

## SCIENTIFIC ARTICLE

## Response of the ornamental bedding plant *Impatiens walleriana* to plug cell volume on a floating system during nursery

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### Abstract

In ornamental plants, the need to optimize nursery management has led to a tendency to decrease plug cell tray volume. However, in ornamental plants such as *Impatiens walleriana*, a lower plug cell volume can negatively affect leaf area expansion and biomass accumulation during the pre-transplant cycle. Because these results have been associated with a decrease in root growth, a floating system where roots come out of the plug cell has been proposed. The aim of this work was to describe pre-transplant biomass accumulation in plants from different plug cell volumes and the response to two propagation systems: media-based plug cell trays and floating system. The relationship found between plug cell volume and growth in both the media-based and floating systems is in agreement with that found in previous reports. With respect to the traditional media-based system, the floating system showed higher leaf area expansion, as a result of higher leaf appearance rate and relative leaf area expansion. Higher fresh and dry weight accumulation were estimated through the relative growth rate (RGR), with a strong relationship with the capacity of photoassimilate production (net assimilation rate) and RGR. However, all these changes cannot be exclusively associated with a higher root growth in the floating system. Our experiments validate the positive results in favor of the use of a floating system and also shows the physiological mechanisms involved.

**Keywords:** abiotic stress, propagation systems, transplant.

### Resumo

#### Resposta da planta ornamental de forração *Impatiens walleriana* ao tamanho de recipiente e sistema de floating durante a fase de produção de mudas.

Em plantas ornamentais, a necessidade de otimizar o manejo na fase de produção de mudas levou a uma tendência de diminuir o volume da célula da bandeja. No entanto, em plantas ornamentais como *Impatiens walleriana*, um volume menor do recipiente de cultivo pode afetar negativamente a expansão da área foliar e o acúmulo de biomassa durante o ciclo pré-transplante. Como esses resultados foram associados a uma diminuição no crescimento radicular, foi proposto um sistema flutuante (floating) onde as raízes saem da célula da bandeja. O objetivo deste trabalho foi descrever o acúmulo de biomassa das plantas na produção de mudas em diferentes volumes de recipientes (tamanho da célula da bandeja) e a resposta a dois sistemas de condução: no sistema tradicional de produção de mudas e em floating. A relação encontrada entre o volume de recipiente e o crescimento em ambos os sistemas está de acordo com o descrito na literatura. Em comparação dos sistemas de cultivo, o floating apresentou maior expansão de área foliar, em função da maior taxa de aparecimento de folhas e expansão relativa da área foliar. Maiores acúmulos de massa fresca e seca foram estimados através da taxa de crescimento relativo (TCR), com forte relação com a capacidade de produção de fotoassimilados (taxa de assimilação líquida) e TCR. No entanto, todas essas alterações não podem estar associadas exclusivamente a um maior crescimento radicular no sistema flutuante. Nossos experimentos validam os resultados positivos apresentados pelo uso do floating e também mostram os mecanismos fisiológicos envolvidos.

**Palavras-chave:** estresse abiótico, sistemas de propagação, transplante.

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## Introduction

The productivity of ornamental pot plants is positively related to the environment but negatively affected by the pot root restriction (Di Benedetto et al., 2020). Previous reports have indicated that root restriction, which is the physical stress imposed on the root system when plants are grown in small containers, leads to a pronounced decrease in root and shoot growth at both the transplant and pot stages (Di Benedetto et al., 2020).

Based on the assumption that plant responses are mainly associated with a negative hormonal signaling from roots when they grow in a limited volume, in ornamental plants such as *Impatiens walleriana*, several strategies have been proposed to optimize plant growth (De Lojo et al., 2017, 2019a, b). Because the “root syndrome” seems to be related to a decrease in both root apical elongation and ramification, plug tray growth in a floating system would be a reasonable alternative to increase bedding pot plant at nursery.

