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Plug Cell Volume, Growing Media Quality and Benzyl Aminopurine (BAP) Spray Effects for Nursery Growth of *Impatiens walleriana*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ADB, CB and EGardina designed the study and wrote the manuscript. Authors JDL and EGandolfo recorded data, performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Plant propagators must take two technological critical decisions: the plug-cell size and the growing medium, both of which have been mentioned as abiotic stress sources for bedding pot plants. However, only a few recent reports on bedding pot plants have simultaneously included limiting and non-limiting plug cell volumes and growing medium during nursery. The aim of this work was to assess the nursery performance of *Impatiens walleriana* seedlings grown in four plug cell volumes and four growing media with significant differences in both physical and chemical properties. Plants were sprayed or not with an early and single benzyl aminopurine (BAP) dose, aiming to understand how they interact on determining biomass accumulation at the pot transplant stage. The hypothesis tested was that, both plug cell volume and growing medium, must be seen as additive abiotic stress sources, which can be partially overridden by exogenous cytokinin supply. The main result was 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

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expansion and photo assimilates partitioning than plug cell volume and constitute an interactive process associated with cytokinin synthesis. From a grower´s point of view, one expensive option to avoid root restriction is to use high quality growing media and increase the plug cell volume. In contrast, a single 100 mg L^{-1} BAP spray can partially override the root restriction symptoms related to abiotic stresses. The novelty of this work is related to the fact that growing media quality would be a more limited factor than plug cell volume for *I. walleriana* seedlings during nursery.

Keywords: Abiotic stress; bedding plants; cytokinin; plug trays.

1. INTRODUCTION

Technological advances in transplanting have contributed to the growth of the bedding plant industry. The plug system for seedling propagation has been adopted by bedding plant growers to minimize labor costs, increase crop uniformity, and reduce cropping duration. Many growers specialized in the young plant stage (plug) know that the time required for seedlings to reach transplantable size determines greenhouse utilization efficiency and greatly affects production costs. A fundamental objective of most commercial bedding plant grower's, including propagators, is to produce a crop that meets the quality standards of the market in the shortest time.

An ornamental crop undergoes different abiotic stresses during commercial production cycle, which decrease both plant growth and quality. Previous reports from our laboratory have shown that plug cell volume [1,2] and growing media quality [3-7] would be considered as abiotic stresses. Negative growth effects on nursery remain and increase during the pot growth phase [3,8,9]. To override the root restriction syndrome related to plug cell volume and growing media quality [9] an early benzyl aminopurine spray (BAP) has been suggested [10-13]. However, quantitative information on the combined effects of these main abiotic stresses during nursery is lacking.

Plant roots can sense adverse soil conditions and, via some internal signal, transmit the condition of the soil to extending leaves, a process named "root restriction syndrome". The typically net result is a decrease in biomass accumulation rates. The primary function of a container is to provide a discrete space for the growth medium, which also affects the physical conditions of the medium. Balancing the air and water content of growing media is of the biggest problem facing plug growers. The second corner of plug air and water content are container size. Plug cells have two basic problems; they are too short and too small.

Soilless substrates are used in horticulture for growing seedlings, plant propagation, and ornamental plant production due to its high physical and chemical stability and low degradation rate, but peat mixes are not necessarily the optimal growth substrates because of their water-logging tendency and low oxygen availability under watering. Alternative soilless growing media can be better than peat for several ornamental bedding plants [6,7,14,15), including *Impatiens walleriana* [4,9, 16,17].

It has been claimed that the close coordination between root and shoot growth is controlled by a hormonal signaling pathway located in the root system. Cytokinins are root-synthesized, which are transported via the xylem to the shoot where they exert a major regulatory influence on growth [18-20]. Increased root growth may lead to a corresponding increase in the synthesis of cytokinins [21-23]. On the other hand, close relationships between root dry weight and physiological processes determining plant growth has been found for ornamentals [6,7,9,24] and vegetables [13] when exogenous cytokinins was applied.

