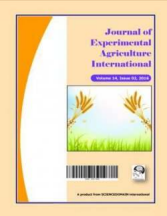

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Root Restriction Effects on the Bedding Pot Plant *Impatiens walleriana*

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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

Pot plant growers must take two technological critical decisions: the pre-transplant plug-cell size and the post-transplant growing medium, both of which have been mentioned as abiotic stress sources for bedding pot plants, including *Impatiens walleriana*. However, only a few recent reports in bedding pot plants have simultaneously included limiting and non-limiting plug cell volumes and growing medium. The aim of this work was to evaluate the response of a wide range of these two abiotic stress sources on *I. walleriana* plant growth on the assumption that the responses are mainly associated with a negative hormonal signaling from roots and that these can be overridden by a pre-transplant benzyl aminopurine (BAP) spray. The main result was that, in *I. walleriana* plants, the abiotic stresses imposed by the plug cell volume and growing medium quality constitute an interactive process associated with cytokinins synthesis. From a grower´s point of view, one expensive option to avoid root restriction is to increase the plug cell volume and the growing

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medium quality. In contrast, a single 100 mg l^{-1} BAP spray can partially override the root restriction symptoms.

Keywords: Bedding plants; cytokinin; growth.

1. INTRODUCTION

The use of transplants is the most reliable method to ensure adequate crop establishment of commercial plantings of most ornamental bedding crops. 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. 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.

The choice of a growing medium, along with that of the container type, is one of the critical decisions that growers must make when starting a pot plant production [1]. In this way, in agreement with recent publications of our laboratory [2,3,4], growers consider the container growth medium combination as a unit, and not as two independent factors.

The effects of the container volume during the propagation stage [5,6] and of the growing medium quality during bedding pot plant production have been previously studied [1,7,8, 9,2]. However, precise information on the effect of these two combined factors is lacking.

Impatiens walleriana (Hook.f.), also known as busy Lizzie (United Kingdom), balsam, sultan or simply impatiens, is a commercially important year-round garden crop for landscape, and the first best-selling bedding plants in both developed and undeveloped countries. Its productivity has been associated with aerial biomass expansion and shoot fresh weight, the latter being mainly determined by the root system size [5].

Root restriction is a physical stress imposed on a root system when plants are grown in small containers, which leads to a pronounced decrease in both root and shoot growth of the plants at the transplant stage. In previous studies on *I. walleriana* we have shown that the stress imposed to root growth during the propagation stage determine a lower increase in total dry weight after transplant [10].

Plant roots can sense adverse soil conditions and, via some internal signal, transmit the condition of the soil to extending leaves, with the typically net result of a decrease in leaf elongation rates [11]. It has been claimed that the close coordination between root and shoot growth is controlled by a signaling pathway which is largely hormonal [12,13], with a major site of control located in the root system, and which involve gene activation [14]. The understanding of the response of bedding pot plants to particular short- and long-term stresses is limited even further by interactions with other stresses such as the root restriction imposed by small plug cell trays or growing medium quality.

The aim of this work was to evaluate the physiological mechanisms involved when *I. walleriana* plants grown in four different pre-transplant cell volume and transplanted to four post-transplant growing media were sprayed with different benzyl aminopurine (BAP) concentrations.

2. MATERIALS AND METHODS

2.1 Plant Material

Two 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): one from October 10 th 2012 to January 24th 2013 and the other from October 16th 2013 to January 15th 2014.

I. walleriana 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown in 50-, 128-, 288- and 512-cell plug trays (55.70, 17.37, 6.18 and 2.50 cm^3 $cell^{-1}$ respectively) in Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany).

Leaves were sprayed at sunset with 0 or 100 mg l^{-1} BAP solutions when the first true leaf pair was developed. The BAP concentration was chosen from previous experiments performed in 2010 and 2011 [5].

When seedlings reached the transplant stage, they were transplanted into 1,200 cm^3 pots filled with four different growing media as follows:

1) Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany): Canadian *Sphagnum* peat moss-perlite-vermiculite (70/20/10 v/v/v) (**K**)
2) *Sphagnum maguellanicum*-perlite (80/20 v/v) (**S**)
3) River waste-perlite (80-20 v/v) (**R**)
4) *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) (**SR**) [15].

2.2 Cultivation and Meteorological Data

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity of 0.486 dS m^{-1}) using intermittent overhead mist. Growing media were weekly fertilized with 1.0:0.5: 1.0:0.5 (v/v/v/v) N: P: K: Ca through the overhead irrigation water according to Styer and Koranski [16] (Stage 2: 50 mg l^{-1} N; Stage 3-4: 100 mg l^{-1} N; pot: 150 mg l^{-1} N) was used.

Daily mean temperatures (18.27 to 25.18 ℃) and daily photosynthetic active radiation (4.24 to 6.14 mole 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 a HOBO H8 data logger. The plants were arranged at a density of 25 plants m^{-2}, which avoided mutual shading.

2.3 Sampling and Growth Evaluations

Samples of each substrate were collected at the beginning of the pot experiments (before transplant to 1,200-cm^3 pots) and total porosity, air-filled porosity, bulk density and container capacity were determined according to Fonteno [17]. Data are indicated in Table 1.

Plants were harvested at the transplant stage and at 15, 30, 45, and 60 days after transplanting. Roots were washed and root, stem and leaf 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 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_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.

