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Changes in Dry Weight Accumulation in the *Impatiens walleriana* Pot Plant in Response to Different Pre-transplant Plug Cell Volume

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Summary

The use of transplants is the most reliable method to ensure adequate crop establishment of commercial plantings of most ornamental bedding crops; however, in plants grown in small pots, root growth restrictions often occur. The aim of this work was to determine the dry weight accumulation processes involved in the root restriction generated by a decrease in plug cell volume under the hypothesis that cytokinins synthesized by roots are part of the mechanism of *Impatiens walleriana* aerial biomass accumulation which can be partially overriding by an early BAP spray during the propagation stage in plug trays. Our results showed that *I. walleriana* aerial biomass productivity is related to root dry weight; the higher total dry weight of the controls grown in 50 plug cell trays is related to higher relative growth rate (RGR) as a result of an increase in net assimilation rate (NAR) and leaf area ratio (LAR). On the other hand, the smaller cell volume the higher photosynthate partitioning to roots. *I. walleriana* dry weight accumulation and photosynthate partitioning were changed in response to different BAP sprays doses (5 to 200 mg L^{-1}) and application stages, but the magnitude of the changes were associated to the cell volume too.

Key words. bedding plant – cytokinin – growth – root restriction

Introduction

Bedding plant producers have progressively adopted plug trays of reduced size leading to a limited substrate volume available for the root system; *I. walleriana* is usually saled in 288 or 512 plug cell trays. Root restriction is a physical stress imposed on a root system when plants are grown in small containers; this situation leads to a pronounced decrease in root and shoot growth, leaf area and height as rooting volume decreases (Dubik et al. 1989; Latimer 1991; van Iersel 1997; Mugnai et al. 2000; Di Benedetto and Klasman 2004, 2007; Di Benedetto 2011).

Root restriction is hypothesized to reduce the supply of photoassimilates to the shoot; an important mediator of shoot physiological processes associated with ornamental biomass production can be the supply of signal molecules (other than water and nutrients) from the root system (Kyozuka 2007). There are strong indications that cytokinins are root factors which are transported via the xylem to the shoot where they exert a major regulatory influence on growth (Chernyad'ev 2005). Increased root growth may lead to a corresponding increase in the synthesis of cytokinins (O'Hare and Turnbull 2004).

In plants grown in small pots, it has been observed that exogenous application of BAP may override shoot growth limitation due to root restriction (Di Benedetto 2011). Haver and Schuch (2001) showed that isopentenyladenosine and zeatin riboside decreased on a whole petunia shoot basis as rooting volume decreased from 162 to 58 cm^3. Di Benedetto et al. (2010) showed that spraying exogenous BAP solutions increased both fresh weight and dry weight for *E. aureum* and *F. benjamina* 'green' and 'variegated' ornamental plants.. Although, it has been well established that root stress induced by volume reduction severely suppresses bedding plants shoot growth, the physiological and biochemical processes involved are largely unknown (Di Benedetto and Klasman 2004, 2007).

The aim of this research was to characterize the changes in dry weigh accumulation in response to the root restriction generated by a decrease in plug cell volume under the hypothesis that cytokinins synthesized by roots are part of the mechanism involved in aerial biomass accumulation in *Impatiens walleriana* which would

be partially overriding by an early BAP spray during the propagation stage in plug trays.

Materials and Methods

The experiment was carried out in the greenhouse facilities of the School of Agriculture, University of Buenos Aires campus (34°28'S), Argentina from 19th September 2010 to 12th December 2010 and repeated once from 1st October 2011 to 30th December 2011.

Plant material

Impatiens walleriana 'Accent White' (Goldsmith Ind., U.S.A.) seeds were germinated and grown in plastic plug trays (Blackmore Inc. U.S.A.) with 50, 128, 288 or 512 plug cells tray^{-1} (55.70; 17.37; 6.18 and 2.50 cm^3 cell^{-1} respectively) using a Fafard Growing Mix 2® media (Canadian *Sphagnum* peat moss-perlite-vermiculite 70:20:10 v/v). Leaves were sprayed, at sunset, with 0, 5, 50, 100 or 200 mg L^{-1} BAP solutions either when the 1st true leaf pair was developed (pre-transplant application) or one week after transplant (post-transplant application).

When plants reached the transplant stage, this is, when the 4th true leaf pair was expanded, plants were transplanted into 1,200 cm^3 pots filled with a Canadian peat-based media.

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity of 0.486 dS m^{-1}) using intermittent overhead mist and a weekly soil fertilization (1N:0.5P:1K:0.5Ca v/v) according to Styer and Koranski (1997) (Stage 2: 50 mg L^{-1} N; Stage 3–4: 100 mg L^{-1} N; pot: 150 mg L^{-1} N) was included.

Daily mean temperatures (22.24 to 24.80 °C) and daily photosynthetic active radiation (8.66 to 10.79 mol photons m^{-2} day^{-1}) for the two experiments were recorded with a HOBO sensor (H08-004-02) connected to HOBO H8 data logger.

Abbreviations

BAP: 6-benzylaminopurine (SIGMA EC 214-927-5); LAR: leaf area ratio; NAR: net assimilation rate; RGR: relative growth rate; RLAER: relative leaf area expansion rate.

Sample and growth evaluations

Plants were harvested at both the transplant and the sales stages (ten weeks after transplant). Roots were washed and both roots and shoots (stems and leaves) were dried at 80 °C for 48 h and weighed. Leaf area was determined with a LI-COR 3000A automatic leaf area meter.

