# Alternative Substrates and Fertilization Routine Relationships for Bedding Pot Plants: Impatiens wallerana.

J. Thibaud<sup>1)</sup>, T. Mc. Loughlin<sup>1)</sup>, A. Pagani<sup>1)</sup>, R. Lavado<sup>2)</sup> and A. Di Benedetto<sup>1, 3)</sup>

(<sup>1</sup>)Facultad de Agronomía (UBA), Cátedra de Floricultura, Argentina, <sup>2</sup>)INBA (CONICET/FAUBA) and Facultad de Agronomía (UBA), Cátedra de Fertilidad y Fertilizantes, Argentine and <sup>3</sup>) Facultad de Ciencias Agrarias (UNMP) Balcarce, Provincia de Buenos Aires, Argentina)

# Summary

This study was carried out searching for alternative substrates to traditional peat based on river waste and *Sphagnum* and *Carex* peat from Argentinean peatlands and used to grow *Impatiens wallerana* bedding plant. The aim of this study was i) to characterize physical and chemical effects from seven growing media on *I. wallerana* plants grown under different fertilization rates and ii) to describe the physiological mechanisms in plants, involved in the use of such substrates. Particle stability was lower for the six alternative substrates compared to the Canadian peat-base control substrate. However, with a high fertilization dose it is possible to reach non significant differences in plant growth compared to the control substrate. It is suggested, for future research, that nitrogen signalling associated to cytokinin synthesis by roots is involved.

Key words. ornamental plant - peat - river waste - nitrogen

# Introduction

Bedding plants growth and their appearance (aesthetic attributes) depend on the quality of growing media, water, fertilizer and the prevailing environmental conditions. Although numerous factors affect the growth and productivity of plants, the quality of growing medium stands out as one of the most important factor especially when they grown in potted culture. That is why growing media selection is an important factor which is influencing plant quality for container cultures. When a pot bedding plant production is started, a critical decision that must be made is the choice of a growing media, along with container type (DI BENEDETTO and KLASMAN 2004, 2007; DI BENEDETTO 2011). Most bedding plants complete their life cycle in a short time period. That is why the knowledge of the growth dynamics of a short-lived plant could be critical. It does not normally have much time to adjust to unsuitable shoot and roots environments. Under poor growing conditions, most bedding plants tend to flower prematurely, giving a poor quality of short-statured plant with small flowers.

In underdeveloped countries a great deal of bedding plants growers uses substrates by mixing two or more components. Components would be often regional and based on local resources available to the nursery operation.

Peat moss is the most common organic substrate used in growing media for propagation and pot plant production; high quality peat mainly consists of incompletely decomposed *Sphagnum*. The stocks of white peat (the most important raw material for substrates in professional horticulture) in most countries of Western and Central Europe are nearly depleted as a result of continuous degradation of the peat-lands. For example, at the Dutch horticulture industry, the percentage of peat used in the commercial standard mixes media was reduced in average from 77 % in early commercial standard mixes to 30 % in the new mixes (BLOK and VERHAGEN 2009).

One consequence of this fact is the increase in recent years of research and development of new substrates to replace conventionally used peat moss substrates. In addition to developing and using new substrates, much work has focused on managing fertility and irrigation programs of these substrates. The aim was to maximize plant performance and minimize nutrient losses which are causing environmental concerns (JACKSON et al. 2008). The need to develop new substrates and fertility management programs for the horticulture industry is an issue that is being addressed by researchers around the world (DI BENEDETTO et al. 2004, 2006a; DI BENEDETTO 2007; CHAVEZ et al. 2008; JACKSON et al. 2009a, b). The possibility of reducing the use of non-renewable sources, along with the need for the suitable disposal of organic residue in the environment, makes nutrient and energy cycling by using organic materials attractive in economic, agricultural and environmental terms.

At present, however, there are no a single peat total substitution alternative. Although some of these alternative substrates are limited in quality in terms of physical and chemical properties and negatively affect the development of plant roots, researchers have developed several commercialized products currently available to growers (JACKSON et al. 2010). Differences in substrate physical properties significantly decreased plant quality (leaf area, plant height and flower number), plant growth and plant productivity (DI BENEDETTO et al. 2006b). A growing media give not only a matrix for water and nutrient absorption so a source of external signaling. Different plants would respond through different physiological mechanism. The most part of them are still no explained for the most ornamental plants. The lack of a clear understanding of plant adaptation to different growing media and the physiological mechanisms involved in this plant growth regulation our efforts to give a genuine alternative to peat moss for bedding pot plants (DI BENEDETTO and PAGANI 2011).

Producing a growing media with a particle size fine enough to possess an adequate water-holding capacity, similar to peat moss, may be too costly. Another approach would be to mix larger media particles with various proportions of fine media particles (less than 0.5 mm), which could result in a growing media with adequate container capacity and substrate air space (GRUDA and SCHNITZLER 2004; BILDERBACK et al. 2005). One of these growing media was obtained by DI BENEDETTO and KLASMAN (2007) mixing the three Argentinean materials tested in the present paper: river waste, Sphagnum maguellanicum peat and Carex sp. peat. Taking into account that finely ground substrate is more costly to produce than coarsely ground growing media, the intermixing of various media particle sizes would result in reduced substrate costs. This is because relatively small amounts (percentage) of finely ground substrate would be needed to obtain desired container capacity of the substrate (JACKSON et al. 2010). However, how will the final yield be (expressed in bedding plants as time to obtain a high quality product or aerial biomass accumulated) is not easy to predict.

