Journal of Horticultural Science & Biotechnology (2015) **90** (6) 643–654

Effects of combined or single exogenous auxin and/or cytokinin applications on growth and leaf area development in *Epipremnum aureum*

By A. DI BENEDETTO[1,2]*, C. GALMARINI[3,4] and J. TOGNETTI[2,5]
[1]Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (C1417DSE), Ciudad Autónoma de Buenos Aires, Argentina
[2]Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Ruta 226, km. 73.5 (B7620ZAA), Balcarce, Provincia de Buenos Aires, Argentina
[3]Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo and CONICET, Alt. Brown 500 (M5528AHB), Chacras de Coria, Provincia de Mendoza, Argentina
[4]Instituto Nacional de Tecnología Agropecuaria EEA La Consulta, CC 8 (5567), La Consulta San Carlos, Provincia de Mendoza, Argentina
[5]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Calle 526 entre 10 y 11 (1900), La Plata, Provincia Buenos Aires, Argentina
(e-mail: dibenede@agro.uba.ar) *(Accepted 13 June 2015)*

SUMMARY
We have analysed the effects of combined auxin and cytokinin treatments on whole plant growth and leaf development in *Epipremnum aureum* and compared both morphological and physiological variables to those obtained with a single hormone application. Rooted cuttings of *E. aureum* were sprayed with 0, 5, 50, or 100 mg l^{-1} indole-3-acetic acid (IAA) 7 d after transplanting. One week later, they were then sprayed with 0, 5, 50, or 100 mg l^{-1} benzylaminopurine (BAP). Whole plant growth, leaf development, carbon fixation, and leaf anatomy were recorded for 6 months after these sequential treatments. Following a single application of IAA or BAP, we observed an increase in the accumulation of whole-plant biomass, which reached a plateau at the highest concentration of either plant hormone. The promotion of growth was associated with increased rates of net C-assimilation and net photosynthesis, as well as with increased leaf thickness and the relative proportion of intracellular spaces in the mesophyll layer. The effect on leaves of applying both hormones, in different combinations and concentrations, resembled the results on whole plants obtained by spraying either the auxin or the cytokinin (at 50 or 100 mg l^{-1} BAP). Similarities in plant and leaf responses to the auxin and/or the cytokinin suggest that both hormones may act via the same pathway, which agreed with the well-known promotional effect of auxins on the development of new lateral roots, as root apices are the main site of cytokinin biosynthesis. Conversely, our results did not support the occurrence of a significant auxin-driven inhibition of cytokinin synthesis in root apices, at least for *E. aureum*, as reported in other species.

The productivity of pot-grown ornamental foliage plants is usually limited by several factors including post-transplant stress, especially the restriction on root development imposed by pot size, which, in turn, affects whole plant growth (Di Benedetto *et al*., 2006; Di Benedetto and Klasman, 2007). Exogenous supplies of auxins or cytokinins have been proposed to overcome the low vigour of root-restricted plants and negative post-transplantation effects (Mobli and Baninasab, 2009), in part by increasing leaf areas, leaf numbers, and leaf dry weights (DW; Di Benedetto *et al*., 2013; 2015).

Root restriction of plants can lower the biosynthesis of root cytokinins (O'Hare and Turnbull, 2004), whereas exogenous application of a cytokinin have been found to be effective at promoting the growth of pot-raised, root-restricted plants (Di Benedetto *et al*., 2010; Di Benedetto and Pagani, 2013). Previous results from our laboratory (Di Benedetto *et al*., 2013; 2015) showed increases in leaf area development and biomass accumulation between 17.2 – 47.9% and 21.8 – 58.8%, respectively, in rooted cuttings of *Epipremnum aureum* sprayed with benzylaminopurine (BAP). In addition, individual leaf areas increased and there were higher rates of leaf appearance in response to the cytokinin (Di Benedetto *et al*., 2013; 2015). Growth promotion by BAP may also result from increased concentrations of chlorophyll and other photosynthetic components (Ookawa *et al*., 2004; Song *et al*., 2013) and changes in leaf anatomy, as described in other species (Li *et al*., 2007; Kakani and Peng, 2011; Su *et al*., 2011; Gandolfo *et al*., 2014).

Another plant hormone used extensively during the propagation of ornamental plants is an auxin [usually indole-3-acetic acid (IAA) for herbaceous plants], because of well-documented effects of auxins on the initiation of new roots (Blythe *et al*., 2004; Guo *et al*., 2009; De Smet, 2011; Laskowski, 2013). However, conflicting reports exist regarding the effect of auxins on leaf area expansion and whole-plant growth. For

*Author for correspondence.

example, petunia plants transformed to overproduce IAA were found to develop smaller leaves than the non-transformed controls (Klee *et al*., 1987). In general, higher leaf concentrations of IAA have consistently been found to inhibit leaf expansion in intact plants (Keller *et al*., 2011). However, auxins have also been shown to promote whole-plant growth in *Vinca* plants (Van Iersel, 1998) and in *Phaseolus vulgaris* (Keller, 2007).

Auxins and cytokinins interact in a complex manner to control many aspects of plant growth and differentiation (Jones and Ljung, 2011). Based on experiments using excised shoots or roots of *Arabidopsis thaliana*, some authors proposed a homeostatic regulatory feedback loop model in which cytokinins functioned as positive regulators of auxin biosynthesis, and auxins repressed cytokinin biosynthesis (Nordstrom *et al*., 2004; Jones *et al*., 2010; Jones and Ljung, 2011). However, it is unclear whether this model can be extrapolated to the functioning of whole plants in which the concentrations of both types of hormone are modified naturally, or in response to exogenous supply. For example, if a strong auxin-driven inhibition of root cytokinin synthesis occurred, auxins might be expected to promote more root than shoot development, which might explain the observed reduction in leaf size on plants that overproduced auxin (Klee *et al*., 1987). On the other hand, increasing cytokinin concentrations by the application of exogenous BAP in plants with a high auxin concentration would be expected to reverse such an effect. Testing these possibilities is relevant for the cultivation of pot-grown ornamental plants in which applications of auxins or cytokinins may be used to enhance root and shoot growth, respectively.

In this work, we analysed the effects of an exogenously shoot-applied auxin and a cytokinin, separately or successively, on root and shoot development and on physiologically related traits in pot-raised rooted cuttings of *E. aureum*. We also analysed the possibility of the combined use of both hormones to improve the growth of *E. aureum* plants during commercial cultivation.

MATERIALS AND METHODS

Plant material and growth conditions

Rooted cuttings of *E. aureum* L. were obtained from a commercial propagator (Vivero Kogiso, Buenos Aires, Argentina) and transplanted into rigid 1,200-cm^3 plastic pots (one plant per pot). At the transplant stage, cuttings had a range of 3.3 ± 0.15 leaves, with an average leaf area of 145.49 ± 24.45 cm^2 and an average fresh weight (FW) of 7.57 ± 0.83 g $cutting^{-1}$. The pots were filled with a 40:40:20 (v/v/v) mix of *Sphagnum maguellanicum* peat:river waste:perlite (Di Benedetto *et al*., 2004). Plants were watered to saturation each day with high quality tap water (pH 6.64; electrical conductivity 0.486 dS m^{-1}) and were fertilised each week with 50 mg l^{-1} N in a 1.0:0.5:1.0:0.5 (v/v/v/v) mix of N:P:K:Ca (nitric acid, phosphorus acid, potassium nitrate, and calcium nitrate; Agroquímica Larocca S.R.L., Buenos Aires, Argentina) via the overhead irrigation system.

The experiment was carried out in a greenhouse at the Faculty of Agronomy, University of Buenos Aires, Argentina (34º 28'S) from 8 September 2007 to 12 March 2008. The greenhouse was covered with a black shade-cloth to deliver 50% of full-sunlight and divided into three blocks for statistical sampling. The red:far red (R:FR) ratio was not significantly modified by the shade-cloth, as shown using a 660/730 sensor (Skye Instruments, Llandrindod Wells, UK). Mean temperatures and light intensities during the experiment were recorded using three HOBO sensors (H08-004-02; Onset Computer Corporation, Bourne, MA, USA), one per block, connected to an HOBO H8 data logger, and are shown in Table I.