RGR was calculated as the slope of the regression of the natural logarithm of whole plant weight vs. time in days while RLAER was calculated as the slope of the regression of natural logarithm total leaf area vs. time in days. Both NAR and LAR were calculated according to Potter and Jones (1977). The allometric relationships between roots and shoots were performed using a straight-line regression analysis between the natural logarithm to root dry weight and the natural logarithm to shoot dry weight during the experiments.

Statistical analysis

The experimental design was a randomized factorial with 3 blocks of 5 single-pot replications of each treatment combination (plug cell volume × BAP concentration). Since there were no significant differences between the two experiments, they were considered together (n = 30). Data were subjected to two way analysis of variance and means were separated by Tukey tests ($P < 0.05$). Equations to predict shoot dry weight relationship to root dry weight were developed via linear regression.

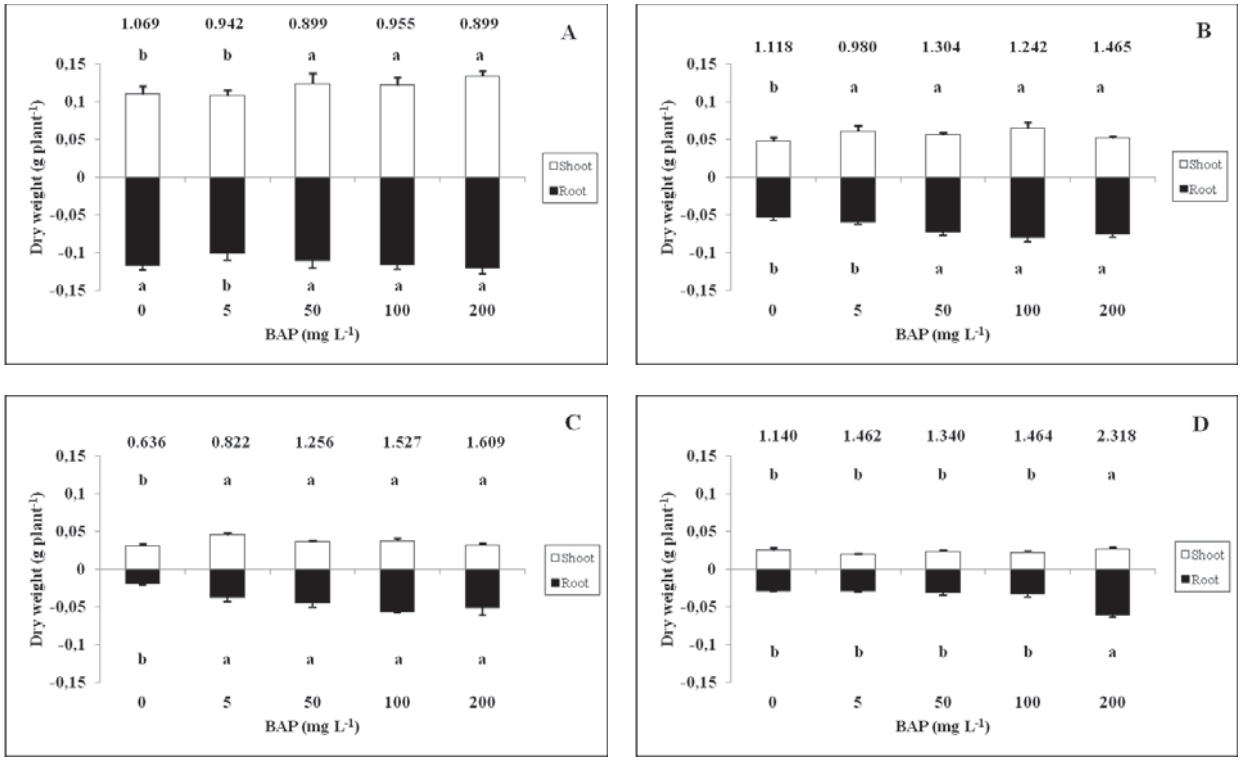
Results

Biomass accumulation

There were no significant differences between both experiments that is why they were considered together. The greenhouse climatic control gives small variations in mean temperature while the fact that experiment was repeated in the same season would explain the lack of main significant difference in the daily photosynthetic active radiation between the two yearly experiments.

A significant decreased in both root and shoot biomass at the transplant stage as plug try volume decreased was found (Fig. 1). A single pre-transplant BAP spray when the 1st true leaf pair was developed did not change root growth although a higher BAP concentration than 50 mg L^{-1} was required for increasing shoot biomass accumulation in plants grown in 50 plug cell trays (Fig. 1A). When trays with128 or 288 plug cell were used, pre-transplant BAP applications stimulated both root and shoot growth (Fig. 1B, C). For trays with 512 plug cell (Fig. 1D), a 200 mg L^{-1} BAP solution was required for significant differences in root and shoot growth. Controls trays with 50 and 288 plug cells showed the higher root/shoot ratio; little changes in 50 plug cell-grown plants but an increase for the rest of plug cell volume tested was observed when different BAP concentrations were used. Main (Plug cell volume or BAP concentration) and interaction (Plug cell volume × BAP concentration) for roots and shoots dry weight effects in the ANOVA test showed highly significant ($P \leq 0.001$) differences. However, there was no main significant difference between the two yearly experiments.

