

2 **Growing media quality and plug cell volume**
3 **would be interactive abiotic stresses for**
4 ***Impatiens walleriana* pot yield**

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8 **ABSTRACT**

9 Higher bedding plant yield per unit greenhouse area was reaching through two grower
10 currently decision-making: plug cell volume during nursery and growing media quality for
11 both nursery and pot cycle. With the goal of maximize bedding plant yield to identify the
12 main limiting factor at the pot stage, we evaluated *I. walleriana* yield at the end of the pot
13 growth stage when four different pre-transplant cell volume and four pre or post-transplant
growing media with different physical properties were used. The hypothesis tested was that
only one of the potentially negative stress source (pre-transplant cell volume or growing
medium quality) is the main responsible for decreasing biomass accumulation at the post-
transplant pot growing cycle. The main result was that, in *I. walleriana* seedlings, the
combining abiotic stresses imposed by both the growing medium quality and nursery plug
cell volume define biomass accumulation (on a fresh and dry base), leaf area expanded and
photo assimilates partitioned as opposed to a previous report, which indicate that that
growing media quality would be a more limited factor than plug cell volume for *I. walleriana*
seedlings during nursery.

14 *Keywords: abiotic stress, aesthetic traits, bedding plants, growth parameters.*

15 **1. INTRODUCTION**

16
17 Bedding plant industry has been exponentially expanded around the world according to
18 significant costs decrease. The last has been related to higher bedding pot plant yield per unit
19 greenhouse area through two grower currently decision-making: plug cell volume during nursery
20 and growing media quality for both nursery and pot cycle. Commercial profits has been related
21 to a decrease in plug cell volume [1] and the use of lower expensive growing media [2,3,4].
22 However, these business choices imply that plants will suffer different root restriction stresses
23 during most growing cycle.

24
25 Usually, the 'root restriction syndrome' has been defined as a physical stress imposed on a root
26 system when plants are grown in small containers, which leads to a pronounced decrease in
27 both root and shoot growth at the transplant stage. The pre- and post-transplant effects of the
28 container volume during nursery [5,6] have been extensively studied by our laboratory and we
29 found that growth restriction would be closely related to endogenous cytokinin synthesis by
30 roots. A limited plug cell volume gives a vertical root restriction when root apical meristem come
31 to the bottom of the cell or the pot. At this moment, both primary root growth and root branching
32 decrease [7] and cytokinins, the main endogenous hormone synthesized by root apical
33 meristems would decrease as well [8,9].
34

35 On the other hand, a growing media quality decrease change their physical properties [3,10],
36 which generally result in decreased pore sizes, and would be taken into account as an abiotic
37 stress [4]. As pore size decreases, total porosity and air-filled porosity decrease as well. Bailey-
38 Serres and Colmer [11] and Voesenek and Sasidharan [12] have indicated that a lack of oxygen
39 inhibits respiration, decrease metabolic plant adaptations to cope with the hypoxic and anoxic
40 conditions and resulting energy deficits, as well as change anatomical and morphological
41 adaptations to improve internal O₂ supply. These metabolic changes would give the same
42 effects on endogenous cytokinin synthesis as plug cell volume [13,14].

43
44 With the goal of maximize bedding plant yield to identify the main limiting factor it is imperative.
45 Previous reports in ornamentals [15,16,17] and vegetables [18,19,20,21] have shown that
46 nursery growth has a significant effect on post-transplant biomass accumulation. The precise
47 effects of combined plug cell volume and growing media quality on nursery has been recently
48 indicated as well [22,23,24]. However, the simultaneously post-transplant interactions between
49 these two stress sources during both nursery and pot growth is lacking.

50
51 *Impatiens walleriana* (Hook.f.) is a commercially important year-round garden crop for
52 landscape, and the first best-selling bedding plants in both developed and undeveloped
53 countries. Most *Impatiens* genotypes produce a compact green foliage and covers itself through
54 extremely uniform growth habit with bright blooms. *Impatiens* F₁ genotypes prefer partial
55 sun/shade (8-25 mol photons m⁻² day⁻¹) [25]. Dry mass and flowering increase from 14 to 28°C
56 [26]. Plants only grow well with 100% evapotranspiration [27]. *I walleriana* has been included
57 in most research from our laboratory for the last decade.

58
59 The aim of this work was to evaluate *I. walleriana* yield at the end of the pot growth stage when
60 four different pre-transplant cell volume and four pre or post-transplant growing media with
61 different physical properties were used. The hypothesis tested was that only one of the
62 potentially negative stress source (pre-transplant cell volume or growing medium quality) is the
63 main responsible for decreasing biomass accumulation at the post-transplant pot growing cycle.

64 65 66 **2. MATERIALS AND METHODS**

67 **2.1 Plant Material**

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69 Experiment were carried out under a greenhouse at the Faculty of Agronomy, University of
70 Buenos Aires, Argentina (34° 35' 59"S, 58° 22' 23"W) from October 10th 2012 to December 9th
71 2013 and repeated from October 16th 2013 to December 15th 2014.

72
73 *I. walleriana* 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown in
74 50-, 128-, 288- and 512-cell plug tray⁻¹ (55.70, 17.37, 6.18 and 2.50 cm³ cell⁻¹ respectively) in
75 four different pre-transplant growing media as follows:

- 76
77 1) Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany): Canadian
78 *Sphagnum* peat moss-perlite-vermiculite (70/20/10 v/v/v) (**K**)
- 79 2) *Sphagnum maguellanicum*-perlite (80/20 v/v) (**S**)
- 80 3) River waste-perlite (80-20 v/v) (**R**)
- 81 4) *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) (**SR**).