Hormone applications

Seven days after transplantation, all leaves were sprayed to run-off at sunset with different concentrations of BAP (0, 5, 50, or 100 mg l^{-1}) or IAA (0, 5, 50, or 100 mg l^{-1}; Sigma-Aldrich Co., St. Louis, MO, USA), or 16 combinations of the same IAA concentrations followed by the same BAP concentrations 7 d later. The IAA and BAP were first diluted in 80% (v/v) ethanol and no surfactants were used. Three blocks were used and 30 plants, in total, were sprayed with each of the 16 combinations of hormones, and distributed at random in each block (ten per block).

Growth measurements and data analysis

At 0, 60, 90, and 120 d after transplanting, two plants treated with each combination of hormones were destructively sampled in each block. The number of leaves on each plant was recorded, and each leaf area was determined using a LI-COR 3000A automatic leaf area meter (LI-COR Inc., Lincoln, NE, USA). The FWs of the different aerial parts (i.e., leaf blades, petioles, and stems) on each plant were determined. Dry weights (DWs) were obtained after drying each aerial and root part to constant weight at 80ºC for 96 h.

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 *vs*. time (in d). The rate of leaf appearance (RLA) was calculated as the slope of the number of visible leaves (including unrolled ones > 1.0 cm) *vs*. time (in weeks). The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm (*ln*) of whole plant DW *vs*. time (in d). The mean net C assimilation rate (NAR) and leaf area ratio (LAR) were calculated according to De Lojo and Di Benedetto (2014).

Net photosynthetic rate of leaves

The net photosynthetic rate was measured at ambient

TABLE I

Daily mean temperatures and mean daily photosynthetically active radiation (PAR) each month during the experiment in 2007–2008

Month (2007–2008)	Mean temperature (ºC)	PAR (mol photons m^{-2} d^{-1})
September	21.8 ± 1.12[†]	14.80 ± 1.13
October	26.3 ± 0.90	19.02 ± 1.26
November	25.7 ± 1.37	24.50 ± 1.28
December	34.3 ± 1.02	25.64 ± 1.15
January	37.5 ± 1.04	24.42 ± 0.94
February	36.8 ± 1.00	20.33 ± 1.03
March	32.0 ± 0.85	15.69 ± 0.67

[†]Values are daily means ± SE of values taken every 30 min.

O_2 and CO_2 concentrations and at a saturating photon flux density (> 1,700 μmol photons m^{-2} s^{-1}) between 11.30 – 13.00 h on the last sunny day before final harvest. The youngest fully-expanded leaf on three plants from each hormone treatment were selected and measured using a portable LICOR LI-6200 photosynthetic system (LI-COR Inc.).

Leaf anatomy

Samples of young, fully-expanded leaves were collected to examine leaf anatomy (i.e., overall leaf thickness, the thicknesses of the mesophyll layer and the epidermal layer, and the volume of intercellular spaces) on the final harvest date (120 d from transplanting). Tissue from the middle region of each lamina was fixed in a mixture of 70% (v/v) ethanol, 5% (v/v) formalin, 5% (v/v) glacial acetic acid, and 20% (v/v) distilled water prior to dehydration in an ethanol and *tert*-butyl alcohol series, following the procedure described by Jensen (1962). Fixed tissues were sectioned (10 – 20 μm thick) on a rotary microtome and stained with safranin-crystal violet-fast green (Gerlach, 1969).

To determine stomatal size on the abaxial surface, and epidermal cell size on the adaxial surface, leaves were boiled in 80% (v/v) ethanol for 15 min., soaked in 5% (w/v) NaOH at 60ºC for 3 d, kept in a choral hydrate solution until clear, dehydrated in an ethanol series (Foster, 1950), stained with 0.05% (v/v) safranin in a 1:1 (v/v) mix of ethanol-xylol, and mounted in Canada balsam (BLC, Buenos Aires, Argentina) according to Foster (1950).

The data presented are the means of three leaves per treatment, using ten cross-sections per leaf. Quantitative anatomical data were obtained using Image Pro Express Version 6.0 (Media Cybernetics, Rockville, MD, USA).

Statistical analysis

The experiment was arranged in a 16-way factorial design, with four concentrations of IAA and four concentrations of BAP. Data were subjected to two-way analysis of variance (ANOVA). STATISTICA 8 (StatSoft, 2013) software was used and the assumptions of ANOVA were checked. Least significant differences (LSD) values were calculated. Two plants from each treatment from each of the three blocks were harvested on each sampling date. Slopes from straight-line regressions of RLAE, RGR, NAR, and LAR values were tested using the SMATR package (Warton *et al.*, 2012).

RESULTS

Dynamics of plant growth after single or sequential hormone applications and hormone concentration effects on plant biomass at final harvest

A single application of IAA or BAP at 50 mg l^{-1} led to a significant increase in FW accumulation observed 60 d after treatment in both roots (Figure 1A) and leaves (Figure 1C), and at 90 d after treatment in stems (Figure 1B) compared with control plants. Supplying both hormones sequentially was generally less effective than supplying either alone. At the end of the experimental period, sequential applications of IAA and BAP resulted in an increase in FW accumulation over the controls which was dependent on the concentrations applied (Figure 1D). Hormone concentrations lower or higher than 50 mg l^{-1} generally resulted in less than the maximum promotion of growth and, in all cases, 100 mg l^{-1} corresponded to a supra-optimal hormone concentration.

In plants treated with IAA and, 7 d later with BAP, addition of the second hormone resulted in only a slight growth promoting effect (generally less than 20%), which tended to be lower with increasing IAA and BAP concentrations (Figure 1E). ANOVA of the data in Figure 1D showed highly significant IAA and BAP main effects ($P \leq 0.001$) and non-significant ($P > 0.05$) IAA × BAP interactions, for both shoots and roots. On the other hand, there were no significant differences in the dry matter content of shoots or roots between control and hormone-treated plants (data not shown).

Leaf growth and development

Total leaf area (Figure 2A) and leaf number per plant (Figure 2B) were significantly higher 90 d after IAA or BAP application at 50 mg l^{-1} compared with control plants. These effects were observed later than those on the accumulation of FW.

Accordingly, total leaf areas at final harvest were higher in plants sprayed with either IAA or BAP than in the controls (Figure 2C). This was the result of a higher RLA (Figure 2E) and larger mean values of individual leaf areas (Figure 2G). The response to either hormone was similar. A 5 mg l^{-1} application of IAA or BAP was enough for a strong response, which reached an apparent plateau at higher concentrations. In plants treated with 5 – 100 mg l^{-1} IAA, subsequent application of BAP had no substantial added effect, regardless of the concentration used (Figure 2D, F, H). ANOVA of the data in Figure 2C, E, G showed highly significant IAA and BAP effects ($P \leq 0.001$ for both hormones) and non-significant ($P > 0.05$) IAA × BAP interactions.

Plant growth analysis

A BAP spray increased both RLAE and RGR values (Figure 3A,B) compared to control plants. The maximum response was observed at 50 mg l^{-1} IAA or BAP. In contrast, in plants sprayed with IAA and then with BAP, the latter had no added promoting effect at 5 or 50 mg l^{-1}, irrespective of the IAA concentration used. In fact, at 50 mg l^{-1} BAP, RGR and RLAE values tended to decrease below those in control plants (Figure 3E, F). The effects of either hormone on the RGR were associated with an increase in NAR and a decrease in LAR values (Figure 3C, D). When plants sprayed with 5 – 100 mg l^{-1} IAA were given a later spray of BAP, only small effects were observed (Figures 3E–H).