This study was carried out searching for alternative substrates based on river waste and *Sphagnum* and *Carex* peat from Argentinean peat-lands and used to grow *Impatiens wallerana* bedding plant. The aim of this study was i) to characterize physical and chemical effects from seven growing media on *I. wallerana* plants grown under different fertilization rates and ii) to describe the physiological mechanisms involved in the use of such substrates.

#### Materials and Methods

## Alternative materials tested for substrate mixes

Six different growing media were formulated using river waste ('temperate peat') which is the result of the accumulation of plant residues under an anaerobic temperate environment, which is dredged from river or lake banks ( $34^\circ$  S to  $27^\circ15'$  S) (DI BENEDETTO and KLASMAN 2007); *Sphagnum maguellanicum* and *Carex* peat from the Southern Argentina peat lands ( $55^\circ$  S to  $52^\circ$  S and  $46^\circ$  S to  $42^\circ$  S respectively). A commercial media high quality peat-base (Fafard Growing Mix  $2^\circ$ ) (Canadian *Sphagnum* peat moss-perlite-vermiculite 70:20:10 v/v) was used as a control. The formulae (v/v) t) tested were:

#### F: Fafard Growing Mix 2<sup>®</sup>

**S**: *Sphagnum maguellanicum* peat (80 %) + Perlite (10 %) + Vermiculite (10 %)

C: Carex peat (80 %) + Perlite (10 %) + Vermiculite (10 %) R: River Waste (80 %) + Perlite (10 %) + Vermiculite (10 %) SR: Sphagnum maguellanicum peat (40 %) + River Waste (40 %) + Perlite (10 %) + Vermiculite (10 %)

**CR**: *Carex* peat (40 %) + River Waste (40 %) + Perlite (10 %) + Vermiculite (10 %)

**SC**: *Sphagnum maguellanicum* peat (40 %) + *Carex* peat (40 %) + Perlite (10 %) + Vermiculite (10 %)

## Fertilization routine

A weekly fertilization of 100, 200 or 400 mg L<sup>-1</sup> N (1N: 0.5P:1K:0.5Ca v/v) from transplant to sale stages was used. All nitrogen was as nitrate. A weekly fertilization volume of 75 ml pot<sup>-1</sup> was applied. Plants were watered daily as needed with tap water (pH: 6.64 and electrical conductivity of 0.486 dS m<sup>-1</sup>) according previous results (CHAVEZ et al. 2008). The fertilization solution for the 100, 200 and 400 mg L<sup>-1</sup> N treatments showed a pH of 5.01, 5.13 and 5.51 and an electrical conductivity of 1.11, 2.41 and 3.53 dS m<sup>-1</sup> respectively.

#### Plant material

Impatiens wallerana 'Accent' seeds (Goldsmith Inc.) were germinated and grown in 200 plastic plug trays at a high Canadian peat-based (Fafard Growing Mix 2<sup>®</sup>) under greenhouse facilities located on School of Agriculture, University of Buenos Aires campus, Argentine (34°28' S). All materials were limed to achieve similar pH's. Mean temperatures (25.1 to 27.9 °C) and photosynthetic active radiation (8.94 to 10.17 mol photons m<sup>-2</sup> day<sup>-1</sup>) for the different experiments were recorded with three HOBO sensors (H08-004-02) connected to HOBO H8 data loggers. At the fourth true leaf stage, one plant per pot was transplanted. The seven soilless media were tested in pots with a volume of 1000 cm<sup>3</sup>.The experiment was repeated twice.

### Sample evaluations

Crops were harvested at the transplant stage and after ten weeks roots were washing and both roots and shoots were dried at 80 °C for 48 h and weighed to determine the dry aerial and root biomass (ten replicates block<sup>-1</sup>). Leaf area was determined with a LI-COR 3000A automatic leaf area meter. The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm of whole plant weight vs. time in days. At the sale stage, nitrogen content for both shoots and roots were determined in duplicate using the Kjeldall method.

Samples of each substrate were collected at transplanting and at the end of the experiment; total porosity, airfilled porosity, density and container capacity were determined according to FONTENO (1996) Samples of air dry media for particle size distribution were passed through a series of sieves from 25 to 2 mm.

Electrical conductivity (EC) and pH were analysed in a 1:5 (v/v) water extract. Nutrient concentration analysis included nitrogen (using the Kjeldall method), phosphorus (colorimetrically), potassium (atomic absorption), calcium and magnesium (atomic absorption). The cationexchange capacity (CEC) was determined with 1 M ammonium acetate at pH = 7. Chemical and physical analyses were performed in duplicate.

# Growth evaluations

The relative growth rate on a dry mass basis (RGR) was calculated as the slope of the regression of the natural logarithm of whole plant fresh mass vs. time. The allometric relationships between roots-shoots and between leaf blades and petioles-stem were performed using a straight-line regression analysis between *ln* roots dry weight and *ln* shoots and between *ln* leaf blades dry weight and *ln* stem dry weight respectively during the experiments.

#### Statistics

The experimental design was a randomized factorial with 3 blocks of 10 single-pot replications of each treatment combination (media × fertility × experiment). Data were subjected to a one way analysis of variance and means were separated by Tukey tests (P < 0.05). Equations to predict dry weight relationship to total porosity or electrical conductivity were developed via linear regression.

