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Pot growing Media Amendment with Calcium Cyanamide and Weed Control Relationships

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this research was to characterize the effect of a pre-transplant calcium cyanamide fertilization on weed control in the potted bedding ornamental plant *Impatiens wallerana* and to describe the physiological mechanisms involved. The positive effect of a calcium cyanamide amendment included both ammonium toxicity on weed seed germination and a decrease of weed rates growth such as relative leaf expansion rate (RLAE) and relative growth rate (RGR). Data showed RGR-NAR (net assimilation rate), RLAE-RGR, RLAE-NAR and RGR-root dry weight relationships, which would explain ammonium toxicity to roots and weed growth responses. These effects probably could be explained by a change in hormonal root synthesis. Our results showed that a pre-transplant calcium cyanamide amendment combining with a transplant routine optimize both *I. wallerana* growth and weed control.

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1. INTRODUCTION

Peat moss is the most common organic substrate used in growing media for propagation and pot plants. The increasing demand of growing media for greenhouse horticultural uses and the scarcity and increasing cost of traditional substrates based on *Sphagnum* peat moss, have raised the interest in new substrates [1,2]. The amount of peat used in the mixes was reduced from 77% on average in the commercial standard mixes to 30% on average in the new mixes and go on [3]. Despite of the physical and chemical limits, one of the main difficulties arises in pot weed control [4] because pre- and post-transplant herbicides in ornamental plants result in phytoxycity [5] or stunting [6,7].

The most popular organic alternatives were coir products [8], which increased from 17 to 40%. Other alternatives used were various barks [9], rice hulls [10], wood fibers [11], composts [12, 13] and river waste ('temperate peat') [1]. Those alternatives were used in mixtures from 5 to 50%. River waste or 'temperate peat' is the result of the accumulation of plants residues under an anaerobic environment, which is dredged from river or lake banks. The sedimentary organic matter is derived from the delta plain vegetation and is highly dominated by phytoplasts (plant debris). The result is a fine-grained, black, oozy sediment deposited in the bottom of the coasts [14] with a high weed seed bank.

Recently, Sala et al. [15] showed that the use of calcium cyanamide to fertilize *Impatiens wallerana* plants in substitution of the traditional liquid fertilization system would increase crop productivity. Calcium cyanamide would be a better alternative than other coated products used as controlled-release fertilizer, especially under a global temperature increase or low environmental greenhouse facilities.

Calcium cyanamide has been one of the potential candidates as soil disinfectant since the restriction of methyl bromide in soil fumigation due to its ecological risk [16,17]. From its introduction in the 1950's, cyanamide was used as a pre-emergence herbicide [18,19]. On the other hand, cyanamide is multifunctional for agricultural purposes because it serves in the soil as an insecticide, fungicide, and herbicide for a time after application, then decomposes to urea in the soil, and finally is absorbed by crops as fertilizer. Calcium cyanamide showed as far as 80% annual weed control [20,21], but the physiological mechanism underlying this phenomenon is still not clearly understood.

The aim of this research was to characterize the effect of a pre-transplant calcium cyanamide fertilization on weed control in the potted bedding ornamental plant *Impatiens wallerana* and to describe the physiological mechanisms involved.

2. MATERIALS AND METHODS

2.1 Plant Material and Treatments

Three experiments were carried out in the Faculty of Agronomy campus, University of Buenos Aires, Argentina (34°35' 59''S, 58°22' 23''W) during December 2015 and repeated during December 2016. Two growth experiments were performed in an acclimatized greenhouse and a germination experiment was carried out at the campus laboratory.

For the first growth experiment (experiment 1), *Impatiens wallerana* 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were grown in 50-plastic plug trays (55.70 cm^3 $cell^{-1}$) in a Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) for 35 days. When seedlings reached the transplant stage, they were transplanted into 1,200 cm^3 pots filled with a *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium. At the beginning of the experiments total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 63.50, 17.06, 10.06 and 0.35 respectively. Weeds were manually removed.

