Original Research Article

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

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ABSTRACT

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Higher bedding plant yield per unit greenhouse area was reaching through two grower currently decision-making: plug cell volume during nursery and growing media quality for both nursery and pot cycle. With the goal of maximize bedding plant yield to identify the main limiting factor at the pot stage, we evaluated *I. walleriana* yield at the end of the pot 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.

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

1. INTRODUCTION

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

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Usually, the 'root restriction syndrome' has been defined as 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 at the transplant stage. The pre- and post-transplant effects of the container volume during nursery [5,6] have been extensively studied by our laboratory and we found that growth restriction would be closely related to endogenous cytokinin synthesis by roots. A limited plug cell volume gives a vertical root restriction when root apical meristem come to the bottom of the cell or the pot. At this moment, both primary root growth and root branching decrease [7] and cytokinins, the main endogenous hormone synthesized by root apical meristems would decrease as well [8,9].

On the other hand, a growing media quality decrease change their physical properties [3,10], which generally result in decreased pore sizes, and would be taken into account as an abiotic stress [4]. As pore size decreases, total porosity and air-filled porosity decrease as well. Bailey-Serres and Colmer [11] and Voesenek and Sasidharan [12] 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_2 supply. These metabolic changes would give the same effects on endogenous cytokinin synthesis as plug cell volume [13,14].

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

Impatiens walleriana (Hook.f.) is a commercially important year-round garden crop for landscape, and the first best-selling bedding plants in both developed and undeveloped countries. Most *Impatiens* genotypes produce a compact green foliage and covers itself through extremely uniform growth habit with bright blooms. *Impatiens* F_1 genotypes prefer partial sun/shade (8-25 mol photons m^{-2} day^{-1}) [25]. Dry mass and flowering increase from 14 to 28°C [26]. Plants only grow well with 100% evapotranspiration [27]. *I wallweriana* has been included in most research from our laboratory for the last decade.

The aim of this work was to evaluate *I. walleriana* yield at the end of the pot 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.

2. MATERIALS AND METHODS

2.1 Plant Material

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

I. walleriana 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown in 50-, 128-, 288- and 512-cell plug $tray^{-1}$ (55.70, 17.37, 6.18 and 2.50 cm^3 $cell^{-1}$ respectively) in four different pre-transplant 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**).

When seedlings reached the transplant stage, they were transplanted into 1,200 cm^3 pots filled with a post-transplant Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany). At the same time, plants grown at the pre-transplant stage in a Klasmann 411® medium (Klasmann-Deilmann, GmbH, Germany) were transplanted to 1,200 cm^3 pots filled with the four different pre-transplant growing media tested, given 32 combinations of plug cell volume-growing media.

2.2 Cultivation and Meteorological Data

Plants were irrigated daily at saturation with high quality tap water 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 (Stage 2: 50 mg L^{-1} N; Stage 3-4: 100 mg L^{-1} N; pot: 150 mg L^{-1} N).

Daily mean temperatures (22.26 to 25.06°C) and daily photosynthetic active radiation (4.24 to 5.03 mol photons m^{-2} day^{-1}) for the 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 [28]. Data are indicated in Table 1 and show significant physical properties differences in of the growing media tested.

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%)]. Standard errors are indicated.

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

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°C for 96 h. The number of leaves was recorded, and each leaf area was determined using the ImageJ® (Image Processing and Analysis in Java) software. The number of stems and nodes has been recorded as well.

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 specific leaf area on a FW basis (SLA) and leaf weight rate (LWR) were calculated as the ratio between the area of the new individual leaf and leaf FW and the ratio between the leaf DW and the total plant DW respectively at the end of the experiments. The relative growth rate (RGR) was calculated as the slope of the regression of the ln of whole plant DW versus time (in days).

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

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

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

where W_0: extrapolated value of total DW (g) at time zero; k_w: RGR (day^{-1}); A_0: extrapolated value of leaf area (cm^2) at time zero; k_a: RLAE (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 were calculated as the slope (**β**) of the straight-line regression of the ln of the root DW versus the ln of the shoot DW. The Root: Shoot ratio (at the end of the experiment) was performed as well.

2.4 Statistical Analysis

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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 ≤ 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

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).