Photosynthetic rates in leaves

In *E. aureum* plants treated with either IAA or BAP, the net photosynthetic rate increased compared to control plants (Figure 4A). The response of the photosynthetic rate to IAA was higher than to BAP at all concentrations tested (Figure 4B). ANOVA showed significant main effects of both hormones ($P \leq 0.001$ and $P \leq 0.01$ for IAA and BAP, respectively), while no significant IAA × BAP interaction was found. In plants first treated with IAA, a subsequent application of BAP

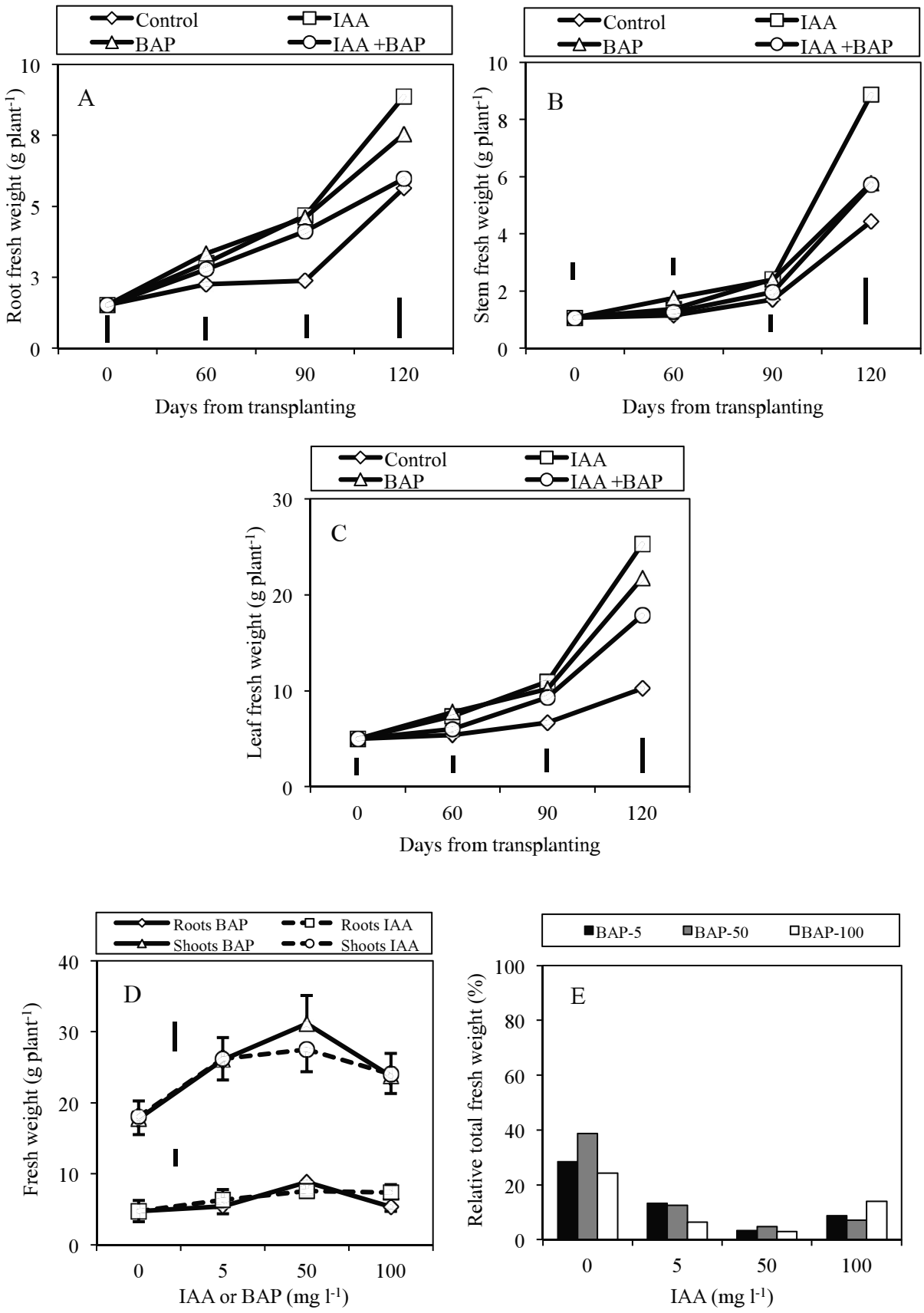

FIG. 1

Changes in root (Panel A), stem (Panel B), and leaf (Panel C) fresh weights (FWs) in *E. aureum* during the 120 d experiment in control plants and in plants which showed the greatest response to 50 mg l^{-1} sprays of IAA or BAP applied either as a single hormone spray or as sequential sprays of IAA + BAP separated by 7 d. Panel D shows whole-plant FW (± standard error) on the final harvest (day-120) in *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as single hormone applications. Vertical lines in Panels A–D indicate least significant differences (LSD). Panel E shows the effect of a 5, 50, or 100 mg l^{-1} BAP spray application to plants sprayed 7 d before with IAA at 0, 5, 50 or 100 mg l^{-1}. Data in Panel E are expressed as percentage changes observed following BAP application at 5, 50, or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