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

Table 1. Physical properties for the growing media tested. K: [Canadian *Sphagnum* peat (70% + Perlite (20%) + Vermiculite (10%)], S: [*Sphagnum maguellanicum* (80%) + Perlite (20%)], R: [River waste (80%) + Perlite (20%)], SR: [*Sphagnum maguellanicum* (40%) + River waste (40%) + perlite (20%)]

Growing media	Total porosity (%)	Air-filled porosity (%)	Bulk density (g dm^{-3})	Container capacity (%)
F	60.00 ± 0.55	12.93 ± 0.98	0.21 ± 0.04	36.89 ± 1.4 6
S	70.67 ± 0.67	29.67 ± 2.15	0.15 ± 0.01	48.00 ± 0.3 8
R	72.67 ± 0.18	44.60 ± 0.95	0.18 ± 0.01	50.22 ± 0.4 4
SR	67.53 ± 0.64	23.27 ± 2.43	0.21 ± 0.01	42.67 ± 0. 38

Glucose content was analysed on each plant organ (roots, shoots and leaves) at the final sampling of the pot experiments using the Nelson-Somogyi method.

2.4 Statistical Analysis

The experimental design was a randomised factorial with three blocks of five single-pot replications of each treatment combination (plug cell volume × growing medium × BAP 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 the ANOVA were checked. Means were separated by Tukey's tests ($P \leq 0.05$). Slopes from straight-line regressions of RLA, RLAE, RGR, NAR, LAR and allometric values were tested using the SMATR package [18].

3. RESULTS

The greenhouse climatic control gives small variations in mean temperature and the fact that the experiment was repeated in the same season would explain the lack of main significant difference in the mean temperature and daily photosynthetic active radiation between the two experiments (data not shown). Thus, since there were no significant differences between both experiments, they were considered together.

3.1 Fresh Weight Accumulation

The FW 60 days after transplanting was higher in control plants from 50- or 128-cell-plug trays although the responses were also related to the growing medium used. The highest FW values were found in plants grown in **R** and **S**. A pre-transplant 100 mg l^{-1} BAP spray increased FW in most of the cell size-growing media combinations tested (Fig. 1A). Main effects (plug cell volume, growing medium and BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for total FW in the ANOVA showed highly significant ($P \leq 0.001$) differences (data not shown). When the mean aerial FW was plotted against the mean root FW (Fig. 1B), a positive correlation was found ($r^2 = 0.647$; $P < 0.001$). Although there was an effect related to the pre-transplant plug cell volume and the growing medium used, a pre-transplant 100 mg l^{-1} spray gave the highest values.

3.2 Leaf Growth and Plant Growth Analysis

Total leaf area at the end of the experiments was higher in control plants from 50- or 128-cell-plug trays, although **R** concentrated the highest values. Although a pre-transplant 100 mg l^{-1} BAP increased the leaf area, the higher responses were found in **SR** and **F**, and in plants propagated in 512- and 288-cell-plug trays^{-1} (Fig. 2A). The mean leaf area of each leaf showed higher differences related to the growing medium than to the pre-transplant plug cell volume or BAP spray (Fig. 2B), but did not entirely explain the highest differences found in total leaf area. Main effects (plug cell volume, growing medium and BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for total leaf area in the ANOVA showed highly significant ($P \leq 0.001$) differences (data not shown).

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

RLA decreased in control plants according to a decrease in the pre-transplant plug cell size, but there were significant differences between the post-transplant growing medium used. A pre-transplant BAP spray increased RLA except in plants propagated in 288- and 512- cell-plug trays^{-1} and in those grown in **SR** (Table 2). Main effects (plug cell volume, growing medium and BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for RLA in the ANOVA showed highly significant ($P \leq 0.001$) differences (data not shown).

Control plants showed higher RLAE, RGR and NAR values and lower LAR values related to a decrease in the pre-transplant plug cell size; **R** and **SR** showed higher differences as well. A pre-transplant 100 mg l^{-1} BAP application significantly increased RLAE, RGR and NAR as compared to the control plants (Table 2). Main effects (plug cell volume, growing medium and