## Results

## Chemical and physical characteristics

Chemical properties of the control substrate (F) and the alternatives growing media tested are shown in Table 1. Both Argentinean peats (Sphagnum maguellanicum and Carex sp.) showed a very low pH value before limed. Both would require a pH adjust using dolomite loam previous to use. Electrical conductivity was very low in both Argentinean peats as compared with the control substrate (F) but river waste was the opposite. Nutrient concentrations were quite different when the control substrate (F) is compared with the alternative materials tested. The control substrate (F) contained the higher nitrogen, potassium and magnesium concentration, while river waste showed the higher phosphorus and potassium concentration and a significant higher cation exchange capacity (CEC). There were no differences in calcium concentration between the growing media tested.

Growing	pH*	EC	N	P	K	Ca	Mg	CEC
media		(dS m⁻¹)	(%)	(mmol L <sup>−⊥</sup> )	(mmol L <sup>−</sup> 1)	(mmol L <sup>−</sup> )	(mmol L <sup>−⊥</sup> )	(meq 100cm <sup>-3</sup> )
F	6.06	0.40	1.71	0.64	1.02	6.26	4.19	1.20
S	4.80	0.11	0.90	0.95	0.78	4.40	1.98	1.09
С	4.51	0.16	1.13	2.08	0.44	5.48	1.50	0.98
R	6.15	0.80	1.14	5.36	1.05	6.40	2.32	4.41
SR	5.38	0.44	1.54	3.47	0.41	6.78	1.05	2.31
CR	4.99	0.66	1.65	3.71	0.39	6.58	1.52	1.89
SC	5.40	0.18	1.90	2.28	0.55	6.73	1.29	1.20

Table 1. Chemical properties of the materials used for the tested growing media.

\* Initial pH before limed adjustment.

F: [Canadian Sphagnum peat (80 %) + Perlite (10 %) + Vermiculite (10 %)], S: [Sphagnum maguellanicum peat (80 %) + Perlite (10 %) + Vermiculite (10 %)], R: [River Waste (80 %) + Perlite (10 %) + Vermiculite (10 %)], R: [River Waste (80 %) + Perlite (10 %) + Vermiculite (10 %)], SR: [Sphagnum maguellanicum peat (40 %) + River Waste (40 %) + Perlite (10 %) + Vermiculite (10 %)], CR: [Carex peat (40 %) + River Waste (40 %) + Perlite (10 %) + Vermiculite (10 %)], SC: [Sphagnum maguellanicum peat (40 %) + Carex peat (40 %) + Perlite (10 %) + Vermiculite (10 %)], SC: [Sphagnum maguellanicum peat (40 %) + Carex peat (40 %) + Perlite (10 %) + Vermiculite (10 %)], SC: [Sphagnum maguellanicum peat (40 %) + Carex peat (40 %) + Perlite (10 %) + Vermiculite (10 %)].

Table 2 showed some physical properties of the seven growing media tested at the beginning and the end of the experiments. The highest initial total porosity and airfilled porosity for Fafard Growing Mix 2<sup>®</sup> were found; both total porosity and air-filled porosity was significantly higher on alternative substrates with the highest proportion of *Sphagnum maguellanicum* peat. Container capacity was similar for Fafard Growing Mix 2<sup>®</sup>, *Sphagnum maguellanicum* and *Carex sp* peat and decreased when two different components was mixed. The highest initial bulk density was found when river waste was used (Table 2).

The differences among the proportion of particle size distribution (Table 3) can be analyzed in different planes (i) the differences among the control substrate (**F**) and the alternative substrates and, (ii) the differences among all the treatments and the differences found on substrates between the beginning the experiments and 70 days later. The control substrate (F) and the Sphagnum magellanicum peat showed a high proportion of relatively small aggregates (< 2.00 mm), with differences between them because the latter had higher proportion of aggregate size ranging from 6.35 to 12.7 mm. Carex peat showed a reverse picture: the size distribution shows the larger proportion of aggregates of higher size when compared with the other substrates. River waste was relatively close to the control substrate (F) and Sphagnum magellanicum but concentrated more than 68 % of the particles in the fraction of > 2.00 mm. The other growing media tested showed an aggregate size which ranged according the original materials mixed. When comparing the particle size distribution in the substrates at the end of experiment with that found at the start, it is clear that the control sub-

Tabla a Dhuu	sign nunnerting .	of the meteriale	used for the tested	arouing modia	Cubetrate abb	roviatione coo Table 1
IADIE Z POV	SIGAL DIODELLIES (	or the marenals.	insection the rested	Prowing media		) EVIATIONS SEE TADIE I
14010 2.1119.	sicul properties .	or the materials		growing means	· substrate upt	

Growing media	Porosity (%)	Air-filled porosity (%)	Container capacity (%)	Bulk density (g cm <sup>-3</sup> )
F	85 72	20.94	22.28	0.14
S	85.50	15.94	25.94	0.14
С	78.56	17.00	23.00	0.23
R	62.83	14.56	15.56	0.55
SR	63.50	17.06	10.06	0.35
CR	60.06	16.67	18.67	0.36
SC	86.22	19.89	20.89	0.19

Table 3. Particle size distribution (%) from different growing media materials at the beginning of the experiments (Initial) and at the sale stage (Final) of *I. wallerana* bedding plants grown during 60 days in pots. Substrate abbreviations see Table 1.

