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Impatiens 'New Guinea' (*Impatiens Hawkeri* Bull) Hormonal Effects During The Post-Transplant Biomass Accumulation

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Abstract

Pot ornamental plant productivity is related to the environmental growth facilities but negatively affected by the pot root restriction syndrome so during nursery as the post-transplant stage. The physiological mechanism involved included both the synthesis and translocation of auxins and cytokinins. However, clear sink-source and dose-response relationships of exogenous plant regulators such as indole acetic acid (AIA) and benzyl amino purine (BAP) and environment on biomass accumulation in most ornamental foliage plants, including New Guinea *Impatiens* (*Impatiens hawkeri*) are lacking. The aim of this work was to analyze the effects of an exogenously shoot-applied auxin and a cytokinin, separately or successively, on the post-transplant biomass accumulation of *I. hawkeri* through the anatomical, morphological and physiological changes observed. Two experiments were performed. The first experiment included the response to only BAP-sprayed plants (0, 5, 50, or 100 mg L^{-1}). For the second experiment, rooting cuttings of *I. hawkeri* were sprayed with different concentrations of IAA (0, 5, 50, or 100 mg L^{-1}) followed by different BAP concentrations (0, 5, 50, or 100 mg L^{-1}) one week later to run-off at sunset. Results showed that (a) a single BAP or AIA dose increased increase post-transplant biomass accumulation through a higher leaf area expansion and photo assimilate production, (b) as a result of both AIA and BAP spray, the higher NAR the higher post-transplant biomass accumulation, (c) leaf anatomical changes (leaf thickness, intercellular spaces) let a higher carbon dioxide diffusion and fixation with a correlative increase in photo assimilates, (d) a higher root system would be related to a higher cytokinin synthesis. In summary, similarities between 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 *I. hawkeri* ornamental plants by using early foliar sprays.

Keywords: auxins, cytokinins, growth, ornamentals

Introduction

Impatiens hawkeri hybrids usually known as New Guinea *Impatiens* are the result of hybridization between three *Impatiens* species from that country (Tabak and von Wettberg, 2008). New Guinea *Impatiens* are a vegetative-propagated cultivar grown as annual ornamental. Much of the popularity of New Guinea *Impatiens* is due to large flower size, wide range of flower colors, and the dark green foliage (Strefeler and Quené, 1985). Flowers appear at the axillar stems, for which a higher leaf appearance rate and ramified stems are required for high commercial acceptance. Additionally, both growth and flowers lifespan are related to available photo assimilates (López and Runkle, 2008; Currey and Lopez, 2015).

Vegetative-propagated herbaceous bedding plants, such as *I. hawkeri*, are produced in two distinct phases: a young plant stage, in which shoot tip cutting is rooted, and a finish plant stage, in which the rooted young plant is transplanted into a bigger pot where they are grown until they become marketable (Hutchinson *et al.*, 2012). This implies that plants needs to develop a new root system with stored reserves consumption (Klopotek *et al.*, 2016). The physiological mechanism involved included both the synthesis and translocation of auxins and cytokinins.

Auxins are synthesized in the shoot apical meristem and move through phloem (Bishop *et al.*, 2011). After detachment from the donor plant, phloem basipetal transport of auxins from mature leaves contributes to auxin accumulation in the stem base (Garrido *et al.*, 2003). Because high concentrations of free auxin during the induction phase of adventitious rooting are needed (Han *et a*l., 2009), the exogenous rise of auxin levels in the basal stem likely contributes to the early events of adventitious root formation (Ahakami *et al.*, 2009). Because cytokinins are mainly synthesized in the roots, there is a close relationship between root growth in general, the number of root meristems and cytokinin production in the root system (Aloni *et al.*, 2005). Antagonic (Muraro *et al.*, 2013) or complementary responses between auxins and cytokinins were found. However, the plant response to different indole acetic acid (IAA) and 6, benzyl amino purine (BAP) concentration sprays have been only recently explored (Di Benedetto *et al.*, 2015b; Molinari *et al.*, 2018), for which the post-transplant response of most ornamental pot plant included *Impatiens* 'New Guinea' (*Impatiens hawkeri* Bull) are lacking.

The aims of this work were to analyze the effects of an exogenously shoot-applied auxin and a cytokinin, separately or successively, on the post-transplant biomass accumulation of *I. hawker* through the anatomical, morphological and physiological changes observed.

Materials and Methods

Plant Material and Treatments

To reach the proposed objectives and validate proposed hypothesis, two experiments were conducted under a greenhouse located at the Faculty of Agronomy campus, University of Buenos Aires, Argentina (34°35’ 59’’S, 58°22’23’’W) from October 15th 2013 to February 20th 2014 (experiment 1) and from October 11th 2014 to February 10th 2015 respectively.