At the sale stage the biomass accumulation differences found at the transplant stage were reduced and more so when the number of plug cell tray increased, although those plants previously grown in 50 plug cell tray showed the highest biomass accumulation for leaves, stems and

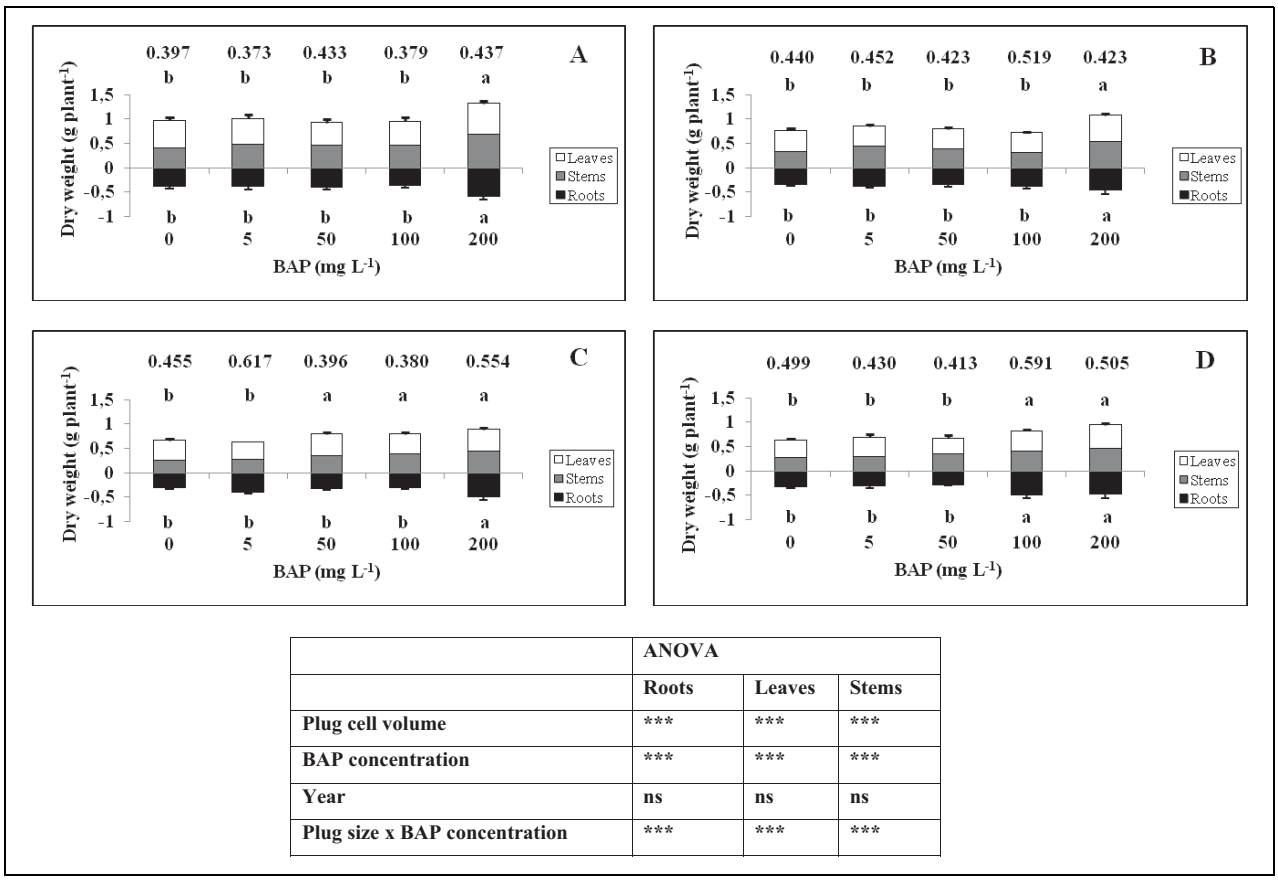


	ANOVA	
	Roots	**Shoots**
Plug cell volume	***	***
BAP concentration	***	***
Year	**ns**	**ns**
Plug size x BAP concentration	***	***

Fig. 1. Dry weight distribution (n = 30) at the transplant stage for *I. walleriana* seedlings grown in four plug cell volumes. A: 50, B: 128, C: 288 and D: 512 plug cell trays and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) solutions at the pre-transplant stage. Bars indicate standard errors. Different lower case letters indicate statistical significance ($P \leq 0.05$) for shoots and roots. Numbers above the bars showed root/shoot ratio. Significance *** 0.001 ** 0.01 * 0.05 'ns' not significant.

roots (Fig. 2A). Pre-transplant BAP-sprayed plants increased root and shoot dry weight but the dose required for significant differences were related to the initial plug cell volume; highest biomass accumulation in plants grown at 50 and 128 cell trays (Fig. 2A, B) was achieved when a 200 mg L^{-1} BAP was used but for 288 and 512 cell trays (Fig. 2C, D) 100 to 200 mg L^{-1} BAP were needed. The use of BAP in the pre-transplant stage on plants grown on 128 cell trays let similar dry weights as the 50 cell plug tray control plants, although the use of BAP sprays increased dry weight accumulation for the 50 cell trays too. There were no root/shoot ratio differences between plug cell volume for the control plants but an increase when BAP sprays were applied. Main (Plug cell volume; BAP concentration) and interaction (Plug cell volume × BAP concentration) for roots, leaves and stem dry weight effects in the ANOVA test showed highly significant ($P \leq 0.001$) differences, with no single significant difference between the two yearly experiments.