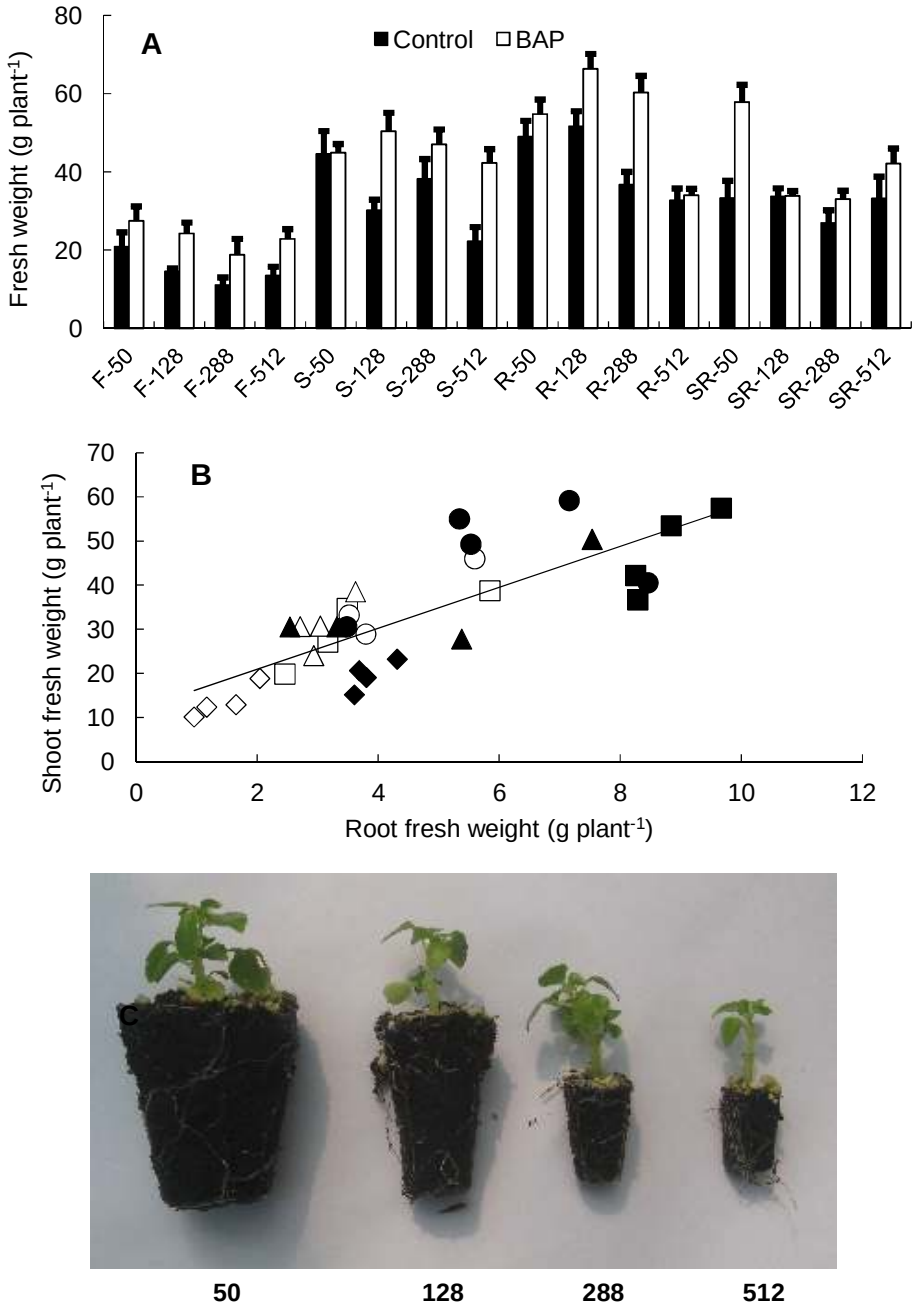

Fig. 1. Panel A. total fresh weight (n = 30) at the end of the experiment (60 days from transplanting) for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants) or 100 mg l^{-1} BAP at the pre-transplant stage and four growing media at the post-transplant stage. Bars indicate standard errors. Panel B. Relationships between shoot and root fresh weight according to four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants, empty symbols) or 100 mg l^{-1} BAP (full symbols) at the pre-transplant stage and four growing media at the post-transplant stage. For substrate abbreviations see Table 1. F: ♦-◊; R: ●-○; S: ▲-Δ; SR: ■-□. The straight-line regression was: Shoot fresh weight = 4.64 Root fresh weight + 11.67 (r^2 = 0.648). The probability of the slope being zero was P ≤ 0.001. Panel C. control seedlings at the transplant stage

BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for all growth parameters in the ANOVA showed highly significant ($P \leq 0.001$) differences (data not shown).

Total glucose content in untreated plants showed no significant differences between plug cell volumes at the end of the experiments. However, values changed depending on the growing medium tested (Fig. 3A). A single 100 mg l^{-1} BAP spray changed total glucose content in plants grown in different plug cell volumes but increased the differences between the growing media. The highest total glucose content was found in **R** (Fig. 3B).

3.3 Photoassimilate Partitioning

When the photo-assimilate partitioning was characterized using root-shoot allometries, control plants from 50-cell-plug tray^{-1} showed almost the same partitioning from roots than from shoots with differences related to the growing medium used. A higher root partition was observed according to an increase in plug number tray^{-1}. When plants were sprayed with a single 100 mg l^{-1} BAP at pre-transplant minor differences were found from 50-cell-plug tray^{-1} but a partition change towards the shoots was