The efficiency of the floating system in optimizing the growth of several types of plants, including vegetables (Özer, 2018; Öztekin et al., 2018; Siaga et al., 2018; Giménez et al., 2019, 2020; Melo et al., 2020; Kanatas, 2020; Monteiro et al., 2020; Tsouvaltzis et al., 2020; Cristofano et al., 2021), cut flowers (Zanin et al., 2003; Barbaro et al., 2017), and aromatic and medicinal plants (Aktsoğlu et al., 2021) has been previously demonstrated. However, data on bedding pot plants are scarce (Cros et al., 2007).

The floating system has many advantages, such as its simplicity, functionality, low operational cost, high plant density and early production, optimal use of water and nutrients, and standardization of the production at an industrial scale. However, data on comparative growth efficiency of media-based and floating systems are scarce and limited to vegetables such as chili pepper, tomato and lettuce (Siaga et al., 2018; Özer, 2018; Giménez et al., 2020).

In all published reports, the responses studied have been limited to yield, root length, root fresh weight, stem height, stem fresh weight and diameter, number of leaves per plant, leaves fresh weight, and total plant fresh weight. An approach based on the analysis of growth during nursery and the physiological mechanisms involved is lacking.

Thus, the aim of this work was to evaluate pre-transplant biomass accumulation in plants grown in different plug cell volumes and the response to two propagation systems (soilless media plug cell trays and a floating system) in the bedding plant *Impatiens walleriana*.

## Materials and Methods

The experiment was carried out inside a greenhouse in the campus of the Faculty of Agronomy, University of Buenos Aires, Argentina (34°35'59"S, 58°22'23"W) between December 3<sup>rd</sup> 2019 and January 9<sup>th</sup> 2020.

To reach the objective proposed, *Impatiens walleriana* ‘Xtreme White’ seeds (Goldsmith Inc., NY, USA) were grown in 50- (55.7 cm<sup>3</sup> cell<sup>-1</sup>), 128- (17.37 cm<sup>3</sup> cell<sup>-1</sup>), 288- (6.18 cm<sup>3</sup> cell<sup>-1</sup>) and 512- (2.50 cm<sup>3</sup> cell<sup>-1</sup>) plug trays in a Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) for 37 days. Plug trays were placed on wooden platforms (media-based system) or in a floating tray system.

After transplant, plants were grown in 3-L pots filled with a *Sphagnum maguellanicum*-river waste-perlite (2-2-1 v/v/v) medium. The pots were arranged at a density of six plants m<sup>-2</sup> to avoid mutual shading.

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity: 0.486 dS m<sup>-1</sup>), using intermittent overhead mist to compensate evapotranspiration losses. The floating system fertilizer solution was composed of 143.0, 62.9 and 118.7 mg L<sup>-1</sup> of N, P and K, respectively. Plants in the soilless media system were fertilized with an equivalent fertilizer solution through the overhead water irrigation.

Daily mean temperatures (21.05 to 25.80 °C) and daily photosynthetic active radiation (10.16 to 11.77 mole photons m<sup>-2</sup> day<sup>-1</sup>) during the experiment were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger.

Plants were harvested at the transplant stage (37 days from 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 hours. The number of leaves was recorded, and each leaf area was determined using the ImageJ® software (Image Processing and Analysis in Java).

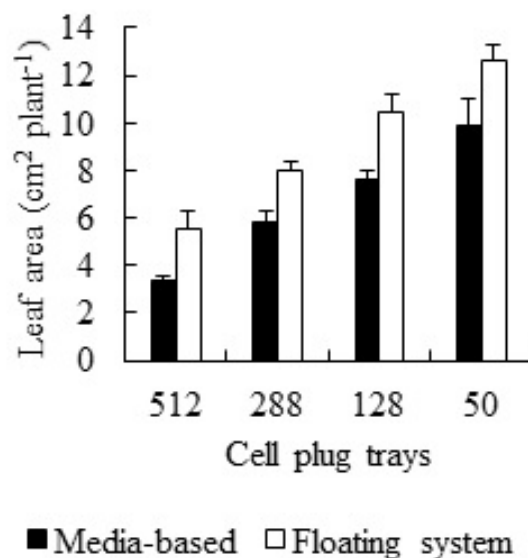
The relative rate of leaf area expansion (RLAE), the rate of leaf appearance (RLA), the relative growth rate (RGR), the mean net assimilation rate (NAR), the leaf area ratio (LAR), the specific leaf area (both on a fresh and a dry weight basis) and the leaf weight ratio (LWR) were calculated according to previous reports (Di Benedetto and Tognetti, 2016).