For both experiments, stem cuttings (30 per treatment) of *Impatiens* 'Nueva Guinea' (*Impatiens hawkeri* Bull) from 128 plug cell trays (17.37 cm^3 $cell^{-1}$) were transplanted into 1.200 cm^3 plastic pots (one cutting per pot) filled with a 2:2:1 (v/v) mix of *Sphagnum maguellanicum* peat, river waste and perlite. Plants were watered daily and fertilized weekly with N, P, K and Ca fertilizer added to the irrigation water (150 mg L^{-1} N) (2:1:2:2 N:P:K:Ca).

For experiment 1, 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}). On the other hand, for experiment 2, 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 days later. The IAA and BAP were first diluted in 80% (v/v) ethanol and no surfactants were used.

Daily mean temperatures (19.73 to 24.90°C and 21.82 to 26.8°C for experiment 1 and 2 respectively) and daily photosynthetic active radiation (8.94 to 10.71 and 10.80 to 12.69 mol photons m^{-2} day^{-1} for experiment 1 and 2 respectively) were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to HOBO H8 data logger. The plants were arranged at a density of 25 plants m^{-2} which avoided mutual shading.

Assessed Variables

At 0, 60, 90, and 120 days after transplanting, five 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 the ImageJ® (Image Processing and Analysis in Java) software.

The Fresh weights (FW$_s$) of the roots, leaf blades and stems on each plant were determined. Dry weights (DWs) were obtained after drying to constant weight at 80°C for 96 hours. The relative rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the natural logarithm (*ln*) of total leaf area versus time (in days). The rate of leaf appearance (RLA) was calculated as the slope of the number of visible leaves versus time (in weeks). The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm of whole plant DW versus time (in days). The mean net carbon assimilation rate (NAR) and leaf area ratio (LAR) as following:

$$NAR = \frac{k_w W_0 e^{k_w t}}{A_0 e^{k_a t}}$$

$$LAR = \frac{k_w}{NAR}$$

where, k_w: RGR (g g^{-1} days^{-1}); W_0: extrapolated value of total dry weight at time zero (g); A_0: extrapolated value of external stem area at time zero (cm^2); k_a: RLAE (cm^2 cm^{-2} days^{-1}); t: time (in days) at the midpoint of the experimental period and e: base of natural logarithms.

The allometric coefficients between root and stems were calculated as the slope (**β**) of the straight-line regression of the natural logarithm of the root DW versus the natural logarithm of the stem DW.

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 of experiment 2 (120 days from transplanting). Tissue from the middle region of the 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. Samples were sectioned at 10 - 20 µm thick with a rotary microtome and stained with safranin-crystal violet-fast green. Data presented are the means of three leaves per treatment using ten leaf cross-sections per leaf. Quantitative anatomical data were obtained using Image Pro Express version 6.0 (Media Cybernetics, MD, USA).

Experimental Design and Statistical Analysis

The experimental design was a completely aleatory design for experiment 1. 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. Slopes from straight-line regressions of RLAE, RGR, NAR, and LAR values were tested using the SMATR package (Warton et al., 2012).

RESULTS

Experiment 1. BAP response

Fresh weight accumulation

At the end of the experiments (120 days from transplant), all BAP-sprayed plants showed significant increase in total fresh weight related to control plants (Fig. 1).

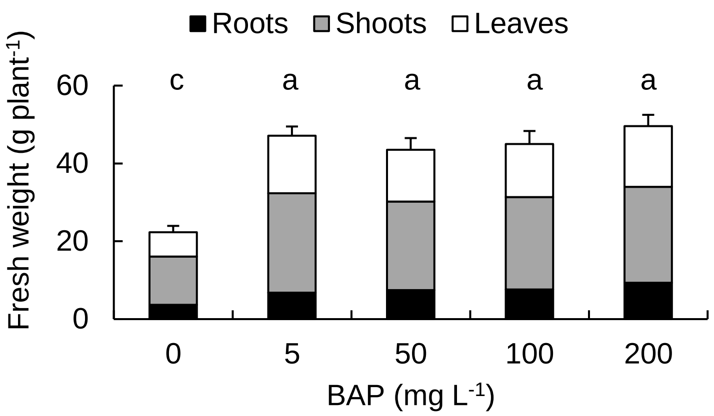


Fig. 1: Fresh weight at the end of the experiment 1 in different plant organs of *I. hawkeri* sprayed with different BAP concentrations. The standard errors over each bar have been indicated. Different lower-case letters indicate significant differences ($P < .05$) between BAP-sprayed plants.