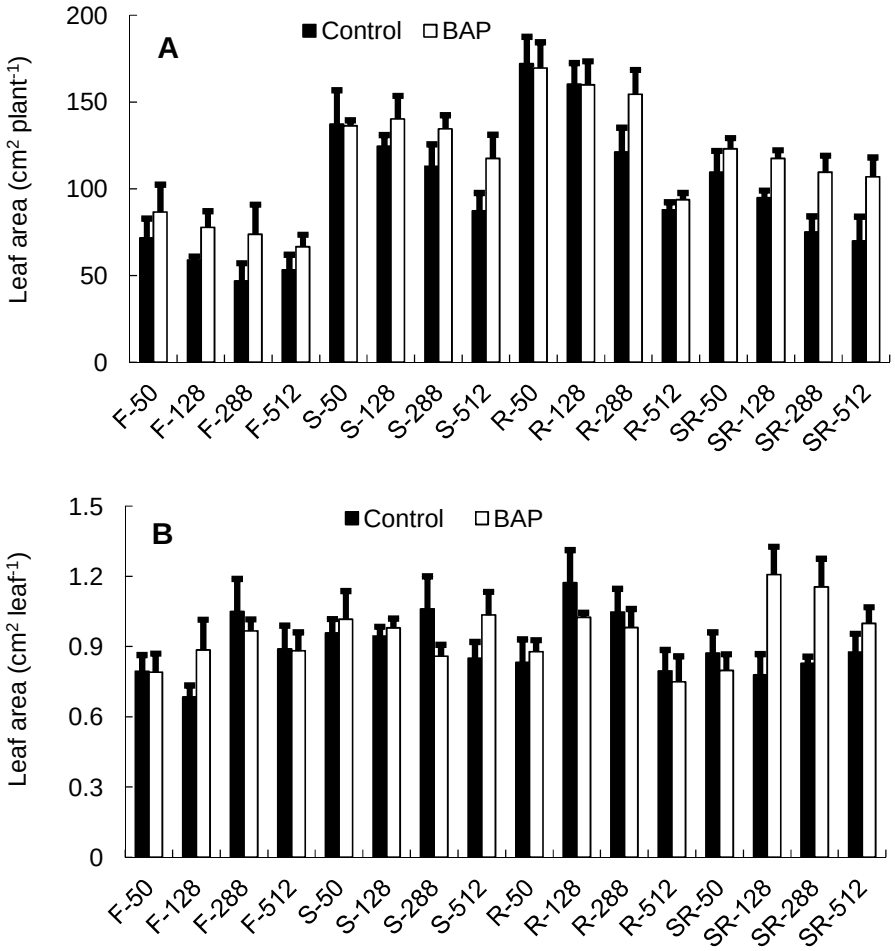

Fig. 2. Total leaf area (A) (n = 30) and individual leaf area (B) (n = 30) at the end of the experiment for *Impatiens walleriana* seedlings grown in four plug-cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants) or 100 mg l^{-1} BAP at the pre-transplant stage and four growing media at the post-transplant stage. For substrate abbreviations see Table 1. Bars indicate standard errors

Table 2. Changes in RLA, RLAE, RGR, NAR and LAR for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants) or 100 mg l^{-1} BAP at the pre-transplant stage and four growing media at the post-transplant stage. For substrate abbreviations see Table 1. Different lowercase letters indicate significant differences (P < 0.05) between control and BAP-sprayed plants, while different capital letters indicate significant differences (P < 0.05) between different substrates for each plant cell size. The probability of the slope being zero for RLA, RLAE, RGR, NAR and LAR was P ≤ 0.001

	RLA (leaves week^{-1})		RLAE (cm^2 cm^{-2} day^{-1})		RGR (g g^{-1} day^{-1})		NAR (g cm^{-2} day^{-1}) (x 10^{-5})		LAR (cm^2 g^{-1})	
	Control	**BAP**	**Control**	**BAP**	**Control**	**BAP**	**Control**	**BAP**	**Control**	**BAP**
F										
50	0.512^{bD}	0.625^{aD}	0.0286^{bC}	0.0333^{aB}	0.0299^{bC}	0.0342^{aC}	41.42^{bC}	53.30^{aC}	72.19^{aA}	64.16^{bA}
128	0.449^{bD}	0.581^{aD}	0.0282^{bB}	0.0337^{aC}	0.0309^{bC}	0.0325^{aB}	41.42^{bB}	55.94^{aB}	74.60^{aB}	58.10^{bB}
288	0.340^{bD}	0.406^{aD}	0.0231^{bC}	0.0279^{aC}	0.0275^{bC}	0.0318^{aC}	32.43^{bB}	44.29^{aC}	84.80^{aA}	71.80^{bA}
512	0.291^{bD}	0.414^{aD}	0.0223^{aC}	0.0244^{aC}	0.0259^{bD}	0.0282^{aC}	37.38^{aB}	40.78^{aB}	69.28^{aB}	69.16^{aB}
S										
50	0.859^{bC}	0.882^{aC}	0.0363^{aB}	0.0377^{aA}	0.0375^{bB}	0.0417^{aB}	59.57^{bA}	73.52^{aB}	62.95^{aB}	56.72^{aA}
128	0.843^{aB}	0.817^{bC}	0.0371^{bA}	0.0406^{aA}	0.0410^{aA}	0.0396^{aA}	49.54^{bA}	56.28^{aB}	82.76^{aA}	70.36^{bA}
288	0.838^{aA}	0.793^{bC}	0.0317^{bA}	0.0333^{aA}	0.0352^{bB}	0.0374^{aB}	48.36^{aA}	52.83^{aB}	72.78^{aB}	70.79^{aA}
512	0.604^{bB}	0.696^{aC}	0.0280^{aA}	0.0287^{aB}	0.0345^{aB}	0.0320^{bB}	42.41^{aB}	42.42^{aB}	81.35^{aA}	75.43^{bA}
R										
50	1.000^{bA}	1.116^{aB}	0.0367^{bB}	0.0381^{aA}	0.0438^{bA}	0.0450^{aA}	66.55^{bA}	84.85^{aA}	65.82^{aA}	53.03^{bB}
128	0.902^{bA}	0.974^{aA}	0.0369^{bA}	0.0423^{aA}	0.0434^{aA}	0.0414^{bA}	49.57^{bA}	67.25^{aA}	87.55^{aA}	61.56^{bB}
288	0.788^{bB}	0.978^{aA}	0.0340^{aA}	0.0344^{aA}	0.0407^{aA}	0.0410^{aA}	52.27^{bA}	60.61^{aA}	77.87^{aB}	67.65^{bA}
512	0.764^{bA}	0.865^{aA}	0.0280^{bA}	0.0307^{aA}	0.0377^{aA}	0.0352^{bA}	53.98^{aA}	49.30^{aA}	69.84^{aB}	71.39^{aA}
SR										
50	0.949^{bB}	1.137^{aA}	0.0400^{aA}	0.0370^{bA}	0.0418^{bA}	0.0430^{aA}	53.77^{bB}	81.63^{aA}	77.73^{aA}	52.68^{bB}
128	0.704^{bC}	0.859^{aB}	0.0352^{bA}	0.0373^{aB}	0.0365^{bB}	0.0404^{aA}	53.30^{bA}	78.62^{aA}	68.48^{aB}	51.39^{bC}
288	0.549^{bC}	0.805^{aB}	0.0296^{bB}	0.0320^{aB}	0.0359^{bB}	0.0373^{aB}	53.33^{bA}	67.96^{aA}	67.32^{aC}	54.88^{bB}
512	0.543^{bC}	0.746^{aB}	0.0258^{bB}	0.0281^{aB}	0.0303^{bC}	0.0336^{aA}	37.99^{aB}	42.67^{aB}	88.44^{aA}	71.01^{bA}