We used a complete aleatory design. Data were subjected to two-way analysis of variance and means were separated by Tukey's test ( $p < 0.05$ ); STATISTICA 8 software (StatSoft) was used. Least significant differences (LSD) values were calculated.

Slopes from straight-line regressions of RLAE, RLAE, RGR and allometric values were tested using the SMATR package.

## Results and Discussion

The results showed that the higher the plug cell volume, the higher the total leaf area at the transplant stage, both in plants grown in the traditional soilless media on wooden platforms and in those grown in the floating system. However, the highest leaf areas for each plug cell volume were found in plants propagated through the floating system (Figure 1).



**Figure 1.** Effect of plug cell volume and propagation system on the leaf area of *Impatiens walleriana* plants at the transplant stage. Vertical lines indicate standard errors. LSD = 2.191 cm<sup>2</sup> plant<sup>-1</sup>.

The results found in the traditional soilless media are in agreement with those of Özer (2018) on tomato and with previous reports performed in *I. walleriana* plants (De Lojo et al., 2017, 2019a, b). In contrast, our results are not in agreement with those of Dintcheva (2017), who suggested that the size of the cell does not significantly affect tomato plant growth, although the plug cell number from her experiments ranged only between 70- and 104-cells per tray.

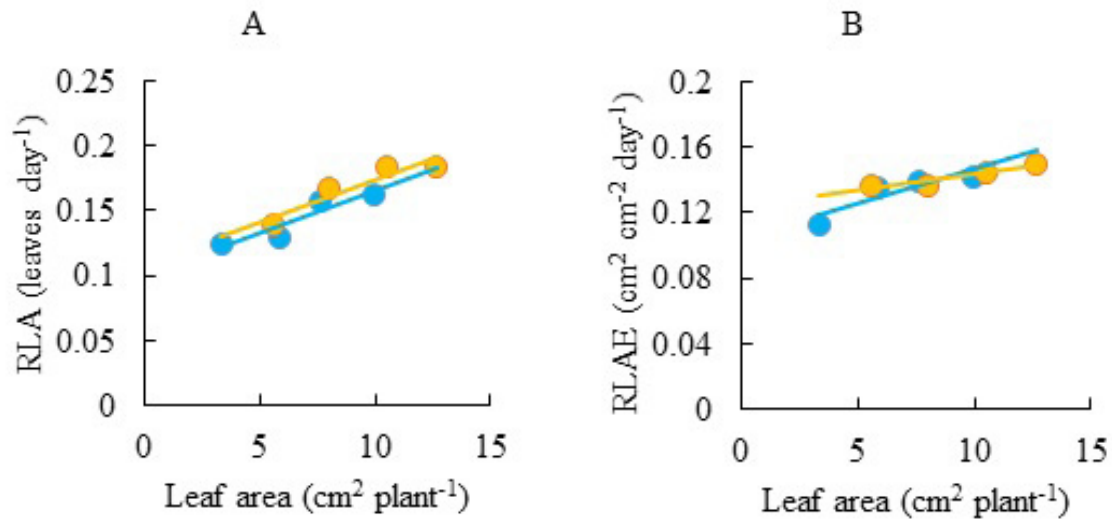
The results also showed close positive relationships between RLA ( $r^2 = 0.860-0.888$ ), RLAE ( $r^2 = 0.903-0.804$ )

and total leaf area, without significant differences between the traditional soilless media on wooden platforms and the floating system (Figure 2).

Differences in total leaf area between plants from different plug cell volumes and propagation systems were the result of higher RLA and RLAE values (Table 1). Previous reports in lettuce have shown that the floating system allows obtaining a high leaf number (Monteiro et al., 2020), but there are no data on leaf area development, which compare the floating system against the traditional media-based plug trays.

