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Physiological Changes Involved in the Use of Calcium Cyanamide as a Slow-release Nitrogen Fertilizer in *Impatiens wallerana* (Hook.f.)

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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

Root growth of bedding plants in small pots is usually restricted and evidently influenced by substrate quality and fertilization routine. Two pre-transplant 50- and 288-plug cells tray^{-1} were used. Plants were grown in two growing media and fertilized with liquid feeding and pre-transplant supply of calcium cyanamide (CC). CC-fertilized plants showed higher fresh-dry weight, glucose content and nitrogen content. The higher dry weight accumulation in CC-fertilized plants was supported by the increase in the relative rate of leaf area expansion (RLAE) and the increase in the rate of leaf appearance (RLA). The changes in leaf area were associated with increase in both leaf number and leaf size. CC-fertilized plants also showed a higher relative growth rate (RGR), mainly associated with higher net assimilation rates (NAR) and a change in photo assimilate partitioning that favoured shoots and specifically stems. From a grower's point of view, the use of calcium cyanamide to fertilize *I. wallerana* plants in substitution of the traditional liquid fertilization system would increase crop productivity. Calcium cyanamide would be a better alternative than other

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coated products used as controlled-release fertilizer, especially under a global temperature increase or low environmental greenhouse facilities.

Keywords: Transplants; plug size; growing media.

1. INTRODUCTION

The marketability of potted plants is greatly influenced by the conditions during their production; include the quality of the substrate [1] and the fertilization program [2,3].

Nutrients for container-grown plants are usually applied by injecting fertilizers into the irrigation systems (liquid feeding) or added to growing media before transplant (controlled-release fertilizer). Container crop production requires frequent irrigation and high fertilization rates, which can result in contamination of both ground and surface water [4]. Nitrate pollution of groundwater is a serious global problem for most countries. It has been indicated that the response to changes in the fertilization routine is a complex phenomenon that depends on many internal and external factors, including soil nitrogen availability, nitrogen uptake and assimilation, photosynthetic carbon and reductant supply, carbon–nitrogen flux, nitrate signalling and regulation by light and hormones [5,6].

The nutrient requirements of container-grown ornamental plants are also influenced by the composition of the medium. Soilless media have a wide range of cation-exchange capacities depending on the ratios and types of components in the mix [1]. The use of controlled-release fertilizers improve the fertilizer nitrogen use efficiency in plants [7,8].

Nitrogen release from coated products may dependent on soil moisture, soil temperature, microbial activity, coating thickness and/or the orifice size in the coating [9,10]. However, the availability of nitrogen from calcium cyanamide is not related to these environmental constraints.

Polymer-coated products are very widely used in bedding plants, including *I. wallerana*, because their release is highly predictable and dependent on temperature and moisture level. In developed countries, both factors can be controlled through a high technology investment but, in developing countries, the increase in temperature related to a changing global environment can limit the use of polymer-coated products.

Calcium cyanamide was the first artificial nitrogen fertilizer to be manufactured industrially. This fertilizer contains approximately 20% nitrogen and 50% calcium and is an environmentally benign way of providing these nutrients to protected crops independently of the greenhouse air temperature. The properties of calcium cyanamide include a gradual release of nitrogen and low leaching and demineralization rates, mainly in acid growing media; consequently, if calcium cyanamide is used as a fertilizer, nitrate pollution is limited and soil health is improved because of the increase in microbial diversity [11]. Calcium cyanamide is essentially insoluble in water, but undergoes a partial hydrolysis to calcium hydrogen cyanamide, a source of cyanamide ions [12]. When applied to soil calcium cyanamide undergoes hydrolysis to cyanamide, then urea and then ammonium. Before hydrolysis is complete, higher rates of the cyanamide anion may be toxic to higher plants [13]; however, at the commonly suggested rates, calcium cyanamide did not toxic to *I. wallerana* plants (unpublished data) or soil microbial community [14].

Bedding plant producers have progressively adopted containers of reduced size, which have a limited soil volume available for the root system. This choice allows an increase in plant density, but has the disadvantage of root restriction in a limited volume, followed by considerable changes in plant growth and physiology. Root restriction stress related to a small pot volume could limit biomass accumulation and negatively interact with the growing media and fertilization [15,16].

The aim of this research was to characterize the effect of two fertilization routines, two pre-transplant plug cell volume and two post-transplant growing media on *Impatiens wallerana* growth and to describe the physiological mechanisms involved.

2. MATERIALS AND METHODS

The experiments were carried out in a greenhouse at the Faculty of Agronomy, University of Buenos Aires, Argentina (34° 35' 59''S, 58° 22' 23''W), from October 15th 2013 to March 29th 2014 and from October 20th 2014 to March 26th 2015.

Impatiens wallerana 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown in 50- and 288-plastic plug trays (55.70 and 6.18 cm^3 $cell^{-1}$ respectively) in a Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany) during 35-30 d for the two experiments respectively. When seedlings reached the transplant stage, they were transplanted into 1,200 cm^3 pots filled with two different growing media as follows:

1) Klasmann 411® medium: Canadian *Sphagnum* peat moss-perlite-vermiculite (70/20/10 v/v/v) (**K**). At the beginning of the experiments total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 85.72, 20.94, 22.78 and 0.14 respectively.
2) *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium (**SR**) [17]. 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.

The two growing media tested were chosen with the aim to compare a based-Canadian peat and an alternative growing medium previously tested in *I. wallerana* and other bedding pot plants. 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 [18].