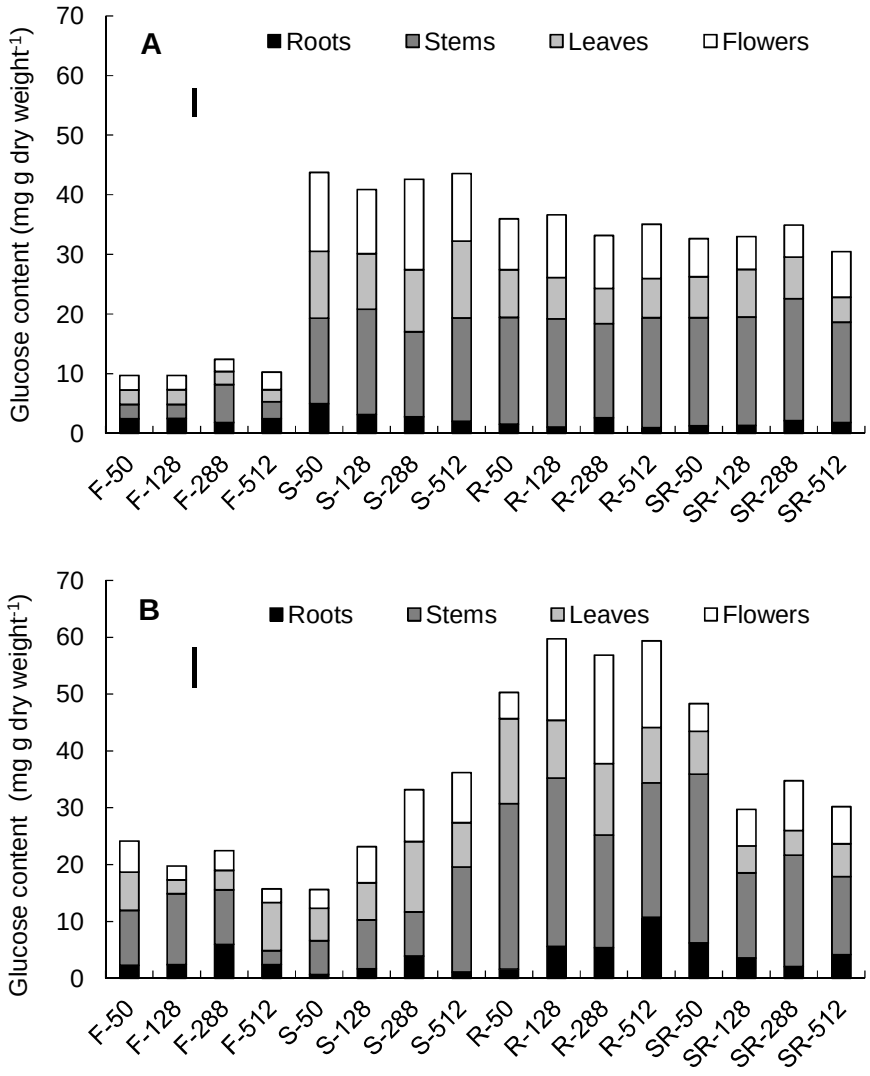

Fig. 3. Total glucose content at the end of the experiments for *Impatiens walleriana* seedlings grown in four plug-cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants) (A) or 100 mg l^{-1} BAP (B) at the pre-transplant stage and four growing media at the post-transplant stage. (n = 6). For substrate abbreviations see Table 1. The vertical line indicates least significant differences (LSD)

found in 128-, 288- and 512-cell-plug tray^{-1} (Table 3). Main effects (plug cell volume, growing medium and BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for root-shoot partitioning in the ANOVA showed highly significant ($P \leq 0.001$) differences (data not shown).

The stem-leaf allometries in control plants showed higher photo-assimilate partitioning toward the stems for most of the growing media tested. Stem partitioning was also higher according to a decrease in plug cell number tray^{-1}. When plants were sprayed with a single 100 mg l^{-1} BAP at the pre-transplant stage, a partition change towards the stems was observed in plants from 50- cell-plug tray^{-1} but a higher photo- assimilate partitioning to leaves was found in 128-, 288- and 512-cell-plug tray^{-1} (Table 3). Main effects (plug cell volume, growing medium and BAP application) and the interactions (plug cell volume × growing medium; plug cell volume × BAP

Table 3. Changes in allometric relationships between roots and shoots and between stem and leaves for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants) or 100 mg l^{-1} BAP at the pre-transplant stage and four growing media at the post-transplant stage. For substrate abbreviations see Table 1. The slope straight-line (β) and the coefficients of determination (r^2) are indicated. The probability of the slope being zero was P ≤ 0.001. Different lowercase letters indicate significant differences (P < 0.05) between control and BAP-sprayed plants, while different capital letters indicate significant differences (P < 0.05) between different substrates for each plant cell size