Treatments included different calcium cyanamide (**CC**) concentrations (0, 1.0, 1.5 and 2.0 kg m^{-3}; equivalent to added 0, 1.2, 1.8 and 2.4 g N pot^{-1}) (Perlka®, AlzChem, Trostberg, Germany) added one week before transplanting and a fertiirrigated control. Additional phosphorus and potassium was added to calcium cyanamide treatments through overhead irrigation water to avoid deficiencies in these nutrients. A weekly fertiirrigated control of 1.0: 0.05: 1.0: 0.5 (v/v/v/v) N: P: K: Ca (nitric acid, phosphorus acid, potassium nitrate, and calcium nitrate; Agroquímica Larocca S.R.L., Buenos Aires, Argentina) through to the overhead irrigation water (150 mg L^{-1} N; equivalent to 2.2 g N pot^{-1}) according to Styer and Koranski [22] was included. This fertilizer combination and nitrogen concentration, neutralized at pH= 5.8, optimize *I. wallerana* growth.

A second growth experiment (experiment 2) was carried out to evaluate the same CC treatments tested in experiment 1 on weed appearance. Twenty 1,200 cm^3 pots filled with a *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium and the same calcium cyanamide concentrations than in experiment 1 were used.

Daily mean temperatures (21.01 to 25.85°C) and daily photosynthetic active radiation (5.63 to 7.01 mol photons m^{-2} day^{-1}) for the two experiments were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to HOBO H8 data logger. The plants were arranged at a density of 25 plants m^{-2}, which avoided mutual shading.

For the germination experiment (experiment 3) four replications of 100 weed seeds from the river waste medium component were uniformly distributed on a single sheet of filter paper adequately wetted with 40 ml of distilled water (and daily rewetted) in transparent polyethylene boxes and incubated in a germination chamber at 25°C.

2.2 Assessed Variables

Impatiens wallerana plants were harvested at the transplant stage and at 15, 30, 45 and 60 days after transplanting (experiments 1 and 2) while at the same time weeds were harvested and identified (experiment 2). Roots were washed and root, stem, leaf and flower fresh weights (FW) were recorded. Dry weights (DW) were obtained after drying roots, stems and leaves to constant weight at 80°C for 96 h. The number of leaves was recorded, and each leaf area was determined using the ImageJ® (Image Processing and Analysis in Java) software. The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm (ln) of whole plant DW versus time (in days). The rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the natural logarithm (ln) of total leaf area versus time (in days). The mean net assimilation rate (NAR) was calculated as follow:

$$NAR = \frac{k_w W_0 e^{k_w t}}{A_0 e^{k_a t}}$$

where W_0: extrapolated value of total DW (g) at time zero; k_w: RGR (g g^{-1} day^{-1}); A_0: extrapolated value of leaf area (cm^2) at time zero; k_a: RLAE (cm cm^{-2} day^{-1}); t: time (days) at the midpoint of the experimental period and e: base of the ln.

Weed germination rates were calculated according to the proposal made by Maguire [23].

2.3 Experimental Design and Statistical Analysis

The experimental designs was a completely randomized block for experiments 1 and 2, while a completely aleatory design for experiment 3 was used. Since there were no significant differences between the two yearly experiments, data were combined (n = 40). Data were subjected to three-way analysis of variance (ANOVA). STATISTICA 8 (StatSoft) software was used for statistical analysis and the assumptions of ANOVA were checked. Least significant differences (LSD) values were calculated as well. Means were separated by Tukey's tests ($P \leq 0.05$). Slopes from straight-line regressions of RGR and RLAE values were tested using the SMATR package [24].

3. RESULTS

3.1 *Impatiens wallerana* Biomass Accumulation

I. wallerana FW (90 days from transplanting) showed significant differences between the fertirrigated control plants (FC) and most CC-fertilized. The CC doses for higher response were 1.5 and 2.0 kg m^{-3} (Fig. 1). The total FW increase was achieved through increases mainly in shoots and in less proportion in leaves.