However, when different BAP concentrations were applied at the post-transplant stage the results showed a negative correlation between 50 to 200 mg L^{-1} BAP doses and biomass accumulation increase for all plug cell volumes tested (Fig. 3). A significant higher biomass was found when 5 mg L^{-1} BAP was used for the 50, 128 and 288 plug cell trays (Fig. 3A, B, C); but plants from the 512 cell trays did not show significant biomass accumulation differences to control plants (Fig. 3D). Root/shoot ratio differences between plug cell volume for the control plants showed little changes but an increase when BAP sprays were applied. Main effects in the ANOVA test showed highly significant ($P \leq 0.001$) differences for Plug cell volume and BAP concentration ($P \leq 0.05$) and for the interaction (Plug cell volume × BAP concentration)



	ANOVA		
	Roots	**Leaves**	**Stems**
Plug cell volume	***	***	***
BAP concentration	***	***	***
Year	**ns**	**ns**	**ns**
Plug size x BAP concentration	***	***	***

Fig. 2. Dry weight distribution (n = 30) at the sale stage for *I. walleriana* seedlings grown in four plug cell volumes. A: 50, B: 128, C: 288 and D: 512 plug cell trays and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) Bars indicate standard errors. Different lower case letters indicate statistical significance ($P \leq 0.05$) for aerial parts (stems + leaves) and roots. Numbers above the bars showed root/shoot ratio. Significance *** 0.001 ** 0.01 * 0.05 'ns' not significant.

($P \leq 0.01$) for roots, leaves and stem dry weight. No main significant difference between the two yearly experiments was found again.

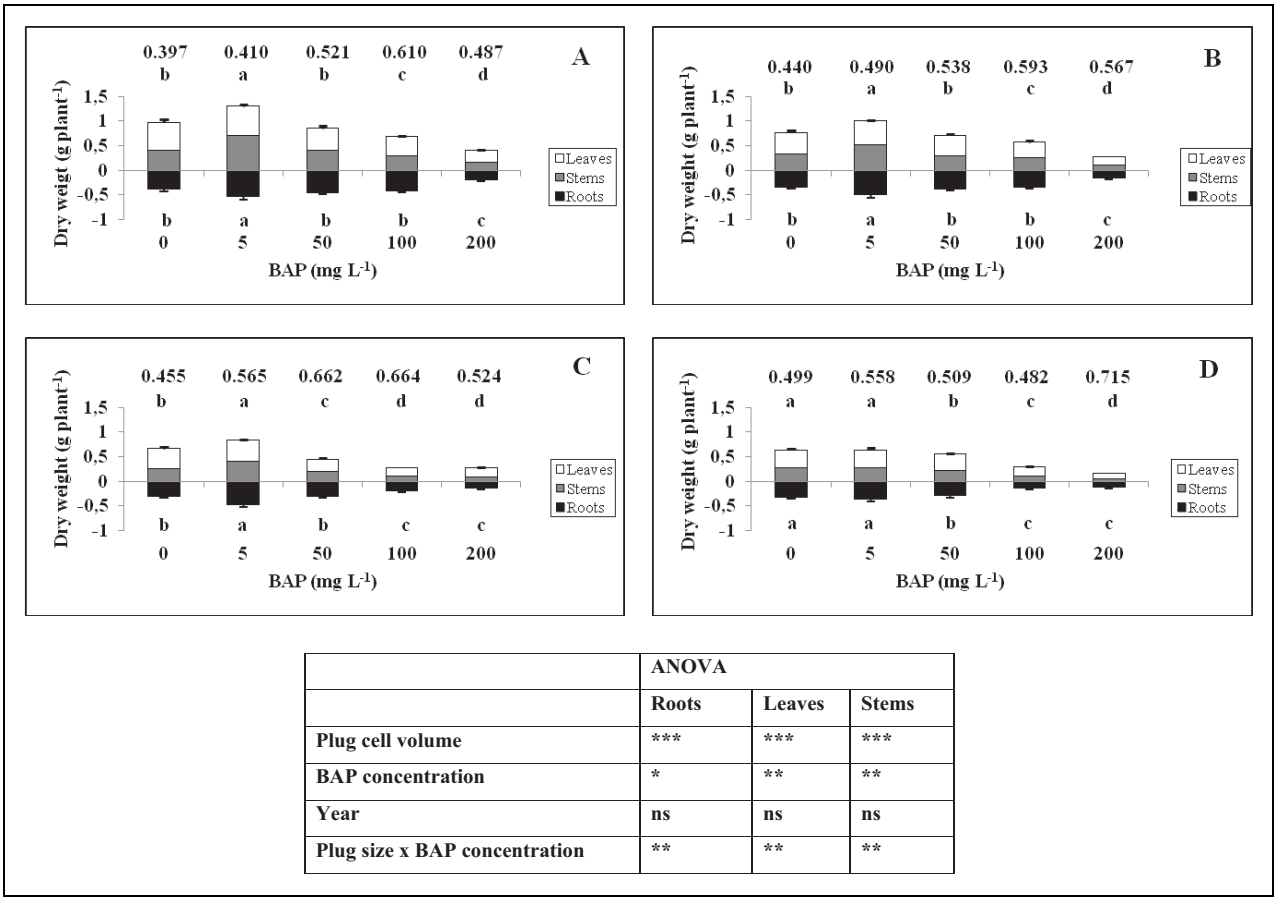
Fig. 4 showed a good correlation between shoot dry weight and root dry weight, although a higher determination coefficient and lower data dispersion were found at the transplant stage ($r^2 = 0.729$; slope standard error = 0.053) (Fig. 4A) than for the sale stage ($r^2 = 0.492$; slope standard error = 0.065). The higher plug cell volume the higher shoot-root correlation (Fig. 4B).