	Roots vs. shoots				Stem vs. leaves			
	Control		BAP		Control		BAP	
	β	r^2	β	r^2	β	r^2	β	r^2
F								
50	1.031^{aA}	0.878	0.842^{bB}	0.859	0.762^{aA}	0.899	0.683^{bB}	0.851
128	1.106^{aA}	0.895	0.884^{bB}	0.917	0.811^{aA}	0.905	0.741^{bB}	0.944
288	1.160^{aA}	0.862	0.985^{bA}	0.899	0.925^{aA}	0.946	0.803^{bB}	0.960
512	1.188^{aA}	0.899	0.993^{bB}	0.890	0.943^{aA}	0.967	0.859^{bB}	0.923
S								
50	1.004^{aB}	0.859	0.875^{bB}	0.833	0.731^{aA}	0.835	0.735^{aA}	0.841
128	1.117^{aA}	0.872	0.869^{bB}	0.949	0.840^{aA}	0.972	0.806^{bA}	0.955
288	1.117^{aB}	0.937	0.907^{bB}	0.953	0.900^{aA}	0.916	0.835^{bB}	0.962
512	1.165^{aA}	0.733	0.992^{bB}	0.968	0.976^{aA}	0.963	0.927^{bA}	0.934
R								
50	1.032^{aA}	0.933	1.013^{bA}	0.941	0.773^{aA}	0.909	0.715^{bA}	0.909
128	1.040^{aB}	0.927	1.006^{bA}	0.956	0.819^{aA}	0.978	0.808^{aA}	0.947
288	1.182^{aA}	0.889	1.096^{bA}	0.972	0.910^{aA}	0.962	0.875^{bA}	0.954
512	1.164^{aA}	0.922	0.911^{bB}	0.965	0.866^{aB}	0.952	0.859^{a}	0.941
SR								
50	1.030^{aA}	0.722	0.993^{bA}	0.931	0.786^{aA}	0.906	0.699^{bB}	0.921
128	1.037^{aB}	0.931	1.009^{bA}	0.792	0.810^{aA}	0.946	0.806^{aA}	0.932
288	1.065^{aA}	0.893	0.974^{bA}	0.831	0.927^{aA}	0.958	0.905^{aAA}	0.855
512	1.086^{aB}	0.963	1.034^{bA}	0.879	0.901^{aB}	0.952	0.924^{aA}	0.959

application; growing medium × BAP application; plug cell volume × growing medium × BAP application) for stem-leaf partitioning in the ANOVA showed highly significant (P ≤ 0.001) differences (data not shown).

3.4 Relationships between Growth Parameters and Root Dry Weight

Positive relationships between RLAE (r^2 = 0.600 P < 0.001) (Fig. 4A), RLA (r^2 = 0.679 P < 0.001) (Fig. 4B), RGR (r^2 = 0.622 P < 0.001) (Fig. 4C), NAR (r^2 = 0.625 P < 0.001) (Fig. 4D), total glucose content (r^2 = 0.668 P < 0.001) (Fig. 4E) and root DW were found. The highest values were those from plants sprayed with BAP, with significant influence of the growing medium used.

4. DISCUSSION

Expansion of the bedding plant industry implies the possibility to offer the best plants at the lowest price. To reach this objective, growers must make two technological critical decisions: the pre-transplant plug-cell size and the post-transplant growing medium, both of which have been previously mentioned as abiotic stress sources for bedding pot plants, including *I. walleriana* [1,7].

Roche et al. [19] showed that the DW content appears to be the best leaf trait to be quantified for plant functional screening. However, the quality of ornamental plants can be appraised with several types of criteria, such as tolerance to biotic and abiotic stresses, development potentialities and aesthetics [20].

Growing plants in containers alters their root growth and function. The effects of substrate aeration and water holding capacity interact with different pot characteristics, resulting in changes in root morphology [21]. However, only a few recent reports have simultaneously studied limiting and non-limiting plug cell volume and growing media [2,3,4].