Treatment	Particle size (mm)					
and sampling	> 24.50	12.70-24.50	6.35-12.70	4.80-6.35	2.00-4.80	> 24.50
Initial						
F	0.00	0.76	4.41	3.15	41.85	49.83
S	0.00	8.90	11.57	2.10	34.20	43.23
С	12.69	36.74	24.29	4.70	13.96	7.62
R	0.00	0.00	2.88	3.72	68.20	25.20
SR	0.00	0.69	1.42	0.37	71.30	26.22
CR	0.00	2.34	1.58	1.88	72.10	22.10
SC	8.30	28.55	17.34	4.02	16.25	25.54
<u>Final</u>						
F	0.00	0.48	3.99	10.86	36.85	47.82
S	0.00	5.91	10.68	3.10	32.18	48.13
С	2.01	21.50	19.10	6.52	21.77	29.10
R	0.00	6.72	13.30	4.70	36.40	38.88
SR	0.00	0.00	0.01	0.60	61.96	37.43
CR	0.00	2.43	7.81	6.30	60.40	23.06
SC	1.58	18.43	15.22	5.05	24.60	35.12

strate (**F**) retained the higher proportion of aggregates on the same size. *Sphagnum maguellanicum* (**S**) retained also a high proportion of its original aggregate size distribution. *Carex sp.* peat (**C**) and river waste (**R**), otherwise, showed the highest changes in aggregates sizes, being the more unstable substrates. The other growing media showed some changes in aggregates sizes.

## Plant growth and substrate-fertility relationships

Fig. 1 shows the Impatiens wallerana dry weight of roots shoots and leaves at the end of the experiments for three fertilization levels. There was no significant difference between both experiments that is why they were considered together. Both the control substrate (F) and river waste (**R**) showed the highest aerial dry weight but the lower root biomass; which determined a decrease in root/shoot ratio according a fertilization increase. The remained of growing media showed a higher total dry weight accumulation but mainly concentrated in roots. That is why the root/shoot ratio was significant higher than for the other two growing media. The fertilization treatments changed the biomass production and the root/shoot ratio. Single (Growing media; Fertilization level) and double (Growing media × Fertilization level) for roots, shoots and leaves dry weight accumulation effects in the ANOVA test showed highly significant ( $P \le 0.001$ ) differences.

The total dry weight at the sale stage was positively related to total porosity in different degree according the growing media tested. All growing media tested, except alternative substrates **C** and **SC**, showed a high positive relation with the initial total porosity as indicated the determination coefficient  $r^2$  of the straight-line regression; the  $r^2$  coefficient ranged from 0.647 and 0.997 between all growing media tested. The dry weight showed a positive relationship to electrical conductivity but no significant differences between growing media tested, except again for the alternative substrates **C** and **SC**; the determination coefficient  $r^2$  ranged from 0.733 and 0.998 (Table 4).

The changes in relative growth rate (RGR) of *I. wallerana* are shown in Table 5. The RGR was higher for the most alternative substrate tested than the control substrate (**F**). The RGR increased as fertilization level increased and was significantly lower for the control substrate (**F**) than for the other growing media tested at all fertilization levels used.

At the lower fertilization rate (100 mg L<sup>-1</sup> N) the higher total leaf area was achieved by plants growing in the alternative substrates **S** and **R**. An increase in fertilization level (200 mg L<sup>-1</sup> N) showed a positive response for all growing media tested. The higher fertilization rate (400 mg L<sup>-1</sup>N) used significantly increased leaf area per plant in the alternative substrates **S**, **SR**, **CR** and **SC** without changes in the rest of the growing media. Single (Growing media; Fertilization level) and double (Growing media × Fertilization level) effects were highly significantly significantly significantly significantly significantly significantly increased leaf area per plant in the alternative substrates **S**, **SR**, **CR** and **SC** without changes in the rest of the growing media.



Fig. 1. Dry weight (g plant<sup>-1</sup>) partitioned in roots, shoots and leaves at the end of the experiment (sale stage) of *l. wallerana* plants grown in seven growing media at 100 (A), 200 (B) or 400 mg L<sup>-1</sup> N (C) fertilization level. Numbers above the bars: root/shoot ratio. Statistical significance: \*\*\* 0.001; \*\* 0.01; \* 0.05; 'ns' not significant. Substrate abbreviations see Table 1.

icant (P  $\leq$  0.001); on the other hand, there was no significant difference related to the two experiments (Fig. 2).

Table 6 shows the changes found in allometric relationships between shoots and roots for *I. wallerana*. The slopes of the straight-lines which related *ln* roots dry weight and *ln* shoots dry weight showed that plants grown in the control substrate (**F**) assigned a balanced photosynthate proportion to root and shoot growth while in the other growing media the plants partitioned a higher photosynthate proportion to roots. The plants grown in Table 4. Straight-line regression equations relating dry weight and total porosity [Dry weight = a + b (total porosity)] or dry weight and electrical conductivity (EC) [Dry weight = a + b (EC)] of *I. wallerana* plants grown in different growing media and fertilized at three levels. The determination coefficient ( $r^2$ ) and the standard error (SE) of the straight-line regression equation slope are indicated. Substrate abbreviations see Table 1.