82
83 When seedlings reached the transplant stage, they were transplanted into 1,200 cm³ pots filled
84 with a post-transplant Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany). At the
85 same time, plants grown at the pre-transplant stage in a Klasmann 411® medium (Klasmann-
86 Deilmann, GmbH, Germany) were transplanted to 1,200 cm³ pots filled with the four different pre-
87 transplant growing media tested, given 32 combinations of plug cell volume-growing media.

88 89 90 **2.2 Cultivation and Meteorological Data**

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92 Plants were irrigated daily at saturation with high quality tap water using intermittent overhead
93 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
94 overhead irrigation water (Stage 2: 50 mg L⁻¹ N; Stage 3-4: 100 mg L⁻¹ N; pot: 150 mg L⁻¹ N).

95

96 Daily mean temperatures (22.26 to 25.06°C) and daily photosynthetic active radiation (4.24 to
97 5.03 mol photons m⁻² day⁻¹) for the experiments were recorded with a HOBO sensor (H08-004-
98 02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The plants
99 were arranged at a density of 25 plants m⁻², which avoided mutual shading.

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102 2.3 Sampling and Growth Evaluations

103

104 Samples of each substrate were collected at the beginning of the pot experiments (before
105 transplant to 1,200-cm³ pots) and total porosity, air-filled porosity, bulk density and container
106 capacity were determined according to Fonteno [28]. Data are indicated in Table 1 and show
107 significant physical properties differences in of the growing media tested.

108

109 **Table 1: Physical properties for the growing media tested. K: [Canadian *Sphagnum* peat
110 (70%) + Perlite (20%) + Vermiculite (10%)], S: [*Sphagnum maguellanicum* (80%) + Perlite
111 (20%)], R: [River waste (80%) + Perlite (20%)], SR: [*Sphagnum maguellanicum* (40%) +
112 River waste (40%) + perlite (20%)]. Standard errors are indicated.**

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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 ± 1.46
S	70.67 ± 0.67	29.67 ± 2.15	0.15 ± 0.01	48.00 ± 0.38
R	72.67 ± 0.18	44.60 ± 0.95	0.18 ± 0.01	50.22 ± 0.44
SR	67.53 ± 0.64	23.27 ± 2.43	0.21 ± 0.01	42.67 ± 0.38

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115 Plants were harvested at the transplant stage and at 15, 30, 45, and 60 days after transplanting.
116 Roots were washed and root, stem and leaf fresh weights (FW) were recorded. Dry weights
117 (DW) were obtained after drying roots, stems and leaves to constant weight at 80°C for 96 h.
118 The number of leaves was recorded, and each leaf area was determined using the ImageJ®
119 (Image Processing and Analysis in Java) software. The number of stems and nodes has been
120 recorded as well.

121

122 The relative rate of leaf area expansion (RLAE) was calculated as the slope of the regression of
123 the natural logarithm (ln) of total leaf area versus time (in days). The rate of leaf appearance
124 (RLA) was calculated as the slope of the number of fully expanded leaves versus time (in
125 weeks). The specific leaf area on a FW basis (SLA) and leaf weight rate (LWR) were calculated
126 as the ratio between the area of the new individual leaf and leaf FW and the ratio between the
127 leaf DW and the total plant DW respectively at the end of the experiments. The relative growth
128 rate (RGR) was calculated as the slope of the regression of the ln of whole plant DW versus
129 time (in days).

130

131 The mean net assimilation rate (NAR) and leaf area ratio (LAR) were calculated according to
132 Potter and Jones [29] as follows:

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

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$$LAR = \frac{k_w}{NAR}$$

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138 where W₀: extrapolated value of total DW (g) at time zero; k_w: RGR (day⁻¹); A₀: extrapolated
139 value of leaf area (cm²) at time zero; k_a: RLAE (day⁻¹); t: time (days) at the midpoint of the
140 experimental period and e: base of the ln.

141

142 The allometric coefficients between root and shoot were calculated as the slope (β) of the
143 straight-line regression of the ln of the root DW versus the ln of the shoot DW. The Root: Shoot
144 ratio (at the end of the experiment) was performed as well.

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2.4 Statistical Analysis

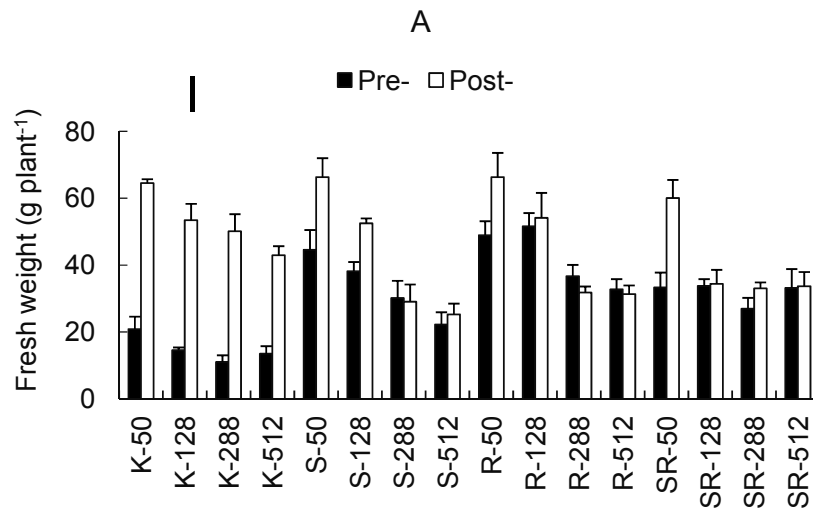
The experimental design was a randomised factorial with three blocks of five single-pot replications of each treatment combination (plug cell volume × growing medium × pre- and post-transplant). Since there were no significant differences between the two experiments, they were considered together (n = 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 and allometric values were tested using the SMATR package [30].