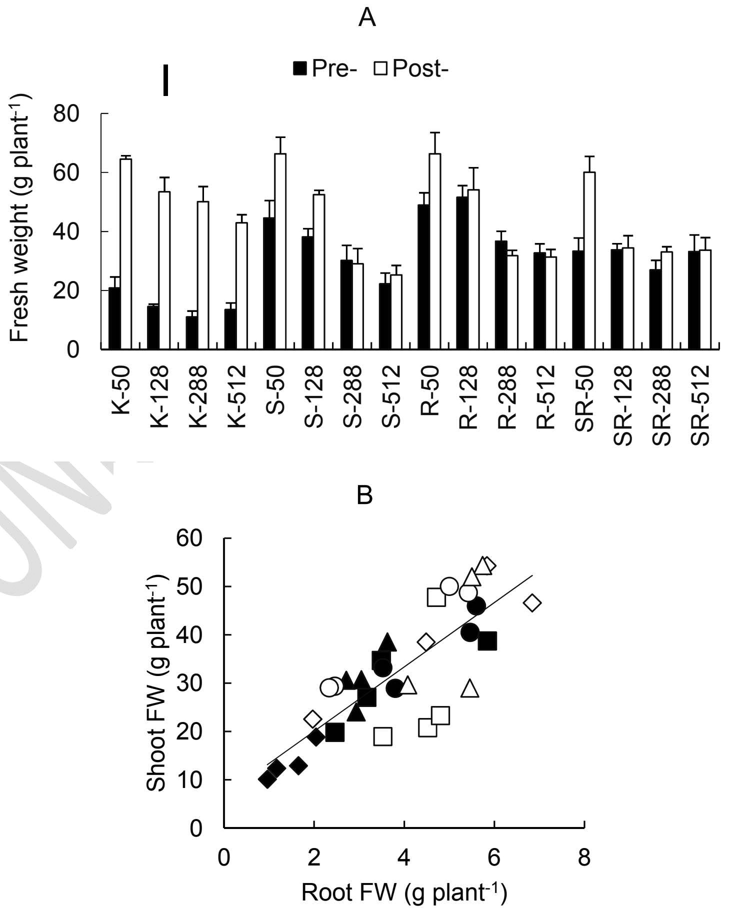

Figure 1. Total fresh weight at the end of the experiment (60 days from transplanting) for *Impatiens walleriana* plants grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- or post-transplant stage. Bars indicate standard errors and vertical line indicate least significant differences (LSD). Panel B. Relationships between shoot and root FW according to four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- (full symbols) or post-transplant stage (empty symbols). For substrate abbreviations see Table 1. F: ♦-◊; R: ■-□; S: ●-○; SR: ▲-Δ. The straight-line regression was: Shoot FW = 6.65 Root FW + 6.73 (r^2 = 0.661). The probability of the slope being zero was P < 0.001.