Growing	Total Po	orosity (x)		Electrical Conductivity (x)			
media	Regression equations	r <sup>2</sup>	Slope SE	Regression equations	r <sup>2</sup>	Slope SE	
F	-6.49 + 0.146x	0.948	0.034	2.00 + 9.917x	0.933	2.655	
S	-1.47 + 0.129x	0.712	0.082	0.77 + 7.061x	0.972	13.108	
С	-15.89 + 0.109x	0.647	0.024	2.43 + 1.334x	0.733	0.391	
R	-3.27 + 0.123x	0.997	0.007	4.60 + 6.312x	0.915	1.924	
SR	-10.70 + 0.133x	0.997	0.012	0.48 + 11.645x	0.927	3.259	
CR	-2.30 + 0.137x	0.699	0.015	3.63 + 8.551x	0.883	3.108	
SC	-32.80 + 0.100x	0.676	0.021	3.56 + 10.429x	0.998	0.449	

Table 5. Changes in relative growth rate (RGR) for plants of *l. wallerana* grown in seven growing media at 100, 200 or 400 mg L<sup>-1</sup> N fertilization level. The standard errors are indicated. Substrate abbreviations see Table 1.

Growing	N fertilization level	RGR
media	(mg L <sup>-1</sup> )	(g g <sup>-1</sup> day <sup>-1</sup> )
-	100	0.0502 + 0.00150
F	100	$0.0593 \pm 0.00159$
	200	$0.0658 \pm 0.00195$
	400	$0.0688 \pm 0.00163$
S	100	0.0675 ± 0.00170
	200	0.0689 ± 0.00183
	400	0.0734 ± 0.00174
С	100	0.0612 ± 0.00181
	200	$0.0710 \pm 0.00174$
	400	$0.0683 \pm 0.00204$
R	100	$0.0681 \pm 0.00136$
	200	0.0687 ± 0.00166
	400	$0.0722 \pm 0.00153$
SR	100	0 0680 + 0 00154
511	200	$0.0723 \pm 0.00134$
	400	0.0723 = 0.00111
	400	$0.0774 \pm 0.00137$
CR	100	0.0674 ± 0.00139
	200	0.0674 ± 0.00192
	400	0.0707 ± 0.00152
sc	100	0 0646 + 0 00154
50	200	$0.0687 \pm 0.00157$
	200	$0.0007 \pm 0.00157$ 0.0729 + 0.00162
	400	0.0729 ± 0.00163



Fig. 2. Total leaf area (cm<sup>2</sup> plant<sup>-1</sup>) at the end of the experiment (sale stage) of *I. wallerana* plants grown in seven growing media at 100, 200 or 400 mg L<sup>-1</sup> N fertilization level. The standard errors were indicated. Statistical significance: \*\*\* 0.001; \*\* 0.01; \* 0.05; 'ns' not significant. Substrate abbreviations see Table 1.

the control substrate (**F**) did not show significant changes in photosynthate partition while the other six alternative substrates tested (**S**, **C**, **R**, **SR**, **CR** and **SC**) decreased partition toward roots as fertilizer rate increased (Table 5). Complementarily, Table 6 shows the changes in allometric relationships between stems and leaves. Plants growing in most of the growing media tested showed a higher photosynthate partition to stems than to leaves but as fertilization level increased, the photosynthate partition to leaves increased.

Table 6. Changes in allometric relationships between shoots and roots resp. stems and roots for plants of <i>I. wallerana</i>
grown in seven growing media at 100, 200 or 400 mg L <sup>-1</sup> N fertilization level using a straight-line regression analysis
between <i>In</i> roots and <i>In</i> shoots dry weight, resp. <i>In</i> stems and <i>In</i> leaves dry weight. Standard errors for the slope ( $\beta$ ) are
indicated. The intercept straight-line ( $\alpha$ ) and the coefficients of determination (r <sup>2</sup> ) are indicated, too. Substrate abbrevi-
ations see Table 1.

Growing	ing N fertilization		Shoots and roots			Stems and leaves			
media	level (mg L⁻¹)	α	β	r <sup>2</sup>	α	β	r <sup>2</sup>		
F	100	-0.215	$1.034 \pm 0.038$	0.960	-0.390	0.914 ± 0.054	0.957		
	200	-0.494	$1.025 \pm 0.030$	0.974	-0.592	0.868 ± 0.045	0.698		
	400	-0.279	$1.012 \pm 0.030$	0.973	-0.659	$1.001 \pm 0.015$	0.997		
S	100	0.254	1.207 ± 0.042	0.958	-0.275	0.910 ± 0.097	0.831		
	200	-0.111	$1.076 \pm 0.041$	0.954	-0.620	0.858 ± 0.091	0.856		
	400	0.031	$1.122 \pm 0.031$	0.975	-0.696	0.964 ± 0.033	0.981		
С	100	0.514	1.299 ± 0.049	0.954	-0.315	1.042 ± 0.088	0.904		
	200	0.265	$1.215 \pm 0.034$	0.978	-0.132	0.980 ± 0.015	0.997		
	400	0.143	$1.164 \pm 0.030$	0.977	-0.408	$1.050 \pm 0.087$	0.891		
R	100	0.226	1.195 ± 0.035	0.971	-0.387	1.042 ± 0.080	0.908		
	200	0.066	$1.141 \pm 0.027$	0.983	0.160	1.174 ± 0.029	0.732		
	400	0.173	$1.171 \pm 0.039$	0.965	-0.528	0.975 ± 0.015	0.996		
SR	100	0.496	1.290 ± 0.044	0.964	-0.193	0.922 ± 0.074	0.918		
	200	0.040	1.129 ± 0.029	0.978	-0.538	0.985 ± 0.030	0.985		
	400	0.025	$1.126 \pm 0.022$	0.986	-0.310	0.651 ± 0.015	0.320		
CR	100	0.490	1.288 ± 0.046	0.953	-0.360	0.843 ± 0.016	0.481		
	200	0.085	1.149 ± 0.037	0.964	-0.173	$1.010 \pm 0.034$	0.979		
	400	-0.124	$1.066 \pm 0.033$	0.961	-0.783	0.956 ± 0.031	0.980		
SC	100	0.226	1.195 ± 0.040	0.963	-0.339	0.857 ± 0.098	0.826		
	200	0.191	1.184 ± 0.029	0.979	-0.384	1.107 ± 0.070	0.933		
	400	-0.010	$1.112 \pm 0.026$	0.981	-0.480	0.938 ± 0.044	0.666		