Both CC-1.5 and CC-2.0 treatments significantly increased *I. wallerana* RGR, RLAE and NAR related to the rest of CC plants or fertiirrigated control plants (Table 1).

3.2 Weed Appearance, Weed Germination and Weed Growth

A CC pre-transplant soil fertilization significantly decrease both dicotyledonous (Fig. 2a) and monocotyledonous (Fig. 2b) weeds per pot. The greater CC concentration the lower total weed appearance (Fig. 2c).

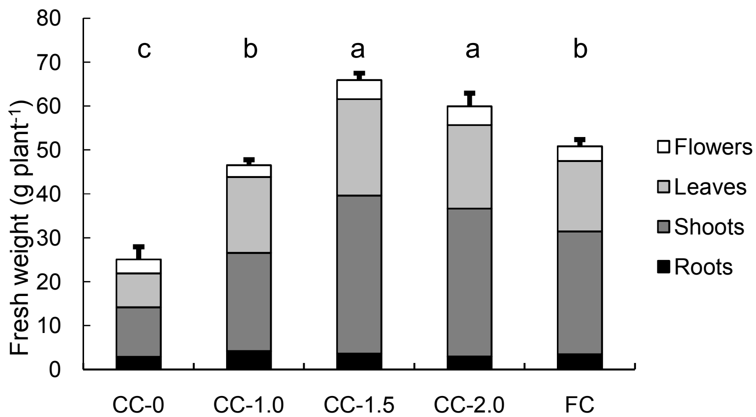

Fig. 1. Fresh weight at the end of the experiment 1 in different plant organs of *Impatiens wallerana* plants fertilized with four calcium cyanamide fertilizer concentrations (0, 1.0, 1.5 and 2.0 Kg m^{-3}) and a fertiirrigated control (FC) (n = 40). The standard errors over each bar have been indicated. Different lower-case letters indicate significant differences (P $\leq$ .05) between CC-fertilized plants