Different calcium cyanamide (**CC**) concentrations (0, 1.0, 1.5 and 2.0 kg m^{-3}) (0, 1.2, 1.8 and 2.4 g pot^{-1}) (Perlka®, AlzChem, Trostberg, Germany) were added at transplanting. A weekly ferti-irrigated control (**F**) 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) according to Styer and Koranski [19] was included. This fertilizer combination and nitrogen concentration, neutralized at pH= 5.8, optimize *I. wallerana* growth. Additional phosphorus and potassium was added to calcium cyanamide treatments through overhead irrigation water to avoid deficiencies in these nutrients.

Daily mean temperatures (21.06 to 26.96℃) and daily photosynthetic active radiation (5.51 to 7.14 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.

Samples of each growing medium were collected at the beginning of the pot experiments (before transplant to the 1,200 cm^3 pots) and total porosity, air-filled porosity, bulk density and container capacity were determined according to Fonteno [20].

Plants were harvested at the transplant stage and at 15, 30, 45, 60 and 90 days after transplanting. 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℃ for 96 h. The number of leaves was recorded, and each leaf area was determined using a LI-COR 3000A automatic leaf area meter (LI-COR, Inc., Lincoln, NE, USA).

The relative 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 rate of leaf appearance (RLA) was calculated as the slope of the number of fully expanded leaves versus time (in weeks). 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 mean net assimilation rate (NAR) and leaf area ratio (LAR) were calculated according to Gandolfo et al. [21]. The specific leaf area (SLA) and the leaf weight ratio (LWR) were calculated as the leaf area: leaf DW ratio and the leaf DW: total DW ratio respectively between the transplant stage and the end of the experiments.

The allometric coefficients between roots and shoots and between leaf blades and the petioles-stems fraction were calculated as the slope (**β**) of the straight-line regression of ln root DW versus ln shoot DW (ln root DW = a **+** b x ln shoot DW), and between ln leaf blade DW versus ln (petiole-stem) DW (ln leaf blade DW = a **+** b x ln petiole-stem DW), respectively.

Glucose and nitrogen concentration were analysed on each plant organ (roots, shoots and leaves) at the final sampling of the pot experiments using the Nelson-Somogyi method and the Kjeldahl method respectively.

The experimental design was a randomized factorial with three blocks of five single-pot replications of each treatment combination (plug cell volume × growing medium × CC concentration). Since there were no significant differences between the two experiments, they were considered together (n = 30). Data were subjected to three-way analysis of variance (ANOVA). STATISTICA 8 (StatSoft) software was used and the assumptions of ANOVA were checked. Least significant differences (LSD) values were calculated. Means were separated by Tukey's tests ($P \leq 0.05$). Slopes from straight-line regressions of RLA, RLAE, RGR, NAR, LAR, SLA, LWR and allometric values were tested using the SMATR package [22].

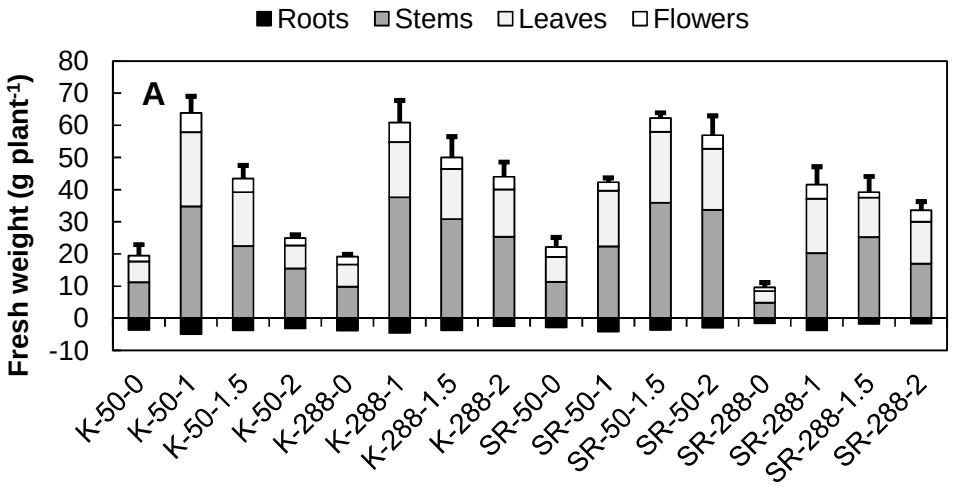
3. RESULTS

FW (90 days from transplanting) showed no significant differences between control plants grown in 50-plug cell trays and those grown in 288-plug cell trays with the K growing medium. In contrast, when plants were grown in SR growing medium, the FW of plants grown in 50-plug cell trays was higher than that of plants grown in 288-cell trays. The use of CC significantly increase FW over controls although the best responses were found when the lowest CC concentration (1 Kg m^{-3}) was used (Fig. 1A). A positive relationship between shoot and root FW was found ($r^2 = 0.688$) with higher values in CC-fertilized plants (Fig. 1B). The experiments showed highly significant differences ($P \leq 0.001$) for the single CC effect and significant differences ($P \leq 0.01$) for the rest of double or triple effects.

The higher total and individual leaf area, the number of leaves and the plant height were mainly related to the use of CC as a fertilizer (the best results were those with 1.0 kg m^{-3} CC) and the quality of growing medium (Table 1).

The use of CC as a controlled-release fertilizer increased RLAE and RLA in both growing medium and cell volumes tested with little differences between them. On the other hand, neither SLA nor LWR showed significant differences (Table 2).

The CC fertilization treatment increased RGR, NAR, LAR and LAP with changes related to the growing medium or the pre-transplant cell volume used (Table 3).