**Table 1.** Effect of plug cell volume and propagation system on the rate of leaf appearance (RLA) and relative leaf area expansion (RLAE) during nursery in *Impatiens walleriana* plants. Different lower case letters indicate significant differences ( $p < 0.05$ ) between different plug cell volumes. Different capital letters indicate significant differences ( $p < 0.05$ ) between nursery growth systems.

| Cell plug trays | RLA (leaves day <sup>-1</sup> ) |                 | RLAE (cm <sup>2</sup> cm <sup>-2</sup> day <sup>-1</sup> ) |                 |
|-----------------|---------------------------------|-----------------|--|-----------------|
|                 | Media-based                     | Floating system | Media-based  | Floating system |
| 512             | 0.1235dB                        | 0.1382cA        | 0.1130cB   | 0.1363cA        |
| 288             | 0.1297cB                        | 0.1676bA        | 0.1356bA   | 0.1365cA        |
| 128             | 0.1568bB                        | 0.1838aA        | 0.1388bB   | 0.1443bA        |
| 50              | 0.1622aB                        | 0.1838aA        | 0.1416aB   | 0.1494aA        |



**Figure 2.** Relationships between the rate of leaf appearance (RLA) (A), relative leaf area expansion (RLAE) (B) and total leaf area (LA) during nursery of *Impatiens walleriana* plants. The straight-line regressions were:

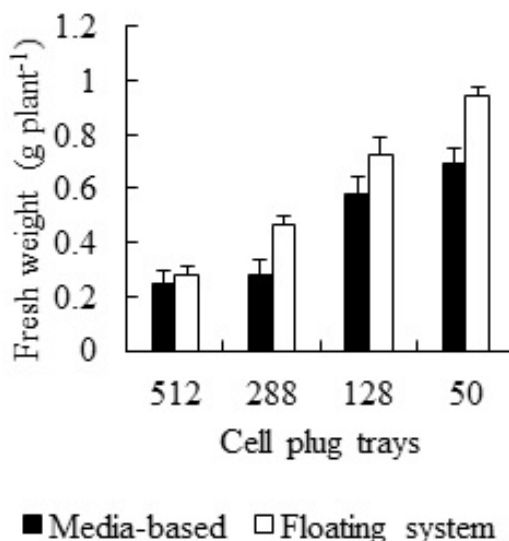
$$\begin{aligned} \text{RLA}_{\text{Media-based}} &= 0.0065 \text{ LA} + 0.1088 \quad (r^2 = 0.860); & \text{RLA}_{\text{Floating system}} &= 0.0065 \text{ LA} + 0.0995 \quad (r^2 = 0.888); \\ \text{RLAE}_{\text{Media-based}} &= 0.002 \text{ LA} + 0.1235 \quad (r^2 = 0.903); & \text{RLAE}_{\text{Floating system}} &= 0.0042 \text{ LA} + 0.1042 \quad (r^2 = 0.804). \end{aligned}$$

Aesthetically, the main trait related to plant quality for commercial acceptance of ornamentals is total leaf area. Regarding this, the results shown in Figure 1 and Table 1 indicate that the floating system would be a technological alternative to obtain plants with a larger leaf area during nursery in all cell sizes tested.

A general statement is that biomass production increases through both leaf appearance and leaf expansion and that photo assimilates from leaves are the force for the initiation and growth of roots, shoots and leaves (Lu et al., 2020). Leaf area development can be characterized by means of three growth parameters: (i) RLA, which is an estimator of leaf initiation and plastochron length, (ii) RLAE, which allows quantifying leaf expansion, and (iii) specific leaf area (SLA), which characterizes leaf thickness and is positively related to the photosynthetic rate (Di Benedetto et al., 2020). The changes in total leaf area are

mainly related to the meristematic shoot apex capacity to initiate and expand leaf primordia. The precedent statement is supported by the high correlation coefficients ( $r^2$  between 0.804 and 0.903) shown in Figure 2. These coefficients also indicate the importance of both RLA and RLAE for the total leaf area expanded during nursery regardless of the culture system used.