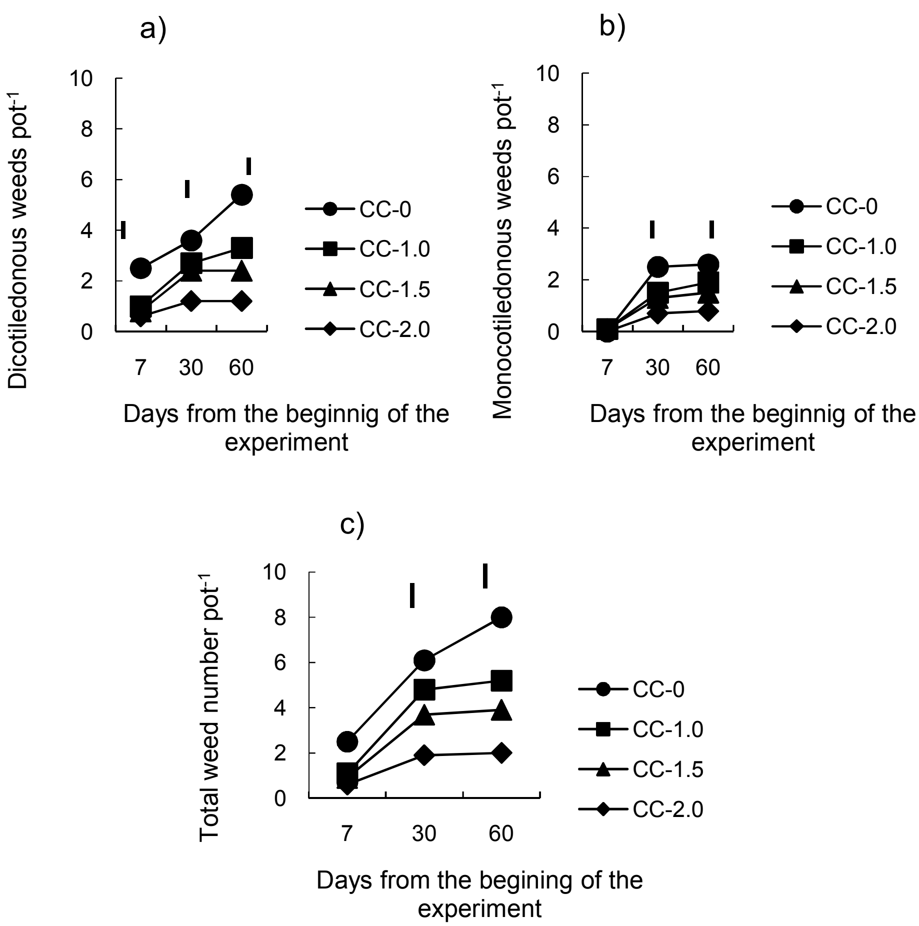

Fig. 2. Changes in dicotyledonous (a), monocotyledonous (b) and total (c) weed number in pots fertilized with four calcium cyanamide fertilizer concentrations (0, 1.0, 1.5 and 2.0 Kg m^{-3}) (n = 40). Vertical lines indicate least significant differences (LSD)

Table 1. Changes in the relative leaf area expansion (RLAE), the relative growth rate (RGR) and the net assimilation rate (NAR) estimated from *Impatiens wallerana* plants fertilized with four calcium cyanamide fertilizer concentrations (0, 1.0, 1.5 and 2.0 Kg m^{-3}) and a fertiirrigated control (FC) (n = 40) (Experiment 1). Different lower-case letters indicate significant differences (P $\leq$ .05) between CC-fertilized plants

Calcium cyanamide (kg m^{-3})	RLAE (cm cm^{-2} day^{-1})	RGR (g g^{-1} day^{-1})	NAR (g cm^{-2} day^{-1}) x 10^{-5}
0	0.047^{b}	0.083^{b}	41.03^{b}
1.0	0.068^{a}	0.099^{ab}	47.96^{a}
1.5	0.072^{a}	0.103^{a}	46.15^{a}
2.0	0.086^{a}	0.104^{a}	47.48^{a}
Fertiirrigated control	0.065^{a}	0.095^{ab}	46.04^{a}

In control pots, RLAE, RGR and NAR values were high and different between weed species, but the higher the CC dose the lower RLAE, RGR and NAR. Higher CC doses (1.5 and 2.0 Kg m^{-3}) were significantly phytotoxic for the most weeds present in the growing media (Table 2).

Weed germination percentages were significantly decreased from near 70% in control plots to 30% with the low CC doses (0.5 kg m^{3}). Higher CC doses (1.5 and 2.0 kg m^{-3}) decrease seed germination until 20% and 4.5% respectively (Fig. 3). When the germination rates were performed, the differences for the higher and the lower CC concentration related to control plots were between 26% and 1.9% respectively.

When plotting the data from all weeds and treatments, we found a direct relationship between RLAE versus RGR (Fig. 4a) and RGR versus root dry weight (Fig. 4d) (r^2=0.781 and 0.703 respectively). On the other hand, a logarithmic relationship between both RGR (Fig. 4b) and RLAE (Fig. 4c) versus NAR (r^2 = 0.834 and 0.733 respectively).

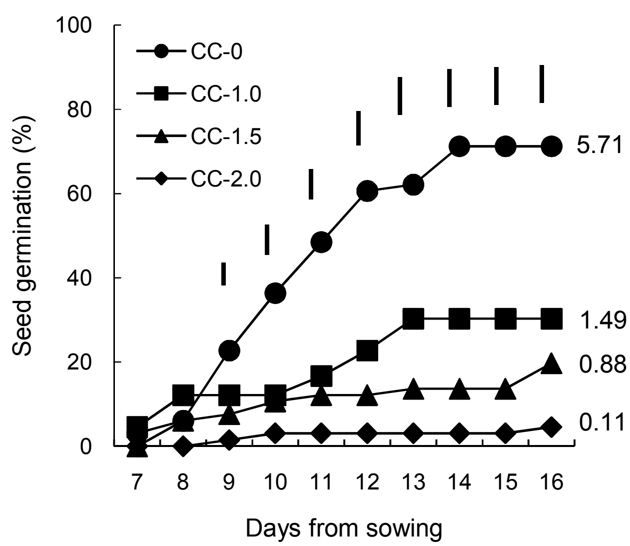

Fig. 3. Effect of four calcium cyanamide fertilizer concentrations (0, 1.0, 1.5 and 2.0 Kg m^{-3}) (n = 40) on weed seed germination. Vertical line indicate least significant differences (LSD). The germination rate values were indicated as well