Growth coefficients

From the leaf area data at the seed emergence, transplant and sale stage harvests, RLAER were calculated (Table 1). At the transplant stage the higher the plug cell volume the higher RLAER. A pre-transplant BAP-spray increased RLAER with a higher plug cell volume with a maximum response for 100–200 mg L^{-1} BAP in 50 cell tray; 50 to 200 mg L^{-1} BAP for 512 cell tray and 5 to 200 mg L^{-1} BAP for 128 and 288 cell tray; RLAER for the control plants, at the emergence-sale stage showed a similar response patterns. A pre-transplant BAP sprays increased RLAER although different BAP doses for maximum increasing were required. A post-transplant BAP application gave, in general, a decreased in RLAER.

Although the lower cell tray number the higher RGR for control plants between the emergence-sale stages was found, the highest RGR ever were found for the emergence-transplant stage. A single pre-transplant BAP-spray increased RGR for both emergence-transplant and emergence-sale stage, although the final responses were related to the plug cell volume. Plants sprayed with different BAP solutions at the post-transplant stage showed a lower increase and, in some cell trays, a decrease in RGR (Table 2).

When RGR was disaggregated in NAR and LAR, significant differences were found (Table 2). During the emergence-transplant stage, control plants showed a decrease in both NAR and LAR as cell tray number increase; a BAP spray increased NAR and LAR too specially in 288- and 512-grown plants. During the emergence-sale stage, controls showed no differences in NAR for the 50-, 128- and 288-grown plants and an increase in 512-grown plants; at the same time, LAR decreased as cell tray number increased. The pre-transplant BAP-sprayed plants



	ANOVA		
	Roots	**Leaves**	**Stems**
Plug cell volume	***	***	***
BAP concentration	*	**	**
Year	**ns**	**ns**	**ns**
Plug size x BAP concentration	**	**	**

Fig. 3. Dry weight distribution (n = 30) at the sale stage for *I. walleriana* seedlings grown in four plug cell volumes. A: 50, B: 128, C: 288 and D: 512 plug cell trays and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) solutions at the post-transplant stage. Bars indicate standard errors. Different lower case letters indicate statistical significance ($P \leq 0.05$) for aerial parts (stems + leaves) and roots. Numbers above the bars showed root/shoot ratio. Significance *** 0.001 ** 0.01 * 0.05 'ns' not significant.

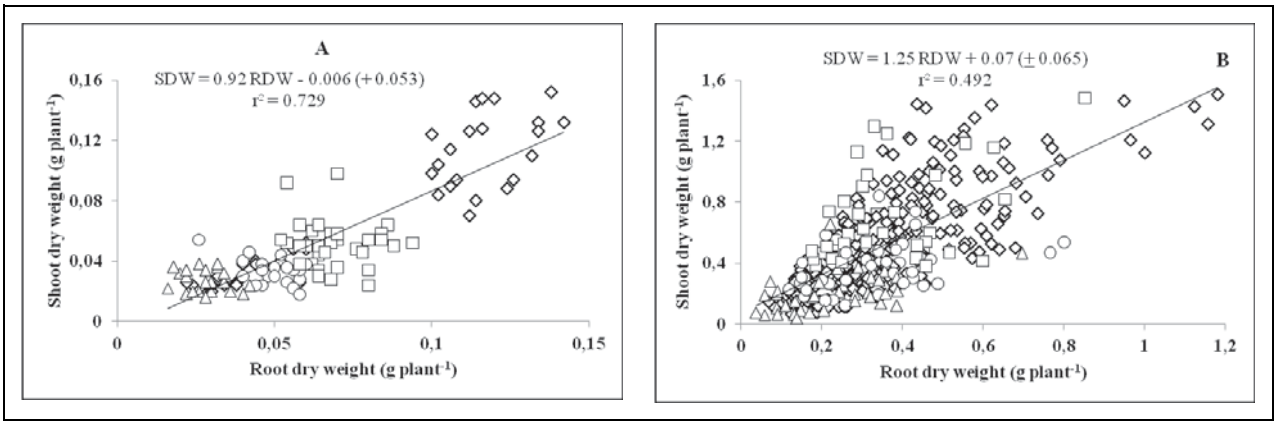


Fig. 4. Root and shoot dry weight relationships at both the transplant stage (A) and the sale stage (B) for *I. walleriana* seedlings grown in different plug cell trays number and sprayed with different BAP solutions at the pre-transplant stage. The straight-line regressions are indicated and the probability of the slope being zero was $p \leq 0.05$ and $p \leq 0.01$ for A and B respectively. SDW: shoot dry weight; RDW: root dry weight. ◊: 50, □: 128, ○: 288 and Δ: 512 plug cell trays.

Table 1. Changes in RLAER ($cm^2\ cm^{-2}\ day^{-1}$) for plants of *I. walleriana* grown in four plug cell volumes (50, 128, 288 and 512 plug cell trays) and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) solutions at the pre- or post-transplant stage. The standard errors are indicated.