	ANOVA
Cell volume (A)	**
Growing media (B)	**
Calcium cyanamide (C)	***
A x B	**
A x C	**
B x C	**
A x B x C	**

*Significance *** $p \leq 0.001$; $p \leq$ ** 0.01*

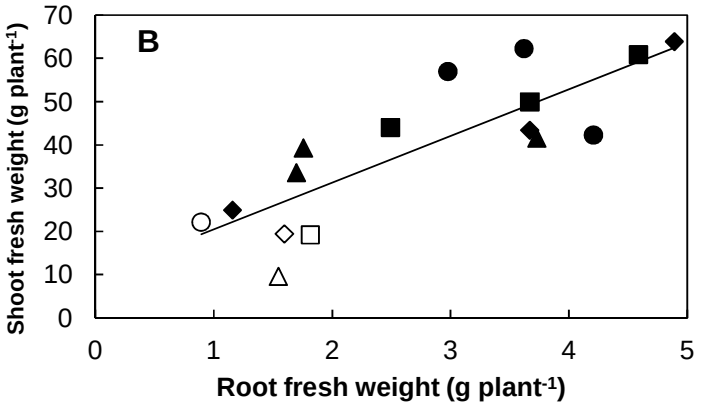


Fig. 1. Mean fresh weight at the end of the experiments from roots, stem, leaves and flowers in *Impatiens wallerana* plants grown in two pre-transplant plug cell size (50- and 288-cell tray^{-1}) but grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}) (A) (n = 30). The standard errors over each bar and the significance of interactions (ANOVA) have been indicated. Shoot-root fresh weight relationships has been showed as well (B). Linear regression equation are: Shoot fresh weight = 10.79 Root fresh weight + 9.67 (r^2 = 0.688, P < 0.001). The probability of the slope being zero was P ≤ 0.001. K: ◊-□; SR: ○-Δ; 50-cells trait: ◊-○; 288-cells trait: □-Δ. Empty or full symbols indicated both controls and CC-fertilized plants respectively

Table 1. Changes in both total and individual leaf area, the number of expanded leaves and plant height in *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cell tray^{-1}), grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}) (n = 30). Different lower-case letters indicate significant differences (P ≤ 0.05) between CC-fertilized plants while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes. The significance of interactions (ANOVA) has been indicated

Calcium cyanamide	Leaf area (cm^2 plant^{-1})		Leaf area (cm^2 leaf^{-1})		Number of leaves (leaves plant^{-1})		Plant height (cm plant^{-1})	
(kg m^{-3})	K	SR	K	SR	K	SR	K	SR
50-cells								
0	67.16^{cA}	79.37^{dA}	0.97^{bA}	0.91^{bA}	69.00^{cB}	87.25^{bA}	16.35^{bA}	14.80^{cA}
1.0	235.81^{aA}	177.35^{cA}	1.55^{aA}	1.97^{aA}	152.00^{aA}	90.25^{bB}	26.75^{aB}	24.48^{bA}
1.5	172.30^{bA}	225.68^{aA}	1.41^{aA}	2.10^{aA}	122.00^{bA}	107.25^{aA}	27.63^{aB}	26.58^{bB}
2.0	73.24^{cB}	195.39^{bA}	0.93^{bB}	1.99^{aA}	78.75^{cB}	98.25^{aA}	26.08^{aA}	34.00^{aA}
288-cells								
0	70.67^{cB}	37.50^{cB}	0.79^{bA}	0.78^{dA}	90.00^{cA}	48.00^{cB}	13.48^{cA}	11.05^{cB}
1.0	176.20^{aB}	172.84^{aA}	1.40^{aA}	1.42^{cB}	125.75^{aB}	121.50^{aA}	30.78^{aA}	21.63^{bB}
1.5	159.76^{bB}	126.15^{bB}	1.56^{aA}	2.13^{aA}	102.25^{bB}	59.25^{bB}	30.83^{aA}	36.30^{aA}
2.0	150.99^{bA}	133.54^{bB}	1.53^{aA}	1.85^{bA}	98.75^{bA}	72.25^{bB}	25.23^{bA}	23.68^{bB}

ANOVA	Total leaf area	Individual leaf area	Number of leaves	Plant height
Cell volume (A)	**	*	**	**
Growing media (B)	**	*	**	**
Calcium cyanamide (C)	***	***	***	***
A x B	*	*	*	*
A x C	**	**	**	**
B x C	**	**	**	**
A x B x C	**	**	**	**

*Significance *** 0.001 ** 0.01 * 0.05 'ns' No significant*

Table 2. Changes in the relative expansion rate (RLAE), the rate of leaf appearance (RLA), the specific leaf area (SLA) and the relative leaf weight (LWR) from *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cell tray^{-1}), grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 K m^{-3}) (n = 30). Different lower-case letters indicate significant differences (P ≤ 0.05) between CC-fertilized plants while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes. The probability of the slope being zero was P ≤ 0.001 for all growth parameters