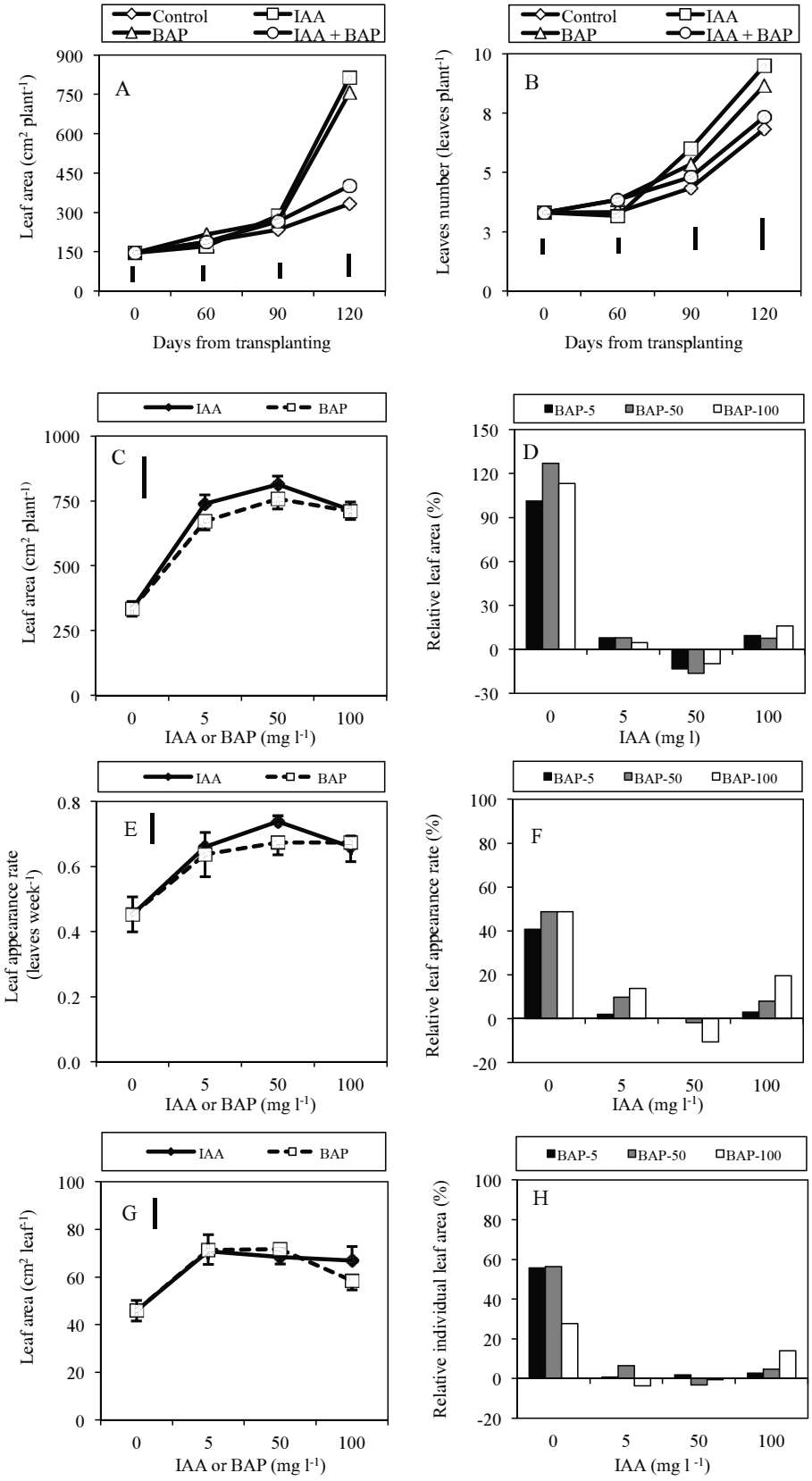

Fig. 2

Changes in mean values (n = 6) of total leaf area (Panel A) and leaf number per *E. aureum* plant (Panel B) during the 120 d experiment in control plants and in plants which showed the greatest response to 50 mg l^{-1} IAA or BAP applied either as a single hormone spray or as sequential sprays of IAA + BAP separated by 7 d. Panels C, E, and G show mean values (n = 6; ± standard error) of total leaf areas, rates of leaf appearance, and individual leaf areas, respectively on the final harvest (day-120) of *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as single hormone applications. Vertical lines in Panels A–C, E, and G indicate least significant differences (LSD). Panels D, F, and H show the effect of a 5, 50, or 100 mg l^{-1} BAP spray application on total leaf areas, rates of leaf appearance, and individual leaf areas, respectively, in plants sprayed 7 d before with IAA at 0, 5, 50 or 100 mg l^{-1}. Data in Panels D, F–H are expressed as the percentage change observed following BAP application at 5, 50 or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

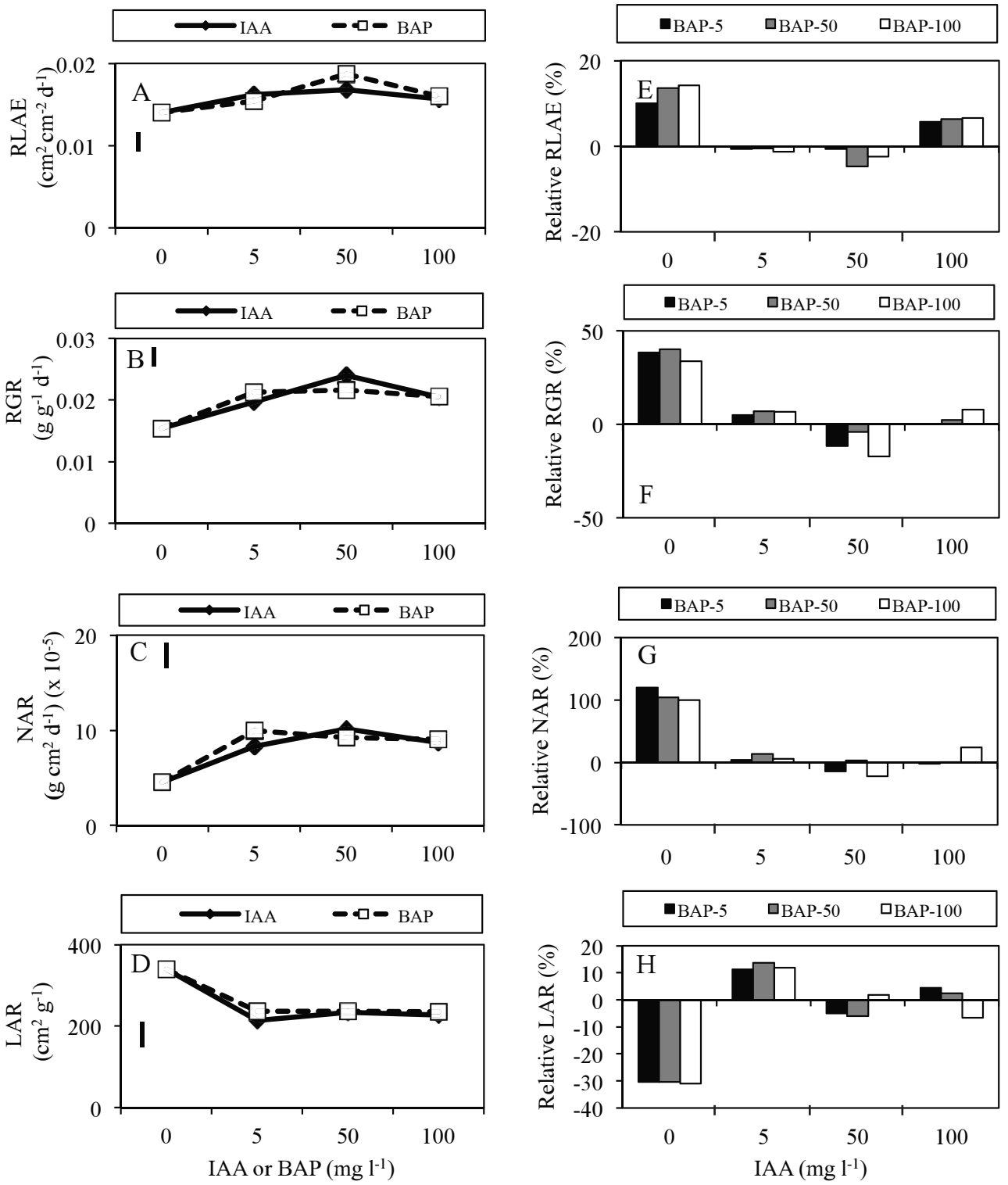

FIG. 3

Mean values (n = 24; ± SE) of the relative rate of leaf area expansion (RLAE; Panel A), relative growth rate (RGR; Panel B), net C-assimilation rate (NAR; Panel C), and leaf area ratio (LAR; Panel D) on the final harvest (day-120) of *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as single hormone applications. Vertical lines in Panels A–D indicate least significant differences (LSD). Panels E, F, G, and H show the effects of a single spray of 5, 50, or 100 mg l^{-1} BAP on RLAE, RGR, NAR and LAR, respectively, in plants sprayed 7 d earlier with IAA at 0, 5, 50 or 100 mg l^{-1}. Data in Panels E–H are expressed as the percentage change observed following the application of BAP at 5, 50 or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

had only a small effect, regardless of the concentration used (Figure 4B).

Leaf anatomy

Increases in overall leaf thickness (Figure 5A), epidermal cell size (Figure 5B), and stomatal pore size (Figure 5C) were observed in plants sprayed with IAA or BAP at 5 mg l^{-1} and 50 mg l^{-1}, while 100 mg l^{-1} IAA or BAP tended to be supra-optimal. In plants supplied with IAA and subsequently with BAP, the latter had no

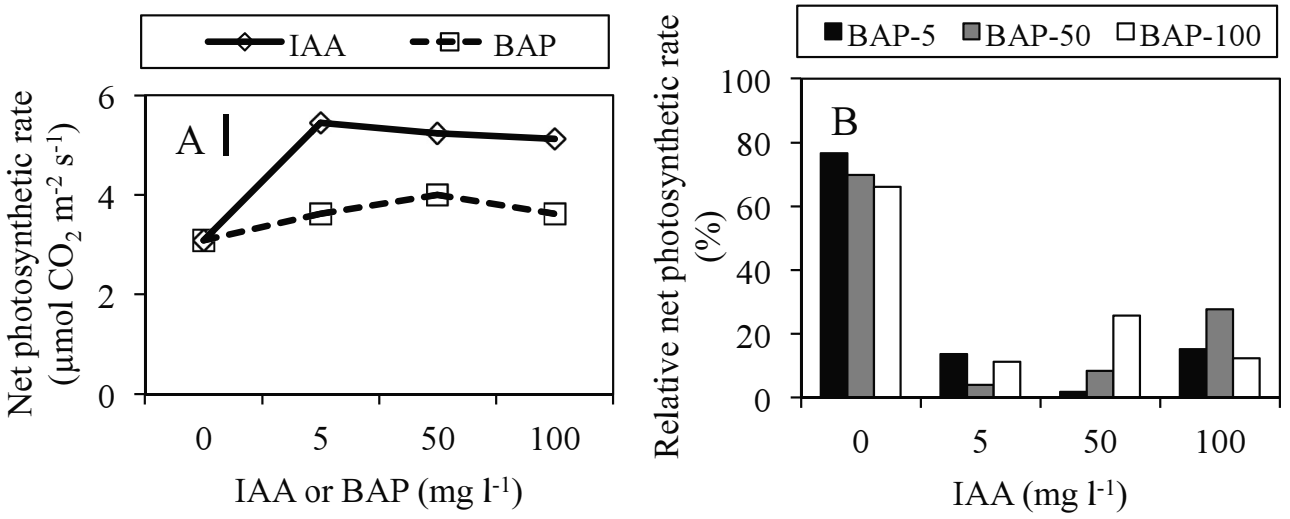

Fig. 4
Net photosynthetic rate (± standard error) in *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as a single hormone application (Panel A). Vertical line indicates least significant difference (LSD). Panel B shows the effect of a 5, 50, or 100 mg l^{-1} BAP spray on plants previously sprayed 7 d before with IAA at 0, 5, 50, or 100 mg l^{-1}. Data in Panel B are expressed as the percentage change observed following BAP application at 5, 50 or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

promoting effects, irrespective of the IAA concentration (Figure 5D–F). ANOVA showed highly significant IAA or BAP effects ($P \leq 0.001$) on all three variables measured, but no significant IAA × BAP interactions.