Leaf area expansion

Control plants showed the lower both total and individual leaf area as the result of lower RLAE and RLA. A single BAP spray significantly increased all these growth parameters, although the higher response was found in 100 and 200 mg L^{-1} BAP sprayed-plants (Table 1).

Table 1: Changes in total and individual leaf area at the end of the experiment 1, in the relative leaf area expansion rate (RLAE) and the rate of leaf appearance (RLA) of *I. hawkeri* plants sprayed with different BAP concentrations (0, 5, 50, 100 or 200 mg L^{-1}). The probability of the slope being zero was $P \leq 0.001$ for RLAE and RLA. Different lower case letters indicate significant differences ($P \leq 0.05$) between control and BAP-sprayed plants.

BAP (mg L^{-1})	Leaf area (cm^2 plant^{-1})	Leaf area (cm^2 leaf^{-1})	RLAE (cm^2 cm^{-2} day^{-1})	RLA (leaves day^{-1} plant^{-1})
0	140.68 c	11.92 c	0.0056 c	0.0605 d
5	277.59 b	15.59 b	0.0109 b	0.1055 c
50	309.84 b	15.79 b	0.0117 b	0.1111 c
100	334.72 a	16.70 a	0.0126 a	0.1279 b
200	354.47 a	16.85 a	0.0129 a	0.1491 a

Dry weight accumulation and partitioning

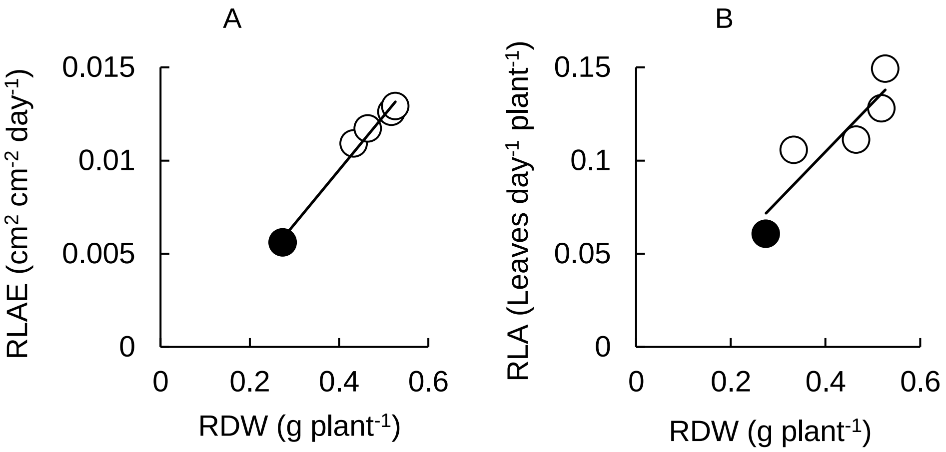
A single BAP spray significantly increased RGR and NAR. The higher response was found in 200 mg L^{-1} BAP sprayed- plants. A similar response from LAR was found as well. The allometric analysis between roots and stems showed a higher photo assimilates partitioning to roots in control plants and a change towards stems in BAP-sprayed plants (Table 2).

Table 2: Changes in the relative growth rate (RGR), the net assimilation rate (NAR), the leaf area ratio (LAR) and the allometric relationships between roots and stems from *I. hawkeri* plants sprayed with different BAP concentrations (0, 5, 50, 100 or 200 mg L^{-1}). The slope straight-line (β) is indicated. The probability of the slope being zero was $P \leq 0.001$ for all growth parameters. Different lower case letters indicate significant differences ($P \leq 0.05$) between control and BAP-sprayed plants.

BAP (mg L^{-1})	RGR (g g^{-1} day^{-1})	NAR (g cm^2 day^{-1})	LAR (cm^2 g)	β
0	0.0081 d	6.69 d	83.69 c	2.297 a
5	0.0114 c	7.11 c	160.34 a	2.017 b
50	0.0129 b	8.26 b	156.17 a	1.965 c
100	0.0141 b	8.50 b	165.88 a	1.951 c
200	0.0142 a	9.70 a	146.39 b	1.930 c

Growth parameters relationships

Positive relationships between RLAE (Fig. 2A), RLA (Fig. 2B), RGR (Fig. 2C), NAR (Fig. 2D) and root DW (r^2 = 0.985, 0.824, 0.914 and 0.844 respectively) were found at the end of the experiment. The lower values belonged to control plants always. On the other hand, an inverse response between the β coefficient and root DW (r^2 = 0.873) (Fig. 2E) with the higher value in control plants was found.



