



## Relationships between Plug Cell Size and Substrate Quality in the Bedding Pot Plant *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

In pot plant production, balancing the air and water content in the medium is one of the largest problems. The 'root restriction' effects of the plug cells on seedling growth during nursery could increase after transplant and thus limits pot plant productivity. Two plug cell size (limiting and non-limiting ones) and two growing media were tested. Different concentrations (0, 1.5 and 3.0 kg m<sup>-3</sup>) of the hydrophilic gel potassium acrylate (Stockosorb®, Evonik, Germany) were added at transplanting to create growing media with different container capacity. The aim of this work was to evaluate the physiological mechanisms involved when two different pre-transplant cell volumes (50- and 288- cells-plug tray<sup>-1</sup>) and two post-transplant growing media amended with different potassium acrylate concentrations (0, 1.5 and 3.0 Kg m<sup>-3</sup>) were used. The hypothesis tested was that the negative effects of combined abiotic stress sources (pre-transplant cell volume and growing medium quality) that reduced air-filled porosity and affect *Impatiens wallerana* growth and yield are

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mainly associated with a decrease in root size. Our results showed that the root restriction related to cell volume and growing medium quality reduced air-filled porosity but in different magnitude according to the growing medium tested. We found a decrease in the relative rate of leaf area expansion (RLAE), the relative growth rate (RGR), the net assimilation rate (NAR) and glucose content, and an increase in water-holding capacity. We also found positive relationships between the previous mentioned growth parameters and an increase in root dry weight. Since the responses to the different plug cell volume and growing medium to overcome the root restriction were the same as those found in other experiments in plants sprayed with exogenous cytokinins, we speculated that endogenous cytokinins are involved in plant growth.

*Keywords: Technological abiotic stress; growth parameters; photo-assimilate partitioning; root restriction.*

## 1. INTRODUCTION

In pot plant production, the primary function of a container is to provide a discrete space for the growing medium but this restricted space also affects the physical conditions of the medium. Balancing the air and water content in the medium is one of the largest problems facing plug growers. Plug cells have two basic problems: they are too short and too small. The 'root restriction' effects of the plug cells on seedling growth during nursery could increase after transplant and thus limits pot plant productivity [1].

Aeration in soilless mixes is often a problem. After the partial saturation and complete drainage of potted media, the water table at the bottom of the pot is very small and perched; resulting in media equilibrated at very high water potentials. Under these conditions, many of the substrate pores tend to remain saturated, further increasing the risk of root asphyxia if the period of saturation of a large proportion of these pores is prolonged. Ensuring proper aeration in soilless mixes is often difficult. At these high water potentials, even if total porosity is very high, the air-filled porosity of these soilless mixes may be very low [2].

The physical properties of substrates initially considered appropriate for plant growth may deteriorate upon ageing of the medium due to several processes [3], which generally result in decreased pore sizes. As pore size decreases, total porosity and air-filled porosity decrease as well. We have previously found [4] that this was true for *I. wallerana* plants grown from 512- and 288-cells-plug trays, but not for those grown from 50-cells-plug trays. The root growth after transplant could reduce the decrease in total porosity and air-filled porosity imposed by the compaction of the medium [5]. Bailey-Serres and

Colmer [6] and Voesenek and Sasidharan [7] have indicated that a lack of oxygen inhibits respiration, decrease metabolic plant adaptations to cope with the hypoxic and anoxic conditions and resulting energy deficits, as well as change anatomical and morphological adaptations to improve internal O<sub>2</sub> supply.

To describe changes in physical properties throughout time, researchers used growing media containing different pore size. Under the growing medium compaction generated, the largest air-filled pores disappear and are replaced by smaller, mainly water-filled pores. A second alternative is to use different water retention additives such as potassium acrylate [8] to increase the water-holding capacity of the medium [9].

We have previously shown that the shoot fresh weight of the bedding plant *I. wallerana* is mainly determined by the root system size [10] and we proposed to consider the growing medium not only as a matrix for water and nutrient availability but also a source of external signaling [11,12]. Similarly, we have also suggested [13] considering the growing medium as an abiotic stress source that would allow changing the medium-based paradigm to optimize both the growth and productivity of bedding pot plants.

The aim of this work was to evaluate the physiological mechanisms involved when two different pre-transplant cell volume and two post-transplant growing media amended with different potassium acrylate concentrations were used. The hypothesis tested was that the negative effects of combined abiotic stress sources (pre-transplant cell volume and growing medium quality) that reduce air-filled porosity and affect *I. wallerana* growth and yield are mainly associated with a decrease in root size.