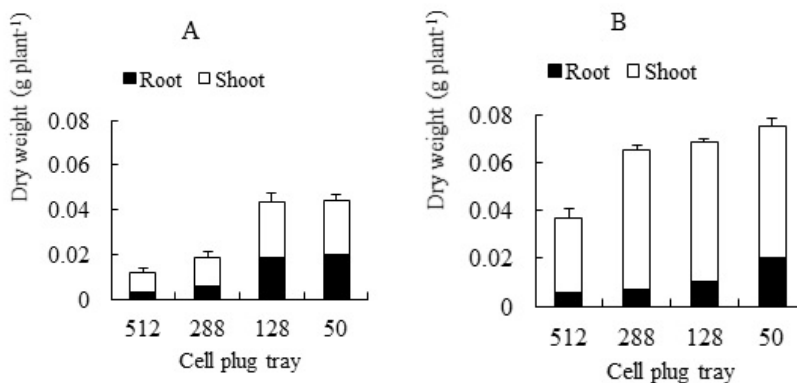
Regarding total fresh weight at the transplant stage, the best results were found in *I. walleriana* plants grown in the larger plug cell volumes (50-cell trays). Plants grown in the floating system showed increasing differences in relation with those grown in the traditional soilless media on wooden platforms (Figure 3). The hypothesis that shoot fresh weight accumulated during nursery is closely related to root fresh weight (Di Benedetto et al., 2020) would be validated by the decrease in the root restriction in plants grown in the floating system.



**Figure 3.** Effect of plug cell volume and propagation system on the total fresh weight at the transplant stage in *Impatiens walleriana* plants. Vertical lines indicate standard errors. LSD = 0.015 g plant<sup>-1</sup>.

When plants were dried to constant weight at 80 °C for 96 hours and dry weight was recorded, statistical differences between treatments increased strongly (Figure 4). In this way, data from both the media-based and floating systems showed a clear effect of plug cell volume on biomass accumulation. The higher the root dry weight, the

higher the shoot dry weight of plants grown both in the media-based (Figure 4A) and in the floating system (Figure 4B). However, the plants from the traditional media-based system showed lower root dry weight (mainly in plants from 128- and 50-cell trays) and lower root: shoot ratio than those from the floating system (Table 2).



**Figure 4.** Effect of plug cell volume on the root and shoot dry weight in *Impatiens walleriana* plants at the transplant stage in media-based (A) and floating (B) systems. Vertical lines indicate standard errors. LSD = 0.005 (root) and 0.011 (shoot) g plant<sup>-1</sup>.

**Table 2.** Effect of plug cell volume and propagation system on the root:shoot ratio and root relative growth rate (RRGR) during nursery in *Impatiens walleriana* plants. Different lower case letters indicate significant differences ( $p < 0.05$ ) between different plug cell volumes. Different capital letters indicate significant differences ( $p < 0.05$ ) between nursery growth systems.

| Cell plug trays | RRGR ( $\text{g g}^{-1} \text{day}^{-1}$ ) |                 | Root:shoot ratio |                 |
|-----------------|--|-----------------|------------------|-----------------|
|                 | Media-based                                | Floating system | Media-based      | Floating system |
| 512             | 0.0986dB                                   | 0.1107dA        | 0.473cA          | 0.376aB         |
| 288             | 0.1211cA                                   | 0.1166cB        | 0.519bA          | 0.376aB         |
| 128             | 0.1477bA                                   | 0.1316bB        | 0.848aA          | 0.161cB         |
| 50              | 0.1454aB                                   | 0.1495aA        | 0.853aA          | 0.193bB         |

The results also showed that as the plug cell volume increased, the relative root growth rate (RRGR) also increased. Although significant, the differences in RRGR between plug cell volumes were small between the two propagation systems tested. The root: shoot ratio was significantly higher for the media-based system regardless of the plug cell volume. The differences in the root: shoot ratio greatly increased in plants from 128- and 50-plug cell trays (Table 2).