Calcium cyanamide (kg m^{-3})	RLAE (cm^2 cm^{-2} day^{-1})		RLA (leaves week^{-1})		SLA (cm^2 g^{-1})		LWR (g g^{-1})	
	K	SR	K	SR	K	SR	K	SR
50-cells								
0	0.0457^{dB}	0.0573^{cA}	0.913^{dB}	1.125^{cA}	195.04^{aA}	150.23^{bA}	0.313^{cB}	0.379^{cA}
1.0	0.0647^{aB}	0.0683^{bA}	1.482^{aB}	1.060^{dB}	173.88^{bA}	176.45^{aA}	0.437^{bA}	0.438^{aA}
1.5	0.0583^{bB}	0.0715^{aA}	1.274^{bA}	1.232^{bA}	185.41^{aA}	177.32^{aB}	0.477^{aA}	0.405^{bA}
2.0	0.0489^{cB}	0.0686^{bA}	1.018^{cA}	1.274^{aA}	189.59^{aA}	168.76^{aB}	0.319^{cB}	0.364^{cB}
288-cells								
0	0.0515^{cA}	0.0401^{cB}	1.000^{dA}	0.587^{dB}	202.07^{aA}	159.67^{cA}	0.352^{bA}	0.379^{cA}
1.0	0.0661^{aA}	0.0630^{aB}	1.586^{aA}	1.172^{aA}	175.62^{cA}	174.20^{bA}	0.337^{cB}	0.449^{bA}
1.5	0.0634^{bA}	0.0548^{bB}	1.205^{bB}	0.619^{cB}	184.07^{bA}	217.50^{aA}	0.352^{bB}	0.361^{cB}
2.0	0.0617^{bA}	0.0551^{bB}	1.020^{cA}	0.815^{bB}	192.65^{aA}	203.19^{aA}	0.398^{aA}	0.496^{aA}

Table 3. Changes in the relative growth rate (RGR), the net assimilation rate (NAR), the leaf area ratio (LAR) and the coefficient of leaf partitioning (LAP) estimated from *Impatiens wallerana* plants grown in two pre-transplant plug cell size (50- and 288-cell tray^{-1}), grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}) (n = 30). Different lower-case letters indicate significant differences (P ≤ 0.05) between CC-fertilized plants while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes. The probability of the slope being zero was P ≤ 0.001 for all growth parameters

Calcium cyanamide (kg m^{-3})	RGR (g g^{-1} day^{-1})		NAR (g cm^{-2} day^{-1}) (x 10^{-5})		LAR (cm^2 g^{-1})		LAP $\frac{\text{cm}^2\ \text{day}^{-1}}{\text{g day}^{-1}}$	
	K	SR	K	SR	K	SR	K	SR
50-cells								
0	0.0699^{dB}	0.0928^{cA}	50.04^{cB}	45.87^{cA}	139.69^{cB}	202.29^{bA}	91.33^{dB}	124.91^{cA}
1.0	0.0927^{aA}	0.0979^{bA}	53.30^{aB}	47.96^{aB}	155.15^{aB}	204.14^{bA}	121.38^{aA}	142.42^{bA}
1.5	0.0749^{bB}	0.1030^{aA}	50.19^{bB}	46.15^{bA}	149.23^{bB}	223.18^{aA}	116.16^{bB}	154.93^{aA}
2.0	0.0724^{cB}	0.1041^{aA}	50.21^{bA}	47.48^{aA}	144.19^{bB}	219.27^{aB}	97.38^{cB}	144.49^{bB}
288-cells								
0	0.0782^{dA}	0.0700^{cB}	54.04^{aA}	42.23^{bB}	144.71^{bA}	165.77^{dB}	95.30^{bA}	94.96^{cB}
1.0	0.0899^{aB}	0.0891^{aB}	55.67^{aA}	51.29^{aA}	167.50^{aA}	188.42^{cB}	123.16^{aA}	133.23^{bB}
1.5	0.0874^{bA}	0.0823^{bB}	51.36^{bA}	39.19^{cB}	170.90^{aA}	210.02^{bB}	123.45^{aA}	139.84^{bB}
2.0	0.0845^{cA}	0.0832^{bB}	49.53^{bA}	33.47^{dB}	170.61^{aA}	248.59^{aA}	124.58^{aA}	164.63^{aA}

Glucose concentrations were higher in both stems and roots than leaves in control plants. CC-fertilization significantly increased glucose concentration. The growing medium had higher effects than the pre-transplant cell volume (Fig. 2A). On the other hand, nitrogen distribution was almost the same in the different plant organs (roots, shoots and leaves). Although there were only little differences related to the growing medium or pre-transplant cell volume used, CC-fertilized plants showed higher nitrogen accumulation than control ones (Fig. 2B).

In control plants, the allometries between roots and shoots showed a balanced DW partitioning, although the higher the cell volume the higher the DW partitioning to shoots. The use of CC as a controlled-release fertilizer significantly increased DW in favour of shoots. On the other hand, the stem-leaf allometries of control plants

did not show a clearly defined response pattern when both growing medium and pre-transplant plug cell volumes were tested. However, a higher DW partitioning to stems was found in CC-fertilized plants (Table 4).

Positive relationships between RLAE (Fig. 3A), RLA (Fig. 3B), RGR (Fig. 3C), NAR (Fig. 3D), glucose concentration (Fig. 3F), nitrogen concentration (Fig. 3G) and root DW (r^2 = 0.599, 0.690, 0.511, 0.570, 0.668 and 0.675 respectively; $P \leq 0.001$ for all relationships) were found at the end of the experiment. The higher control values belonged to plants grown in the K growing medium and transplanted from 50-cell trays. The higher responses were found when plants were CC-fertilized. A negative relationship between SLA and root DW was found as well (r^2 = 0.754; $P \leq 0.001$) (Fig. 3E).

4. DISCUSSION

Impatiens wallerana bedding plant productivity was associated with both aerial biomass expansion and shoot FW (Fig. 1A); the latter was mainly determined by the root system size (Fig. 1B) in agreement with previous reports [4,15,16]. Growth response differences between the two growing media tested would be associated to their both physical and chemical properties [23].