Impatiens walleriana (Hook.f.), also known as busy Lizzie (United Kingdom), balsam, sultan or simply impatiens, is a commercially important year-round garden crop for landscape, and the first best-selling bedding plants in both developed and undeveloped countries. The Xtreme *Impatiens* White produce a compact green foliage and covers itself through extremely uniform growth habit with bright blooms. This is a great annual for spring, Mother's Day, summer, and fall. *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].

A previous report [9,28] has shown the significant influence of plug cell volume and growing media quality on *I. walleriana* growth, but there is no report, until today, on the combined effects of them during nursery.

The aim of this work was to assess the nursery performance of *I. walleriana* seedlings grown in four plug cell volumes and four growing media with significant differences in both physical and chemical properties. Plants were sprayed or not with an early and single benzyl aminopurine (BAP) dose, aiming to understand how they interaction determining biomass accumulation at the pot transplant stage. The hypothesis tested was that both plug cell volume and growing medium, must be seen as additive abiotic stress sources, which can be partially overridden by exogenous cytokinin supply.

2. MATERIALS AND METHODS

2.1 Plant Material

Experiment were carried out in 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.

Impatiens walleriana 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were germinated and grown (one seed by cell) 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 growing media as follows:

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

Leaves were sprayed at sunset with 0 (control plants) or 100 mg L^{-1} BAP solutions when the first true leaf pair was developed. The BAP concentration was chosen from previous experiments [25].

2.2 Cultivation and Meteorological Data

Plants were irrigated as needed 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 two experiments were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The plants were arranged at a density of 25 plants m^{-2}, which avoided mutual shading.

2.3 Sampling and Growth Evaluations

Samples of each substrate were collected at the beginning of the experiments (before transplant) and total porosity, air-filled porosity, bulk density and container capacity were determined according to Fonteno [29]. Data are indicated in Table 1 and show significant physical properties differences in of the growing media tested.

Plants were harvested at the transplant stage. Roots were washed and root, stem and leaf fresh weights (FW) were recorded. Dry weights (DW)

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%)]. The 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

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® (Image Processing and Analysis in Java) software.

The relative rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the natural logarithm (ln) of total leaf area versus time (in days). The rate of leaf appearance (RLA) was calculated as the slope of the number of fully expanded leaves versus time (in weeks). The relative growth rate (RGR) was calculated as the slope of the regression of the 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 [30] 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 and between leaf blades and the stem fraction 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 and between the ln of the leaf blade DW and the ln of the stem DW respectively.

2.4 Statistical Analysis

The experimental design was a randomised factorial with three blocks of five single-pot replications of each treatment combination (plug cell volume × growing medium × BAP concentration). Since there were no significant differences between the two experiments, they were considered together (n = 30). Data were subjected to three-way analysis of variance (ANOVA). STATISTICA 8 (StatSoft) software was used and the assumptions of the ANOVA were checked. Means were separated by Tukey's tests ($P \leq 0.05$). Slopes from straight-line regressions of RLA, RLAE, RGR and allometric values were tested using the SMATR package [31].

3. RESULTS

3.1 Fresh and Dry Weight Accumulation

The lower total FW values were found in plants grown on the K-growing media for most treatments. A single BAP spray when the first true leaf pair was developed significantly increased FW in some of the plug cell volume tested (Fig. 1A). When the mean shoot FW was plotted against the mean root FW (Fig. 1B), a positive correlation was found (r^2 = 0.910; $P < .001$).

When biomass accumulation was plotted on a DW base of different plant organs, lower values were found in control plants (Fig. 2A).The higher DW was found in plants in S-, R- and SR-growing media grown in 50-plug cell $tray^{-1}$. An early BAP spray increased DW differences in each plant organ (Fig. 2B). Leaves showed the highest DW biomass accumulation in all treatments tested and organs.

3.2 Leaf Growth and Plant Growth Analysis

The total leaf area at the transplant stage (60 days from the beginning of the experiment) showed similar trend to the total FW, however the BAP spray in 50-cells $tray^{-1}$ failed to increase leaf area in some of the treatments (Fig. 3).