When seedlings are grown in typical plug cell trays (media-based system), growth tends to be proportional to the volume of the container because the roots that come out through the drainage hole usually die. Additionally, roots from the floating system remain functional until transplantation. This visual effect suggests that the plants from the floating system have a larger root system. On the other hand, previous reports in *I. walleriana* have suggested a close direct relationship between growth parameters and root dry weight (De Lojo et al., 2017). However, results shown in Figure 4 are not in agreement with the previous hypothesis when the floating systems were included.

In previous studies, we found that endogenous cytokinins synthesized in roots are transported to shoots via the xylem but controlled both by environmental and endogenous signals (Di Benedetto et al., 2020). Although the net cytokinin concentration that reaches the apical meristem is the result of both its synthesis and degradation rates, cytokinin synthesis increases with the root size.

In the media-based system, the limited plug cell volume restricts vertical root growth when the root apical meristem reaches the bottom of the cell or the pot (Figure 5A). Thus, both primary root growth and root branching decrease and, presumably, the concentration of cytokinin, the main endogenous hormone synthesized by the root apical meristem, probably decreases as well (Kotov and Kotova, 2015). Bedding plants grown in plug trays may show a well-developed root system with white roots and without damage, but with a root girdling growth around the cell (De Lojo et al., 2017), which can limit cytokinin translocation. The absence of the latter effect in floating plants (Figure 5B) would explain the differences in leaf area and biomass accumulation in relation to the traditional system.



**Figure 5.** Effect of a media-based system (A) and floating system (B) on root appearance through the drainage hole in *Impatiens walleriana* plants.

Although the leaf area determines the plant capacity of light interception, the RGR, which may vary as a result of the 'physiological component' NAR (net assimilation rate) and the 'morphological component' LAR (leaf area ratio) and which ultimately quantifies biomass accumulation, is also greatly influenced by the photosynthetic efficiency (Demura and Ye, 2010).

Differences in total dry weight at the transplant stage seem to be explained by higher RGR due to differences in the cell volume and culture system. When RGR values were disaggregated in NAR and LAR, both increased according to the plug cell volume. On the other hand, the higher values in each case were found in plants from the floating system (Table 3).

**Table 3.** Effect of plug cell volume and propagation system on the relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) during nursery in *Impatiens walleriana* plants. Different lower case letters indicate significant differences ( $p < 0.05$ ) between different plug cell volumes. Different capital letters indicate significant differences ( $p < 0.05$ ) between nursery growth systems.

| Cell plug trays | RGR ( $\text{g g}^{-1} \text{day}^{-1}$ ) |                 | NAR ( $\text{g cm}^{-2} \text{day}^{-1}$ ) $\times 10^{-5}$ |                 | LAR ( $\text{cm}^2 \text{g}^{-1}$ ) |                 |
|-----------------|---|-----------------|---|-----------------|-------------------------------------|-----------------|
|                 | Media-based                               | Floating system | Media-based   | Floating system | Media-based                         | Floating system |
| 512             | 0.0701cB                                  | 0.1023dA        | 4.36cB  | 7.75dA          | 135.37cA                            | 105.21cB        |
| 288             | 0.0887bB                                  | 0.1136cA        | 4.60bB  | 10.49cA         | 139.32cA                            | 108.29bB        |
| 128             | 0.1066aB                                  | 0.1226bA        | 7.77aB  | 11.39bA         | 160.61bA                            | 110.39bB        |
| 50              | 0.1082aB                                  | 0.1257aA        | 7.87aB  | 11.65aA         | 192.64aA                            | 132.05aB        |

When LAR was disaggregated in SLA (on a dry weight basis) and LWR, both growth parameters increased according to the plug cell volume, with the higher values in plants from the floating system (Table 4). When SLA