3. RESULTS

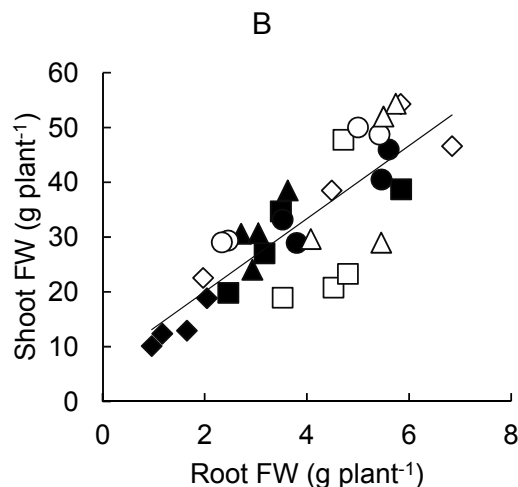
3.1. Biomass accumulation

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Total fresh weight at the end of the pot growth cycle (60 days from transplant) was higher in plants from 50-plug tray⁻¹ and decreased according cell number increased in all growing media tested. Anyway, growing media significantly change post- transplant biomass accumulation on a FW base as during the pre- as the post-transplant stage, but especially during nursery. The higher FW was found in plants grown in **R**- and **S**-growing media (Fig. 1A). When the mean aerial FW was plotted against the mean root FW (Fig. 1B), a positive correlation was found ($r^2 = 0.661$; $P < 0.001$).



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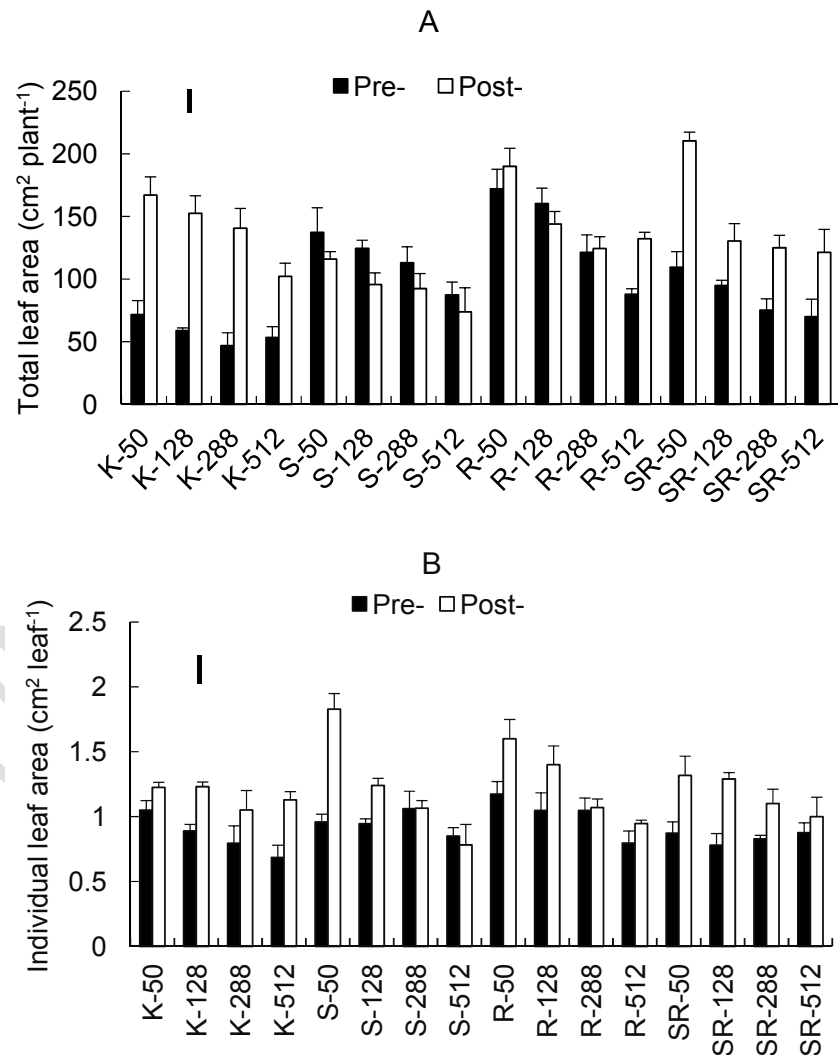
170 **Figure 1. Total fresh weight at the end of the experiment (60 days from transplanting) for**
 171 ***Impatiens walleriana* plants grown in four plug cell volumes (50-, 128-, 288-, and 512-cell**
 172 **tray⁻¹) at the pre-transplant stage and four growing media at the pre- or post-transplant**
 173 **stage. Bars indicate standard errors and vertical line indicate least significant differences**
 174 **(LSD). Panel B. Relationships between shoot and root FW according to four plug cell**
 175 **volumes (50-, 128-, 288-, and 512-cell tray⁻¹) at the pre-transplant stage and four growing**
 176 **media at the pre- (full symbols) or post-transplant stage (empty symbols). For substrate**
 177 **abbreviations see Table 1. F: ◆-◇; R: ■-□; S: ●-○; SR: ▲-△. The straight-line regression**
 178 **was: Shoot FW = 6.65 Root FW + 6.73 (r² = 0.661). The probability of the slope being zero**
 179 **was P < 0.001.**

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182 3.2. Leaf area expansion

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184 Total leaf area at the end of the experiment were once again higher in plants from 50-plug cells
 185 and in those grown at different growing media during the pot grow stages (Fig. 2A). Although
 186 there were significant differences in individual leaf area according to different pre-transplant cell
 187 volume and pre- or post-transplant growing media, changes were smaller than in total leaf area
 188 (Fig. 2B).