R- and SR-growing media showed, during nursery, higher RLA values under the control plants and the BAP-sprayed ones than their corresponding K- and S-growing media. The higher the plug cell volume the higher the RLA in most growing media tested. RLAE differences were quite lower in K-growing media but showed the same response as the RLA in the rest of growing media tested. An early BAP spray increased both RLA and RLAE. Plants grown in 50-plug cell $tray^{-1}$ showed the higher RGR values in all growing media tested. BAP-sprayed plants increased RGR only in S-, R- and SR-growing media. Changes in RGR were related to higher NAR values and lower LAR ones (Table 2).

3.3 Photo Assimilates Partitioning

Allometries from roots, shoots and from leaves: stems showed that the lower the plug cell volume the higher the β coefficients (photo assimilates

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

	RLA (leaves week^{-1} plant^{-1})		RLAE (cm^2 cm^{-2} day^{-1})		RGR (g g^{-1} day^{-1})		NAR (g cm^{-2} day^{-1}) (x 10^{-5})		LAR (cm^2 g^{-1})	
	Control	**BAP**	**Control**	**BAP**	**Control**	**BAP**	**Control**	**BAP**	**Control**	**BAP**
K										
50	0.087^{aD}	0.100^{aD}	0.046^{aB}	0.063^{aB}	0.0632^{aC}	0.0627^{aD}	10.57^{bA}	17.57^{aA}	56.22^{aB}	43.07^{bB}
128	0.087^{aC}	0.113^{aC}	0.040^{aB}	0.048^{aB}	0.0489^{aC}	0.0468^{aD}	8.39^{bA}	14.60^{aA}	70.52^{aC}	46.80^{bB}
288	0.080^{aA}	0.093^{aB}	0.034^{aB}	0.043^{aB}	0.0469^{aC}	0.0403^{bD}	6.12^{bA}	12.30^{aA}	95.88^{aB}	7.25^{bC}
512	0.067^{aB}	0.083^{aB}	0.030^{aA}	0.032^{aB}	0.0428^{aB}	0.0406^{aC}	3.02^{bB}	7.20^{aA}	172.91^{aA}	85.53^{bB}
S										
50	0.139^{aC}	0.139^{aC}	0.079^{aA}	0.080^{aA}	0.0724^{aB}	0.0751^{aB}	8.45^{aB}	6.39^{bD}	69.83^{bA}	92.50^{aA}
128	0.067^{aC}	0.080^{aD}	0.042^{aB}	0.057^{aB}	0.0394^{bD}	0.0563^{aC}	8.09^{aA}	7.46^{aB}	64.39^{aC}	73.59^{aA}
288	0.063^{aB}	0.070^{aC}	0.031^{aB}	0.049^{aB}	0.0363^{bD}	0.0516^{aC}	6.66^{aA}	5.94^{aB}	70.87^{bC}	103.76^{aA}
512	0.033^{bC}	0.077^{aC}	0.019^{bB}	0.044^{aA}	0.0290^{bC}	0.0508^{aB}	5.70^{aA}	5.81^{aB}	78.45^{aB}	80.92^{aB}
R										
50	0.210^{bA}	0.278^{aB}	0.081^{aA}	0.090^{aA}	0.0781^{bA}	0.0852^{aA}	9.89^{bA}	15.06^{aB}	51.66^{aB}	34.46^{bB}
128	0.133^{bA}	0.153^{aB}	0.065^{aA}	0.076^{aA}	0.0618^{bB}	0.0714^{aB}	2.53^{bC}	8.45^{aB}	212.60^{aA}	62.85^{bA}
288	0.094^{bA}	0.197^{aA}	0.056^{aA}	0.071^{aA}	0.0607^{bA}	0.0706^{aA}	3.45^{bB}	6.02^{aB}	134.08^{aA}	84.94^{bB}
512	0.087^{bA}	0.117^{aA}	0.044^{aA}	0.056^{aA}	0.0545^{bA}	0.0600^{aA}	1.10^{bC}	7.02^{aA}	42.79^{bC}	89.95^{aB}
SR										
50	0.183^{bB}	0.390^{aA}	0.072^{bA}	0.092^{aA}	0.0764^{aA}	0.0679^{aC}	6.92^{bC}	11.96^{aC}	75.43^{aA}	49.07^{bB}
128	0.117^{bB}	0.200^{aA}	0.068^{aA}	0.077^{aA}	0.0684^{bA}	0.0839^{aA}	5.40^{bB}	13.17^{aA}	97.46^{aB}	42.14^{bB}
288	0.087^{aA}	0.096^{aB}	0.058^{aA}	0.060^{aA}	0.0588^{bB}	0.0651^{aB}	5.31^{bB}	7.63^{aB}	89.47^{aB}	67.20^{bB}
512	0.070^{aB}	0.083^{aB}	0.037^{bA}	0.053^{aA}	0.0428^{bB}	0.0577^{aA}	3.00^{aB}	2.52^{aC}	175.18^{aA}	206.44^{aA}