Table 2. Effect of four calcium cyanamide fertilizer concentrations (0, 1.0, 1.5 and 2.0 Kg m^{-3}) on the rate of leaf area expansion (RLAE), the relative growth rate (RGR) and the net assimilation rate (NAR) of the weeds in the growing media used for the experiments. Different lower-case letters indicate significant differences (P $\leq$ .05) between different plant species from control pots while different capital letters indicate significant differences (P $\leq$ .05) between CC doses. The probability of the slope being zero was P < .001 for RLAE and RGR

	RLAE (cm^2 cm^{-2} day^{-1})				RGR (g g^{-1} day^{-1})				NAR (g cm^{-2} day^{-1})			
	Calcium cyanamide (Kg m^{-3})				Calcium cyanamide (Kg m^{-3})				Calcium cyanamide (Kg m^{-3})			
	0	**1.0**	**1.5**	**2.0**	**0**	**1.0**	**1.5**	**2.0**	**0**	**1.0**	**1.5**	**2.0**
Althaea officinalis	0.287^{aA}	0.271^{A}	0.153^{B}	0.145^{B}	0.482^{aA}	0.434^{B}	0.359^{C}	0.321^{C}	10.31^{aA}	$6,10^{B}$	3.58^{C}	1.42^{D}
Amaranthus palmeri	0.086^{cA}	0.073^{A}	0.027^{B}		0.149^{dA}	0.085^{B}	0.038^{C}		0.07486^{dA}	0.00521^{B}	0.00195^{C}	
Amaranthus quitensis	0.100^{cA}	0.085^{A}			0.287^{cA}	0.144^{B}			0.03737^{eA}	0.00185^{B}		
Cardus acanthoides	0.283^{aA}	0.260^{A}			0.426^{aA}	0.340^{B}			0.01482^{eA}	0.00871^{B}		
Conyza bonariensis	0.153^{b}				0.444^{a}				0.50129^{c}			
Cyperus odoratus	0.174^{bA}	0.157^{A}	0.153^{A}	0.067^{B}	0.394^{bA}	0.258^{B}	0.183^{C}	0.102^{D}	0.61666^{cA}	0.16311^{B}	0.02771^{C}	0.00644^{D}
Eupatorium hecatanthum	0.175^{bA}	0.071^{B}	0.047^{C}		0.266^{cA}	0.083^{B}	0.078^{B}		0.02902^{eA}	0.00961^{B}	0.00211^{C}	
Fumaria capreolata	0.100^{c}				0.183^{d}				0.0745^{1e}			
Ipomoea sp.	0.074^{cA}	0.071^{A}			0.231^{cA}	0.125^{B}			0.01475^{eA}	0.00336^{B}		
Polygonum punctatum	0.070^{cA}	0.064^{A}	0.054^{A}		0.228^{cA}	0.140^{B}	0.105^{C}		0.01031^{eA}	0.00462^{B}	0.00122^{C}	
Sorghum halepense	0.073^{cA}	0.058^{A}	0.049^{A}		0.242^{cA}	0.104^{B}	0.109^{B}		0.01526^{eA}	0.00775^{B}	0.00472^{C}	
Spaeralcea bonariensis	0.074^{cA}	0.068^{A}			0.141^{dA}	0.123^{B}			0.00512^{fA}	0.00483^{A}		
Stellaria media	0.271^{a}				0.480^{a}				12.73^{a}			
Taraxacum officinale	0.081^{cA}	0.067^{A}	0.061^{A}		0.198^{dA}	0.097^{B}	0.094^{B}		0.04344^{A}	0.00813^{B}	0.00754^{B}	
Wedelia glauca	0.278^{aA}	0.169^{B}	0.154^{B}	0.054^{C}	0.472^{aA}	0.371^{B}	0.121^{C}	0.120^{C}	$7.97b^{A}$	5.35^{B}	0.33487^{C}	0.05381^{D}