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191 **Figure 2. Total (A) and individual (B) leaf area at the end of the experiments (60 days from**
 192 **transplanting) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-,**
 193 **and 512-cell tray⁻¹) at the pre-transplant stage and four growing media at the pre- or post-**

194 transplant stage. Bars indicate standard errors and vertical line indicate least significant
 195 differences (LSD). For substrate abbreviations see Table 1.

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Table 2: Changes in RLA, RLAE and SLA for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray⁻¹) at the pre-transplant stage and four growing media at the pre- or post-transplant stage. Different lower-case letters indicate significant differences (P < 0.05) between pre-transplant plug cell volumes, while different capital letters indicate significant differences (P < 0.05) between pre- and post-growing media. For substrate abbreviations see Table 1. The probability of the RLA and RLAE slope being zero was P < 0.001.

	RLA (leaves week ⁻¹ plant ⁻¹)		RLAE (cm ² cm ⁻² day ⁻¹)		SLA (cm ² g ⁻¹)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
F						
50	0.512 ^{aB}	0.972 ^{aA}	0.0286 ^{aB}	0.0635 ^{aA}	188.43 ^{CA}	195.89 ^{bA}
128	0.449 ^{bB}	0.861 ^{bA}	0.0282 ^{aB}	0.0622 ^{aA}	230.92 ^{aA}	197.49 ^{bB}
288	0.340 ^{cB}	0.744 ^{CA}	0.0231 ^{bB}	0.0616 ^{aA}	238.04 ^{aA}	197.35 ^{bB}
512	0.291 ^{dB}	0.648 ^{dA}	0.0223 ^{bB}	0.0508 ^{bA}	215.16 ^{bA}	215.78 ^{aA}
S						
50	0.859 ^{aA}	0.627 ^{aB}	0.0363 ^{aB}	0.0567 ^{aA}	156.39 ^{CB}	204.58 ^{dA}
128	0.843 ^{aA}	0.624 ^{aB}	0.0371 ^{aB}	0.0552 ^{aA}	185.15 ^{bB}	226.07 ^{CA}
288	0.838 ^{aA}	0.547 ^{bB}	0.0317 ^{bB}	0.0543 ^{aA}	220.20 ^{aA}	224.42 ^{bA}
512	0.604 ^{bA}	0.518 ^{bB}	0.0280 ^{CB}	0.0441 ^{bA}	200.77 ^{aA}	294.62 ^{aA}
R						
50	1.000 ^{aA}	0.950 ^{aB}	0.0367 ^{aB}	0.0575 ^{aA}	179.64 ^{bB}	200.44 ^{aA}
128	0.902 ^{bA}	0.752 ^{bB}	0.0369 ^{aB}	0.0509 ^{bA}	184.61 ^{aA}	198.96 ^{aA}
288	0.788 ^{CA}	0.666 ^{CB}	0.0340 ^{bB}	0.0505 ^{bA}	186.52 ^{aA}	201.48 ^{aA}
512	0.764 ^{CA}	0.619 ^{dB}	0.0280 ^{CB}	0.0481 ^{CA}	183.03 ^{aA}	201.23 ^{aA}
SR						
50	0.949 ^{aA}	0.987 ^{aA}	0.0400 ^{aB}	0.0586 ^{aA}	176.86 ^{bB}	220.88 ^{bA}
128	0.704 ^{bA}	0.745 ^{bA}	0.0352 ^{bB}	0.0569 ^{bA}	179.18 ^{bB}	227.05 ^{bA}
288	0.549 ^{CB}	0.741 ^{bA}	0.0296 ^{CB}	0.0531 ^{CA}	187.80 ^{aB}	225.63 ^{bA}
512	0.543 ^{CB}	0.671 ^{CA}	0.0258 ^{dB}	0.0440 ^{dA}	189.70 ^{aB}	240.21 ^{aA}

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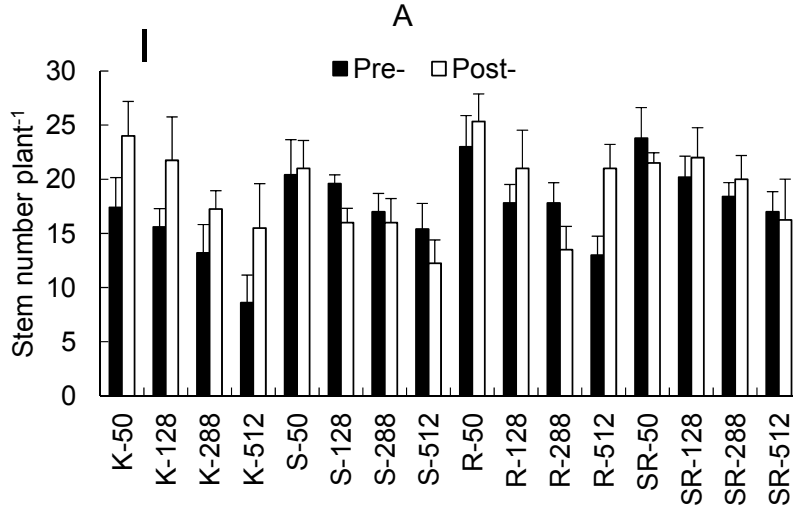
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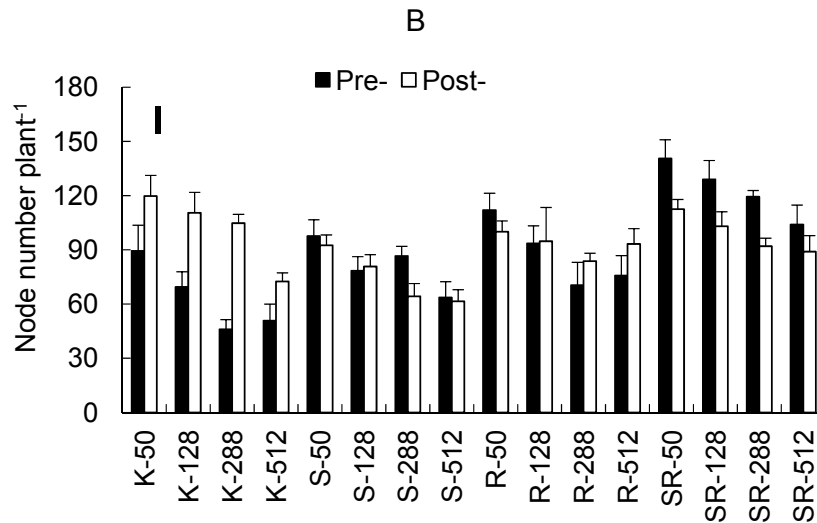
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The higher plug cell volume the higher RLA and RLAE but the lower SLA (leaves has a higher thickness). The response to a change in growing media quality at the pre- or post-transplant changed according pre-transplant plug cell volume and growing media tested in the same way that total leaf area (Table 2).