## 2. MATERIALS AND METHODS

The experiments were carried out in a greenhouse at the Faculty of Agronomy, University of Buenos Aires, Argentina (34°28'S), from October 15<sup>th</sup> 2013 to March 29<sup>th</sup> 2014 and from October 20<sup>th</sup> 2014 to March 26<sup>th</sup> 2015.

*Impatiens wallerana* 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown in 50- and 288-cells-plug trays (55.70 and 6.18 cm<sup>3</sup> cell<sup>-1</sup> respectively) in Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany) for 35-30 days for the two experiments respectively. When seedlings reached the transplant stage, they were transplanted into 1,200-cm<sup>3</sup> 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) (S<sub>1</sub>). At the beginning of the experiments, total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm<sup>-3</sup>) were 85.72, 20.94, 53.00 and 0.14 respectively.
- 2) *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium (S<sub>2</sub>) [14]. At the beginning of the experiments, total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm<sup>-3</sup>) were 63.50, 17.06, 51.40 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 phytoplants (plant debris). The result is a fine-grained, black, oozy sediment deposited in the bottom of the coasts [15].

Different concentrations (0, 1.5 and 3.0 kg m<sup>-3</sup>) of the hydrophilic gel potassium acrylate (Stockosorb®, Evonik, Germany) were added at transplanting to create growing media with different container capacity.

A weekly ferti-irrigated solution with 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 Larrocca S.R.L., Buenos Aires, Argentina) through the overhead irrigation water (150 mg L<sup>-1</sup> N) was included.

Daily mean temperatures (21.06 to 26.96°C) and daily photosynthetic active radiation (5.51 to 7.14 mole photons m<sup>-2</sup> day<sup>-1</sup>) for the two experiments were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The plants were arranged at a density of 25 plants m<sup>-2</sup>, 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<sup>3</sup> pots) and both air-filled porosity and container capacity were determined according to Fonteno [16].

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°C for 96 hours. 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 ln of the whole plant DW versus time (in days).

The mean net assimilation rate (NAR) and leaf area ratio (LAR) were calculated as follows:

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

$$LAR = k_a / \frac{A_a e^{k_a t}}{k_w W_0 e^{k_w t}}$$

where W<sub>0</sub>: extrapolated value of total DW (g) at time zero; k<sub>w</sub>: RGR (day<sup>-1</sup>); A<sub>0</sub>: extrapolated value of leaf area (cm<sup>2</sup>) at time zero; k<sub>a</sub>: RLAE (day<sup>-1</sup>); t: time (days) at the midpoint of the experimental period and e: base of the ln.

The specific leaf area (SLA) was calculated as the leaf area: leaf DW ratio between the transplant stage and the end of the experiments.

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

Glucose concentration was analyzed at the final sampling of the pot experiments using the Nelson-Somogyi method.

The experimental design was a randomized factorial with three blocks of 20 single-pot replications of each treatment combination (plug cell volume  $\times$  growing medium). Since there were no significant differences between the two experiments, they were considered together ( $n = 6$ ). 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, and allometric values were tested using the SMATR package [17].