The final nitrogen content for the growing media tested at the end of the experiments was compared with a determination at the beginning of its. The dynamic of nitrogen accumulation in the control substrate (F) showed significant differences as compared with the other alternative substrates tested. At the end of the experiments, treatments with 100 mg L<sup>-1</sup> N fertilization showed the lowest N concentration in all growing media tested; at 200 mg L<sup>-1</sup> N fertilization level, N content increased with some differences among growing media and the highest N content in all growing media was found with the highest fertilization level but again with some differences among treatments. Single (Growing media; Fertilization level) and double (Growing media × Fertilization level) effects in the ANOVA test showed highly significant ( $P \le 0.001$ ) differences, with no significant differences related to different experiments (Fig. 3). There was an inverse relationship between root N concentration and the shoots N concentration at our low fertilization rate (100 mg L<sup>-1</sup> N) (Fig. 4A). Nitrogen accumulation by shoots increased as fertilization rate increased (200 and 400 mg L<sup>-1</sup> N). These changes were more significant for the alternative substrates than the control substrate (F) (Fig. 4B and C). Single (Growing media; Fertilization level) and double (Growing media × Fertilization level) for roots and shoots nitrogen accumulation effects in the ANOVA test showed highly significant ( $P \le 0.001$ ) differences. There was no significant difference related to different experiments.

## Discussion

Differences of chemical and physical properties

With the merely exception of phosphorus content only the river waste (**R**) approximated the quality of the control



Fig. 3. Changes in nitrogen content (%) in the seven growing media tested fertilized weekly with 100 (A), 200 (B) or 400 (C) mg L<sup>-1</sup> N related to the beginning of the experiment (initial). Data are mean of two replicates. Statistical significance: \*\*\* 0.001; \*\* 0.01; \* 0.05; 'ns' not significant. Substrate abbreviations see Table 1.

substrate (**F**). There were also great differences in physical properties between the control substrate (**F**) and the six alternative substrates tested, particularly when the Argentinean *Carex* sp. peat was used (Table 1 and 2).

Substrate particle sizes are commonly grouped into categories/classes such as: coarse, medium and fine (DRZAL et al. 1999; RICHARDS et al. 1986). The reduction of large particles and an increase in fine particles before and after crop production is an estimation of substrate breakdown, which is known to occur in organic substrates (BILDERBACK et al. 2005). Present data and previously published results from DI BENEDETTO (2007) and DI BENEDETTO and PAGANI (2011) assert the low quality of Sphagnum maguellanicum and Carex sp. Argentinean peat. However, present results showed that I. wallerana growth would be associated to the presence of higher particle sizes which quickly break downed in smaller particles (Table 3, Fig. 1 and 2). The increased proportion of smaller particles would bock growing media macro pores, reducing air-filled porosity. The resulting lower oxygen availability would decrease both root length and root branch; this latter would finally reduce cytokinin synthesis plant ability.

The differences between the control substrate (**F**) and the six alternative substrates tested in our experiments would be associated to changes in the proportion of particle sizes in the different growing media at the beginning the experiments and the changes observed at the end of it, 70 days after (Table 3). The amount of pore space and the continuity of pores in container media are the most



Fig. 4. Nitrogen content (g plant<sup>-1</sup>) at the end of the experiment (sale stage) for shoots (stems and leaves) and roots of *I. wallerana* plants grown in seven growing media at 100 (A), 200 (B) or 400 (C) mg L<sup>-1</sup> N fertilization level. Standard errors are indicated. Statistical significance: \*\*\* 0.001; \*\* 0.01; \* 0.05; 'ns' not significant. Substrate abbreviations see Table 1.

critical physical characteristics which influence water and nutrient absorption and gas exchange by the root system (GRUDA and SCHNITZLER 2004).