Stem number per plant showed significant differences in plants grown at nursery in different both plug cell volume and growing media; they were positively stimulated by a change in post-transplant growing media in plants from K-growing media. An inverse or no significant result was found when S-, R- or SR-growing media were used at the post-transplant stage (Fig. 3A). A similar response in the number node per plant was found (Fig. 3B).



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218 **Fig. 3. Stem (A) and node (B) number at the end of the experiments (60 days from**
 219 **transplanting) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-,**
 220 **and 512-cell tray⁻¹) at the pre-transplant stage and four growing media at the pre- or post-**
 221 **transplant stage. Bars indicate standard errors and vertical line indicate least significant**
 222 **differences (LSD). For substrate abbreviations see Table 1.**
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3.3. Dry weight accumulation

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Due there is no DW significant differences between treatments (data not shown), the traditional growth analysis approach would be performed. During the experiments, RGR values were significantly different for plants grown in different plug cell volume although data from post-transplant growing media were higher than those from plants grown at the same growing media at the pre-transplant stage. When RGR was separating in their 'physiological' (NAR) and 'morphological' (LAR) components, NAR decreased according plug cell volume decrease with significant differences between growing media tested and time (lesser pre-transplanted plants than post-transplanted ones). Quite opposite responses were found for LAR (Table 3).

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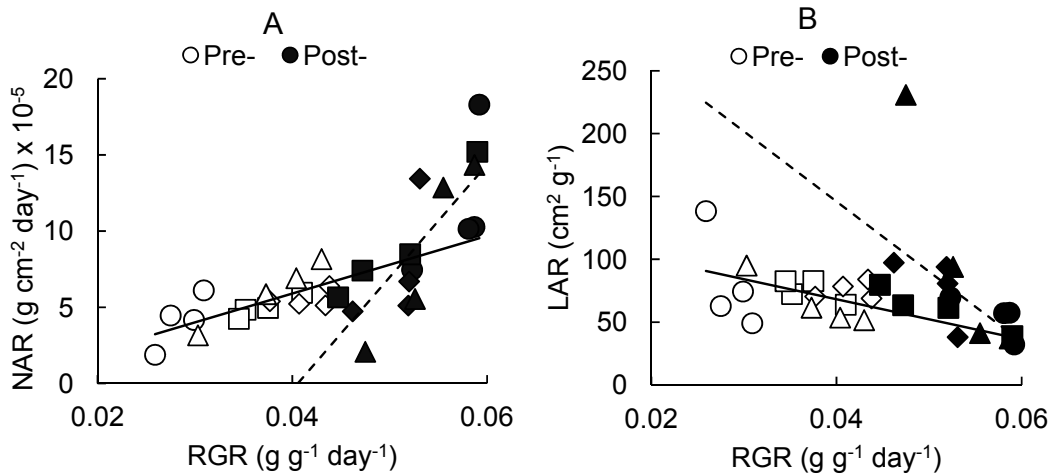
Table 3: Changes in RGR, NAR and LAR for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray⁻¹) at the pre-transplant stage and four growing media at the pre- or post-transplant stage. Different lower-case letters indicate significant differences (P < 0.05) between pre-transplant plug cell volumes, while different capital letters indicate significant differences (P < 0.05) between pre- and post- growing media. For substrate abbreviations see Table 1. The probability of the RGR slope being zero was P < 0.001.