A decrease in the contribution of the epidermal layer to overall leaf thickness was observed following either IAA or BAP treatment (Figure 6A). Conversely, a significant increase in the proportion of intercellular spaces was observed (Figure 6C), while only slight changes were seen in the proportion of the parenchymal layer (Figure 6B). In plants treated with 5 – 100 mg l^{-1} IAA, a later application of BAP had no consistent effect on these variables (Figures 6D–F), although, in general, BAP contributed to a decrease the proportion of the epidermal layer, while increases in the percentage of intercellular spaces were lower as the BAP concentration increased.

Relationships between photosynthesis, NAR, and changes in leaf anatomy

When the net photosynthetic rate was plotted as a function of the percentage of intercellular spaces in the leaf lamina (Figure 7A), or as a function of overall leaf thickness (Figure 7B), direct linear relationships were observed ($r^2 = 0.523$ and $r^2 = 0.548$, respectively; $P < 0.001$). Similar results were obtained when NAR was plotted against these variables in leaf anatomy (Figure 7C, D; $r^2 = 0.696$ and $r^2 = 0.569$, respectively; $P \leq 0.001$).

DISCUSSION

The application of exogenous hormones is a common practice during ornamental foliage plant propagation. Pre-transplant shoot treatments with auxins have long been used to induce adventitious roots (Agulló *et al.*, 2014; Pacurar *et al.*, 2014). More recently, post-transplant applications of cytokinin to the foliage have been proposed to promote shoot development and thus to overcome, in part, root restriction due to limited pot volume (Di Benedetto, 2011; Di Benedetto and Pagani, 2013; Di Benedetto *et al.*, 2013; 2015).

In the present work, we have shown that, despite the reported inhibitory action of an auxin on cytokinin biosynthesis (Jones *et al.*, 2010; Bishop *et al.*, 2011; Jones and Ljung, 2011), a single post-transplant application of exogenous auxin on rooted *E. aureum* cuttings promoted not only root, but also shoot growth. Auxin appeared similar to cytokinin in most of the anatomical, morphological, and physiological responses examined. No interactions were found, but the effects of both hormones, in different concentrations and combinations, resembled the results obtained by spraying a single hormone (either an auxin or a cytokinin) at higher doses.

Previous results from our laboratory (Di Benedetto *et al.*, 2010; 2013; 2015) have shown that a foliar spray of BAP, just after transplanting, increased whole plant biomass and the accumulation of leaf area in *E. aureum* as a result of higher individual leaf areas and higher rates of leaf appearance (RLA). This effect was attributable to a decreased phyllochron, since no branching was observed in *E. aureum*.

In the present work, we showed that the application of IAA had an essentially similar effect to that obtained by BAP (i.e., promoting growth responses that reached an apparent plateau at a hormone concentration of approx. 50 mg l^{-1}). In addition, no further promotion of growth was observed by applying a second hormone to plants that had been sprayed with either 50 mg l^{-1} IAA or BAP 7 d previously (Figure 1; Figure 2). The growth promoting effect of either hormone on biomass accumulation could be explained by a large increase in NAR (Figure 3), which in turn was associated with a higher net photosynthetic rate (Figure 4). Increased accumulation of biomass by either hormone was observed earlier than the effects on leaf development (Figure 1; Figure 3).

It has been shown that cytokinins increased photosynthetic capacity (Song *et al.*, 2013; Ookawa *et al.*, 2004) and the activity of the main photosynthetic enzyme (ribulose-1, 5-bisphosphate carboxylase/

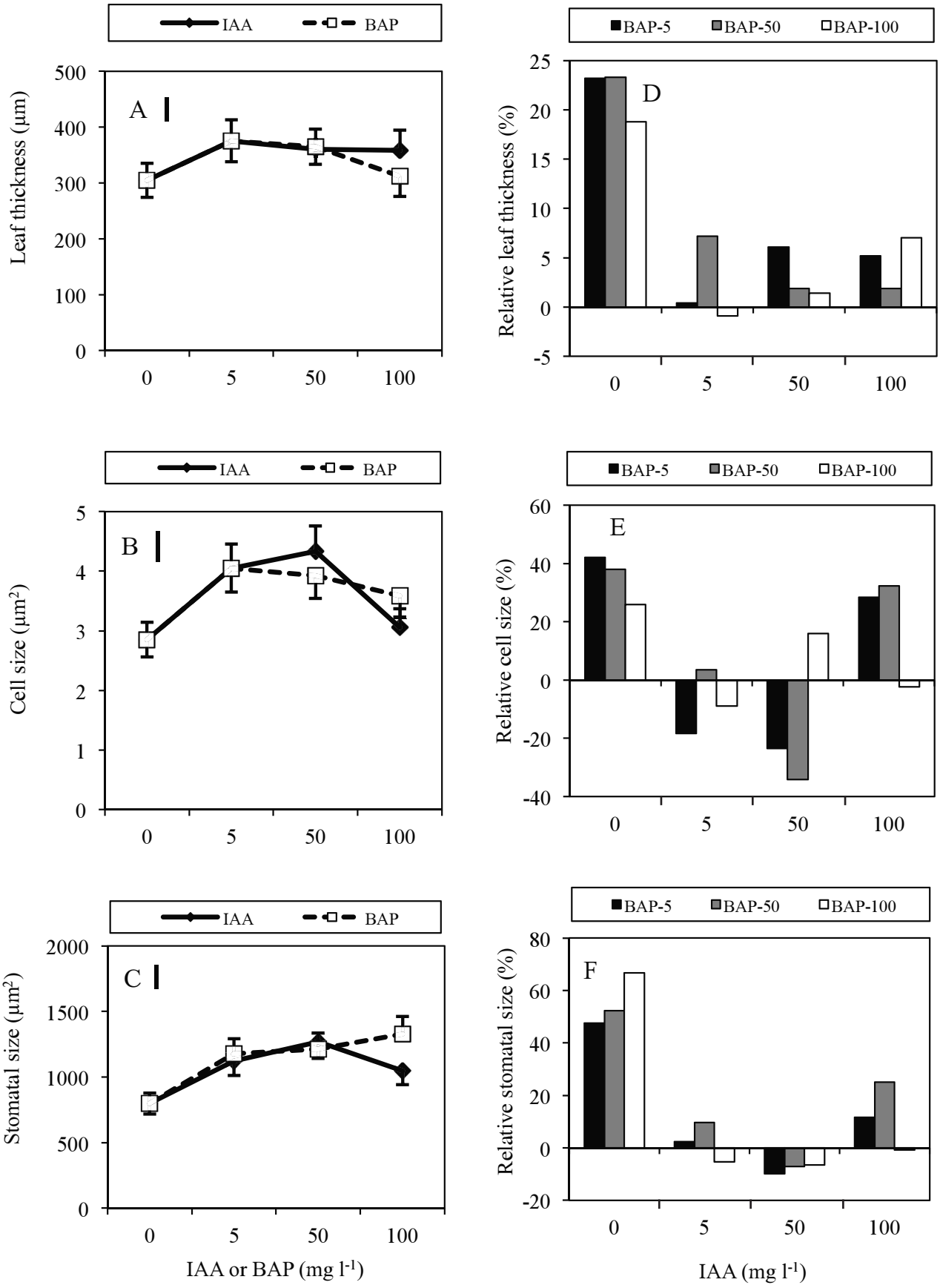

FIG. 5

Epidermal cell size, stomatal size, and overall leaf thickness [mean values (n = 6) ± standard error] in Panels A–C, respectively, of leaves from *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as a single hormone application. Vertical lines in Panels A–C indicate least significant differences (LSD). Panels D–F show the effect of a 5, 50, or 100 mg l^{-1} BAP application on leaf epidermal cell size, stomatal size, and overall leaf thickness, respectively, in plants sprayed 7 d before with IAA at 0, 5, 50, or 100 mg l^{-1}. Data in Panels D–F are expressed as the percentage change observed following BAP application at 5, 50 or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