Plug cell volume/ BAP (mg L^{-1})	BAP at pre-transplant stage		BAP at post-transplant stage
	Emergence-Transplant	Emergence-Sale	Emergence-Sale
50 cell trays			
0	0.0661 ± 0.0044	0.0461 ± 0.0052	0.0461 ± 0.0052
5	0.0627 ± 0.0056	0.0427 ± 0.0064	0.0487 ± 0.0035
50	0.0665 ± 0.0039	0.0453 ± 0.0024	0.0437 ± 0.0025
100	0.0682 ± 0.0029	0.0439 ± 0.0024	0.0435 ± 0.0023
200	0.0685 ± 0.0034	0.0474 ± 0.0022	0.0352 ± 0.0039
128 cell trays			
0	0.0442 ± 0.0044	0.0418 ± 0.0045	0.0418 ± 0.0045
5	0.0483 ± 0.0029	0.0439 ± 0.0035	0.0465 ± 0.0033
50	0.0496 ± 0.0020	0.0418 ± 0.0036	0.0423 ± 0.0034
100	0.0527 ± 0.0015	0.0439 ± 0.0022	0.0410 ± 0.0028
200	0.0542 ± 0.0032	0.0456 ± 0.0032	0.0324 ± 0.0018
288 cell trays			
0	0.0236 ± 0.0025	0.0401 ± 0.0030	0.0401 ± 0.0030
5	0.0387 ± 0.0060	0.0414 ± 0.0008	0.0446 ± 0.0026
50	0.0392 ± 0.0035	0.0422 ± 0.0039	0.0364 ± 0.0020
100	0.0437 ± 0.0027	0.0429 ± 0.0027	0.0310 ± 0.0040
200	0.0414 ± 0.0055	0.0423 ± 0.0054	0.0294 ± 0.0049
512 cell trays			
0	0.0240 ± 0.0027	0.0404 ± 0.0037	0.0404 ± 0.0037
5	0.0230 ± 0.0024	0.0380 ± 0.0052	0.0416 ± 0.0021
50	0.0277 ± 0.0053	0.0375 ± 0.0035	0.0396 ± 0.0024
100	0.0288 ± 0.0045	0.0483 ± 0.0009	0.0303 ± 0.0045
200	0.0379 ± 0.0026	0.0458 ± 0.0040	0.0275 ± 0.0084

showed a LAR increase and a NAR decrease with highest changes in the 512-grown plants. However, for the post-transplant BAP-sprayed plants there were an increase in NAR at lower BAP doses (5–50 mg L^{-1}) and a NAR decrease at higher BAP doses (100–200 mg L^{-1}) for 50-, 128- and 288-grown plants; a post-transplant BAP spray always decreased 512-grown plants NAR. LAR decreased with a single post-transplant BAP-spray in 50-, 128- and 288-grown plants but showed an opposite pattern for 512-grown plants.

The allometric relationships used to estimate the effect of cell tray number and BAP sprays on dry weight partitioning between roots and shoots during the experiments (Table 3) showed that the highest plug cell volume partitioned more photoassimilate towards shoots than roots; a BAP spray in the pre-transplant stage slightly changed dry weight partitioning between roots and shoots in 50- and 128-grown plants but significantly increased partitioning towards shoots in 288- and 512-grown plants. A post-transplant BAP spray significantly changed dry weight partitioning towards roots.

Discussion

Plant productivity

I. walleriana bedding plant productivity has been associated with aerial biomass expansion and shoot fresh weight; by the other hand the last was mainly determined by the root system size (Di Benedetto 2011; Thibaud et al. 2012). The same conclusion was achieved when dry weight was used because there were no significant differences for dry matter content (pre-transplant: 0.068 ± 0.0036 %; post-transplant: 0.063 ± 0.0038 %) (data not shown), nevertheless a higher linear regression fitness for emergence-transplant stage than for transplant-sale stage was found (Fig. 4).

Table 2. Changes in RGR (g g^{-1} day^{-1}), NAR (g cm^{-2} day^{-1} × 10^{-5}) and LAR (cm^2 g^{-1}) for plants of *I. walleriana* grown in four plug cell volumes (50, 128, 288 and 512 plug cell trays) and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) solutions at the pre- and post-transplant stage. The standard errors for RGR are indicated.

Plug cell volume/ BAP (mg L^{-1})	BAP at pre-transplant stage						BAP at post-transplant stage		
	Emergence-Transplant			Emergence-Sale			Emergence-Sale		
	RGR	NAR	LAR	RGR	NAR	LAR	RGR	NAR	LAR
50 cell trays									
0	0.1313 ± 0.0030	20.65	635.75	0.0872 ± 0.0049	19.60	444.88	0.0872 ± 0.0049	19.60	444.88
5	0.1256 ± 0.0053	18.83	667.21	0.0875 ± 0.0066	19.89	428.38	0.0918 ± 0.0029	22.44	409.03
50	0.1320 ± 0.0035	20.89	631.75	0.0884 ± 0.0022	21.61	409.03	0.0874 ± 0.0029	21.91	398.86
100	0.1324 ± 0.0035	20.39	649.24	0.0870 ± 0.0029	21.27	409.03	0.0858 ± 0.0024	20.28	423.01
200	0.1339 ± 0.0032	21.15	633.08	0.0912 ± 0.0018	23.45	390.57	0.0772 ± 0.0041	18.02	428.38
128 cell trays									
0	0.1122 ± 0.0042	18.72	599.44	0.0840 ± 0.0044	19.70	428.38	0.0844 ± 0.0044	19.70	428.38
5	0.1164 ± 0.0033	19.46	598.19	0.0865 ± 0.0031	20.71	417.72	0.0894 ± 0.0037	21.67	412.49
50	0.1181 ± 0.0027	19.91	593.18	0.0842 ± 0.0032	20.37	417.72	0.0848 ± 0.0025	20.23	421.24
100	0.1207 ± 0.0025	20.14	599.44	0.0854 ± 0.0025	19.52	437.47	0.0833 ± 0.0025	19.69	423.01
200	0.1178 ± 0.0021	17.92	657.47	0.0875 ± 0.0034	21.34	414.22	0.0749 ± 0.0021	17.86	419.47
200 cell trays									
0	0.0961 ± 0.0040	17.62	545.39	0.0822 ± 0.0031	19.67	426.58	0.0822 ± 0.0029	19.67	426.58
5	0.1073 ± 0.0052	18.13	591.94	0.0852 ± 0.0014	21.45	397.18	0.0882 ± 0.0017	22.02	400.53
50	0.1073 ± 0.0036	17.94	598.19	0.0848 ± 0.0041	20.30	417.72	0.0807 ± 0.0029	20.75	388.93
100	0.1108 ± 0.0025	18.14	610.88	0.0846 ± 0.0031	19.50	433.81	0.0734 ± 0.0036	17.42	421.24
200	0.1076 ± 0.0060	17.28	622.54	0.0867 ± 0.0064	22.39	387.30	0.0710 ± 0.0047	16.30	435.63
512 cell trays									
0	0.0976 ± 0.0034	16.31	532.96	0.0834 ± 0.0032	20.30	410.76	0.0834 ± 0.0032	20.30	410.76
5	0.0951 ± 0.0034	17.29	549.99	0.0813 ± 0.0057	20.04	405.61	0.0842 ± 0.0026	20.16	417.72
50	0.0983 ± 0.0027	17.32	567.59	0.0821 ± 0.0039	21.38	384.06	0.0810 ± 0.0028	18.44	439.31
100	0.0983 ± 0.0032	16.92	580.85	0.0885 ± 0.0028	19.16	462.02	0.0713 ± 0.0046	15.96	446.75
200	0.1090 ± 0.0035	19.41	561.66	0.0892 ± 0.0032	22.08	403.91	0.0677 ± 0.0055	14.65	462.02