	RGR (g g ⁻¹ día ⁻¹)		NAR (g cm ⁻² día ⁻¹) x 10 ⁻⁵		LAR (cm ² g ⁻¹)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
F						
50	0.030 ^{aB}	0.059 ^{aA}	6.11 ^{aB}	18.30 ^{aA}	49.10 ^{dA}	32.24 ^{cB}
128	0.031 ^{aB}	0.059 ^{aA}	4.17 ^{bB}	10.27 ^{bA}	74.34 ^{bA}	57.45 ^{bB}
288	0.028 ^{bB}	0.058 ^{aA}	4.46 ^{bB}	10.15 ^{bA}	62.78 ^{cA}	57.14 ^{bA}
512	0.026 ^{bB}	0.052 ^{aA}	1.88 ^{cB}	7.46 ^{cA}	138.30 ^{aA}	69.71 ^{aB}
S						
50	0.038 ^{aB}	0.059 ^{aA}	5.95 ^{aB}	15.20 ^{aA}	63.87 ^{cA}	38.82 ^{cB}
128	0.041 ^{aB}	0.052 ^{aA}	4.96 ^{bB}	8.48 ^{bA}	82.66 ^{aA}	61.32 ^{bB}
288	0.035 ^{bB}	0.047 ^{bA}	4.84 ^{bB}	7.41 ^{cA}	72.31 ^{bA}	63.43 ^{bB}
512	0.035 ^{bB}	0.045 ^{bA}	4.24 ^{cB}	5.65 ^{dA}	82.55 ^{aA}	79.65 ^{aA}
R						
50	0.044 ^{aA}	0.051 ^{aA}	6.39 ^{aB}	13.44 ^{aA}	68.86 ^{dA}	37.95 ^{cB}
128	0.043 ^{aA}	0.054 ^{aA}	5.12 ^{bB}	6.69 ^{bA}	83.98 ^{aA}	80.72 ^{bA}
288	0.041 ^{bA}	0.048 ^{aA}	5.23 ^{bA}	5.11 ^{cA}	78.39 ^{bB}	93.93 ^{aA}
512	0.038 ^{cB}	0.046 ^{aA}	5.40 ^{bA}	4.73 ^{dB}	70.37 ^{cB}	97.25 ^{aA}
SR						
50	0.042 ^{aB}	0.053 ^{aA}	8.16 ^{aB}	14.34 ^{aA}	51.47 ^{cA}	36.96 ^{cB}
128	0.037 ^{bB}	0.053 ^{aA}	6.91 ^{bB}	12.86 ^{bA}	53.55 ^{cA}	41.21 ^{cB}
288	0.036 ^{bB}	0.052 ^{aA}	5.86 ^{cA}	5.54 ^{cA}	61.43 ^{bB}	93.86 ^{bA}
512	0.030 ^{cB}	0.048 ^{aA}	3.16 ^{dA}	2.08 ^{dB}	94.94 ^{aB}	230.77 ^{aA}

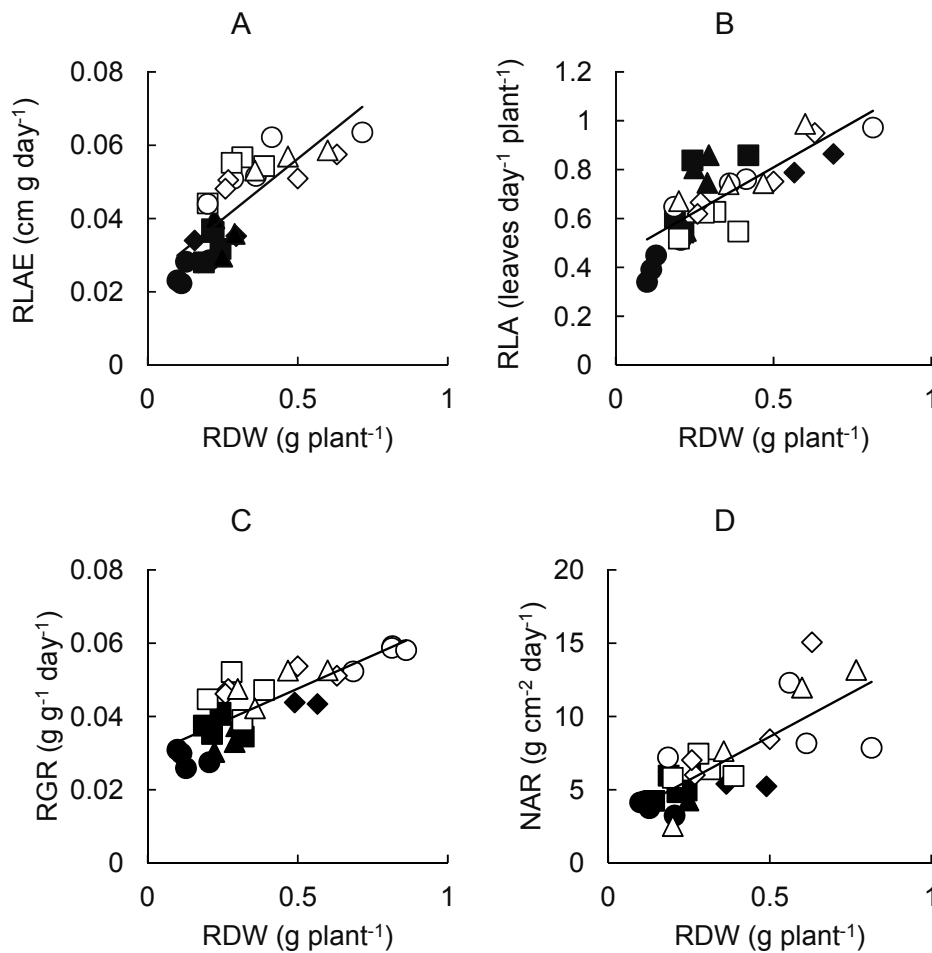
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When plotting the data from all treatments, we found a close direct relationship ($r^2 = 0.656$ and 0.635) for pre- and post-transplant values respectively between RGR and NAR (Fig. 4A) and an inverse relationship between RGR and LAR ($r^2 = 0.191$ and 0.328) (Fig. 4B).