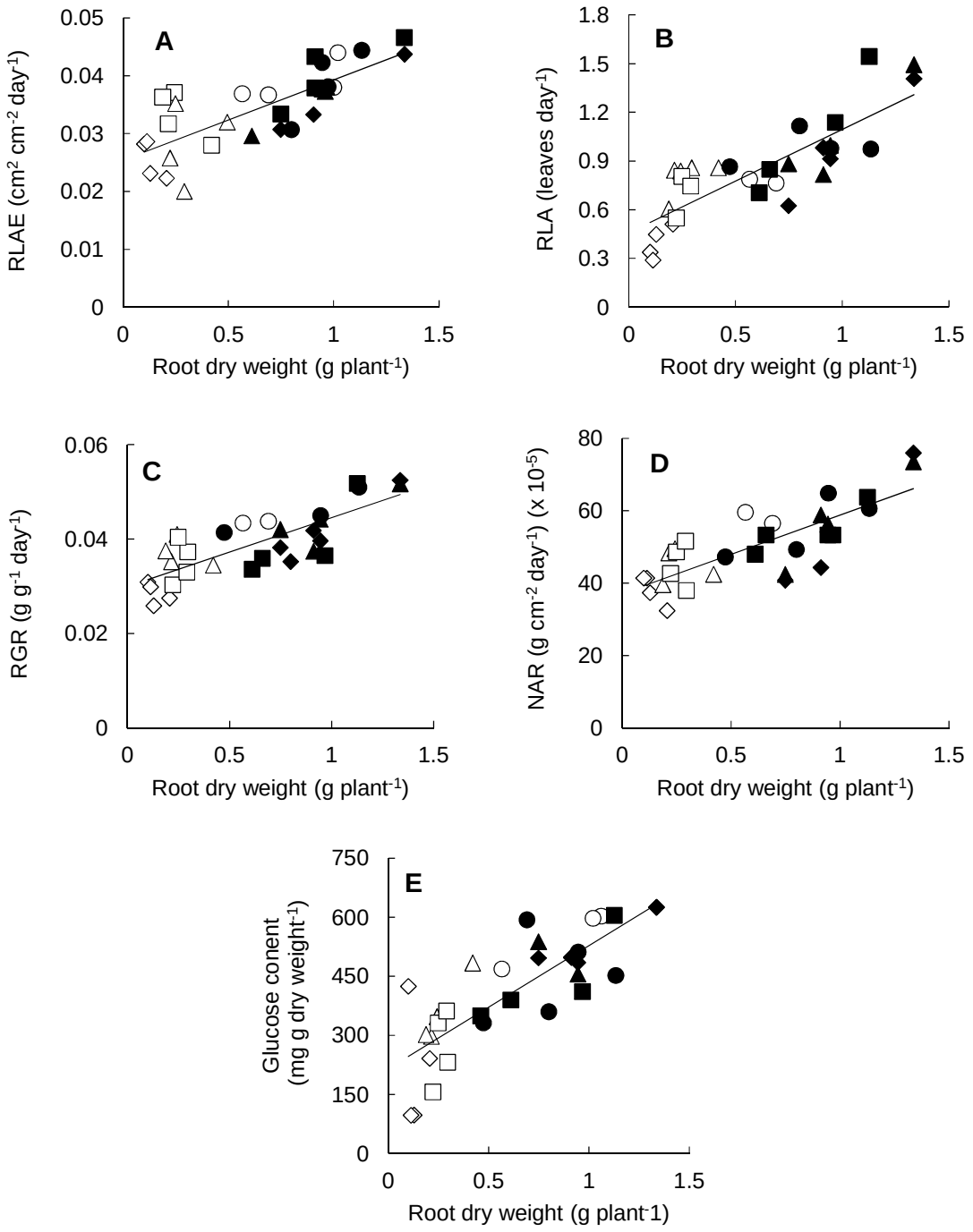

Fig. 4. Relationship between RLAE (A), RLA (B), RGR (C), NAR (D) and glucose content (E) (n = 30) in plants of *Impatiens walleriana* grown in four plug-cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}), sprayed with zero (control plants, empty symbols) or 100 mg l^{-1} BAP (full symbols) at the pre-transplant stage and four growing media at the post-transplant stage. The straight-line regressions were RLAE = 0.014 root DW + 0.025 (r^2 = 0.600 P < 0.001), RLA = 0.637 root DW + 0.46 (r^2 = 0.679 P < 0.001), RGR = 0.015 root DW + 0.03 (r^2 = 0.622 P < 0.001). NAR = 21.74 root DW + 37.1 (r^2 = 0.625 P < 0.001), glucose content = 318.78 root DW + 215.13 (r^2 = 0.668 P < 0.001). F: ♦-◊; R: ●-○; S: ▲-Δ; SR: ■-□

The hypothesis tested in this work was that the negative effects of these combined abiotic stress sources (pre-transplant cell volume and post-transplant growing medium quality) on *I. walleriana* growth and yield are mainly associated with a decrease in the hormonal signaling from roots, which can be overridden by a pre-transplant BAP spray.

Our results showed that the higher the cell-plug size the higher the total *I. walleriana* FW accumulation (Fig. 1A), which is in agreement with our previous results in ornamentals [5,6,10], and vegetables [22]. Although total FW decrease in limiting plug-cell sizes, the response was not the same in all the growing media tested, which indicate a clear relationship between them.

Kawasaki et al. [23] indicated that oxygen availability is an important factor in photosynthesis, photorespiration, and respiration and suggested that long-term hypoxia treatment affects leaf expansion and leaf nitrogen and carbon contents. Data from Table 1 show that the main differences between the growing media tested are related to air-filled porosity.

On the other hand, we found a positive relationship between the aerial FW and the root FW, in agreement with our previous results [5]. We have indicated that both cytokinins (in a direct way) and auxins (indirectly through a stimulation of root branching) increase plant growth in ornamental foliage plants [24,25,26].

Bartoli et al. [12] suggested that the ability of plants to respond to a wide range of environmental stresses is highly flexible and finely balanced through the interaction of hormonal plant growth regulators. Rahayu et al. [27] hypothesised that a decrease in cytokinin supply from the root to the shoot may inhibit leaf growth while a low cytokinin content would promote root growth and thus the root-shoot ratio. Alternatively, cytokinins seem better candidates to explain shoot growth impairment and changes in biomass partitioning [25,28].

Endogenous cytokinins synthesised in roots are transported into shoots via the xylem [29] but controlled both by environmental and endogenous signals [30]. Although Kotov and Kotova [31] indicated that the higher the root system the higher the cytokinin-ribosides synthesised, not all the numerous zeatin riboside isomers show the same biological activity [32]. Because the biological activity of all cytokinin-like compounds is not uniform, it normally depends on several structural aspects [33]. In the present study, when the root system increased, positive relationships with RLAE (Fig. 4A), RLA (Fig. 4B), RGR (Fig. 4C), NAR (Fig. 4D) and glucose content (Fig. 4E) were found.