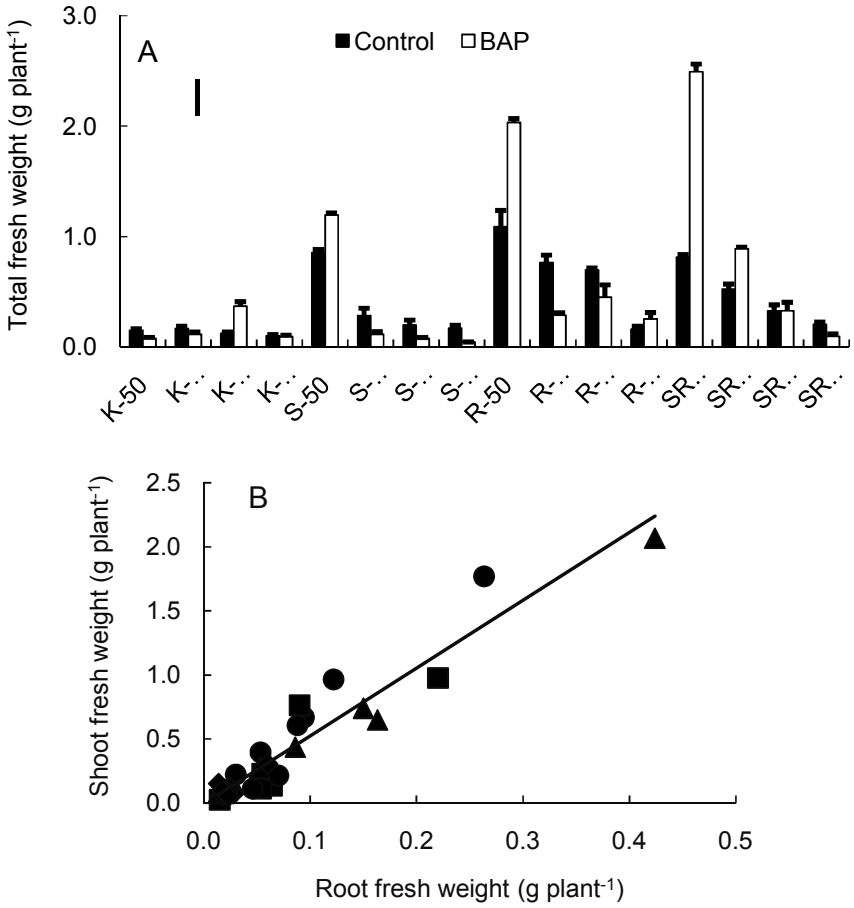

Fig. 1. Total FW (n = 30) at the end of the experiments (near 60 days from sowing) for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) and four growing media, sprayed with zero (control plants) or 100 mg L^{-1} BAP. Bars indicate standard errors 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}) and sprayed with zero or 100 mg L^{-1} BAP at the pre-transplant stage. For substrate abbreviations see Table 1. F:♦; R:●; S:▲; SR:■. The straight-line regression was: Shoot FW = 5.29 Root FW - 0.006 (r^2 = 0.910).The probability of the slope being zero was P < 0.001

would be partitioned preferably to roots and leaves respectively) for all the growing media tested. The BAP spray increased photo assimilates partitioning to shoots and stems respectively (lowered β coefficients) (Table 3).