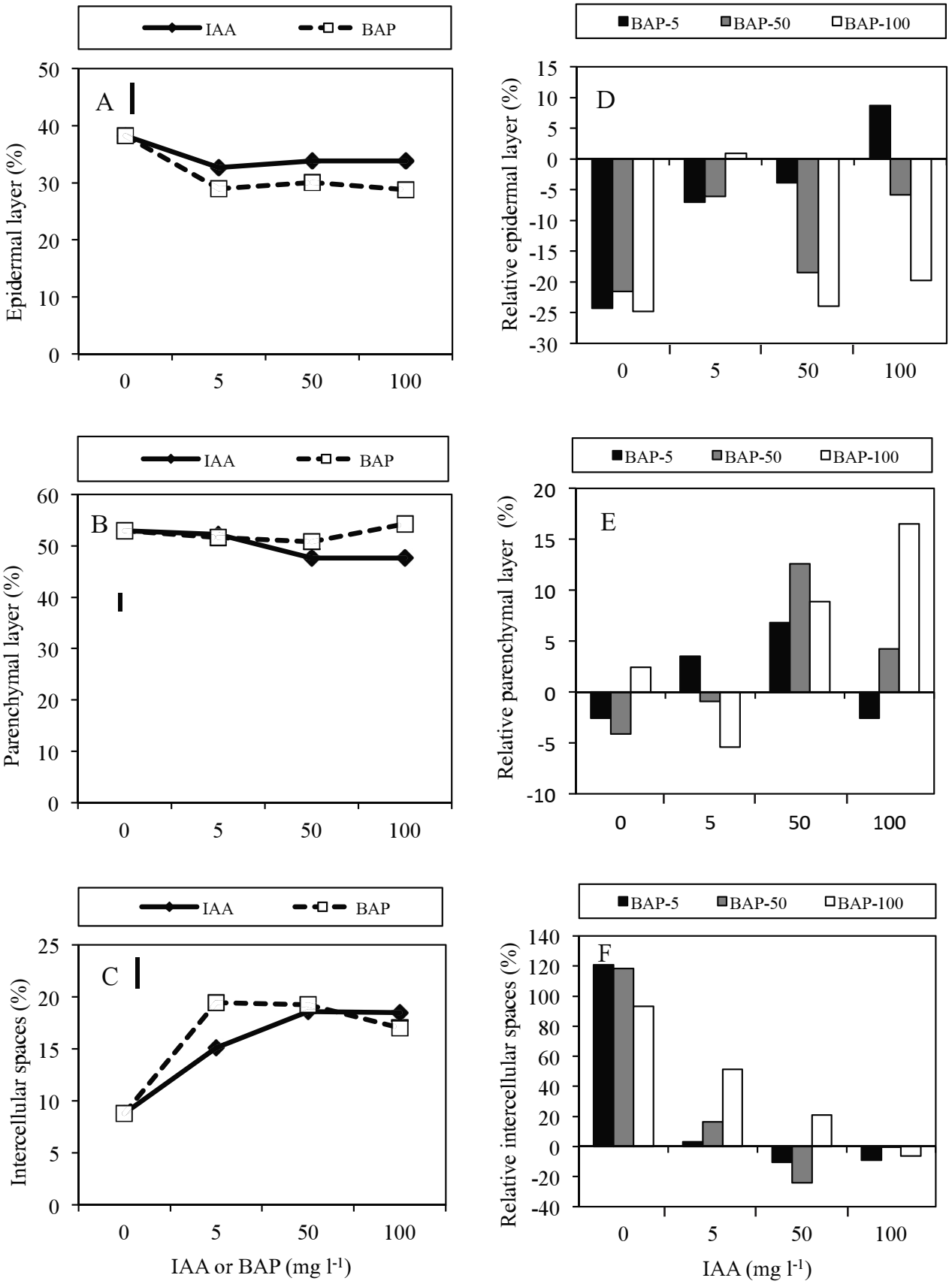

Fig. 6
Contributions (expressed as percentages) of the epidermal layer, parenchymal layer, and intercellular spaces (Panels A–C, respectively) to the overall thickness of leaves on *E. aureum* plants sprayed with 0, 5, 50, or 100 mg l^{-1} IAA or BAP as a single hormone application. Vertical lines indicate least significant differences (LSD). Panels D–F show the relative effect of a 5, 50, or 100 mg l^{-1} BAP spray application on the proportions of the epidermal layer, parenchymal layer, and intercellular spaces, respectively, in leaves on plants sprayed 7 d earlier with IAA at 0, 5, 50 or 100 mg l^{-1}. Data in Panels D–F are expressed as the percentage change observed following BAP application at 5, 50, or 100 mg l^{-1} relative to plants sprayed with 0 mg l^{-1} BAP.