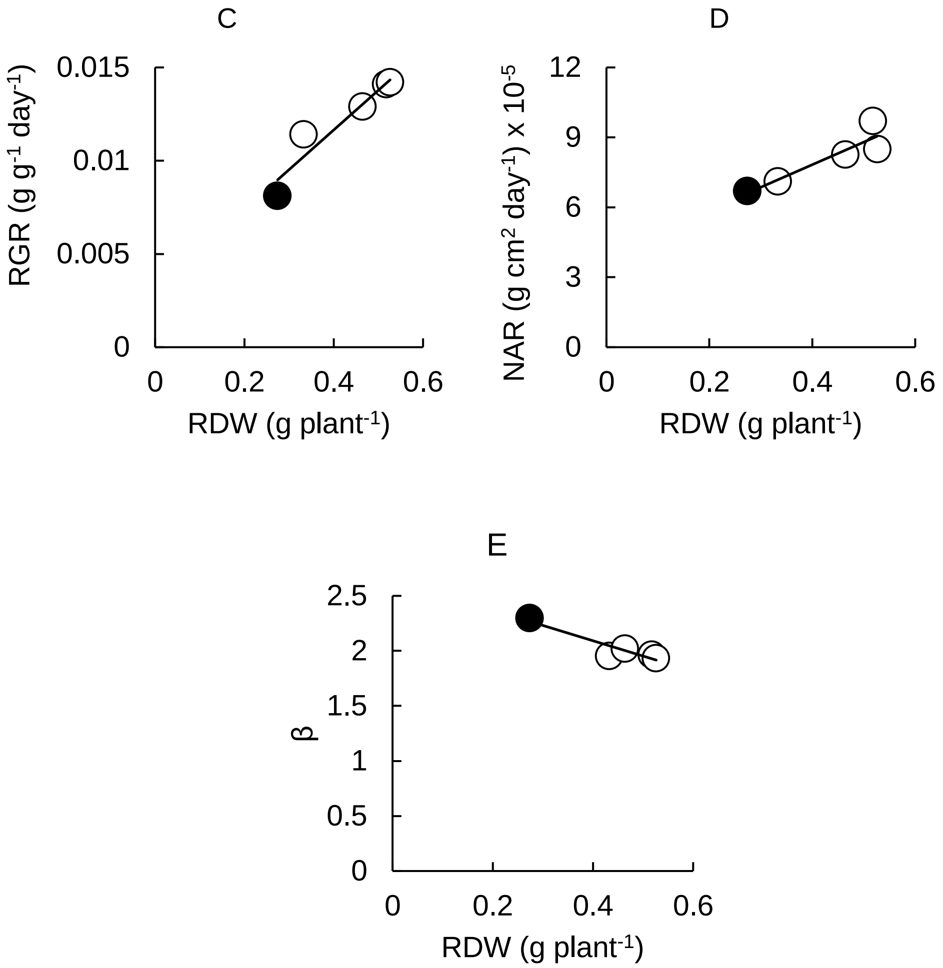


Fig. 2: The relative leaf area expansion rate (RLAE) (A), the rate of leaf appearance (RLA) (B), the relative growth rate (RGR) (C), the net assimilation rate (D) and the partition coefficient β (E) related to the relative root DW (RDW). The straight-line regressions were RLAE = 0.029 RDW - 0.002 (r^2 = 0.985; $P \leq 0.001$), RLA = 0.263 RDW - 0.0004 (r^2 = 0.824; $P \leq 0.001$), RGR = 0.021 RDW - 0.003 (r^2 = 0.914; $P \leq 0.001$), NAR = 9.671 x 10^{-5} RDW + 3.96 (r^2 = 0.844; $P \leq 0.001$), β = -1.319 RDW + 2.65 (r^2 = 0.873; $P \leq 0.001$).

Experiment 2. IAA-BAP treatments

Fresh weight accumulation

A single application of IAA or BAP at 50 mg L^{-1} led to a significant increase in total FW accumulation observed 90 days after treatment (Fig. 3A) compared with control plants. In plants treated with IAA and, 7 days 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 (Fig. 3B).