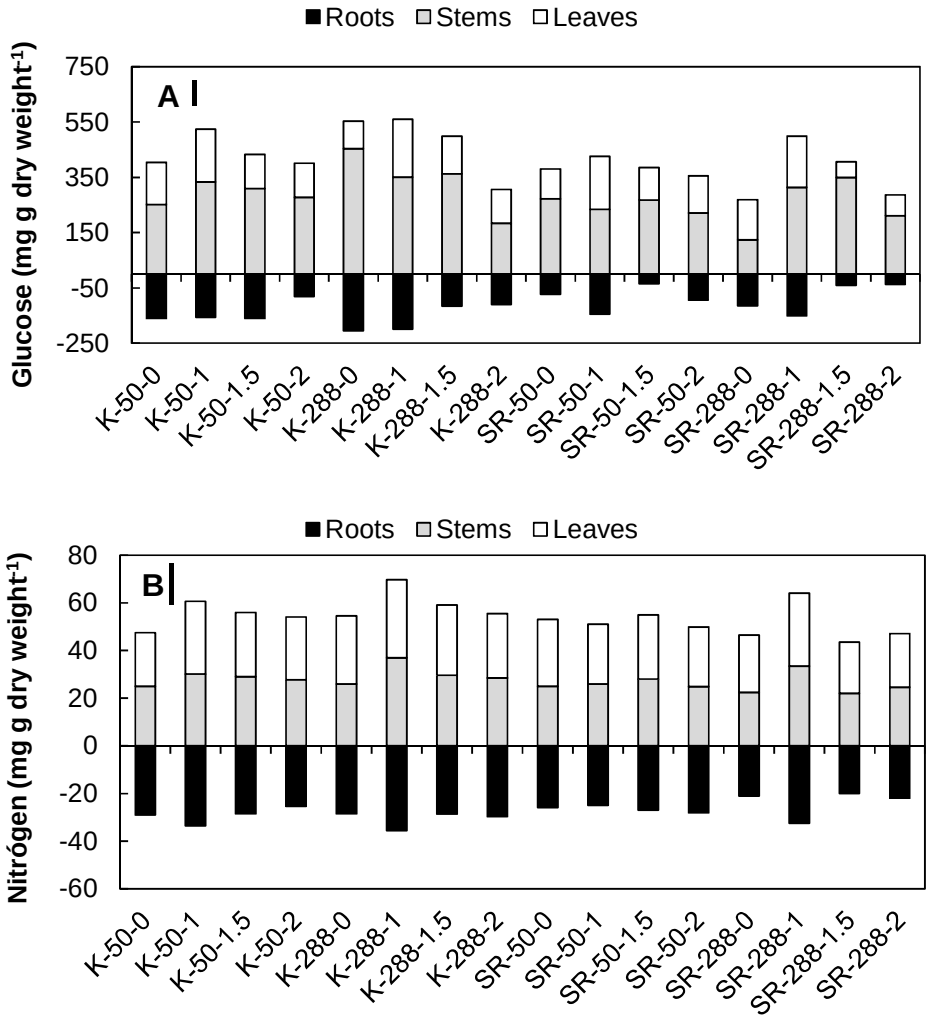


Fig. 2. Glucose (A) and nitrogen (B) contents at the end of the experiments in different plant organs of *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cell tray^{-1}), grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}) (n = 3). Vertical lines indicate least significant differences (LSD)

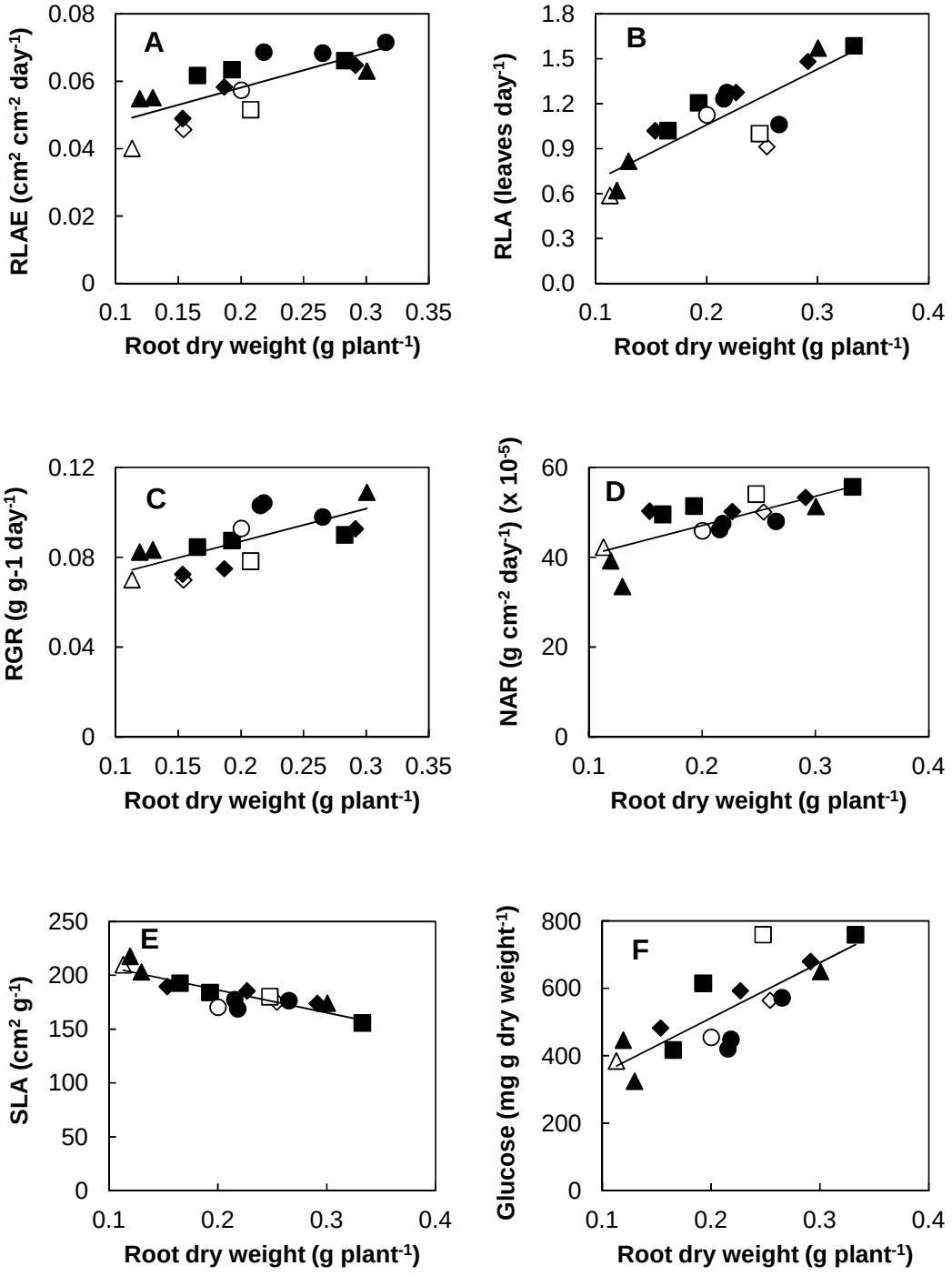
The growth of plants and their quality are mainly a function of the availability of nutrients. Data from Fig. 1A agree with this general point. Therefore, it is very important to improve the use of fertilizer nutrients; one method of reducing fertilizer nutrient losses involves the use of slow- or controlled-release fertilizers [24,25]. In this way, calcium cyanamide offer a gradual release of nitrogen regardless of the temperature [11], a very important matter that make difficult greenhouse acclimatization associated with the changing global environment which, could be limit the use of polymer-coated products. Although the effects of manipulating nutrient supply on plant growth and development have long been known, these effects consist of more than simply relieving nutrient-limited growth. It is now well documented that several nutrients and metabolites act as signalling molecules in multiple pathways that co-ordinately regulate patterns of gene expression [26].