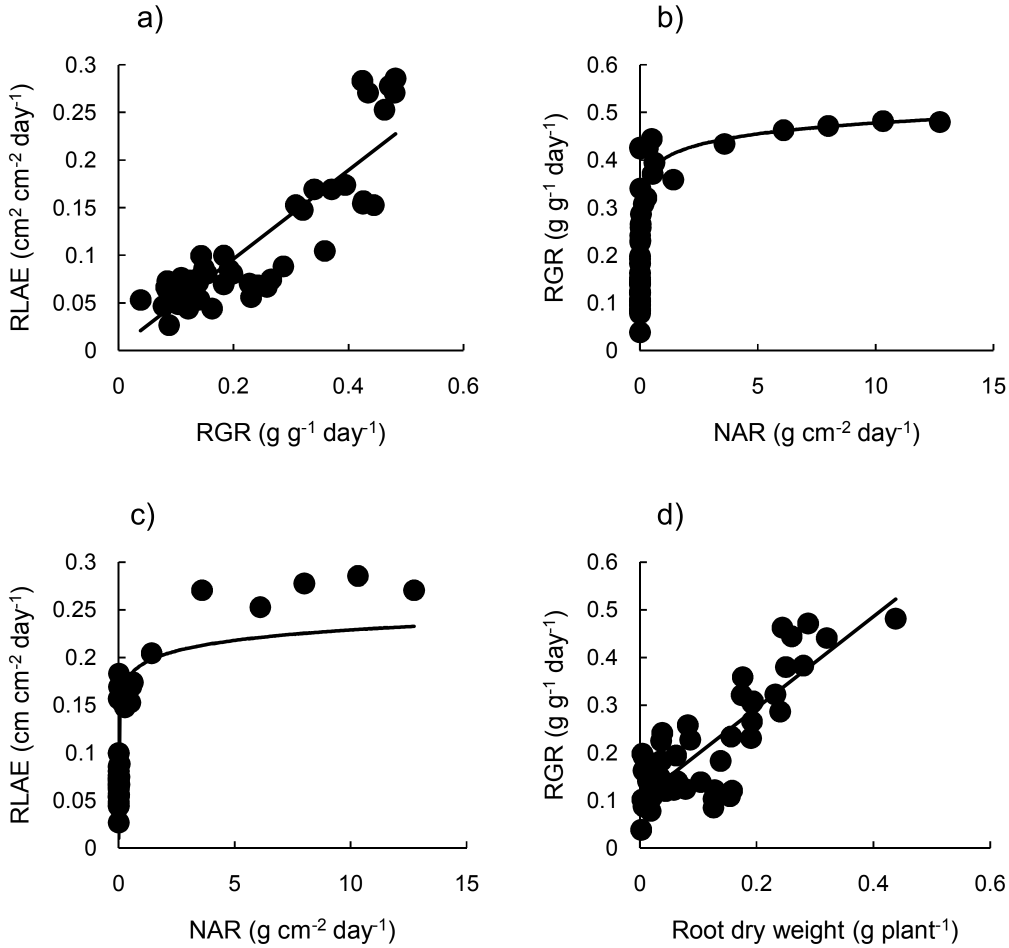

Fig. 4. Relationship between RLAE vs. RGR (a), RGR vs. NAR (b), RLAE vs. NAR (c) and RGR vs. root dry weight (RDW) (d) for dicotyledonous and monocotyledonous weeds in pots fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}). The relations were RLAE = 0.47 RGR + 0.003 (r^2= 0.781 P < 0.001), RGR = 0.033 ln NAR + 0.40 (r^2 = 0.834 P < 0.001), RLAE = 0.016 ln NAR + 0.19 (r^2 = 0.733 P < 0.001), RGR = 0.96 RDW + 0.10 (r^2 = 0.703 P < 0.001)