3.2. Leaf area expansion

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

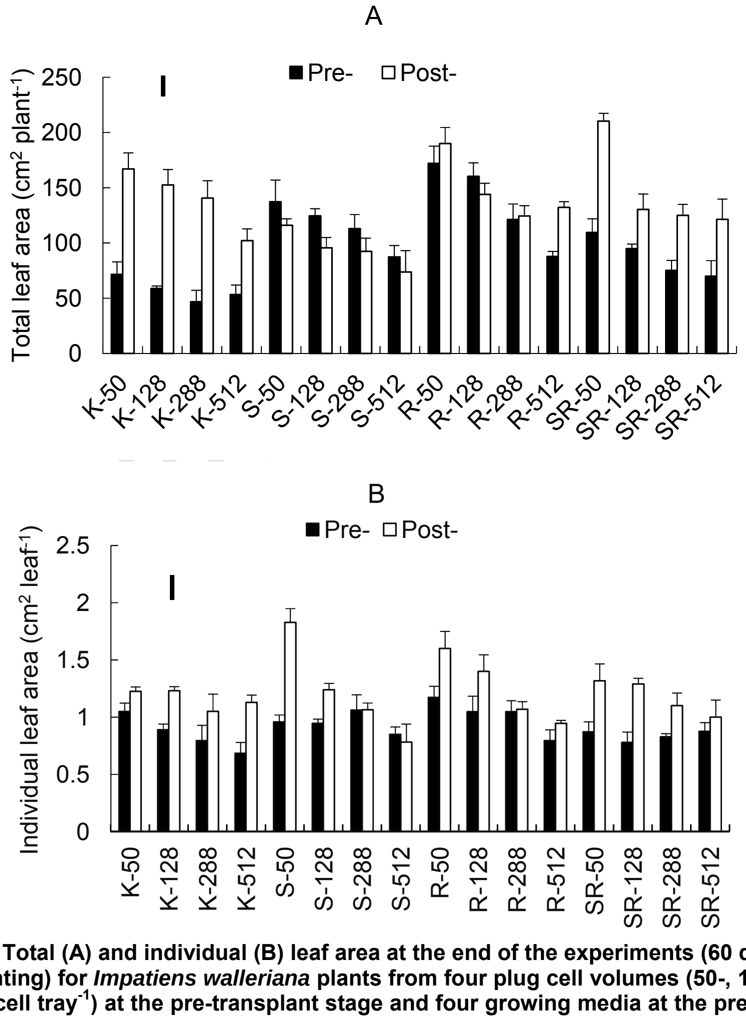

Figure 2. Total (A) and individual (B) leaf area at the end of the experiments (60 days from transplanting) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- or post-

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

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}

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).

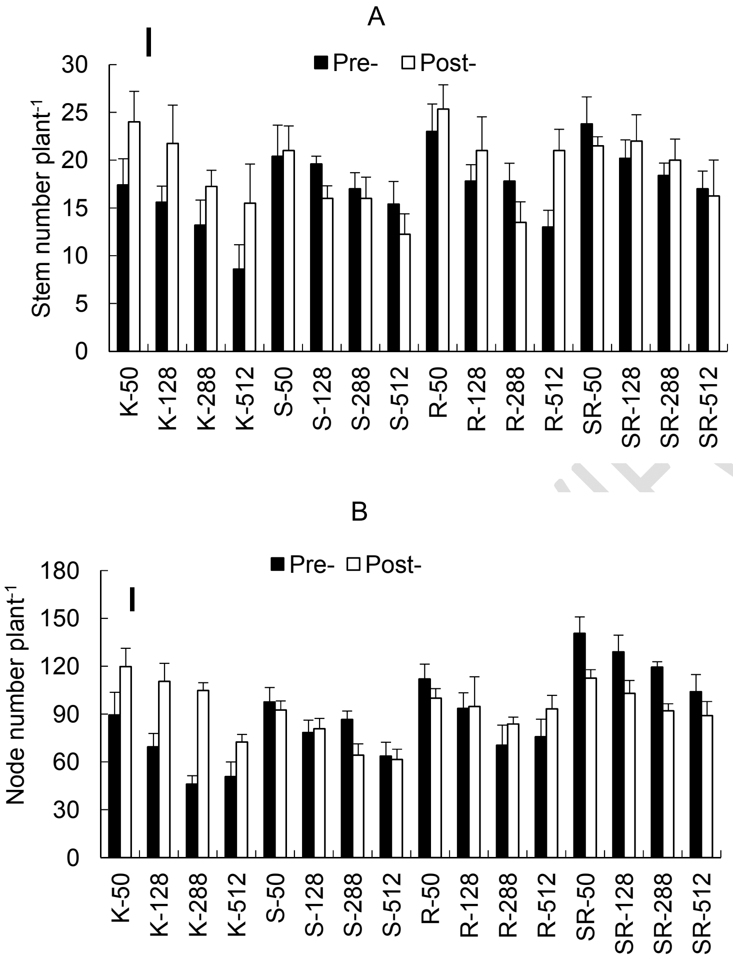

Fig. 3. Stem (A) and node (B) number at the end of the experiments (60 days from transplanting) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- or post-transplant stage. Bars indicate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

3.3. Dry weight accumulation

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).

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}

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.

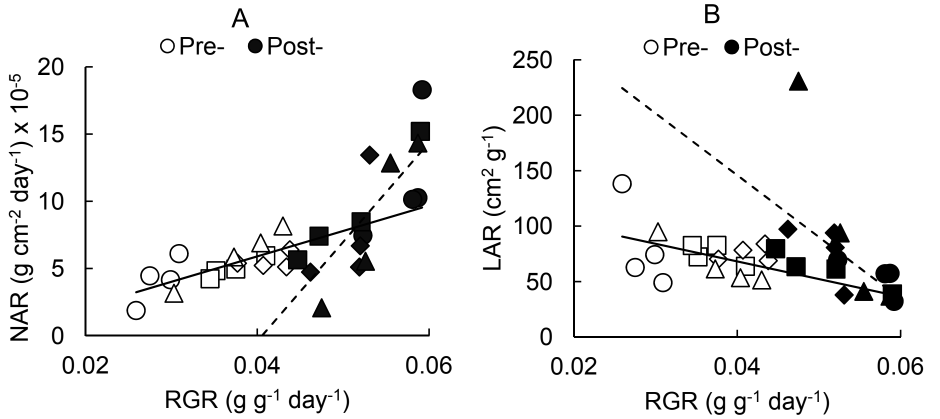

Figure 4: The net assimilation rate (NAR) (A) and the leaf area ratio (LAR) (B) related to the relative growth rate (RGR) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- or post-transplant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