Positive relationships between RLAE ($r^2 = 0.645$ P < 0.001) (Fig. 5A), RLA ($r^2 = 0.627$ P < 0.001) (Fig. 5B), RGR ($r^2 = 0.665$ P < 0.001) (Fig. 5C), NAR ($r^2 = 0.602$ P < 0.001) (Fig. 5D), and root DW were found. The higher values were those from plants grown in different growing media at the post-transplant stage.



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 260 **Figure 4: The net assimilation rate (NAR) (A) and the leaf area ratio (LAR) (B) related to**
 261 **the relative growth rate (RGR) for *Impatiens walleriana* plants from four plug cell volumes**
 262 **(50-, 128-, 288-, and 512-cell tray⁻¹) at the pre-transplant stage and four growing media at**
 263 **the pre- or post-transplant stage. The probability of the NAR and LAR slope being zero**
 264 **was P < 0.001. For substrate abbreviations see Table 1. F: ●-○; R: ■-□; S: ◆-◇; SR: ▲-△.**
 265 **The straight-line regressions were: $NAR_{Pre} = 189.25 RGR - 1.67$ ($r^2 = 0.656$); $NAR_{Post} =$**
 266 **$739.27 RGR - 29.99$ ($r^2 = 0.635$); $LAR_{Pre} = -1,596.20 RGR + 132.07$ ($r^2 = 0.191$) and $LAR_{Post} =$**
 267 **$5,572.40 RGR + 368.89$ ($r^2 = 0.328$).**



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270 Fig. 5. Relationship between RLAE (A), RLA (B), RGR (C), NAR (D) and root dry weight (RDW)
 271 for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell
 272 tray⁻¹) at the pre-transplant stage and four growing media at the pre- (full symbols) or
 273 post-transplant stage (empty symbols). The straight-line regressions were: RLAE = 0.067
 274 RDW + 0.023 ($r^2 = 0.645$ P < 0.001), RLA = 0.735 root DW + 0.44 ($r^2 = 0.627$ P < 0.001), RGR =
 275 0.036 RDW + 0.03 ($r^2 = 0.665$ P < 0.001), NAR = 11.86 RDW + 2.69 ($r^2 = 0.602$ P < 0.001). The
 276 probability of the slopes being zero was P < 0.001. F: \blacklozenge - \diamond ; R: \blacksquare - \square ; S: \bullet - \circ ; SR: \blacktriangle - \triangle .

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279 3.4. Photo assimilate partitioning

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281 The higher plug cell volume the lower β coefficient for root: shoot allometries for all growing
 282 media at the pre-transplant, which showed a higher photo assimilates partitioning to roots. The
 283 same β response pattern was found during the post-transplant but absolute values were even
 284 lower than for the pre-transplant growing media. An increase in root: shoot ratio according plug
 285 cell volume decrease were found as well with significant differences between growing media
 286 (Table 4).

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288 **Table 4: Changes in allometric relationships between roots and shoots for *Impatiens***
 289 ***walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray⁻¹) at the**
 290 **pre-transplant stage and four growing media at the pre- or post-transplant stage.**
 291 **Different lowercase letters indicate significant differences (P < 0.05) between pre-**
 292 **transplant plug cell volumes, while different capital letters indicate significant differences**
 293 **(P < 0.05) between pre- and post- growing media. For substrate abbreviations see Table**
 294 **1. The probability of the slopes being zero was P < 0.001.**

295

	Roots versus Shoots		Root: Shoot	
	Pre- β	Post- β	Pre-	Post-
F				
50	1.031 ^{dA}	0.889 ^{CB}	0.215 ^{bA}	0.147 ^{bB}
128	1.106 ^{cA}	0.911 ^{bB}	0.218 ^{bA}	0.163 ^{bB}
288	1.160 ^{bA}	0.987 ^{aB}	0.233 ^{aB}	0.298 ^{aA}
512	1.188 ^{aA}	0.965 ^{aB}	0.243 ^{aB}	0.317 ^{aA}
S				
50	1.004 ^{cA}	0.773 ^{dB}	0.163 ^{cB}	0.212 ^{cA}
128	1.117 ^{bA}	0.841 ^{cB}	0.173 ^{cB}	0.255 ^{bA}
288	1.117 ^{bA}	0.900 ^{bB}	0.207 ^{bB}	0.270 ^{bA}
512	1.165 ^{aA}	1.157 ^{aB}	0.264 ^{aB}	0.311 ^{aA}
R				
50	1.032 ^{bA}	0.795 ^{cB}	0.360 ^{cA}	0.178 ^{cB}
128	1.040 ^{bA}	0.623 ^{dB}	0.458 ^{bA}	0.181 ^{cB}
288	1.182 ^{aA}	0.905 ^{aB}	0.460 ^{bA}	0.223 ^{bB}
512	1.164 ^{aA}	0.871 ^{bB}	0.556 ^{aA}	0.298 ^{aB}
SR				
50	1.030 ^{bA}	0.853 ^{cB}	0.155 ^{cB}	0.185 ^{bA}
128	1.037 ^{bA}	0.961 ^{bB}	0.165 ^{bB}	0.195 ^{bA}
288	1.065 ^{aA}	0.981 ^{bB}	0.188 ^{bB}	0.208 ^{bA}
512	1.086 ^{aA}	1.002 ^{aB}	0.227 ^{aB}	0.274 ^{aA}

296

297

298 4. DISCUSSION

299

300 In a recent previous report [24], we have found that, in *I. walleriana* seedlings, the abiotic stress
301 imposed by the growing medium quality during nursery had a higher effect on biomass
302 accumulation (on both fresh and dry base), leaf area expansion and photo assimilates
303 partitioning than plug cell volume and constitute an interactive process associated with cytokinin
304 synthesis. However, this novelty approach did not exclude the plug cell volume involvement as
305 a limiting abiotic stress source during other parts of the *I. walleriana* growth cycle. In this context
306 we have evaluated pot biomass accumulation of this bedding plant to four growing media at
307 both nursery and pot stage in plants propagated in four different plug cell volumes.