Physical properties of substrates considered appropriate for plant growth at planting may change over time in containers as a result of several processes (JACKSON et al. 2009a). Some of the chemical and physical parameters of the growth media are usually analyzed at the beginning and end of cultivation; it must be engineered for optimal characteristics for nursery and greenhouse crop production. It has been claimed that development of alternative potting substrates needs optimal properties to avoid decreases in plant quality (leaf area, plant height and flower number), plant growth and plant productivity (DI BENEDETTO et al. 2006b). Although there is no relationship between growth and both physical and chemical properties for some bedding pot plant growth (DI BENEDETTO and PAGANI 2011), Table 3 showed significant determination coefficients (r<sup>2</sup>) between I. wallerana total dry weight and both pH and electrical conductivity at the end (sale stage) of the experiments 70 days after transplant. Globally the r<sup>2</sup> are higher when electrical conductivity, which reflects fertilization, is considered. The possibility of a peat substitution has been associated to both aerial leaf area expansion and dry weight accumulation because the visual impact of the former and the positive post-sale life effect of the latter (ARMITAGE 1993). Alternative substrates such as that tested in this work, showed a lower aerial dry weight at the end of the experiments (Fig. 1) but not lower relative growth rate (RGR) on a dry weight-base (Table 5) than the control substrate (F). A higher root/shoot ratio was associated to a higher root partition for plants grown under river waste, Sphagnum maguellanicum and Carex sp. peats. This showed by the allometric measures between roots and shoots (Table 6).

Fertilization rate was a main factor for the I. wallerana growth when the alternative substrates were tested. The best dry weight accumulation for Canadian peat-base growing media was achieved with a 200 mg L<sup>-1</sup> N, with little changes when 400 mg L<sup>-1</sup> N was used (Fig. 1); this fertilization rate decreased root/shoot ratio but increase RGR (Table 5). Nitrogen media concentration was lower when 100 or 200 mg  $L^{-1}$  fertilization rate was used (Fig. 3). It has been indicated that highly concentrated fertilization solution decreased the substrate quality parameters (nutrient immobilization) and plant growth when a high quality peat was used (JACKSON et al. 2008). This was not the case for I. wallerana bedding plants in the six alternative substrate tested; the best leaf area for the I. wallerana was found when a 400 mg L<sup>-1</sup> N fertilization rate was used (Fig. 2). CHAVEZ et al. (2008) showed that nitrate leaching from the alternative substrates containing river waste was lower than the standard peat-based materials, which makes it desirable from a sustainable pot production system perspective, but highly concentrated fertigation solution decreased the substrate Petunia × hybrid quality parameters and plant growth.

DI BENEDETTO and PAGANI (2011) showed that shoot fresh weight for *I. wallerana* bedding plant was mainly determined by the root system size. This is in agreement with the fact that there is a close coordination between roots and shoots growth, controlled by a signalling 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 corresponding increase in the synthesis of cytokinins (O'HARE and TURNBULL 2004). By the other hand, plant roots favours colonization of the growing media and molecular genetics evidences demonstrated that roots sense and responded to local and global concentrations of inorganic nitrate, in a fashion that depends on the shoot nutrient status; Fig. 4 showed that the alternative substrates accumulated more nitrogen in plants with higher total biomass accumulation. The higher root photoassimilate partition for the alternative substrates used was changed in favor to shoots (and in a higher proportion to stems than leaves) with a fertilization rate increase (Table 6). The lower root systems would not be a limited factor due to the constant water supply under commercial facilities.

A key concept underpinning current understanding of the carbon/nitrogen interaction in plants is that the capacity for nitrogen assimilation is aligned to nutrient availability and requirements by the integrated perception of signals from hormones. The long-distance signals mediating the shoot response to nitrate perception in roots seem to involve cytokinins (HERMANS et al. 2006; RUBIO et al. 2009). There are several reports suggesting that the accumulation of cytokinins is closely correlated with the nitrogen status of the plants (TAKEI et al. 2002). Nutrient deficiencies usually restrain the use of some composted materials as peat-substitute for growing media even if some fertilizer is applied to the media (CABALLERO et al. 2009). Due the proportion of nitrogen in the shoots changed in the alternative growing media (mainly Sphagnum maguellanicum-, Carex sp.- and river waste-base) we can hypothesize it is possible that the decrease in shoot growth would be associated to this endogenous signal. By the other hand, a higher nutrient supply would decrease the negative impact of a substrate quality loss. Although, this investigation line needs for additional experiments, some of it are in progress.

## Conclusions

Our results showed that both particle stability and substrate quality properties (chemical and physical properties) are lower for the six alternative substrates related to the Canadian peat-base control substrate. However, with high fertilization doses it is possible to reach non significant differences in plant growth related to the control substrate. It has been suggested, for future research, that the nitrogen signalling associated to cytokinin synthesis by roots would be involved.

#### Acknowledgements

This work was supported by University of Buenos Aires Science Program 2008–2010 (G054) and University of Mar del Plata 2010–2011 Science Program (AGR 287/ 09).