4. DISCUSSION

Container bedding plant production has been the fastest growing sector in the nursery industry and the growth is expected to continue. The choice of a growing medium is one of the critical decisions that must be made when a grower starting a bedding pot plant production [2]. Peat moss is the most common organic substrate used in growing media for propagation and pot plants. White peat mainly consists of incompletely decomposed *Sphagnum* plants under low temperatures. Due to its mode of formation, peat is free of pests and pathogens and under circumstances of controlled production; it is free of weed seeds as well. The need for alternative substrates in response to decreased peat use resulting from environmental regulations on the mining of peat bogs [25]. On the other hand, the increasing demand of growing media for greenhouse horticultural uses, the rising news uses of substrates, and the scarcity and cost of traditional sources, such as white *Sphagnum* peat moss, have focused new peat sources [1] or research on new substrate materials [26].

The effectiveness of hydrogen cyanamide on weed control was attributable in part of the action of moist soil in hydrolyzing calcium cyanamide to hydrogen cyanamide and in partially to the soil pH decrease. Then, hydrogen cyanamide decomposes to urea, followed by additional breakdown to ammoniac forms of nitrogen and carbon dioxide. In the reaction, two moles of ammonia were formed for each mole of cyanamide consumed. Calcium cyanamide it is not toxic to fish and bees, and does not leave residues in the soil, since it is metabolized to nitrogen and calcium oxide [27]. These metabolites become plant nutrients with their occurrence mediated by biological organisms and physical processes [28], but results mechanically phytotoxic to seeds and plants right now the calcium cyanamide amendment [18,19]. Date from Fig. 2 showed a meaningful seed germination percentage and a slowly germination rate between control pots with an increase in calcium cyanamide concentration amended. These results would indicate a main decrease in weed seed bank. Bremner and Krogmeier [29] provide evidence on the adverse effect of urea fertilizer on seed germination due to ammonia formed through hydrolysis by soil urease. On the other hand, Roem et al. [30] showed that the germination of several heathland species was significantly reduced in plots with a pH below 5 and that acidification was the most important factor in reducing species diversity.

When applied to fields, cyanamide usually disappears within a couple of days, depending on the soil and its moisture content [31], through the enzyme cyanamide hydratase [32]. The main reason why the use of transplant is the most reliable method to ensure adequate crop establishment of commercial plantings of the most ornamental bedding crops is due plants are in contact with the pot substrate after cyanamide disappearance. At this time, cyanamide act as a fertilizer and improve *I. wallerana* growth (Fig. 1) in agreement with Sala et al. [15]. The second reason is that transplant put off plant-weed relationships until plants began their exponential growth, keeping in mind that control bedding plant RLAE and RGR (Table 1) are significant lower than most weed RLAE and RGR (Table 2).

Weed control in container-grown nursery stock is a particularly serious problem because the extent of damage caused by weeds is often underestimated. Various researchers have found that as little as one weed in a pot (around 0.38 m^3 pot^{-1}) affect the growth of a crop [33,34]. The mix used in our experiments showed as much as eight weeds pot^{-1} (25 weeds m^{-3} substrate) in control pots but near two weeds pot^{-1} (6 weeds m^{-3} substrate) in the higher concentration of calcium cyanamide-treated pots (Fig. 2). Although calcium cyanamide drastically reduced seed germination (Fig. 3), the growth of remained emerged weeds can be a serious biotic stress for the bedding pot plants.