Plug cell volume responses

In plants grown in small pots, root growth restrictions often occur. At the transplant stage, ornamental plants grown in plug trays may show a well developed root system with white roots and without damage but with a root guirdling growth around the cell (Di Benedetto and Klasman 2004, 2007; Di Benedetto 2011). The root restriction effects remain for the following weeks although a new root system may develop through the lateral roots that arise adventitiously. The same picture is repeated when root systems from transplanted seedlings which are grown in larger containers, reach the pot base (Di Benedetto et al. 2006, 2010). A limited plug cell or pot volume gives a vertical root restriction even under a wide water, nutrient and oxygen supply; the same effect would be seen when higher containers was used. 'Accent' *I. walleriana* exhibits, at the post-transplant stage, similar morphological changes as observed in other herbaceous plants (Haver and Schuch 2001): a decrease in leaf area as a result of lower RLAER (Table 1) and dry weight accumulation (Fig. 1, 2 and 3).

In the most of the experiments, plants were monitored frequently to prevent drought stress, since the different flats dried at different rates and usually there were no visual evidence of nutrient deficiency in any of the containers. Krizek et al. (1985) reported, on soybean, that inadvertent drought stress in small root volumes was not necessarily responsible for reduced shoot growth and that nutrient distribution was not affected by root restriction. Therefore, water and nutrient stress were not considered to be determinants of plant size under commercial environmental facilities.

Di Benedetto and Pagani (2011) showed a close coordination between roots and shoots growth, controlled by a signaling pathway which is largely hormonal in nature with a major site of control located in the root system (Hirose et al. 2008). Increased root growth may lead to a

Table 3. Changes in the allometric relationships between shoots and roots for *I. walleriana* plants grown in four plug cell volumes (50, 128, 288 and 512 cell trays) and sprayed with different BAP (0, 5, 50, 100 and 200 mg L^{-1}) solutions at the pre- and post-transplant stage using a straight-line regression analysis between the natural logarithm of roots dry weight and the natural logarithm to shoot dry weight (ln Root dry weight = $\alpha + \beta \times$ ln Shoot dry weight). Standard errors for the slope (β) are indicated. The intercept straight-line (α) and the coefficients of determination (r^2) are indicated too.

Plug cell volume/	BAP at pre-transplant stage			BAP at post-transplant stage		
BAP (mg L^{-1})	α	β	r^2	α	β	r^2
50 cell trays						
0	−0.74	0.65 ± 0.061	0.883			
5	−0.68	0.69 ± 0.057	0.902	−0.77	0.93 ± 0.070	0.918
50	−0.72	0.73 ± 0.096	0.806	−0.74	0.94 ± 0.099	0.856
100	−0.66	0.80 ± 0.092	0.854	−0.53	1.10 ± 0.072	0.947
200	−0.62	0.78 ± 0.057	0.921	−0.67	0.95 ± 0.081	0.896
128 cell trays						
0	−0.66	0.77 ± 0.048	0.942			
5	−0.61	0.80 ± 0.060	0.926	−0.70	1.01 ± 0.053	0.962
50	−0.75	0.70 ± 0.058	0.907	−0.63	0.93 ± 0.061	0.941
100	−0.56	0.74 ± 0.065	0.904	−0.59	0.97 ± 0.100	0.864
200	−0.60	0.72 ± 0.064	0.890	−0.59	0.98 ± 0.103	0.860
288 cell trays						
0	−0.49	0.99 ± 0.038	0.977			
5	−0.33	0.98 ± 0.063	0.968	−0.56	1.10 ± 0.081	0.925
50	−0.61	0.78 ± 0.068	0.910	−0.49	1.01 ± 0.067	0.935
100	−0.69	0.69 ± 0.073	0.891	−0.47	0.90 ± 0.111	0.816
200	−0.44	0.77 ± 0.055	0.943	−0.55	0.90 ± 0.084	0.892
512 cell trays						
0	−0.61	0.81 ± 0.041	0.962			
5	−0.34	0.85 ± 0.059	0.950	−0.61	0.97 ± 0.055	0.955
50	−0.50	0.82 ± 0.055	0.953	−0.69	0.95 ± 0.061	0.942
100	−0.59	0.76 ± 0.041	0.961	−0.47	1.00 ± 0.073	0.923
200	−0.45	0.66 ± 0.067	0.900	−0.15	1.05 ± 0.101	0.877

corresponding increase in the synthesis of cytokinins (O'Hare and Turnbull 2004). Root/shoot ratio from Fig. 1 to 3 are, in general, in agreement with previous reports. Although the physiological processes involved in root restriction are largely unknown, our results showed that the higher control plants total dry weight which were grown in 50 plug cell trays (Fig. 1) was be related to higher RGR as between the emergence-transplant as between the emergence-sale stage (Table 2). When RGR was disaggregated as the product of NAR and LAR, an increased as in the 'physiological component' (NAR) as in the 'morphological component' (LAR) related to higher cell volume between emergence and transplant stage were found. If we used the data plotted between emergence and sale stage, the RGR increase in response to a cell volume increase was mainly related to changes in LAR which are in agreement with previous results for *Salvia splendens* (van Iersel 1997).