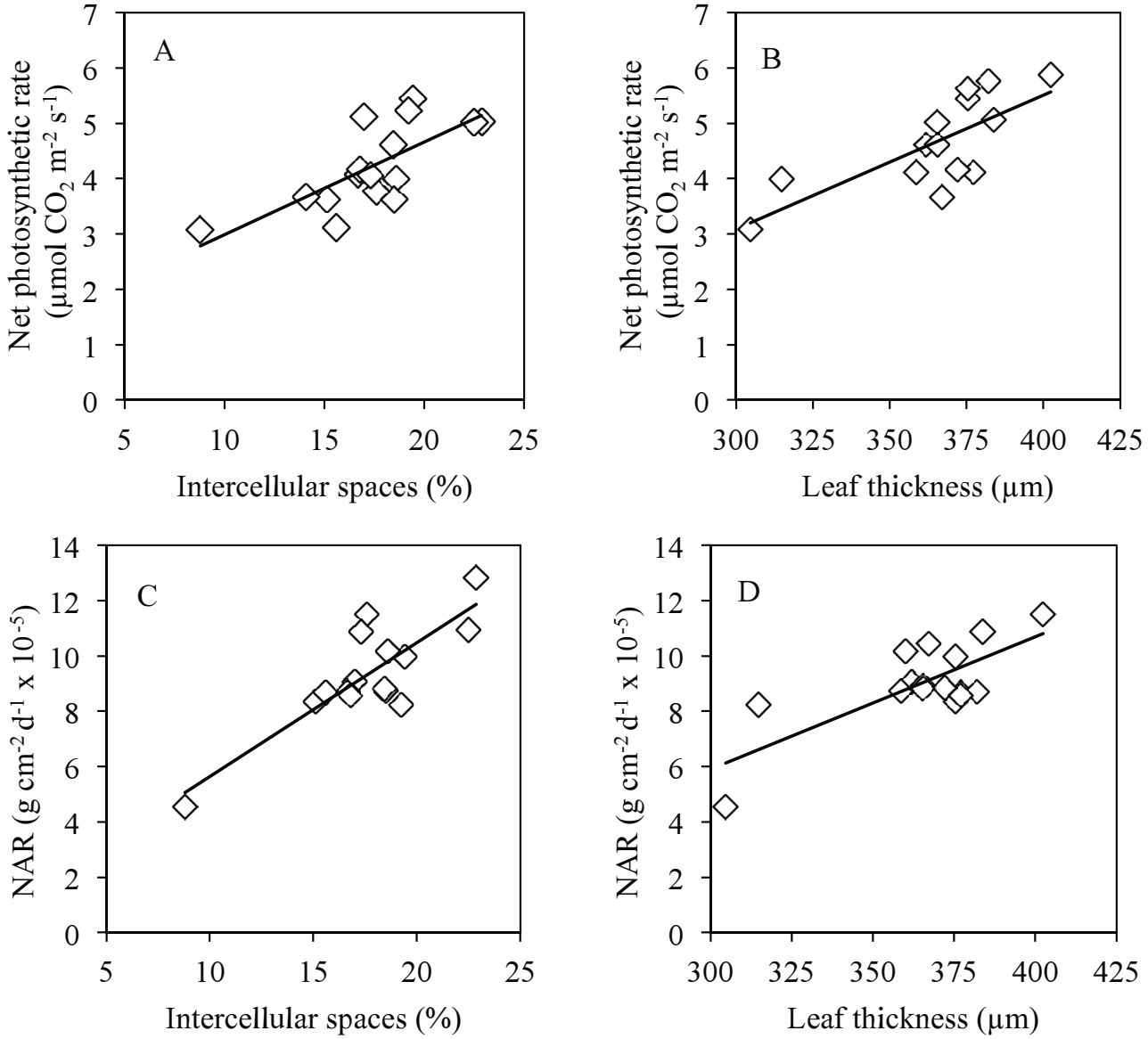

FIG. 7
Inter-relationships between the net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}; Panels A, B) or the net C-assimilation rate (g cm^{-2} d^{-1}; Panels C, D) and the percentage of leaf intercellular spaces (Panels A, C) or overall leaf thickness (µm; Panels B, D). Linear regression equations were as follows:
(Panel A) Net photosynthetic rate = 0.17 Intercellular spaces + 1.31 (r^2 = 0.523; $P \leq 0.001$)
(Panel B) Net photosynthetic rate = 0.024 Leaf thickness – 4.18 (r^2 = 0.548; $P \leq 0.001$)
(Panel C) NAR = 0.484 intercellular spaces (%) + 0.80 (r^2 = 0.696; $P \leq 0.001$)
(Panel D) NAR = 0.0479 leaf thickness – 8.47 (r^2 = 0.569; $P \leq 0.001$).
Data corresponding to all 16 IAA-BAP spray combinations were used for the regression analysis.

oxygenase) in rice and in *Arabidopsis* (Boonman, 2007; 2009). In addition, cytokinins increase tetrapyrrole biosynthesis (Yaronskaya *et al*., 2006) and often increase chlorophyll concentrations. Information on the direct effect of auxins on the photosynthetic rate is scarce. Shah (2011) showed that *Nigella sativa* plants sprayed with 4-Cl-IAA (an analogue of IAA) or kinetin exhibited an increase in their net photosynthetic rate. This effect could be a consequence of the direct stimulation of photosynthesis by the hormone, or due to more actively growing C-sinks.

In addition to the effects of IAA and BAP sprays on leaf area development, we also observed significant changes in overall leaf thickness (Figure 5C) and in the distribution of leaf tissue (Figure 6). Many auxin- and cytokinin-regulated genes are involved in leaf development (Li *et al*., 2007; Kakani and Peng, 2011; Su *et al*., 2011). It is known that the high photosynthetic capacity of sun leaves is supported by the development of a thick lamina (Terashima *et al*., 2001). Strong correlations are commonly found between photosynthetic capacity and overall leaf thickness, between photosynthetic capacity and the mesophyll cell surface area, and between the internal conductance of CO_2 and the surface area of chloroplasts facing the intercellular space in full-sun plants (Oguchi *et al*., 2003).

In our experiments, we found positive relationships between NAR or net photosynthetic rate and leaf intercellular spaces or overall leaf thickness (Figure 7). These effects of an auxin or a cytokinin agree with recently published data on *Impatiens wallerana* (Gandolfo *et al*., 2014). IAA or BAP sprays also increased

epidermal cell and stomatal sizes. However, the fact that the main effect of an IAA spray on leaf anatomy was to increase the volume of intercellular spaces does not support the proposal by Savaldi-Goldstein and Chory (2008), who suggested that the epidermal layer was the preferred target for auxin action on leaves.

The striking similarities in the responses to an auxin or a cytokinin strongly suggest that both hormones act via the same signalling cascade. A possible explanation for this result is that because auxins are known to promote lateral root development, and cytokinins are synthesised in root apical meristems (Aloni *et al*., 2005; Doerner, 2007), then an exogenous auxin might, in fact, also result in increased endogenous cytokinin levels in plants. Although Jones *et al*. (2010) and Jones and Ljung (2011) suggested a homeostatic regulatory feedback loop model in which an auxin down-regulated cytokinin synthesis, our results suggest that any auxin-driven inhibition of cytokinin synthesis might be overcome by the effect of an auxin on stimulating the development of potential sites of cytokinin production (i.e., root tips). Whether this explains the similar responses of *E. aureum* plants to IAA and BAP in the present work, and whether this is a common effect in most species, or if it is unique to *E. aureum* should be addressed in future experiments.

In summary, an increase in biomass accumulation in whole-plants of *E. aureum* was observed as a consequence of either a single IAA or BAP spray. This was associated with an increased NAR and net photosynthetic rate. Changes in leaf anatomy induced by either hormone (including increased stomatal size, overall leaf thickness, and leaf intercellular spaces) may facilitate the absorption and fixation of CO_2. The similarities between the responses to either hormone, together with the lack of any IAA × BAP interaction, provide two independent routes for commercial growers to increase the productivity of ornamental foliage plants by using early foliar sprays, once a precise dose-response relationship for the particular environment in which the *E. aureum* is grown has been established.

This work formed part of a Ph.D. thesis by A.H. Di Benedetto at the Universidad Nacional de Cuyo, supported by the University of Buenos Aires Science Programme 2008-2011 (Grant No. G054) and the University of Mar del Plata 2008-2010 Science Programme (Grant No. AGR 259/08 and AGR 287/09).

REFERENCES

Agulló-Antón, M. A., Ferrández-Ayela, A., Fernández-García, N., Nicolás, C., Albacete, A., Pérez-Alfocea, F., Sánchez-Bravo, J., Pérez-Pérez, J. M. and Acosta, M. (2014). Early steps of adventitious rooting: morphology, hormonal profiling and carbohydrate turnover in carnation stem cuttings. *Physiologia Plantarum*, **150,** 446–462.

Aloni, R., Langhans, M., Aloni, E., Dreieicher, E. and Ullrich, C. I. (2005). Root-synthesized cytokinin in *Arabidopsis* is distributed in the shoot by the transpiration stream. *Journal of Experimental Botany*, **56,** 1535–1544.

Bishopp, A., Benková, E. and Helariutta, Y. (2011). Sending mixed messages: auxin-cytokinin crosstalk in roots. *Current Opinion in Plant Biology*, **14,** 10–16.

Blythe, E. K., Sibley, J. L., Ruter, J. M. and Tilt, K. M. (2004). Cutting propagation of foliage crops using a foliar application of auxin. *Scientia Horticulturae*, **103,** 31–37.

Boonman, A., Prinsen, E., Gilmer, F., Schurr, U., Peeters, A. J. M., Voesenek, L. A. C. J. and Pons, T. L. (2007). Cytokinin import rate as a signal for photosynthetic acclimation to canopy light gradients. *Plant Physiology*, **143,** 1841–1852.

Boonman, A., Prinsen, E., Voesenek, L. A. C. J. and Pons, T. L. (2009). Redundant roles of photoreceptors and cytokinins in regulating photosynthetic acclimation to canopy density. *Journal of Experimental Botany*, **60,** 1179–1190.

De Lojo, J. and Di Benedetto, A. (2014). Biomass accumulation and leaf shape can be modulated by an exogenous spray of 6-benzylaminopurine in the ornamental foliage plant *Monstera deliciosa* (Liebm.). *Journal of Horticultural Science & Biotechnology*, **89,** 136–140.

De Smet, I. (2012). Lateral root initiation: one step at a time. *New Phytologist*, **193,** 867–873.