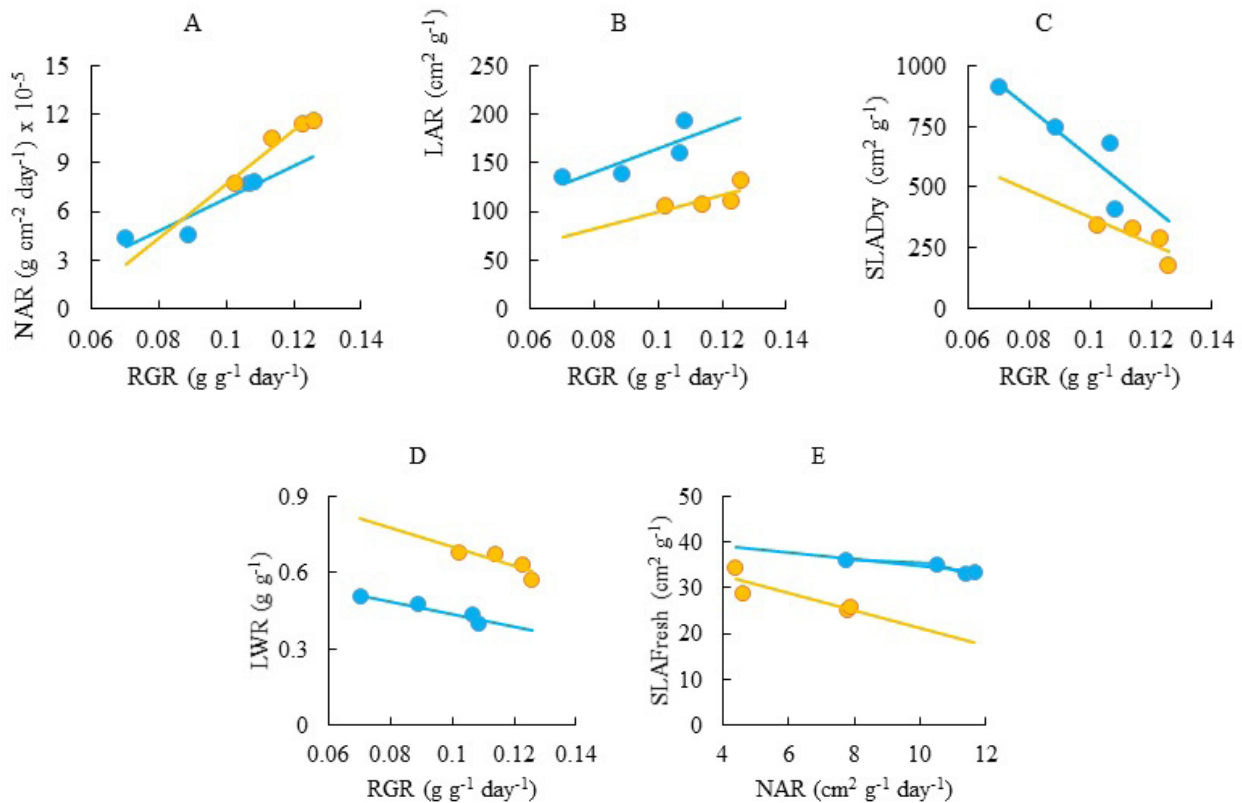
values were calculated on a fresh weight basis, which is an estimation to leaf thickness, they increased significantly as the plug cell volume increased and in plants from the floating system (Table 4).

**Table 4.** Effect of plug cell volume and propagation system on the specific leaf area (both on a dry and fresh weight basis) and leaf weight ratio (LWR) during nursery in *Impatiens walleriana* plants. Different lower case letters indicate significant differences ( $p < 0.05$ ) between different plug cell volumes. Different capital letters indicate significant differences ( $p < 0.05$ ) between nursery growth systems.

| Cell plug trays | SLA <sub>dry</sub> ( $\text{cm}^2 \text{g}^{-1}$ ) |                 | SLA <sub>fresh</sub> ( $\text{cm}^2 \text{g}^{-1}$ ) |                 | LWR ( $\text{g g}^{-1}$ ) |                 |
|-----------------|--|-----------------|--|-----------------|---------------------------|-----------------|
|                 | Media-based  | Floating system | Media-based  | Floating system | Media-based               | Floating system |
| 512             | 914.55aA   | 341.97aB        | 36.16aA  | 34.36aB         | 0.505aB                   | 0.676aA         |
| 288             | 748.16bA   | 330.94bB        | 35.19aA  | 28.66bB         | 0.478bB                   | 0.673aA         |
| 128             | 680.90bA   | 294.49cB        | 33.06bA  | 25.03cB         | 0.432cB                   | 0.632bA         |
| 50              | 413.20dA   | 181.63dB        | 33.32bA  | 25.71cB         | 0.397dB                   | 0.574cA         |