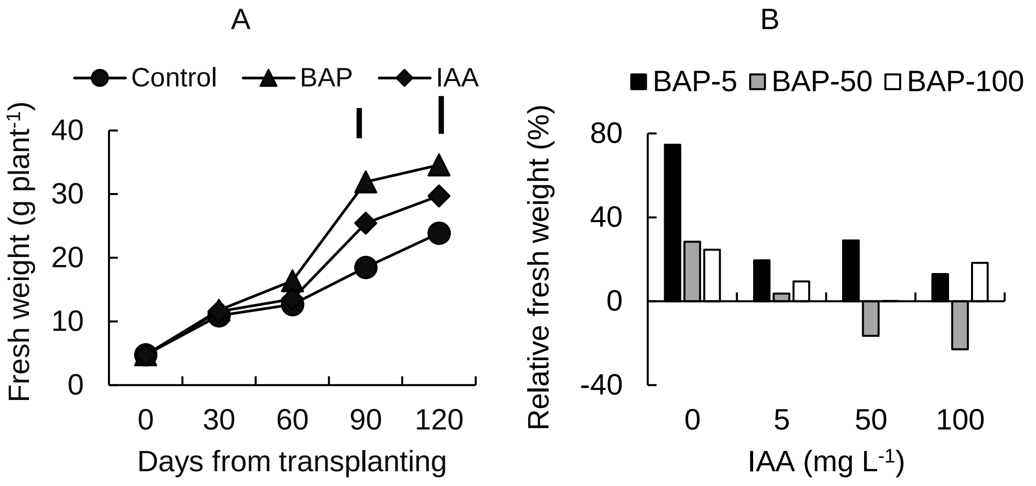


Fig. 3: Total fresh weights (A) in *I. hawkeri* during the 120 days experiment on control plants and in plants which showed the greatest response to 100 mg L^{-1} sprays of IAA or BAP applied as a single hormone spray. Panels B shows the effect of a 5, 50, or 100 mg L^{-1} BAP spray application on total fresh weight in plants sprayed 7 days before with IAA at 0, 5, 50 or 100 mg L^{-1}. Data are expressed as the percentage change observed following BAP application of 5, 50 or 100 mg L^{-1} relative to plants sprayed with 0 mg L^{-1} BAP. Vertical lines indicate least significant differences (LSD).

Leaf area expansion

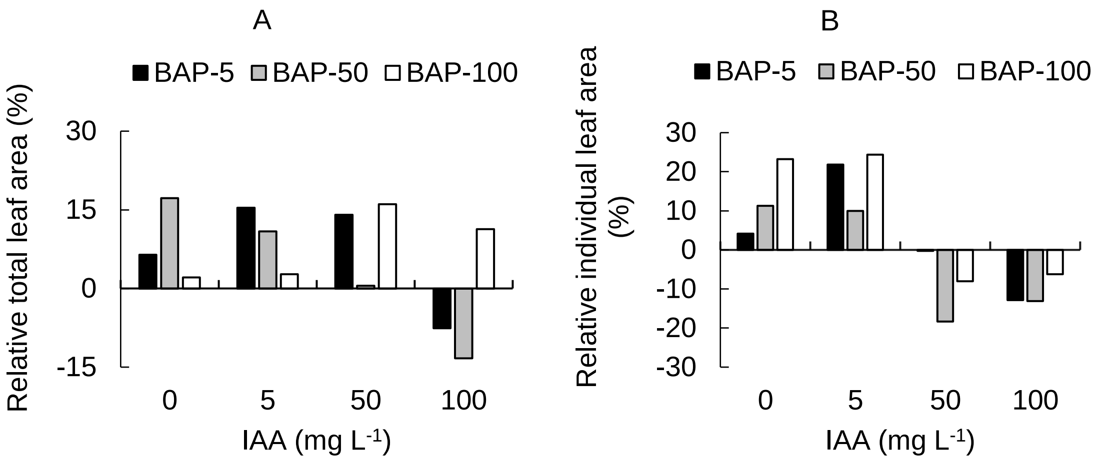
Total leaf area and RLAE at final harvest were higher in plants sprayed with either IAA or BAP than in the controls. These were the result of a larger mean values of individual leaf areas and higher RLA. The response to either hormone was similar (Table 3).

At the end of the experimental period, sequential applications of IAA and BAP resulted in an increase in both total and individual leaf area, RLAE and RLA values over the controls which was dependent on the concentrations applied. In plants treated with IAA and, 7 days later with BAP, addition of the second hormone resulted in only a slight growth promoting effect (generally less than 20%) for leaf areas (Figs. 4A and B) and RLAE (Fig. 4C), which tended to be lower with increasing IAA and BAP concentrations. However, RLA showed a strong response when 50 mg L^{-1} IAA where BAP sprayed (Fig. 4D).

Table 3: Changes in total and individual leaf area at the end of the experiment 2, in the relative leaf area expansion rate (RLAE) and the rate of leaf appearance (RLA) of *I. hawkeri* plants sprayed with different IAA-BAP combinations. The probability of the slope being zero was $P \leq 0.001$ for RLAE and RLA. Different lower case letters indicate significant differences ($P \leq 0.05$) between control and IAA-BAP sprayed plants.