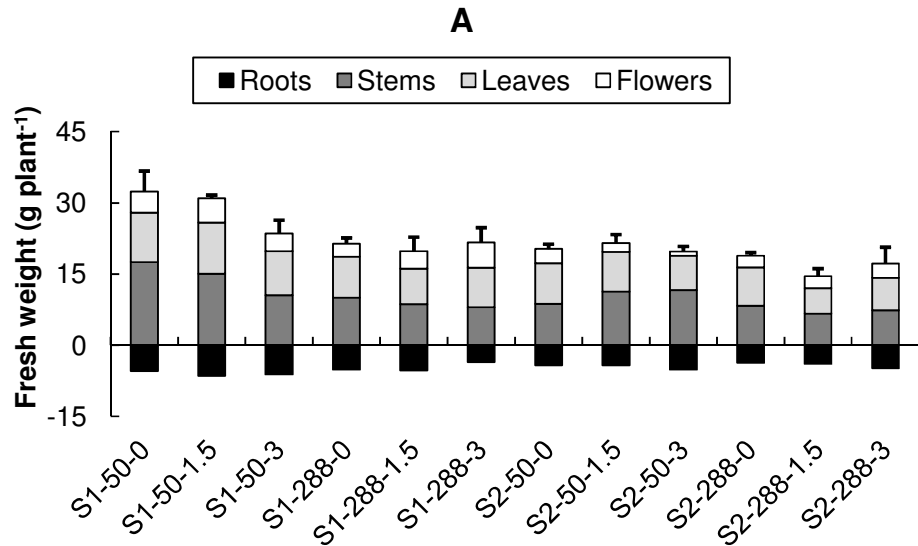
### 3. RESULTS

Control plants showed no significant differences in container capacity between both cell sizes and growing media at the beginning of the experiments; however, the  $S_1$  growing medium showed higher air-filled porosity than  $S_2$ . Anyway, plants grown in 50-cell plug trays showed higher air-filled porosity than those in 288-cells plug trays in both growing media tested. The amendment of the growing medium with the hydrophilic gel potassium acrylate increased container capacity in  $S_2$ . At the end of the experiments,  $S_1$  had higher container capacity; however, air-filled porosity decreased in both growing media. The pattern of electrical conductivity was similar to that found for container capacity was found (Table 1).

The FW accumulated at the end of the experiments was higher in plants grown in  $S_1$  than in those grown in  $S_2$  (Fig. 1A). Control 50-cells plants grown in  $S_1$  showed the higher FW accumulation but no significant differences were found in plants grown in 288-cells-plug trays. Anyway, a positive relationship between shoot FW and root FW ( $r^2 = 0.688$ ) was found (Fig. 1B).

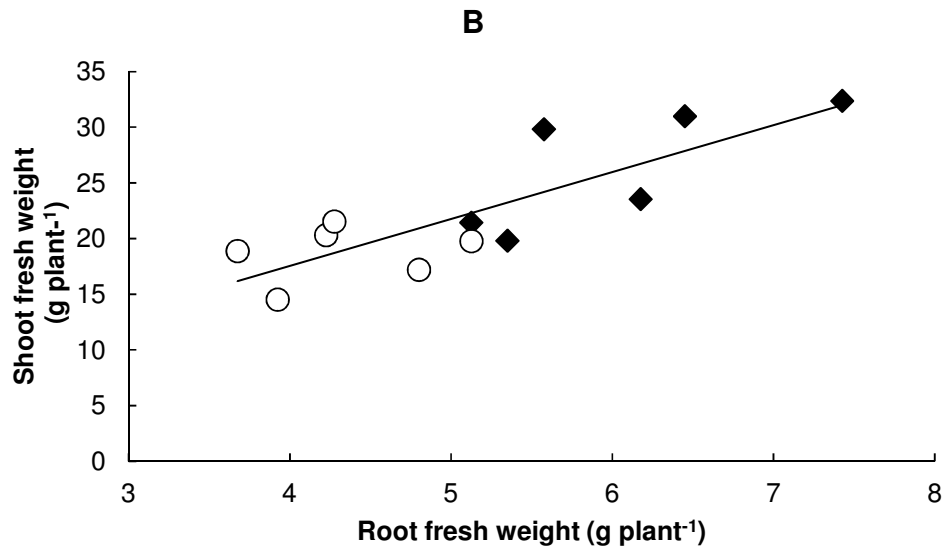
**Table 1. Changes in air-filled porosity (%), container capacity (%) and electric conductivity ( $dS m^{-1}$ ) at the transplant stage (initial) and at the end of the experiments (final) in the two cell size, the two growing media and three potassium acrylate concentrations used**

Treatments	Air-filled porosity (%)		Container capacity (%)		EC ( $dS m^{-1}$ )	
	Initial	Final	Initial	Final	Initial	Final
<b><math>S_1</math> 50-cells</b>						
0	30.00	29.52	53.00	54.08	0.055	0.041
1.5	28.80	21.09	56.35	58.19	0.086	0.046
3.0	10.60	17.47	51.29	70.33	0.083	0.061
<b><math>S_1</math> 288-cells</b>						
0	30.00	22.83	53.00	49.67	0.055	0.048
1.5	28.80	20.26	56.35	55.29	0.086	0.041
3.0	10.60	15.00	51.29	68.50	0.083	0.052
<b><math>S_2</math> 50-cells</b>						
0	19.80	17.25	51.40	60.00	0.030	0.038
1.5	15.20	13.33	69.49	40.88	0.038	0.033
3.0	11.20	8.04	87.40	48.66	0.052	0.033
<b><math>S_2</math> 288-cells</b>						
0	17.80	16.44	51.40	55.11	0.030	0.028
1.5	15.20	12.67	69.49	41.56	0.038	0.027
3.0	11.20	7.48	87.40	35.00	0.052	0.031