308
309 A 'root restriction' syndrome related to either plug cell volume or growing media quality is an
310 exogenous signal, which let plants to sense the volume space and decrease or increase root
311 system accordingly [31,32]. Having in mind that roots are a major source of cytokinins [33],
312 which control the source of biomass accumulation such as the shoot apical meristem [34], the
313 similarity between *I. walleriana* plants grown in the best growing conditions and exogenous
314 cytokinin-sprayed plants [23,24,35], it is not unexpected. Our results from Fig. 5, which shown a
315 positive relationships between the most growing parameters performed (RLAE, RLA, RGE,
316 NAR) according to a root dry weight increases are in agreement with this previous reports. On
317 the other hand, a positive relationship between shoot and root fresh weight (there were not
318 significant differences between fresh and dry weight) was found (Fig. 1B) in agreement with
319 previous reports [4,15,17,21,22,23,24].

320
321 Although growing media performed through a high quality *Sphagnum sp.* peat base (Klasman®
322 commercial growing media) has been indicated as the best for pot plants [1], *I. walleriana*
323 previous results indicated that better growing media for this bedding plant can be found [36].

324
325 Results showed significant fresh weight changes at the end of the experiments (Fig. 1A)
326 according a decrease in plug cell volume and a change in growing media quality, which are in
327 agreement to RGR changes (Table 3). Methodology usually used to describe changes in
328 biomass accumulation on both fresh and dry weight included: (i) stems appearance; (ii) leaf
329 area expansion; (iii) photosynthetic capacity and; (iv) photo assimilate partitioning. In
330 ornamental bedding plants, additional traits such as tolerance to biotic and abiotic stresses and
331 aesthetics must be included [37].

332
333 Stem branching is an important aspect to consider in ornamental bedding plants because it take part
334 as the biomass accumulation as the aesthetic appearance. Results from Fig. 3 indicated that both
335 shoot number and node number decreased according to plug cell volume decrease, which indicates
336 a desirable commercial ideotype with a lower branching and compact growth habit. However,
337 growing media quality change the impact of the response to different pre-transplant plug cell volume.

338
339 Aesthetically, total leaf area is the main trait related to plant quality for commercial acceptance
340 of ornamentals and it determines the time of plant sale and at the same time, leaves are the
341 plant organs responsible to light interception. In physiological terms, it implies to expand leaf
342 area at the higher growth rate which included both individual leaf size and leaf number. Data
343 from Fig. 2A shown that the total leaf area, with minor effects on individual leaf area (Fig. 2B)
344 was significantly affect by both plug cell volume and growing media quality. Three growth
345 parameters can be used to characterize leaf area development: (i) RLA, which is an estimator of
346 leaf initiation and plastochron length, (ii) RLAE, which let to quantify leaf expansion and, (iii)
347 SLA, which characterize leaf thickness. Data from Table 2 showed that a decrease in plug cell
348 volume and a change in growing media quality decreased RLA and RLAE while increased SLA.
349 These results implies that the changes in total leaf area are mainly related to the meristematic
350 shoot apex capacity to initiate and to expand leaf primordia [38]. Both processes are mediated
351 by the down regulation of *KNOTTED* and *WUSCHEL* genes [39] associated to a high cytokinin:
352 low gibberellin ratio [40]. On the other hand, the lower SLA the higher leaf thickness, which it is
353 a pre-requisite for a high photosynthetic rate [41]. In this way, Gandolfo et al. [9] found positive
354 relationships between leaf thickness, intercellular spaces and NAR in *I. walleriana* root-
355 restricted plants. When the mesophyll thickness of the leaf is increased, the maximum
356 photosynthetic rate increased as well. This probably explains the strong relationship between
357 NAR and mesophyll thickness.

358
359 Variation in RGR has the result of two key traits: the 'physiological component' NAR and the
360 'morphological component' LAR. RGR, which ultimate quantify biomass accumulation, is

361 greatly influenced by photosynthetic efficiency. Although the higher the plug cell volume the
362 higher the RGR and NAR, growing media quality at the post-transplant stage increased both
363 growth parameters (Table 3). Shipley [42] indicated that, in general, NAR was the best general
364 predictor of variation in RGR, in agreement with our results from Fig. 4. Root restriction often
365 depresses photosynthetic capacity [44] and decreased energy synthesis [45]. The positive
366 relationships between NAR and RGR (Figure 4A) are in agreement with Shi et al. [44,45].
367

368 Root-restricted plants change photo assimilates partition as a response to abiotic stresses
369 (Table 4). At the end of the experiments, the higher root restriction the higher root: shoot ratio.
370 Root: shoot allometries let to explain these results because showed a higher photo assimilate
371 partitioning to roots (lower β coefficients) during the greater part of the experiments in root-
372 limited treatments.
373

374 As opposed to a previous report [23], which indicate that that growing media quality would be a
375 more limited factor than plug cell volume for *I. walleriana* seedlings during nursery, our results
376 showed that both abiotic stresses would be interactive restricting technological factors during the
377 post-transplant pot stage.
378

379

380 5. CONCLUSIONS

381

382 The effect of an abiotic stress and the relationships between multiples stress sources is not the
383 same according to the plant growth stage. In this context, different *I. walleriana* growth
384 responses to both plug cell volume and growing media found in our experiments would not be
385 unexpected results but to extend to other ornamental bedding plants and to perform a
386 commercial suggestion much more research must be required.
387

388

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