3.4 Relationships between Growth Parameters and Root Dry Weight

Positive relationships between RLAE (r^2 = 0.653 P < 0.001) (Fig. 4A), RLA (r^2 = 0.775 P < 0.001) (Fig. 4B), RGR (r^2 = 0.637 P < 0.001) (Fig. 4C), NAR (r^2 = 0.730 P < 0.001) (Fig. 4D) and root DW were found. The highest values were found from plants sprayed with BAP, with a significant influence of the growing medium used.

4. DISCUSSION

For bedding ornamental plants, quality at the transplant stage is related to a squat, compact seedling and profusely branching with large growing points. A well-developed rooted system with white roots and without damage is needed as well. A decrease in plug cell volume increases plant density and lower labor costs, however, it gives a vertical root restriction, which leads to a pronounced decrease in both root and shoot

growth of the early vegetative stages [3]. A non-settle question until today is the impossibility to choose a growing media without regarding for the container size but at the same time it is not possible to choose a plug cell size without regarding the growing media used. Decisions such as the choice of container cell size and growing media quality before transplanting are frequently made on the basis of economics [8]. When seedlings are grown in typical transplant containers, growth tends to be proportional to the volume of the container cell. The more the space available to the plant from the nursery, the larger it becomes after transplant and the more quickly it attains particular growth stages. Both fresh (Fig. 1) and dry (Fig. 2) weight analyses are in agreement with this previous statements. However, in contrast with the previous reports, which indicated the plug cell size as the main abiotic stress during nursery [1,2], growing media quality would be a more restrictive abiotic stress at that growth stage that was previously supposed.

Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels [32]. Abiotic stress responses in plants occur to various organ levels among which the root specific processes are of particular importance [33].The growth dynamics of nursery

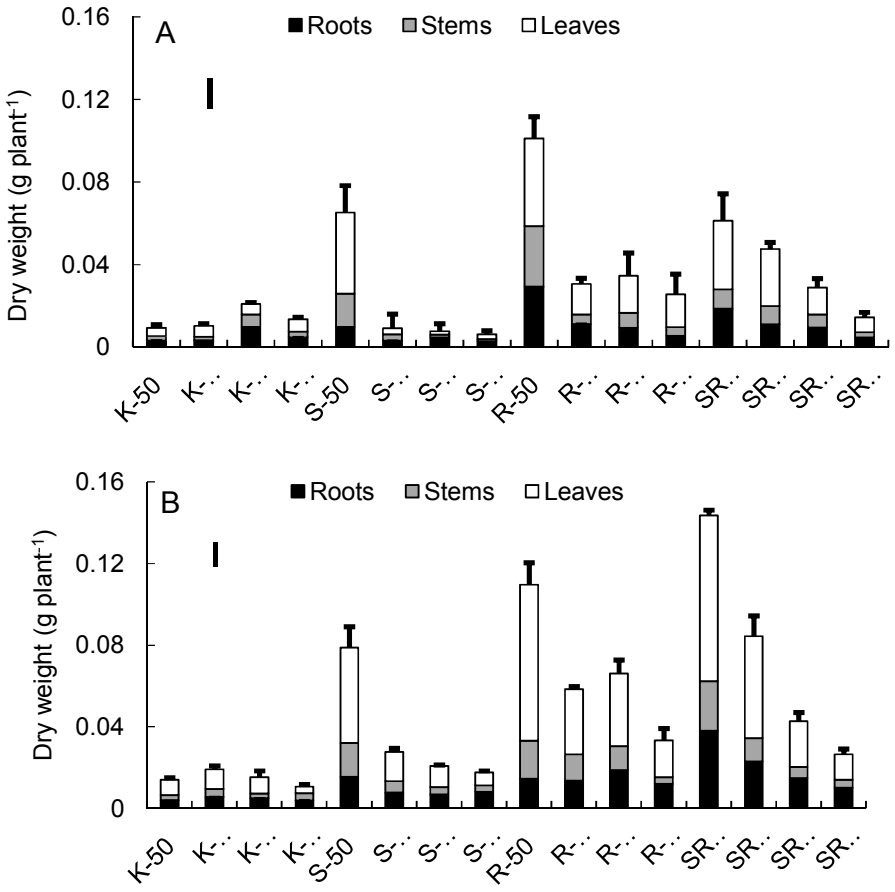

Fig. 2. Dry weight for roots, stems and leaves (n = 30) at the end of the experiments (near 60 days from seeding) for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) and four growing media, sprayed with zero (control plants) or 100 mg L^{-1} BAP. Bars indicate standard errors and vertical line indicate least significant differences (LSD). For substrate abbreviations see Table 1. Panel A showed control plants results while panel B showed BAP-sprayed ones