ANOVA	
Cell volume (A)	**
Growing medium (B)	***
A x B	**

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



**Fig. 1.** Mean fresh weight at the end of the experiments from roots, stems, leaves and flowers in *Impatiens wallerana* plants grown in two pre-transplant plug cell size (50- and 288-cells-plug trays<sup>-1</sup>), two post-transplant growing medium (S<sub>1</sub> and S<sub>2</sub>) and amended with three different potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>) (A) (n = 6). The standard errors over each bar and the significance of interactions (ANOVA) are indicated. Shoot-root fresh weight relationships are shown as well (B). The linear regression equation is 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  $P \leq 0.001$ . S<sub>1</sub>: ♦; S<sub>2</sub>: ○

Control plants showed no changes in RLAE, but significant differences in RLA and SLA related to the pre-transplant cell size or growing medium used. The amendment with potassium acrylate decreased both RLAE and RLA but increased SLA; the response depended on both, the cell size and the growing medium (Table 2).

The highest RGR control values were found in the pre-transplant plants grown in the 50-cells-plug trays. As the potassium acrylate concentrations in the growing medium increased, RGR decreased mainly because of a combination of lower NAR and higher LAR values (Table 3).

Glucose content changed in different plant organs (roots, stems and leaves) according to the treatment. Total glucose content of plants grown in 50-cell plants was higher and decreased with the increase in potassium acrylate concentration when S<sub>1</sub> was used (Fig. 3).

The allometric relationships between roots and shoots for *I. wallerana* plants showed higher photo-assimilate partitioning to shoot when the potassium acrylate concentration increased or the cell volume decreased in both growing media tested (Table 4).

**Table 2. Changes in RLAE, RLA and SLA in *Impatiens wallerana* plants grown in two pre-transplant plug cell size (50- and 288-cells-plug trays<sup>-1</sup>), two post-transplant growing medium (S<sub>1</sub> and S<sub>2</sub>) and amended with three different potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>) (A) (n = 120). Different lower-case letters indicate significant differences (P ≤ 0.05) between potassium acrylate concentrations for each growing medium while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes for each potassium acrylate concentration. The probability of the slope being zero was P ≤ 0.001 for all the growth parameters calculated**

Treatments	RLAE (cm <sup>2</sup> cm <sup>-2</sup> day <sup>-1</sup> )		RLA (leaves week <sup>-1</sup> )		SLA (cm <sup>2</sup> g <sup>-1</sup> )	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>
<b>50-cells</b>						
0	0.058 <sup>aA</sup>	0.057 <sup>aA</sup>	1.232 <sup>aA</sup>	1.258 <sup>aA</sup>	116.32 <sup>bA</sup>	115.24 <sup>cB</sup>
1.5	0.060 <sup>aA</sup>	0.057 <sup>aA</sup>	1.164 <sup>bB</sup>	1.236 <sup>bA</sup>	120.19 <sup>bA</sup>	149.74 <sup>bB</sup>
3.0	0.044 <sup>bA</sup>	0.045 <sup>bA</sup>	1.198 <sup>bA</sup>	1.216 <sup>cA</sup>	151.60 <sup>aA</sup>	160.31 <sup>aA</sup>
<b>288-cells</b>						
0	0.051 <sup>bB</sup>	0.051 <sup>aB</sup>	1.234 <sup>aA</sup>	1.019 <sup>aB</sup>	119.39 <sup>bA</sup>	162.06 <sup>aA</sup>
1.5	0.049 <sup>aB</sup>	0.046 <sup>bB</sup>	1.129 <sup>bA</sup>	1.017 <sup>aB</sup>	124.27 <sup>bA</sup>	161.23 <sup>aA</sup>
3.0	0.045 <sup>aA</sup>	0.041 <sup>bB</sup>	1.023 <sup>cB</sup>	1.022 <sup>aB</sup>	148.76 <sup>aA</sup>	161.73 <sup>aA</sup>

**Table 3. Changes in RGR, NAR and LAR estimated from *Impatiens wallerana* plants grown in two pre-transplant plug cell size (50- and 288-cells-plug trays<sup>-1</sup>), two post-transplant growing medium (S<sub>1</sub> and S<sub>2</sub>) amended with three different potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>) (A) (n = 120). Different lower-case letters indicate significant differences (P ≤ 0.05) between potassium acrylate concentrations for each growing medium while different capital letters indicate significant differences (P ≤ 0.05) between different pre-transplant cell volumes for each potassium acrylate concentration. The probability of the slope being zero was P ≤ 0.001 for all the growth parameters estimated**