Plant organs interact with each other to optimize both metabolic and developmental processes to allow the organism to accommodate to the environment. For these mutual interactions, local and long-distance communication among cells and organs are essential [27]. Molecular genetics evidences have demonstrated that roots sense and respond to local and global concentrations of inorganic nitrate, in a fashion that depends on the shoot nutrient status [28]. Nitrate availability and distribution affect the nitrate control of the root system architecture [29]. It has been suggested [3,4,30] that the nitrogen signalling associated with cytokinin synthesis by roots would be involved in the adaptation of *I. wallerana* plants to different growing media. The same response would be involved when a root restriction related to the pre-transplant plug cell volume is imposed [15]. All abiotic stresses reduce plant growth and yield [31].

A key concept underpinning the current understanding of the carbon/nitrogen interaction in plants is that the capacity for nitrogen assimilation is related to nutrient availability and requirements by the integrated perception of signals from hormones, nitrate and sugars [32]. Studies on the nature and integration of these signals have revealed a complex network, which interplays with carbon and nitrogen signals [27,33,34]. These controls not only act to orchestrate the relative rates of carbon and nitrogen assimilation and carbohydrate production, but also have a significant influence on plant development. The signal transduction network that coordinates information from carbohydrate metabolism and nitrogen assimilation is under phytohormone regulation [35-37]. In this way, our results showed that CC-fertilized plants significantly increased glucose concentration (Fig. 2A).

Table 4. Changes in allometric relationships between roots and shoots or between stems and leaves for *Impatiens wallerana* plants using a straight-line regression analysis between the natural logarithm of root, shoot and leaves dry weight. Treatments included two pre-transplant plug cell size (50- and 288-cell tray^{-1}), two post-transplant growing media (K and SR) and four calcium cyanamide fertilization concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}) (n = 30). The straight-line regression slopes (β) and the coefficients of determination r^2 are indicated. Different lower-case letters indicate significant differences (P ≤ 0.05) between CC-fertilized plants while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes. The probability of the slope being zero was P ≤ 0.001 for all allometric relationships

Calcium cyanamide (kg m^{-3})	Roots vs. Shoots				Stems vs. Leaves			
	K		SR		K		SR	
	β	r^2	β	r^2	β	r^2	β	r^2
50-cells								
0	0.825^{aB}	0.759	0.698^{aB}	0.897	0.630^{cB}	0.876	0.991^{bA}	0.856
1.0	0.649^{dB}	0.882	0.632^{bB}	0.880	0.775^{bA}	0.856	1.012^{aA}	0.837
1.5	0.689^{cB}	0.805	0.545^{cB}	0.896	0.847^{aA}	0.898	0.982^{bA}	0.859
2.0	0.746^{bA}	0.824	0.569^{cB}	0.872	0.624^{cB}	0.854	0.993^{bA}	0.863
288-cells								
0	0.985^{aA}	0.924	0.813^{aA}	0.852	0.743^{cA}	0.949	0.814^{cB}	0.751
1.0	0.768^{bA}	0.891	0.777^{bA}	0.871	0.728^{dB}	0.955	0.898^{aB}	0.877
1.5	0.767^{bA}	0.854	0.630^{dA}	0.718	0.813^{aB}	0.911	0.740^{dB}	0.822
2.0	0.744^{cA}	0.845	0.689^{cA}	0.561	0.769^{bA}	0.924	0.842^{bB}	0.769


