roots. In this work, however, no significant differences were found in \(\text{K}^+\) content of plant tops from

![Figure 8](image_url)

**FIGURE 8.** Effect of \(\text{Li}^+\) and \(\text{La}^{3+}\) on MTZ-induced hormesis in corn on leaf area and dry weights of tops. The error bars represent the standard error of the means.
EFFECT OF LITHIUM AND LANTHANUM

Each treatment. Calcium content was slightly depressed with the La$^{3+}$ treatment only (5810 µg/g, s.e. 575) compared with the controls 6920 µg/g, s.e. 521). Only small differences in the micronutrients were observed in the treatments.

Metribuzin at hormetic levels increased the concentration of Li$^+$ from an average of 152.3 µg/g (s.e. 14.8) to 180.7 µg/g (s.e. 6.7) for Li$^+$ plus MTZ treated corn plants - an increase of 18% compared with the control plants. A small amount of La$^{3+}$ (9 µg/g, s.e. 1.8) was also found in La$^{3+}$ plus MTZ treated plants. The La$^{3+}$ is considered to cross the membrane slowly, if at all (Thomson et al., 1973; Nagahashi and Thomson, 1974); and so its detection in the corn tops was unexpected. Research carried out on La$^{3+}$ treated corn roots showed that La$^{3+}$ is confined to the apoplast and does not appear to penetrate the PM (Leonard et al., 1975; Zee and O'Brien, 1971). A probable explanation for the uptake found in this study is the increased permeability of the cell membrane (Kennedy, 1971; Kennedy and Harvey, 1972) in the presence of hormetic levels of herbicide, thus permitting a small amount of La$^{3+}$ ion uptake into the plant tops. Thus, if some La$^{3+}$ reaches the cytoplasmic side of the membrane it could directly decrease Ca$^{2+}$ (Weiss, 1974), rather than indirectly at the casparian strip or membrane (Weiss, 1974; Kauss, 1987).

Effect of Calcium Antagonists on 2,4-D and MTZ-Induced Hormesis

Figure 7 shows leaf area and growth responses of cotton plants to treatment with Li$^+$, La$^{3+}$, and 2,4-D. Lithium and La$^{3+}$ inhibited 2,4-D induced hormetic growth of leaves and tops in cotton (p<0.01). A comparison of the corn treatments using ANOVA showed a highly significant variance ratio for leaf areas (p<0.001) at 95% confidence level. Figure 8 shows the effect of Li$^+$ and La$^{3+}$ on MTZ induced hormesis in corn plant leaf area and dry weight yields of the cotton plant tops. Lithium proved to be a more effective inhibitor of hormesis growth. Lithium is known to influence the movement of free Ca$^{2+}$ in the cytoplasm which is implicated in the control of a range of intracellular and membrane-related processes in plants (Kauss, 1987; Hepler and Wayne, 1985). Calcium acts as an intracellular messenger, conveying information about the nature of a particular stimulus or stress impinging on the cell to mobilize proteins that guide the cellular response such as growth (Rasmussen et al., 1990). Also Li$^+$ shares common physiochemical properties with Ca$^{2+}$ and readily crosses cell membranes (Hinz and Fisher, 1975; Eisenmann, 1976; Aldenhoff and Lux, 1985).

Lanthanum, on the other hand, has a relatively high affinity for specific sites in various types of Ca$^{2+}$ channels, and can displace Ca$^{2+}$ from the cell wall, but cannot penetrate the cell membrane via the channel pore. Thus it acts as an inhibitor of Ca$^{2+}$ channel activity and regulation (Das et al., 1988; Hurwitz, 1991; Aldenhoff and Lux, 1985).

Zocchi and Hanson (1982) showed that there was a 20% increase in $^{45}$Ca$^{2+}$ influx into corn roots as a result of sublethal (2°C) cold shock. Recent research...
has shown that Ca\(^{2+}\) influx is via ion carriers (or pumps) (Wilkinson et al., 1991) and ion channels (Reid and Smith, 1992; Wilkinson et al., 1992; Wilkinson and Duncan, 1992; Wilkinson, 1995). Many other experiments have shown that both Ca\(^{2+}\) across the PM and cellular responses to stimuli could be inhibited with Ca\(^{2+}\) antagonists (Das et al., 1988; Bush, 1993).

This work has shown indirectly that Ca\(^{2+}\) antagonists, Li\(^+\) and La\(^{3+}\), inhibit herbicide induced hormetic growth in cotton and corn by interfering with Ca\(^{2+}\) metabolism, probably by affecting Ca\(^{2+}\) influx across the PM. Further research is underway to determine the mechanisms involved.

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EFFECT OF LITHIUM AND LANTHANUM


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EFFECT OF LITHIUM AND LANTHANUM