Treatments	RGR (g g <sup>-1</sup> day <sup>-1</sup> )		NAR (g cm <sup>-2</sup> day <sup>-1</sup> ) (x 10 <sup>-5</sup> )		LAR (cm <sup>2</sup> g <sup>-1</sup> )	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>
<b>50-cells</b>						
0	0.0875 <sup>aA</sup>	0.0882 <sup>aC</sup>	59.51 <sup>aA</sup>	60.64 <sup>aB</sup>	147.04 <sup>aB</sup>	145.45 <sup>aA</sup>
1.5	0.0873 <sup>aA</sup>	0.0868 <sup>bB</sup>	61.50 <sup>aA</sup>	66.23 <sup>bA</sup>	141.95 <sup>aB</sup>	131.05 <sup>bB</sup>
3.0	0.0849 <sup>bA</sup>	0.0832 <sup>cA</sup>	49.17 <sup>bB</sup>	60.04 <sup>aA</sup>	172.65 <sup>aA</sup>	137.74 <sup>bB</sup>
<b>288-cells</b>						
0	0.0764 <sup>aB</sup>	0.0857 <sup>aC</sup>	57.26 <sup>aB</sup>	48.39 <sup>bA</sup>	133.42 <sup>bA</sup>	177.08 <sup>aB</sup>
1.5	0.0762 <sup>aB</sup>	0.0860 <sup>aA</sup>	45.71 <sup>aA</sup>	45.71 <sup>bB</sup>	127.57 <sup>bA</sup>	188.14 <sup>aA</sup>
3.0	0.0719 <sup>bB</sup>	0.0818 <sup>bB</sup>	54.59 <sup>aA</sup>	44.09 <sup>bA</sup>	131.72 <sup>bA</sup>	185.51 <sup>aA</sup>

**Table 4. Changes in allometric relationships between roots and shoots for *Imatiens wallerana* plants using a straight-line regression analysis between the natural logarithm of root and shoot dry weight.**

Treatments included two pre-transplant plug cell size (50- and 288-cells plugtrays<sup>-1</sup>), two post-transplant growing medium (S<sub>1</sub> and S<sub>2</sub>) and amended with three different potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>) (n = 120). The straight-line regression slopes ( $\beta$ ) and the coefficients of determination  $r^2$  are indicated. Different lower-case letters indicate significant differences ( $P \leq 0.05$ ) between potassium acrylate concentrations for each growing medium while different capital letters indicate significant differences ( $P \leq 0.05$ ) between different pre-transplant cell volumes for each potassium acrylate concentration. The probability of the slope being zero was  $P \leq 0.001$  for all growth parameters

Treatments	S <sub>1</sub>		S <sub>2</sub>	
	$\beta$	$r^2$	$\beta$	$r^2$
<b>50-cells</b>				
0	0.812 <sup>bB</sup>	0.875	0.895 <sup>cA</sup>	0.905
1.5	0.958 <sup>aA</sup>	0.891	0.942 <sup>bA</sup>	0.913
3.0	0.956 <sup>aA</sup>	0.921	0.991 <sup>aA</sup>	0.898
<b>288-cells</b>				
0	0.905 <sup>bA</sup>	0.961	0.886 <sup>cA</sup>	0.884
1.5	0.900 <sup>bB</sup>	0.923	0.924 <sup>bB</sup>	0.899
3.0	0.927 <sup>aB</sup>	0.928	0.983 <sup>aA</sup>	0.923

Positive relationships between RLA (Fig. 3A), RGR (Fig. 3B), NAR (Fig. 3C), glucose content (Fig. 3D),  $\beta$  partition coefficient (E) and root DW ( $r^2 = 0.605, 0.581, 0.636, 0.601, 0.754$  and  $0.813$  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 S<sub>1</sub>. A negative relationship between SLA and root DW was found as well ( $r^2 = 0.754$ ;  $P \leq 0.001$ ) (Fig. 3F).