Weeds present in the river waste used in our experiments showed different response pattern to calcium cyanamide amendment. On the one hand, *Conyza bonariensis*, *Fumaria capreolata* and *Stellaria media* disappear even with the lowest calcium cyanamide concentrations. A second group (*Amaranthus quitensis*, *Carduus acanthoides* and *Spaeralcea bonariensis*) failed to emerge with the two higher calcium cyanamide doses. A third group (*Amaranthus palmeri*, *Eupatorium hecatanthum*, *Polygonum punctatum*, *Sorghum halepense* and *Taraxacum officinale*) only disappear when 2 kg m^{-3} calcium cyanamide was used. Finally, *Althaea officinalis*, *Cyperus odoratus* and *Wedelia glauca* are present and growth regardless calcium cyanamide concentration (Table 2). Soltys et al. [35] found that the concentration-dependent phytotoxic effects of cyanamide were noted during seed germination and in the root growth of the tested plants. They concluded that the monocotyledonous plants generally were less sensitive to cyanamide treatment than the dicotyledonous ones. Our results from Table 2 did not agree with this previous suggestion.

The toxic action of ammonium to plants has been explained by several mechanisms, which include reduced plant growth, changes in root architecture and decreases in the root/shoot ratio [36]. The disruption of hormonal homeostasis increased oxidative stress as well [37]. The ability of plants to tolerate high ammonium concentrations depends on several main drivers such as root carbon metabolism and the ability to maintain high respiration rates [38], tolerance of acidification of the root zone [39] and the capacity to restrict ammonium accumulation inside tissues [40]. However, data from Table 2 indicate that even though significant weed specie differences, the higher calcium cyanamide concentration the lower RLAE, RGR and NAR (a growth parameter related to photosynthetic carbon fixation).

Roots constitute the first ammonium sensor and the initial signals of ammonium toxicity appear at root level with a severe modification of the root system architecture. Commonly observed modifications include shorter primary root systems; the inhibition of root elongation, embracing primary and lateral roots; the stimulation of lateral root branching, with changes in the insertion of lateral roots in the main root [41,42,43]. Growth of roots and its architecture is under control of phytohormones, mainly auxins [44] and cytokinins [45]. Roots (both main and lateral) grew more slowly on ammonium-grown plants, and the weight of the plant was positively correlated with auxin content, although no recovery was detected when auxin was applied externally to ammonium-grown plants [37]. Di Benedetto et al. [46] showed that following a single application of the auxin indole acetic acid (IAA) or the cytokinin benzyl amino purine (BAP) in the ornamental foliage plant *Epipremnum aureum*, an increase in the accumulation of root biomass was found. The promotion of *E. aureum* growth was associated with increases in RLAE, RGR, NAR and net photosynthesis. The positive relationships between RLAE vs. RGR (Fig. 4a), RGR vs. NAR (Fig. 4b), RLAE vs. NAR (Fig. 4c), and RGR vs. root dry weight (Fig. 4d) when all weed data were plotted together, are in agreement with Di Benedetto et al. [46] results. These relationships suggest that both weed leaf area expansion and dry weight accumulation are under control of signals roots-induced.

The use of transplant is the most reliable method to ensure adequate crop establishment of commercial plantings of the most ornamental bedding crops. Technological advances in transplanting have contributed to the growth of the bedding plant industry by reducing costs and increasing production reliability. On the other hand, sowing and growth ornamental plants in plug tray before transplant would avoid the phytotoxic calcium cyanamide substrate amendment (Fig. 1) and other pre-emergent herbicides [5,6,7].

Because of the Montreal Protocol on substances that deplete atmospheric ozone which agreed for a progressively decrease of substances toxic to humans and animals such as methyl bromide [47,16], the calcium cyanamide use for weed control would be a global alternative [48,17]. However, weed sensitivity to ammonium toxicity must be specifically researched.

5. CONCLUSIONS

Alternative substrates to white peat renew the abiotic stress in pot ornamental plants related to weed control, which can be expensive (stream boiler) or phytotoxic (herbicides). The effectiveness of calcium cyanamide on weed control was attributable in part of the action of hydrogen cyanamide on weed seed germination. The novelty data is that calcium cyanamide decrease weed relative growth rates as well. The last effect would be associated to root toxicity and probably explained by a change in hormonal root synthesis. Our results showed that a pre-transplant calcium cyanamide amendment combining with a transplant routine optimize both *I. wallerana* growth and weed control.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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