Positive relationships between NAR (Figure 6A), LAR (Figure 6B) and RGR were found in *I. walleriana* plants, with significant differences between the two propagation systems tested. On the other hand, negative relationships were found between SLA (both on a dry (Figure 6C) and

fresh (Figure 6E) weight basis), LWR (Figure 6D) and RGR, with significant differences between propagation systems. Data are in agreement with previous reports from our laboratory in *I. walleriana* plants from plug trays on wooden platforms (De Lojo et al., 2017; 2019a, b).



**Figure 6.** Relationships between the net assimilation rate (NAR) (A), leaf area ratio (LAR) (B), leaf specific leaf area on a dry weight basis ( $SLA_{dry}$ ) (C), leaf weight ratio (LWR) and relative growth rate (RGR) during nursery in *Impatiens walleriana* plants. The straight-line regressions were:  $NAR_{Media-based} = 99.89 RGR - 3.18$  ( $r^2 = 0.857$ );  $NAR_{Floating system} = 99.89 RGR - 8.89$  ( $r^2 = 0.949$ );  $LAR_{Media-based} = 1212.80 RGR + 43.71$  ( $r^2 = 0.683$ );  $LAR_{Floating system} = 861.83 RGR + 13.97$  ( $r^2 = 0.548$ );  $SLA_{Dry-Media-based} = -5531.00 RGR + 1636.80$  ( $r^2 = 0.756$ );  $SLA_{Dry-Floating system} = -5532.00 RGR + 929.13$  ( $r^2 = 0.629$ );  $LWR_{Media-based} = -2.58 RGR + 0.69$  ( $r^2 = 0.895$ );  $LWR_{Floating system} = -3.82 RGR + 1.08$  ( $r^2 = 0.714$ ). Sub-plate E shows the relationship between the specific leaf area on a fresh weight basis ( $SLA_{fresh}$ ) and the straight-line regressions were:  $SLA_{Fresh-media-based} = -0.75 NAR + 42.27$  ( $r^2 = 0.825$ );  $SLA_{Fresh-Floating system} = -1.89 NAR + 40.08$  ( $r^2 = 0.739$ ).

Although meristematic activities and cell elongation are two crucial factors determining the rate of vegetative growth, the total biomass production of a particular ornamental plant is greatly influenced by the efficiency of photosynthesis, which supplies raw materials for vegetative growth. In turn, the photosynthetic efficiency greatly influences RGR, which ultimately quantifies biomass accumulation. In *I. walleriana*, when the mesophyll thickness of the leaf increases, the maximum photosynthetic rate increases as well (Gandolfo et al., 2014), which partially explains the higher biomass accumulation in floating plants, regardless of the cell size used.

Although previous studies have demonstrated the efficiency of the floating system for different species under intensive cropping, there are few comparisons between this and the traditional media-based system. In addition, the methodological approach only contemplates changes in leaf number or fresh-dry weight at the time of transplantation. In contrast, the growth analysis methodology used in our experiments validates the positive results in favor of the

use of a floating system and also shows the physiological mechanisms involved.

## Conclusions

Our results showed that the floating system is able to reduce root restriction in such a way as to increase biomass accumulation per unit area and time during nursery in relation to the traditional countertop conduction system. The mechanisms involved included leaf initiation and expansion, plant photosynthetic efficiency, and differential partitioning of photo assimilates between different potential sinks.

## Author contribution

**MS, GH, EG:** provided the structure and conditions to develop the experiments and conducted its. **ADB, EG, JDL:** wrote the manuscript, carried out the statistical analysis and contributed to the discussion of results. All the authors read and approved the final version of the paper.



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