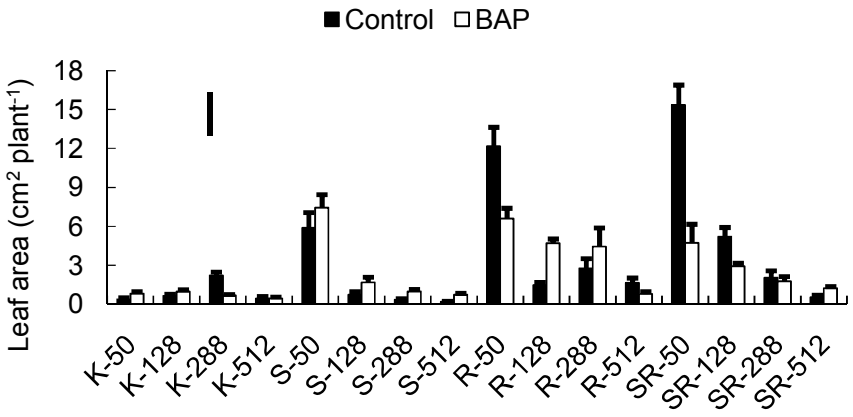

Fig. 3.Total leaf area (n = 30) at the end of the experiments (near 60 days from seeding) for *Impatiens walleriana* seedlings grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) and four growing media, sprayed with zero (control plants) or 100 mg L^{-1} BAP. Bars indicate standard errors and vertical line indicate least significant differences (LSD). For substrate abbreviations see Table 1

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

	Roots: Shoots		Leaves: Stems	
	β		β	
	Control	BAP	Control	BAP
K				
50	0.889^{aD}	0.834^{bB}	0.941^{aC}	0.803^{bC}
128	0.911^{aC}	0.906^{aA}	0.995^{aB}	0.828^{bB}
288	0.987^{aA}	0.812^{bC}	1.104^{aB}	0.823^{bB}
512	0.965^{aB}	0.803^{bC}	1.121^{aA}	0.921^{bA}
S				
50	1.157^{aA}	0.966^{bA}	1.006^{aD}	1.022^{aA}
128	0.900^{aB}	0.865^{bB}	1.024^{aC}	0.822^{bC}
288	0.841^{aC}	0.739^{bC}	1.068^{aB}	0.782^{bD}
512	0.773^{aD}	0.682^{bD}	1.117^{aA}	0.974^{bB}
R				
50	0.905^{aA}	0.908^{aA}	1.058^{aC}	0.676^{bC}
128	0.871^{aB}	0.790^{bB}	1.125^{aB}	0.789^{bA}
288	0.817^{aC}	0.795^{bB}	1.151^{aA}	0.773^{bB}
512	0.923^{aA}	0.626^{bC}	1.162^{aA}	0.786^{bA}
SR				
50	0.961^{aB}	0.826^{bC}	1.115^{aD}	0.852^{bA}
128	0.981^{aB}	0.957^{bA}	1.157^{aC}	0.632^{bC}
288	0.853^{aC}	0.852^{aB}	1.297^{aB}	0.667^{bB}
512	1.002^{aA}	0.729^{bD}	1.386^{aA}	0.855^{bA}