A
RLAE (cm² cm⁻² day⁻¹)
0.08
0.06
0.04
0.02
0
0.1 0.15 0.2 0.25 0.3 0.35
Root dry weight (g plant⁻¹)
B
RLA (leaves day⁻¹)
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0.1 0.2 0.3 0.4
Root dry weight (g plant⁻¹)
C
RGR (g g-1 day⁻¹)
0.12
0.08
0.04
0
0.1 0.15 0.2 0.25 0.3 0.35
Root dry weight (g plant⁻¹)
D
NAR (g cm⁻² day⁻¹) (x 10⁻⁵)
60
40
20
0
0.1 0.2 0.3 0.4
Root dry weight (g plant⁻¹)
E
SLA (cm² g⁻¹)
250
200
150
100
50
0
0.1 0.2 0.3 0.4
Root dry weight (g plant⁻¹)
F
Glucose (mg g dry weight⁻¹)
800
600
400
200
0
0.1 0.2 0.3 0.4
Root dry weight (g plant⁻¹)

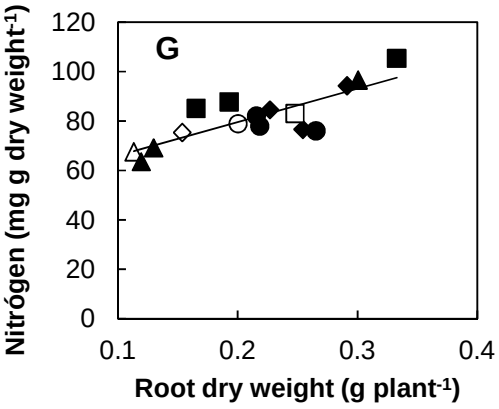


Fig. 3. Relationships between the relative leaf expansion rate (RLAE) (A), the rate of leaf appearance (RLA) (B), the relative growth rate (RGR) (C), the net assimilation rate (NAR) (D), the specific leaf area (SLA) (E), the glucose content (F), the nitrogen content (G) and the root dry weight (RDW). Treatments included *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cell tray^{-1}), grown in two post-transplant growing media (K and SR) and fertilized with four calcium cyanamide fertilizer concentrations (0; 1.0; 1.5 and 2.0 Kg m^{-3}). Linear regression equations are: RLAE = 0.10 RDW + 0.038 (r^2 = 0.699; P ≤ 0.001); RLA = 3.722 RDW + 0.31 (r^2 = 0.690; P ≤ 0.001); RGR = 0.146 RDW + 0.06 (r^2 = 0.711; P ≤ 0.001); NAR = 65.13 RDW + 34.05 (r^2 = 0.770; P ≤ 0.001); SLA = - 210.23 RDW + 228.37 (r^2 = 0.754; P ≤ 0.001); Glucose content = 1,637.80 RDW + 184.68 (r^2 = 0.668; P ≤ 0.001); Nitrogen content = 135.67 RDW + 52.39 (r^2 = 0.675; P ≤ 0.001). K: ◊-ϒ; SR: ○-Δ; 50-cells trait: ◊-○; 288-cells trait: ϒ-Δ. Empty or full symbols indicated both controls and CC-fertilized plants respectively

Several reports have suggested that the accumulation of cytokinins is closely correlated with the nitrogen status of the plants and suggested that cytokinin metabolism and translocation could be modulated by the nitrogen nutritional status [38]. Namely, cytokinin accumulation and translocation occurred after sensing a change in nitrogen availability. Pagani et al. [3] have recently shown that there is a close relationship between *I. wallerana* DW accumulation and nitrogen content. Since these authors found that the alternative growing medium tested (mainly the *Sphagnum maguellanicum*- and the *Carex sp.*-based one) changed the proportion of nitrogen in the shoots they hypothesized that the decrease in shoot growth would be associated with this endogenous signal.

A bedding pot plant can be sold only when it accumulates a FW between 30 and 70 grams [39]. In the present study, the *I. wallerana* plants that received liquid fertilization (control plants) reached the sale stage 90 days after transplanting. However, the CC-fertilized plants showed an increase in both FW (Fig. 1A) and DW (data not shown) at the end of the experiments. It would be speculate that the same result would be achieved increasing the water-soluble fertilizer rate, however, this alternative can result in contamination of both ground and surface water in a high quality peat-based media or increase the electrical conductivity and pH in alternative growing media [4,30].

The higher the plant weight the higher the total leaf area because of a higher leaf number and a higher individual leaf area (Table 1). Therefore, the use of CC as a controlled-release fertilizer increased both RLAE and RLA, which suggest deep changes in the vegetative apex of the plants (Table 2). The phytohormone cytokinin interacts with other systemic signals and is a key regulator of meristem size and functions [40].

Some authors have claimed that the plastochron (i.e. the time between successive leaf initiation events) may be altered in transgenic plants with reduced cytokinin levels [41,42]. However, the possibility that exogenous application of cytokinins may affect the plastochron has attracted little attention. Cytokinins have a strong

influence on many aspects of shoot development and metabolism, including leaf initiation [43]. On the other hand, the possibility that the rate at which leaves appear in a vegetative meristem could be regulated by exogenous application of 6, benzyl aminopurine (BAP) has been explored only recently [44-48]. Plants need nutrient to grow and plant cells need nutrient to divide; however, the extent to which nutrient sensing might be operating in meristems has not been well described [49]. Nitrate addition up-regulates cytokinin levels in part by inducing expression of cytokinin biosynthetic genes [50]. A decrease in the plastochron needs an increase in apex size [39] and the presence of non-limiting sugar availability [51,52]. A higher glucose content in stems of CC-fertilized plants (Fig. 2A) and a higher DW partitioning to stems from the stem-leaf allometries (Table 4) are in agreement with these previous assumptions and support RLA changes (Table 2). In addition, the final size of plant organs such as leaves is tightly controlled by environmental and genetic factors that spatially and temporally co-ordinate cell expansion and cell cycle activity [53-55]. It has been suggested that cytokinins are involved in the maintenance of the vegetative apex and in cell differentiation [56,57]. Cytokinins can promote leaf unfolding and expansion in whole plants of several species, including ornamentals [16,44-47,58]. Any perturbation in one of these processes by modifications in the expression of specific *KNOX* genes [43] might affect the final leaf area [59].