Di Benedetto, A. (2011). Root restriction and post-transplant effects for bedding pot plants. In: *Ornamental Plants: Types, Cultivation and Nutrition*. (Aquino, J.C., Ed.). Nova Science Publishers Inc., New York, NY, USA. 47–79.

Di Benedetto, A. and Klasman, R. (2007). River waste as a potentially amendment for low quality *Sphagnum* peat. *European Journal of Horticultural Science*, **72,** 260–261.

Di Benedetto, A. and Pagani, A. (2013). Dry weight accumulation in the *Impatiens walleriana* pot plant in responses to different pre-transplant plug cell volume. *European Journal of Horticultural Science*, **78,** 76–85.

Di Benedetto, A., Klasman, R. and Boschi, C. (2004). Use of river waste in growing media for ornamental herbaceous perennials. *Journal of Horticultural Science & Biotechnology*, **79,** 119–124.

Di Benedetto, A., Molinari, J., Boschi, C., Benedicto, D., Cerrotta, M. and Cerrotta, G. (2006). Estimating crop productivity for three ornamental foliage plants. *International Journal of Agricultural Research*, **1,** 522–533.

Di Benedetto, A., Tognetti, J. and Galmarini, C. (2010). Biomass production in ornamental foliage plants: Crop productivity and mechanisms associated to exogenous cytokinin supply. *The Americas Journal of Plant Science & Biotechnology*, **4,** 1–22.

Di Benedetto, A., Galmarini, C. and Tognetti, J. (2013). Contribution of changes in leaf size and leaf production rate to cytokinin-mediated growth promotion in *Epipremnum aureum* L. *Journal of Horticultural Science & Biotechnology*, **88,** 179–186.

Di Benedetto, A., Galmarini, C. and Tognetti, J. (2015). Exogenous cytokinin promotes *Epipremnum aureum* L. growth through enhanced dry weight assimilation rather than through changes in partitioning. *American Journal of Experimental Agriculture*, **5,** 419–434.

Doerner, P. (2007). Plant meristems: cytokinins – the alpha and omega of the meristem. *Current Biology*, **17,** R321–R323.

Foster, A. S. (1950). Techniques for the study of venation patterns in the leaves of angiosperms. *Proceedings of the 7th International Botany Congress*. Stockholm, Sweden. 586–587.

Gandolfo, E., De Lojo, J., Gómez, D., Pagani, A., Molinari, J. and Di Benedetto, A. (2014): Anatomical changes involved in the response of *Impatiens wallerana* to different pre-transplant plug cell volumes and BAP sprays. *European Journal of Horticultural Science*, **79,** 226–232.

Gerlach, D. (1969). A rapid safranin–crystal violet–light green staining sequence for paraffin sections of plant materials. *Stain Technology*, **44,** 201–211.

Guo, X., Fu, X., Zang, D. and Ma, Y. (2009). Effect of auxin treatments, cuttings' collection date, and initial characteristics on *Paeonia* 'Yang Fei Chu Yu' cutting propagation. *Scientia Horticulturae*, **119,** 177–181.

Jensen, W. A. (1962). *Botanical Histochemistry*. W. H. Freeman, San Francisco, CA, USA. 408 pp.

Jones, B. and Ljung, K. (2011). Auxin and cytokinin regulate each other's levels via a metabolic feedback loop. *Plant Signaling & Behavior*, **6,** 901–904.

Jones, B., Andersson Gunnera, S., Petersson, S. V., Tarkowski, P., Graham, N., May, S., Dolezal, K., Sandberg, G. and Ljung, K. (2010). Cytokinin regulation of auxin synthesis in *Arabidopsis* involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *The Plant Cell*, **22,** 2956–2969.

Kakani, A. and Peng, Z. (2011). ARR5 and ARR6 mediate tissue specific cross-talk between auxin and cytokinin in *Arabidopsis*. *American Journal of Plant Sciences*, **2,** 549–553.

Keller, C. P. (2007). Leaf expansion in *Phaseolus*: transient auxin induced growth increase. *Physiologia Plantarum*, **130,** 580–589.

Keller, C. P., Grundstad, M. L., Evanoff, M. A., Keith, J. D., Lentz, D. S., Wagner, S. L., Culler, A. H. and Cohen, J. D. (2011). Auxin-induced leaf blade expansion in *Arabidopsis* requires both wounding and detachment. *Plant Signaling & Behavior*, **6,** 1997–2007.

Klee, H. J., Horsch, R. B., Hinchee, M. A., Hein, M. B. and Hoffman, N. L. (1987). The effects of overproduction of two *Agrobacerium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic *Petunia* plants. *Genes and Development*, **1,** 86–96.

Laskowski, M. (2013). Lateral root initiation is a probabilistic event whose frequency is set by fluctuating levels of auxin response. *Journal of Experimental Botany*, **64,** 2609–2617.

Li, L. C., Kang, D. M., Chen, Z. L. and Qu, L. J. (2007). Hormonal regulation of leaf morphogenesis in *Arabidopsis*. *Journal of Integrative Plant Biology*, **49,** 75–80.

Mobli, M. and Baninasab, B. (2009). Effect of indole-butyric acid on root regeneration and seedling survival after transplanting of three *Pistacia* species. *Journal of Fruit and Ornamental Plant Research*, **17,** 5–13.

Nordström, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K. and Sandberg, G. (2004). Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: A factor of potential importance for auxin–cytokinin-regulated development. *Proceedings of the National Academy of Sciences of the USA*, **101,** 8039–8044.

Oguchi, R., Hikosaka, K. and Hirose, T. (2003). Does the photosynthetic light-acclimation need change in leaf anatomy? *Plant, Cell and Environment*, **26,** 505–512.

O'Hare, T. J. and Turnbull, C. G. N. (2004). Root growth, cytokinin and shoot dormancy in lychee (*Litchi chinensis* Sonn.). *Scientia Horticulturae*, **102,** 257–266.

Ookawa, T., Naruoka, Y., Sayama, A. and Hirasawa, T. (2004). Cytokinin effects on ribulose-1,5-bisphosphate carboxylase/oxygenase and nitrogen partitioning in rice during ripening. *Crop Science*, **44,** 2107–2115.

Pacurar, D. I., Perrone, I. and Bellini, C. (2014). Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiologia Plantarum*, **151,** 83–96.

Savaldi-Goldstein, S. and Chory, J. (2008). Growth coordination and the shoot epidermis. *Current Opinion in Plant Biology*, **11,** 42–48.

Shah, S. H. (2011). Comparative effects of 4-Cl-IAA and kinetin on photosynthesis, nitrogen metabolism and yield of black cumin (*Nigella sativa* L.). *Acta Botanica Croatica*, **70,** 91–97.

Song, W., Li, J., Sun, H., Huang, S., Gong, X., Ma, Q., Zhang, Y. and Xu, G. (2013). Increased photosynthetic capacity in response to nitrate is correlated with enhanced cytokinin levels in rice cultivar with high responsiveness to nitrogen nutrients. *Plant and Soil*, **373,** 981–993.

Statsoft (2013). *Electronic Statistics Textbook*. Tulsa, OK, USA. http://www.statsoft.com/textbook/.

Su, Y. H., Liu, Y. B. and Zhang, X. S. (2011). Auxin-cytokinin interaction regulates meristem development. *Molecular Plant*, **4,** 616–625.

Terashima, I., Miyazawa, S. I. and Hanba, Y. T. (2001). Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. *Journal of Plant Research*, **114,** 93–105.

Van Iersel, M. (1998). Auxins affect post-transplant shoot and root growth of *Vinca* seedlings. *HortScience*, **33,** 1210–1214.

Warton, D. I., Duursma, R. A., Falster, D. S. and Taskinen, S. (2012). SMATR 3 – an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution*, **3,** 257–259.

Yaronskaya, E., Vershilovskaya, I., Poers, Y., Alawady, A. E., Averina, N. and Grimm, B. (2006). Cytokinin effects on tetrapyrrole biosynthesis and photosynthetic activity in barley seedlings. *Planta*, **224,** 700–709.