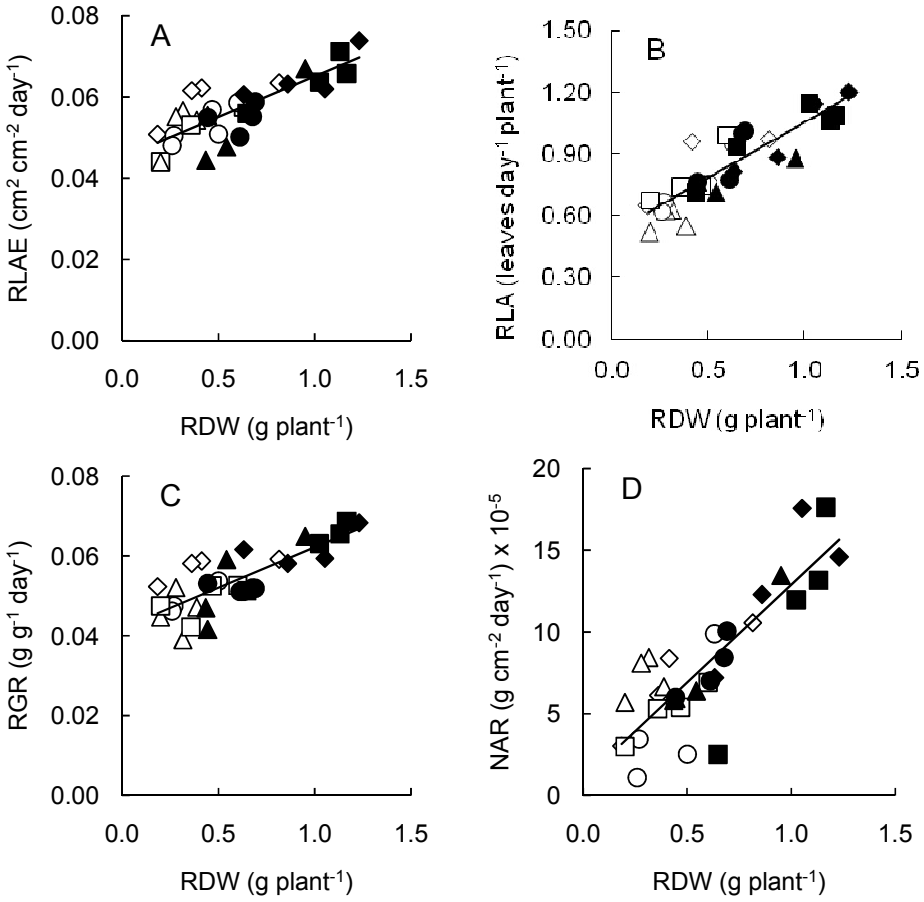

Fig. 4. Relationship between RLAE (A), RLA (B), RGR (C), NAR (D) and root dry weight (RDW) (n = 30) in plants of *Impatiens walleriana* grown in four plug cell volumes (50-, 128-, 288-, and 512-cell tray^{-1}) and four growing media sprayed with zero (control plants, empty symbols) or 100 mg L^{-1} BAP (full symbols) at the pre-transplant stage. The straight-line regressions were RLAE = 0.02 RDW + 0.045 (r^2 = 0.653 P < 0.001), RLA = 0.539 RDW + 0.52 (r^2 = 0.775 P < 0.001), RGR = 0.02 RDW + 0.042 (r^2 = 0.637 P < 0.001), NAR = 11.94 RDW + 0.95 (r^2 = 0.730 P < 0.001). F: ♦-◊; R: ●-○; S: ▲-Δ; SR: ■-□

plants is critical because seedlings complete their life cycle in a short time and normally do not have enough time to adjust to unfavorable environmental conditions. On the other hand, Pagani et al. [17] have previously suggested that to consider the growing medium as an abiotic stress source would allow changing the media-based paradigm to optimize both the growth and productivity of bedding pot plants. Our previous and present results are in agreement with this previous statements.

A general statement showed that plants increased biomass production through both shoots appearance and leaves expansion; photo assimilates from leaves are the force for the roots, shoots and leaves initiation and growth. The size of the different plant sinks determines the partition of photo assimilates between the plant organs.

A meta-analysis on the effects of pot size showed that root growth responds directly to impedance and decrease root growth and leaf area [34-36]. On the other hand, Puig et al. [37] and Chen et al. [38] have concluded that plants can sense the volume of the rooting space available and respond accordingly, having in mind that roots are a major source of cytokinins in plants, which are synthesized in roots and transported to shoots [39]. In this way, the significantly higher shoot FW related to plug cell

volume of *I. walleriana* seedlings from Figs. 1 and 3 would not be an unexpected result, although shoot FW increase in plug-grown vegetables exogenously BAP-sprayed has been previously indicated [11,12,40,41]. However, the most significant effect of a BAP spray on both FW and leaf area by growing media than plug cell volume is clearly a novelty result.