Theoretical plant growth models postulate that the relative rates of shoot and root growth are largely modulated by signals related to the carbon and nitrogen status of the plant. Both whole-plant biomass accumulation and carbon and nitrogen contents, are highly responsive to light and nitrogen availability. Some aspect of the plant carbon controls root-to-shoot biomass partitioning and nitrogen balance [60]. Since in the present study we found no significant differences in the DW content of control and CC-fertilized plants (data not shown), it is possible to describe the photo assimilate acquisition and partitioning rates on a DW base. The higher biomass accumulation in CC-fertilized plants was a result of higher RGR and NAR (Table 3). Besides, the decrease in SLA in CC-fertilized plants (Table 3) suggests an increase in leaf thickness. The negative relationship between RGR and SLA (data not shown) would explain part of the NAR increases [21]. It has been indicated that leaf thickness may be correlated with nutrient availability [61] as well.

Plants constantly sense the changes in their environment; when mineral elements are scarce, they often allocate a greater proportion of their biomass to the root system [36]. Root: shoot partitioning has been extensively studied, and several types of models and partitioning mechanisms in which partitioning is governed by the relative levels of carbon and nitrogen in the storage pools have been developed [62]. Adjustments in root and shoot growth are often assumed to be a fundamental facet of a plant's phenotypic plasticity in response to its environment [63,64]. The changes in LAR and LAP (Table 3) and the allometries between roots and shoots (Table 4) showed a higher DW partition in CC-fertilized plants and are in agreement with previous reports.

The environment of plants is composed of a complex set of both abiotic and biotic stresses; plant responses to these stresses are equally complex [65]. Although the root system architecture is known to be highly plastic and strongly affected by environmental conditions, little is known about the underlying mechanisms controlling root system development. Hess and Kroon [66] and Puig et al. [67] have concluded that plants can sense the volume of the available rooting space, and a limited number of studies on individual roots have shown that plant roots may sense the identity of neighbouring roots and respond accordingly. Intrinsic, hormone-mediated pathways that perceive and respond to external, environmental signals modulate root architecture [68]. Cytokinins are root-synthesized molecules, which are transported via the xylem to the shoot [69-71].

The root restriction related to a low pre-transplant cell volume [15,16], growing medium quality [3,4,23,72] and fertilization routine [30] in bedding ornamental plants has been recently explored. On the other hand, Ouma [73] showed that the interaction between container volume and nitrogen fertilizer levels was significant for both roots and shoots weight and for whole plants. In the present study, increasing nitrogen fertilizer levels increased the growth parameters as the container volumes increased.

Although the higher the root system the higher the zeatin ribosides [74], it is no easy to show quantitative changes in endogenous cytokinin concentration [75] because plants synthesize different cytokinin-ribosides and not all have biological activity. Nevertheless, in the present study, when the root system increased positive

relationships with RLAE (Fig. 3A), RLA (Fig. 3B), RGR (Fig. 3C), NAR (Fig. 3D), glucose content (Fig. 3F) and nitrogen content (Fig. 3G) were found (r^2 = 0.699; 0.690; 0.711; 0.770; 0.668 and 0.675 respectively). At the same time, SLA (a growth parameter that allows estimating leaf thickness) showed a negative relationship with an increase in root DW (Fig. 3E).

The level of nitrate available to the plant regulates the endogenous concentration of cytokinins. Plants grown on low levels of nitrogen show reduced levels of cytokinin, and the addition of nitrate leads to an increase in the levels of various cytokinin species. Nitrate addition up-regulates cytokinin levels in part by inducing expression of cytokinin biosynthetic genes. The balance of different cytokinin species, perceived by two-component elements, would signal the availability of nitrate forms, and would ultimately lead to the expression of cytokinin-responsive and, potentially, also nitrate-responsive genes to regulate nitrogen metabolism in the plant [59,76]. It has been indicated that the fertilizer source, the rate, and the soil type [6] or growing medium [2,3,77] significantly influence the accumulation of nitrogen in shoots. Results from Fig. 3B showed an increase in nitrogen content in CC-fertilized plants, although with a similar accumulation in roots, stems and leaves.

The percentage of the fertilizer dose recovered by plants when applied in conventional forms may amount up to only 30 to 50%; but when slow-release fertilizers are used, the nitrogen losses are lower [78]. Calcium cyanamide, which contains both nitrogen and calcium, plays a role as an important signalling molecule as well. The use of calcium cyanamide is set into the context of needs to increase plant growth, reduce the intensity with which resources are used, enhance natural biodiversity and provide for environmental sustainability [79]. In agreement with that found by Puig et al. [67], our results showed that *I. wallerana* plants adjust their development in relation to the availability of nitrogen.

5. CONCLUSIONS

We considered growing medium, cell volume or nutrient supply as a plant signalling for which, this paper containing new information on the physiological mechanisms involved. From a grower's point of view, the use of calcium cyanamide to fertilize *I. 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 (with lower cost), especially under a global temperature increase or low environmental greenhouse facilities. From an eco-physiological perspective, changes in these technological supplies would be seen as a stimulus, which develop different endogenous signalling in the plant; this approach is extremely new and although it is supported by many previous reports in different plants, the information in ornamentals is scarce. On the other hand, this eco-physiological approach let to point many futures research lines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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