IAA-BAP (mg L^{-1})	Leaf area (cm^2 plant^{-1})	Leaf area (cm^2 leaf^{-1})	RLAE (cm^2 cm^{-2} day^{-1})	RLA (leaves day^{-1})
0-0	310.88 b	14.96 c	0.0080 c	0.1242 d
0-5	371.13 a	14.57 c	0.0102 b	0.1605 b
0-50	311.44 b	16.93 a	0.0092 c	0.1220 d
0-100	373.84 a	17.40 a	0.0102 b	0.1420 c

5-0	330.84 a	15.59 b	0.0086 c	0.1236 d
5-5	428.27 a	17.76 a	0.0118 a	0.1563 b
5-50	436.19 a	16.89 b	0.0124 a	0.1943 a
5-100	345.39 a	15.16 b	0.0099 b	0.1457 c
50-0	364.34 a	15.28 b	0.0104 b	0.1857 a
50-5	411.58 a	16.02 b	0.0116 a	0.1815 a
50-50	313.14 b	13.82 c	0.0086 c	0.1599 b
50-100	286.55 b	15.13 b	0.0072 c	0.1671 b
100-0	317.38 b	18.44 a	0.0084 c	0.1413 c
100-5	381.31 a	18.12 a	0.0104 b	0.1528 b
100-50	361.51 a	15.57 b	0.0097 b	0,1472 b
100-100	416.11 a	16.31 b	0.0105 b	0.1613 b



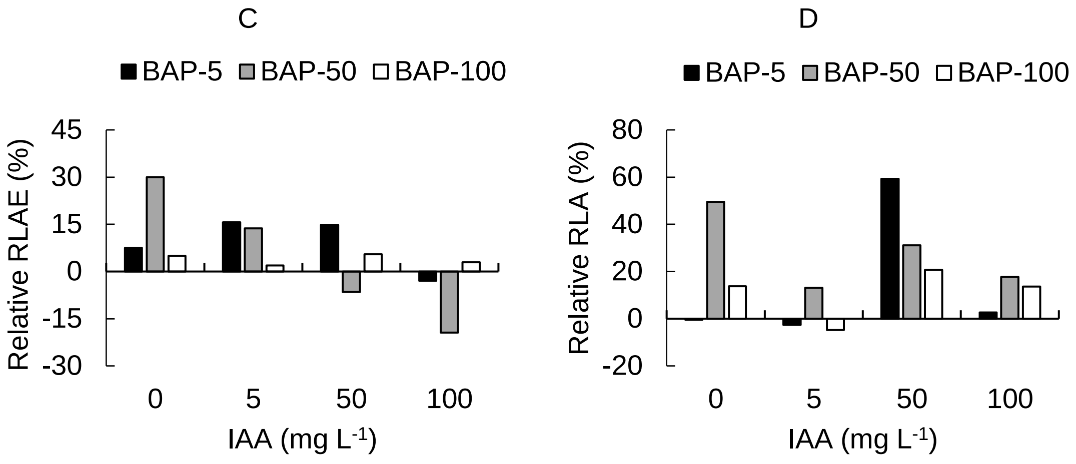
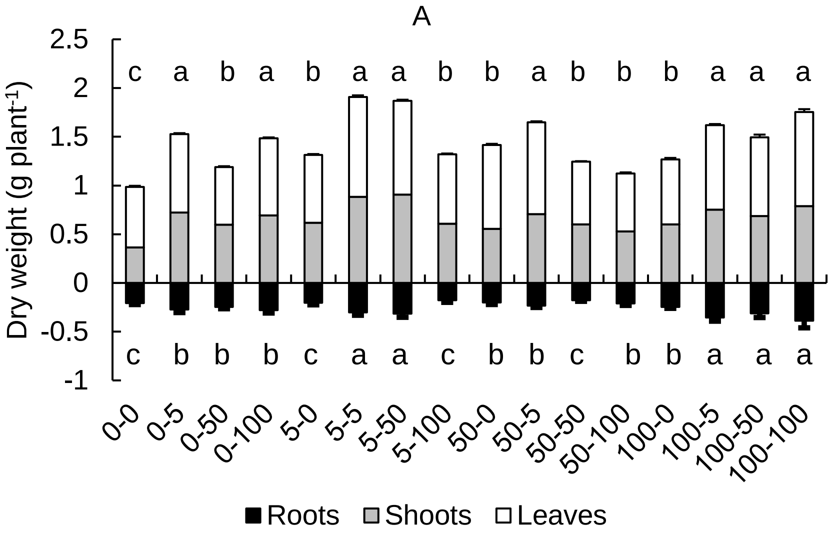


Fig. 4: The effect of a 5, 50, or 100 mg L^{-1} BAP spray application on total leaf area, individual leaf area, RLAE and RLA in plants sprayed 7 days before with IAA at 0, 5, 50 or 100 mg L^{-1}. Data are expressed as the percentage change observed following BAP application of 5, 50 or 100 mg L^{-1} relative to plants sprayed with 0 mg L^{-1} BAP.