Photoassimilates from leaves are used for root, stem and leaf initiation and growth; by the other hand, the size of the different plant sinks determines the photoassimilates partition between the plant organs. Although our results would show that the smaller the cell volume the higher the percentage of dry matter allocated to roots (Table 3), complementary information about sugar contents in different organs of the plant are needed.

BAP concentration responses

An important mediator of shoot biomass production can be the supply of signal molecules from the root system (Jackson 2002; Kudo et al. 2010). It has been indicated that the availability of hormones synthesized in the root apex and reallocated to shoots would be reduced when the vertical root growth was impeded by the container base (Di Benedetto et al. 2006, 2010). Our results (Table 1)

are in agreement with Carmi and Heuer (1981) and Haver and Schuch (2001) who showed that the reduction of leaf area, observed in bean under root restricting conditions, could be partially reversed by the application of benzyladenine. It has been indicated that an increase in root growth may lead to a corresponding increase in the synthesis of cytokinins (O'Hare and Turnbull 2004); exogenous cytokinin supply to ornamental pot plants specially favors the development of axillary shoots and tends to increase leaf biomass (Zieslin and Algom 2004; Araki et al. 2007; Di Benedetto et al. 2010; Di Benedetto 2011).

I. walleriana dry weight distribution was changed in response to different BAP sprays doses and application stage (Fig. 1, 2 and 3), but the magnitude of the changes were mainly associated with the plug cell volume. When BAP was applied at the pre-transplant stage significantly increased root and shoot dry weight at the transplant stage (Fig. 1) and at the sale stage; BAP dose for maximum response ranged between 50 to 200 mg L^{-1} (Fig. 2). On the other hand, a BAP spray (50, 100 or 200 mg L^{-1}) at the post-transplant stage decreased dry weight at the sale stage (Fig. 3) which is in agreement with Araki et al. (2007) results on lettuce. Although the net cytokinin concentration reaches apical meristem is the result of both the synthesis and the degradation rates, the higher roots size the higher cytokinin synthesis. When plants are transplanted to higher pots from a plug cell tray quickly developed an adventitious root system (Di Benedetto et al. 2006) and it is possible that net cytokinin concentration at the shoot apex increase too. Due that a dual mechanism which can stimulate a process at low hormone concentration and limit it at relatively high hormone concentration has been usually proposed, an exogenous BAP spray would increase plant growth at the pre-transplant stage (under restricted root growth) but would decrease it when BAP is applied at the post-transplant stage (with a root system to a greater degree development).

Di Benedetto et al. (2010) showed that an exogenous BAP spray increased both fresh weight and dry weight for the *E. aureum*, *F. benjamina* 'green' and 'variegated' ornamental plants tested through higher RGR, RLAER, NAR and LAR); these responses are supported by a fast leaf area expansion as a result of a decrease in the leaf plastochron, an increase in leaf expansion through changes in both leaf thickness and intercellular spaces extension. The increase of photosynthate partitioning behalf the shoot apical meristem has been associated to a positive feed-back during the first 30 days after the exogenous BAP spray too. Our results, on bedding plants with a shorter lives, are partially in agreement with these previous published results because the effects of a BAP spray on the changes in RLAER (Table 1), RGR, NAR and LAR (Table 2) were mainly associated to plug cell volume (with lower responses for the highest plug cell volume), application stage (pre- or post-transplant) and BAP dose. On the other hand, the changes in the allometric relationships between shoots and roots for *I. walleriana* plants sprayed with different BAP solutions were application time- and plug cell volume-dependent (Table 3).

BAP can be considered to be an endogenous-like compound which provides very little risk to the environment, it is not toxic to several important predatory mite species used for biological control of phytophagous mites on apple and its toxicity to mammalian and arthropod species is low (Bound et al. 1997).

In summary, optimization of *I. walleriana* commercial productivity is limited by root restriction imposed by small plug cell volumes; it can be counteracted by large plug cell volumes and/or a single pre-transplant BAP application.

Conclusions

Decisions such as the choice of container plug cell size or seedbed spacing before transplanting are frequently made on the basis of economics; since the cost of small transplants is lower than that of large, their advantage would be lower establishment costs. When seedlings are grown in typical transplant containers, growth tends to be proportional to the volume of the container cell; the more space available to the plant from the nursery the larger it becomes after transplanting and the more quickly it attains particular growth stages. The only way to be economically competitive in the future is to decrease our costs and increase our efficiency but it does not mean necessarily to use more cells per tray or smaller plug cell volumes for bedding pot plant propagation. Our results showed that the common root restriction effects related to a decrease in plug cell volume can be partially overcoming through a single and early pre-transplant BAP application; although 5 to 200 mg L^{-1} BAP for the best results were needed. Hormonal manipulation was a potential tool for improving plant productivity but, a precise calibration for different bedding pot plants is required.

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