#### 4. DISCUSSION

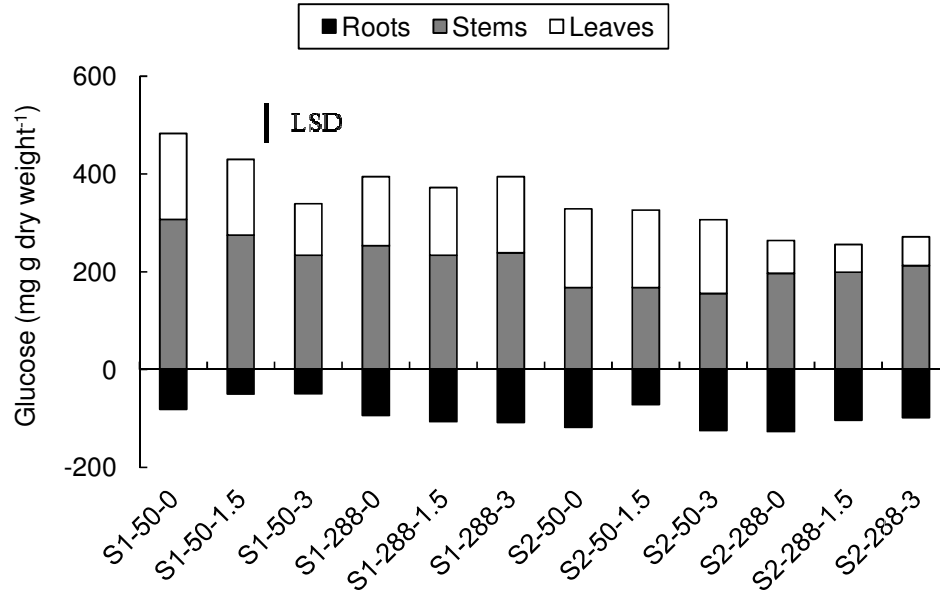
The potential for bedding pot plant quality and maximum longevity is determined during the production period. Decisions of bedding plant grower's such as the choice of the container cell size before transplanting or the post-transplant growing medium are frequently based on economics. When seedlings are grown in typical transplant containers, growth tends to be proportional to the volume of the container cell;

the more space available to the plant from the nursery, the larger it becomes after transplanting and the more quickly it attains a certain growth stages [10]. High quality growing media stimulate plant growth [13] but also increase costs [18].

The effects of container cell volume are currently studied using different plug tray, where those of the growing medium are studied through mixes of different organic and inorganic materials, which change their physical properties. For the first, in our present experiments, we included two plug cell trays, which represent limiting (288-cells-plug trays) and non-limiting (50-cells-plug trays) options for *I. wallerana* plants [10]. On the other hand, we used the hydrophilic gel potassium acrylate, which increases the water-holding capacity of the medium without modifying the proportion of the medium components. Our results showed that the higher the water-holding capacity due to the lower plug cell volume or potassium acrylate concentration, the lower the air-filled porosity of the medium (Table 1). A second expected result was that the effect of an increase in the water holding (because an increase in the potassium acrylate concentration e) on air-filled porosity was not the same in the two growing media tested (Table 1).

From a physiological point of view, both the container cell volume and the growing medium quality can be considered as abiotic stresses. Cramer et al. [19] defined abiotic stress as environmental conditions that reduce growth and yield below optimum levels. In a previous study, we have clearly shown that cell volume is an abiotic stress for *I. wallerana* bedding plants [4]. In the same way, we have also have indicated that growing quality would be considered as an abiotic stress for pansy bedding plants [13]. Bailey-Serres and Colmer [6] indicated that excess water results in complex changes in several environmental parameters, caused by impeded gas exchange. Soil waterlogging leads to hypoxia and progressively to anoxia and high CO<sub>2</sub> in the root zone. Fig. 1A shows that total FW decreased according to a decrease in cell volume and an increase in the water holding capacity of the growing medium. On the other hand, Fig. 1B shows that shoot FW of *I. wallerana* bedding plant was mainly determined by the root system size ( $r^2 = 0.699$ ) in agreement with previous reports [10,18].