Dry weight accumulation and partitioning

I. hawkeri plants IAA and BAP single or sequentially sprayed showed significant DW differences with control ones as a result of both shoots and roots DW increase (Fig. 5A). In plants treated with IAA and, 7 days later with BAP, addition of the second hormone resulted in only a slight growth promoting effect (near 20%), which tended to be lower with increasing IAA and BAP concentrations (Fig. 5B). A positive relationship between root DW and shoot DW was found when all recorded DW data were plotted in Fig. 5C.



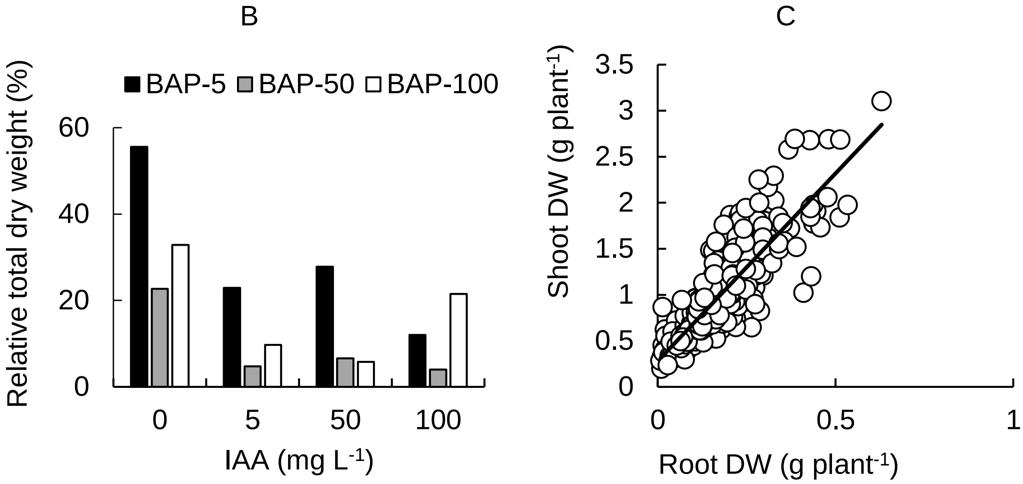


Fig. 5: Roots, shoots and leaves DWs at the end of the experiment 2 of *I. hawkeri* plants sprayed with different IAA-BAP combinations (A). Panel B shows the effect of a 5, 50, or 100 mg L^{-1} BAP spray application on relative total DW in plants sprayed 7 days before with IAA at 0, 5, 50 or 100 mg L^{-1}. Data are expressed as the percentage change observed following BAP application of 5, 50 or 100 mg L^{-1} relative to plants sprayed with 0 mg L^{-1} BAP. Panel C indicated root: shoot DW relationships.

Table 4: Changes in RGR, NAR, LAR and β partition coefficient at the end of experiment 2 of *I. hawkeri* plants sprayed with different IAA-BAP combinations. The probability of the slope being zero was $P \leq 0.001$ for RLAE and RLA. Different lower case letters indicate significant differences ($P \leq 0.05$) between control and IAA-BAP sprayed plants.

IAA-BAP (mg L^{-1})	RGR (g g^{-1} day^{-1})	NAR (g cm^{-2} day^{-1}) (x 10^{-5})	LAR (cm^2 g^{-1})	β
0-0	0.0117 c	4.13 c	283.35 a	0.408 a
0-5	0.0158 a	5.68 a	278.11 a	0.402 a
0-50	0.0147 a	5.34 a	275.45 a	0.446 a
0-100	0.0159 a	5.66 a	281.13 a	0.393 a
5-0	0.0137 b	4.97 b	275.56 a	0.449 a
5-5	0.0179 a	6.73 a	265.89 a	0.415 a
5-50	0.0179 a	6.30 a	284.12 a	0.424 a
5-100	0.0143 b	5.18 a	276.00 a	0.437 a
50-0	0.0152 a	5.47 a	277.83 a	0,409 a
50-5	0.0166 a	5.99 a	277.02 a	0.347 b