The primary shoot apical meristem is responsible for generating all aboveground organs [34] and is controlled by hormones, which regulate biosynthesis and transport of other hormones, and by hormone interactions. The latter regulate particular transcription factors, which integrate and coordinate the developmental response. Several hormones, including auxins, citokinins, and gibberellins, act both independently and in combination to regulate meristem function [24, 25,26,35]. The main function of endogenous cytokinins is to control the cell cycle and shoot apical meristem growth [36,37].

The role of cytokinins has been inferred mostly from the effects of cytokinin application, in other words, from the effects of extra cytokinins added to internal cytokinins [38]. Cytokinins represent a class of phytohormones, which are key players not only in many processes important for plant growth and development, but also in the response to changes in their environment [39]. Holst et al. [40] have indicated that although cytokinins are essential for cell proliferation in the shoot apical meristem, the cytokinin-mediated control of shoot development and growth is not complete.

We can estimate this process through the plastochron (i.e. the time between successive leaf initiation events). In the present study, we found that RLA decreased as the plug-cell volume decreased but the changes were different in most of the growing media tested. When plants were sprayed with a single 100 mg l^{-1} BAP, the negative effects of the 'root restriction' response were partially overridden (Table 2). We have recently published similar results both in ornamentals [24,41] and in vegetables [22,42,43]. The total leaf area expanded by *I. walleriana* plants (Fig. 2A) is the result of RLA and the individual leaf area (Fig. 2B).

Gonzalez et al. [44] claimed that more research is needed to establish whether a causal relationship exists between shoot apical meristem size and leaf size. Our results showed that the plug-cell volume would have a higher

limiting effect on RLA than on individual leaf area. A larger or a smaller shoot apical meristem size is associated with a shorter or prolonged plastochron respectively. A decrease in the plastochron needs an increase in apex size [45], the presence of non-limiting sugar availability [46], and the regulation of relative assimilate allocation [47]. Hepworth and Lenhard [48] indicated that the final size of plant organs must be regulated in response to the developmental stage and the environment to optimally exploit the plant's surroundings.

Although meristematic activities and cell elongation are two crucial factors determining the rate of vegetative growth, the ultimate biomass production of a particular plant is greatly influenced by the efficiency of photosynthesis, which supplies raw materials for vegetative growth [49]. Poorter et al. [50] pointed out that reduced growth in smaller pots is caused mainly by a reduction in photosynthesis per unit leaf area. The increase in NAR when the plug cell volume increased, the quality of the growing medium changed, or a BAP spray was used is in agreement with that observed by these authors (Table 2).

Yang and Midmore [51] indicated that plants preferentially allocate biomass to acquire the resource that most limits growth and that they are capable of modifying their resource allocation to favour the growth of their growing parts. In this sense, Kobe et al. [52] indicated that plant interactions with the biotic and abiotic environment enhanced the carbon balance. In agreement with these authors, in the present study, untreated *I. walleriana* plants showed a higher photo-assimilate partitioning towards roots as the plug-cell volume or growing medium quality decreased. On the other hand, a single 100 mg l^{-1} BAP spray increased the photo-assimilate partitioning towards shoots (Table 3). When the stem-leaves allometries were analyzed, we found a partition towards leaves in control plants and an increase in stem partitioning in BAP-treated ones (Table 3). Benincasa et al. [53] indicated that crop management and environmental factors modify the source-sink relationships, including abiotic and biotic stresses.

In the present study, we found significant changes in total glucose content but related to the quality of the different growing media in control plants (Fig. 3A). Although plants sprayed with a single 100 mg l^{-1} BAP showed a significant increase in glucose content, significant differences were found only between the four growing media tested (and not between the different pre-transplant plug-cell volumes) (Fig. 3B). Biswall et al. [54] indicated that, in addition to its role as a major source of energy, sugar acts as a signalling molecule. However, cellular sugar signaling must be integrated with other growth regulatory pathways such as phytohormone signaling [55,56]. Sugar production in different environmental conditions determine the growth and stress response. Environmental stress can modulate the regulatory processes and results in metabolic changes at the cellular and the whole-plant level, specifically during source-sink transition [57,58].

Cramer et al. [59] defined abiotic stress as an environmental condition that reduces growth and yield below optimal levels. Puig et al. [60] and Chen et al. [61] concluded that plants can sense the volume of the rooting space available, and a limited number of studies on individual roots have shown that plant roots may sense the identity of neighbouring roots and respond accordingly [62, 63]. Abiotic stresses caused by these conditions trigger a wide range of local and long-distance signals such as cytokinins [64], which must be coordinated and integrated into whole-plant processes [13].

5. CONCLUSIONS

The root restriction associated with plug-cell volume has been previously documented in bedding pot plants. Our previous results have shown that the quality of the growing medium can be considered as an abiotic stress and indicated a feasible interaction between plug cell volume and growing medium quality. Our present results are in agreement with this previous information but also indicate that the impact of both stress sources is not precisely the same.

From a grower´s point of view, limiting root restriction is essential for crop productivity. Because our results in *I. walleriana* plants showed that the abiotic stresses imposed by the plug-cell volume and growing medium quality constitute an interactive process associated with the cytokinins synthesis, one expensive option is to increase the plug-cell volume and growing medium quality. In contrast, a single 100 mg l^{-1} BAP spray can partially override root restriction.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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