To characterize leaf area development, two growth parameters can be analyzed: first, the rate of leaf appearance (RLA) as an estimator of plastochron length, which involve leaf initiation rates. Second, the rate of leaf area expansion (RLAE), which quantify leaf growth. Our results showed that the higher the plug cell volume the higher the RLA and RLAE. It has been shown that plastochron may be altered in transgenic plants with reduced cytokinin levels [42], which explains the effect of a single BAP spray to override root restrictions. In the same way, Shani et al. [43] showed the effect of cytokinin on leaf growth rates. Both a decrease in the plastochron length and an increase in leaf expansion are accompanied by the SAM size increase through the synthesis of high-molecular weight substances essential for cell growth. Plant tissues and organs rich in cytokinins are known to attract the assimilate translocation and increase the sink capacity of the benzyl adenine-treated leaves [44].Our results showed that limiting both plug cell volume and growing media quality decreased RLA more than RLAE (Table 2), which would condition post-transplant growth rates during the pot-grown stages.

Variation in RGR has the result of two key traits: the 'physiological component' NAR and the 'morphological component' LAR [45]. Although leaf area determines plant capacity of light interception, RGR, which ultimate quantify biomass accumulation, is greatly influenced by photosynthetic efficiency [46]. Although the higher the plug cell volume the higher the RGR and NAR, growing media quality and exogenous BAP increase both growth parameters (Table 2). Shipley [47] indicated that, in general, NAR was the best general predictor of variation in RGR, in agreement with our results from Table 3. On the other hand, Shi et al. [34] showed that root restriction often depresses photosynthetic capacity but indicated that the mechanism for this reduction remains unclear. However, cytokinin is known to stimulate the expression of photosynthetic enzymes like Rubisco [48,49]. Plants synthesize different cytokinin-ribosides but not all have biological activity [50], although the higher the root system, the higher the zeatin ribosides[21-23]. We have shown positive relationships between RLAE (Fig. 4A), RLA (Fig. 4B), RGR (Fig. 4C) and NAR (Fig. 4D) and root DW.

Poorter and Sack [51] indicated that sink organs can potentially stimulate sugar supply by activating their consumption rate, thereby increasing their sink strength. The relative carbon allocation to a particular organ must be regarded as a function of source and sink activities of all parts of the plant regulate by the relative photo-assimilates allocation [52]. Benincasa et al.[53] indicated that crop management and environmental factors modify the source-sink relationships, including abiotic and biotic stresses.Changes in the allometric slopes (β) reflect variations in carbon partitioning in response to biotic and abiotic environment interactions [54]. Carbohydrate partitioning between competing sites is explained by the fact that plants are capable of modifying their resource allocation to favor the growth of their growing parts [55,56]. Differences in plant allometries shown in Table 3 are in agreement with these assumptions and indicate that a root restriction by plug cell volume or low growing media quality partition photo assimilates to roots and leaves while non limited plug cell volume and high growing media quality or exogenous BAP applications changes photo assimilates partitioning to shoots and stems respectively. The lower root systems would not be a limited factor due to the constant water supply under commercial facilities. In agreement with Zwack and Rashotte [57], who indicate that multiple factors influence how cytokinin treatment affects stress signaling and that the spatial, temporal, and developmental context may be important factors in the downstream stress response. The response to an exogenous BAP supply from our results was quantitatively different when plug cell volume or growing media quality abiotic stress sourceswere analyzed.

5. CONCLUSIONS

The only way to be economically competitive in the future during nursery is to decrease labor costs and increase business efficiency. However, our results indicated that it does not mean necessarilyto use exclusively more cells per tray and decrease growing media quality for bedding pot plant propagation in plug trays. Nursery optimization needshigher cell tray volume and high growing media quality relationships as much

as to identify the differentornamental bedding plant environmental requirements, which is the subject for future research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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