The productivity of ornamental plants is closely associated with an increase in their total leaf area over time, which can be estimated through



**Fig. 2. Glucose content at the end of the experiments in different plant organs of *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cells-plug trays<sup>-1</sup>), two post-transplant growing media (S<sub>1</sub> and S<sub>2</sub>) and amended with three different potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>) (n = 6). Vertical lines indicate least significant differences (LSD)**

RLAE, and is the result of the product of the individual leaf area by the leaf number. On the other hand, the expected leaf number would be estimated through RLA, which is an estimation of the plastochron length, i.e. the time between successive leaf initiation events. An increase in oxygen deprivation decreased both RLAE and RLA (Table 2). In the present study, an increase in the plastochron would be associated with a lower increase in apex size [20], due to the presence of limiting sugar availability [21] or a change in the relative assimilation allocation between roots and shoots [22]. On the other hand, the capacity of leaf photo-assimilate acquisition is associated with leaf thickness and estimated through SLA. Our results showed an increase in SLA with an increase in water holding capacity (Table 2), that is, a decrease in leaf thickness. Voesenek and Sasidharan [7] indicated that flooding restricts gas diffusion underwater and that this hampers the gas exchange needed for the critical processes of photosynthesis and respiration.

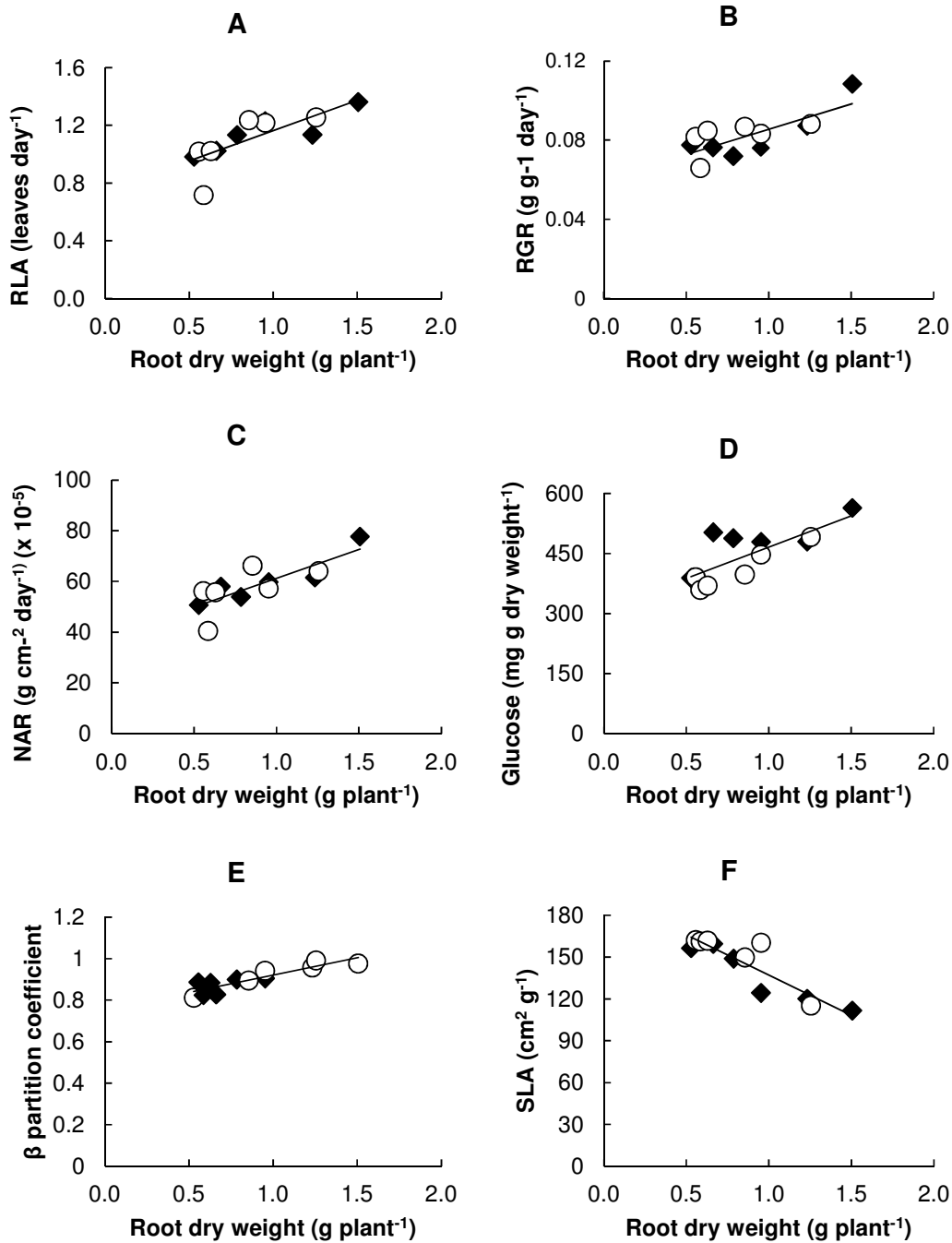
Since, there were no significant differences in DW content between control and treated plants (data not shown), it is possible to describe the photo-assimilate acquisition and partition rates on a dry weight basis. We found that the

decrease in oxygen availability in plants grown under limiting (288-plug-cells trays) or non-limiting (50-plug-cells-trays) cell volume decrease RGR (Table 3) and glucose content (above Fig. 2). The RGR results were explained through lower NAR and higher LAR values (Table 3).