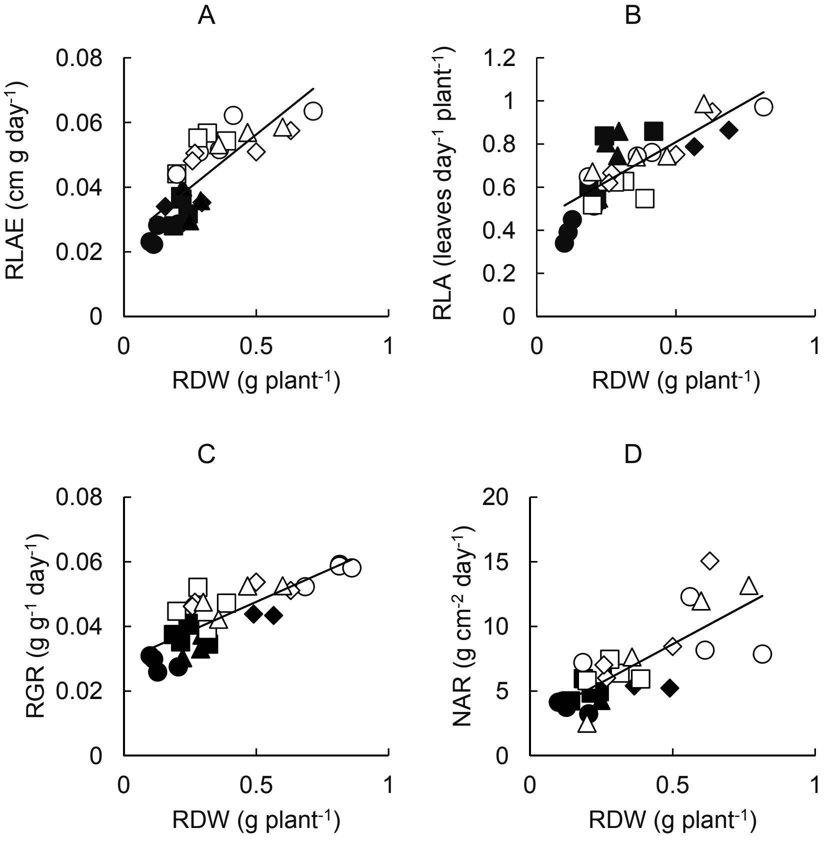

Fig. 5. Relationship between RLAE (A), RLA (B), RGR (C), NAR (D) and root dry weight (RDW) for *Impatiens walleriana* plants from four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) at the pre-transplant stage and four growing media at the pre- (full symbols) or post-transplant stage (empty symbols). The straight-line regressions were: RLAE = 0.067 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 = 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 probability of the slopes being zero was P < 0.001. F: ♦-◊; R: ■-□; S: ●-○; SR: ▲-Δ.

3.4. Photo assimilate partitioning

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

Table 4: Changes in allometric relationships between roots and shoots 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 lowercase 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 slopes being zero was P < 0.001.

	Roots versus Shoots		Root: Shoot	
	Pre-	Post-	Pre-	Post-
	β	β		
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}

4. DISCUSSION

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

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

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

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

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

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

Variation in RGR has the result of two key traits: the ‘physiological component’ NAR and the ‘morphological component’ LAR. RGR, which ultimate quantify biomass accumulation, is greatly influenced by photosynthetic efficiency. Although the higher the plug cell volume the higher the RGR and NAR, growing media quality at the post-transplant stage increased both growth parameters (Table 3). Shipley [42] indicated that, in general, NAR was the best general predictor of variation in RGR, in agreement with our results from Fig. 4. Root restriction often depresses photosynthetic capacity [44] and decreased energy synthesis [45]. The positive relationships between NAR and RGR (Figure 4A) are in agreement with Shi et al. [44.45].

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

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

5. CONCLUSIONS

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

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