## References

- ARMITAGE, A.M. 1993: Bedding plants. Prolonging shelf performance: Postproduction care and handling. Ball Publishing, Batavia, Illinois, USA, 71 pp.
- BILDERBACK, T.E., S.L. WARREN, J.S. JR. OWEN and J.P. ALBANO 2005: Healthy substrates need physicals too! HortTechnol. **15**, 747–751.
- BLOK, C. and J.B.G.M. VERHAGEN 2009: Trends in rooting media in Dutch horticulture during the period 2001–2005: The new growing media project. Acta Hortic. **819**, 47–58.
- CABALLERO, R., P. PAJUELO, J. ORDOVÁS, E. CARMONA and A. DELGADO 2009: Evaluation and correction of nutrient availability to *Gerbera jamesonii* H. Bolus in various compost-based growing media. Scientia Hortic. **122**, 244– 250.
- CHAVEZ, W., A. DI BENEDETTO, G. CIVEIRA and R. LAVADO 2008: Alternative soilless media for *Petunia* × *hybrida* and *Impatiens wallerana*: physical behaviour, effect of fertilization and nitrate losses. Biores. Technol. **99**, 8082–8087.
- DI BENEDETTO A. AND R. KLASMAN 2004: The effect of plug cell volume on the post-transplant growth for *Impatiens walleriana* pot plant. Europ. J. Hort. Sci. **69**, 82–86.
- DI BENEDETTO, A., C. BOSCHI and R. KLASMAN 2004: Use of river waste in growing media for growing ornamental herbaceous perennials. J. Hort. Sci. Biotechnol. **79**, 119–124.
- DI BENEDETTO, A., R. KLASMAN and C. BOSCHI 2006a: Argentinean peat: a poor substitute for Canadian Sphagnum peat for ornamental bedding plants. Europ. J. Hort. Sci. **71**, 69–72.
- DI BENEDETTO, A., J.C. PETRACCHI, G. MARCELLA, P. MONTARON and W. CHAVEZ 2006b: Evaluation of alternative substrates for bedding plants. Int. J. Agric. Res. 1, 545–554.
- DI BENEDETTO, A. 2007: Alternative substrates for potted ornamental plants based on Argentinean peat and Argentinean river waste: a review. Floric. Plant Ornamental Biotechnol. **1**, 90–101.
- DI BENEDETTO, A. and R. KLASMAN 2007: River waste as a potentially amendment for low quality Sphagnum peat. Europ. J. Hort. Sci. **72**, 193–197.
- DI BENEDETTO, A. 2011: Root restriction and post-transplant effects for bedding pot plants. In: AQUINO, J.C. (ed.): Ornamental plants: Types, cultivation and nutrition. Nova Science Publishers, Inc. NY, USA, 47–79.
- DI BENEDETTO, A. and A. PAGANI 2011: Difficulties and possibilities of alternative substrates for ornamental bedding plants: An ecophysiological approach. In: DRAGUHN, C. and N. CIARIMBOLI (eds): Peat: Formation, uses and biological effects. Nova Science Publishers, Inc. NY, USA, 1–34.
- DRZAL, M.S. W.C. Fonteno and K.D. CASSEL 1999: Pore fraction analysis: A new tool for substrate testing. Acta Hortic. **481**, 43–54.
- FONTENO, W.C. 1996: Growing media types and physical/ chemical properties. In: REED, D.W. (ed.): Water, media

and nutrition for greenhouse crops. A grower's guide. Ball Publishing, Batavia, Illinois, U.S.A., 93–122.

- GRUDA, N. and W.H. SCHNITZLER 2004: Suitability of wood fibre substrates for production of vegetable transplants.I. Physical properties of wood fibre substrates. Scientia Hort. 100, 309–322.
- HERMANS, C., J.P. HAMMOND, P.J. WHITE and N. VERBRUGGEN 2006: How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. **11**, 610–617.
- HIROSE, N., K. TAKEI, T. KUROHA, T. KAMADA-NOBUSADA, H. HAYASHI and H. SAKAKIBARA 2008: Regulation of cytokinin biosynthesis, compartmentalization and translocation. J. Exp. Bot. 59, 75–83.
- JACKSON, B.E., R.D. WRIGHT, J.F. BROWDER, J.R. HARRIS and A.X. NIEMIERA 2008: Effect of fertilizer rate on growth of azalea and holly in pine bark and pine tree substrates. HortSci. **43**, 1561–1568.
- JACKSON, B.E., R.D. WRIGHT and J.R. SEILER 2009a: Comparison of fertilizer nitrogen availability, nitrogen immobilization, substrate carbon dioxide efflux, and nutrient leaching in peat-liter, pine bark, and pine tree substrates. HortSci. **44**, 781–790.
- JACKSON, B.E., R.D. WRIGHT and J.R. SEILER 2009b: Changes in chemical and physical properties of pine tree substrate and pine bark during long-term nursery crop production. HortSci. 44, 791–799.
- JACKSON, B.E., R.D. WRIGHT and M.C. BARNES 2010: Methods of constructing a pine tree substrate from various wood particle sizes, organic amendments, and sand for desired physical properties and plant growth. HortSci. **45**, 103–112.
- O'HARE, T.J. and C.G.N. TURNBULL 2004: Root growth, cytokinin and shoot dormancy in lychee (*Litchi chinensis* Sonn.). Scientia Hort. **102**, 257–266.
- RICHARDS, D., M. LANE and D.V. BEARDSELL 1986: The influernce of particle-size distribution in pinebark:sand: brown coal potting mixes on water supply, aeration, and plant growth. Scientia Hort. **29**, 1–14.
- RUBIO, V., R. BUSTOS, M.L. IRIGOYEN, X. CARDONA-LÓPEZ, M. ROJAS-TRIANA and J. PAZ-ARES 2009: Plant hormones and nutrient signaling. Plant Molec. Biol. 69, 361–373.
- TAKEI, K., T. TAKAHASHI, T. SUGIYAMA, T. YAMAYA and H. SAKAKIBARA 2002: Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. J. Exp. Bot. 53, 971–977.

Received 01/16/2012 / Accepted 06/08/2012

Addresses of authors: Javier Thibaud, Tomás Mc. Loughlin, Alberto Pagani, Raúl Lavado and Adalberto Di Benedetto (corresponding author), University of Buenos Aires, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentine, e-mail (corresponding author): dibenede@agro.uba.ar.