Feller et al. [22] showed that for an optimal development of the plant as a whole, both root and shoot biomass must be balanced. Our results showed that the photo-assimilates partitioned favored shoots than roots under water holding increase (Table 4). Bailey-Serres and Chang [23] and Balderas-Hernandez et al. [24] indicated that aerial organs and roots respond differently to oxygen deprivation through changes in mobilization of carbohydrate reserves and long-distance signalling.

Ghosh and Xu [25] suggested that abiotic stress responses in plants occur at various organ levels among which root specific processes are of particular importance. Our results showed positive relationships between RLA, RGR, NAR, glucose content and  $\beta$  partitioning coefficients and root DW (Fig. A, B, C, D and E respectively), and a negative relationship between SLA and root DW (Fig. 3F). In previous reports from our





**Fig. 3. Relationships between RLA (A), RGR (B), NAR (C), glucose content (D),  $\beta$  partition coefficient (E), SLA (E) and root dry weight (RDW). Treatments included *Impatiens wallerana* plants from two pre-transplant plug cell size (50- and 288-cells-plug trays<sup>-1</sup>), two post-transplant growing medium (S<sub>1</sub> and S<sub>2</sub>) and three potassium acrylate concentrations (-0; -1.5 and -3.0 Kg m<sup>-3</sup>). Linear regression equations are, RLA = 0.42 RDW + 0.74 ( $r^2 = 0.605$ ;  $P \leq 0.001$ ); RGR = 0.0256 RDW + 0.06 ( $r^2 = 0.581$ ;  $P \leq 0.001$ ); NAR = 22.72 RDW + 38.57 ( $r^2 = 0.636$ ;  $P \leq 0.001$ ); glucose content = 156.77 RDW + 309.15 ( $r^2 = 0.601$ ;  $P \leq 0.001$ );  $\beta$  partition coefficient = 0.167 RDW + 0.754 ( $r^2 = 0.794$ ;  $P \leq 0.001$ ) and SLA = - 55.44 RDW + 194.50 ( $r^2 = 0.813$ ;  $P \leq 0.001$ ).**

S<sub>1</sub>: ♦; S<sub>2</sub>: ○

laboratory, we found similar changes in the plant growth parameters when plants were sprayed with increasing 6, benzyl aminopurine (BAP) concentrations both in ornamentals [10,26,27,28, 29, 30,13] and in vegetables [31,32,33].

Zwack and Rashotte [34] suggested that plants have evolved elaborate mechanisms to sense and respond to sub-optimal environmental conditions; while Bartoli et al. [35] pointed out that, the ability of plants to respond to an abiotic stress, involves the interaction with endogenous hormonal plant growth regulators. While O'Hare and Turnbull [36] showed that, an increase in root growth might lead to a corresponding increase in the synthesis of cytokinins, Thibaud et al. [11] showed that lower oxygen availability decrease both root length and root branch, and suggested that this would finally reduce cytokinin synthesis. Although, cytokinin is a hormone well known for its role in numerous aspects of growth and development, recent evidence indicates that cytokinin functions in stress responses as well. By contrast, interactions between cytokinin signalling, environmental stimuli and stress have only recently begun to be well characterized [37,38].

## 5. CONCLUSIONS

Our results showed that the response of *I. wallerana* plants to limiting cell volume and growing medium quality are associated with different physiological changes such as both FW and DW accumulation, leaf area expansion, photosynthetic fixation, carbohydrate availability and photo-assimilate partitioning. They showed that both pre-transplant plug cell volume and growing medium quality can be considered as an abiotic stress and indicate a feasible interaction between them. The hypothesis that endogenous cytokinins are involved in these responses is supported by the fact that the mentioned physiological changes are similar those found when ornamentals and vegetables were sprayed with exogenous cytokinins. However, validating the influence of cytokinins in cell volume- and quality medium-restricted plants requires another experimental design, although critical experiments are already in progress.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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