50-50	0.0134 b	4.92 b	272.60 a	0.323 b
50-100	0.0126 b	4.56 b	276.08 a	0.364 b
100-0	0.0134 b	4.78 b	280.09 a	0.360 b
100-5	0.0167 a	6.27 a	266.16 a	0.363 b
100-50	0.0157 a	5.72 a	274.71 a	0.311 b
100-100	0.0160 a	5.84 a	273.96 a	0.318 b

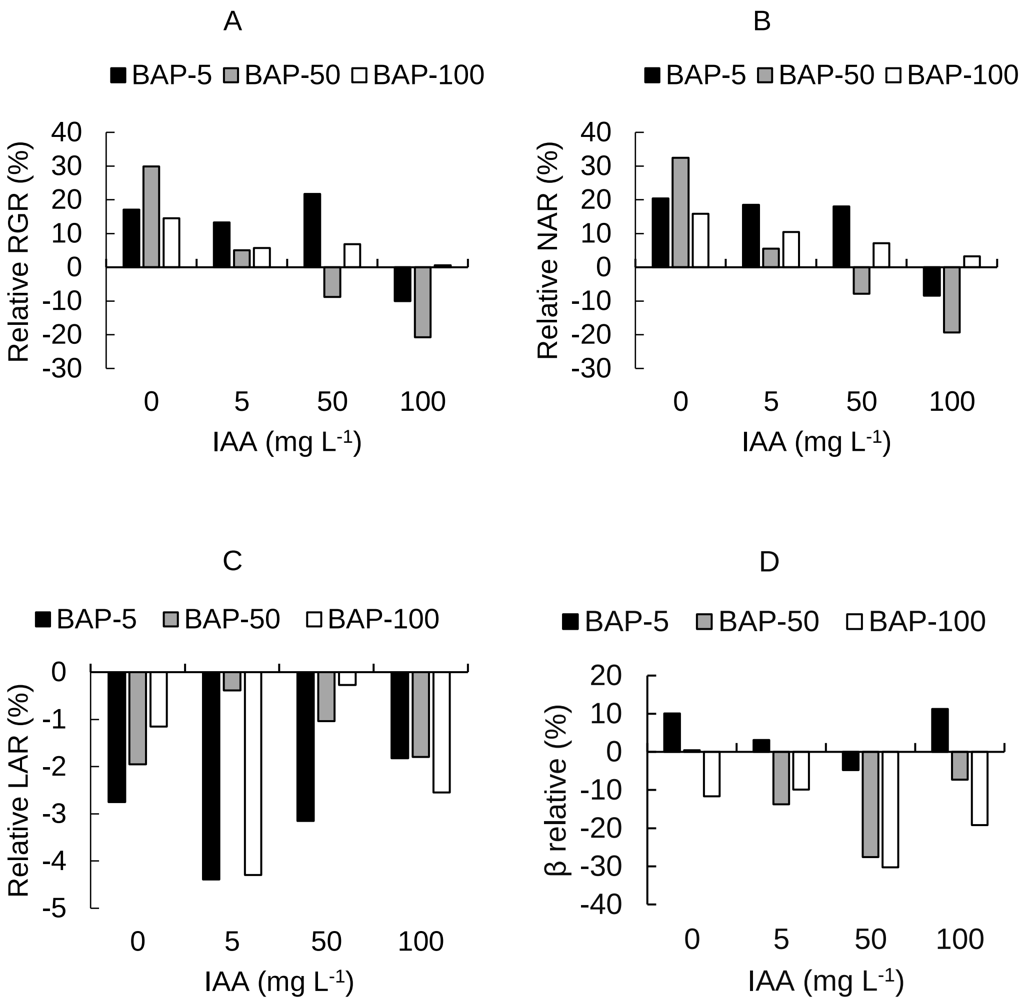


Fig. 6: The effect of a 5, 50, or 100 mg L^{-1} BAP spray application on RGR, NAR, LAR and β partition coefficient in plants sprayed 7 days before with IAA at 0, 5, 50 or 100 mg L^{-1}. Data are expressed as the percentage change observed following BAP application of 5, 50 or 100 mg L^{-1} relative to plants sprayed with 0 mg L^{-1} BAP.

Plants sprayed with different IAA-BAP combinations increased RGR and NAR with only few changes in LAR when compared with control ones. The β partition coefficient decreased as soon as IAA concentration increased to 50 and 100 mg L^{-1} (Table 4).

In plants treated with IAA and, 7 days later with BAP, addition of the second hormone resulted in a near 30% growth promoting effect for RGR (Figure 6A) and NAR (Figure 6B) with minor changes in LAR (Figure 6C). However, the β partition coefficient showed a 30% decrease (Figure 6D).

When plotting the data from all treatments, we found a close direct relationship (r^2= 0.977) between RGR and NAR (Figure 7A) and a weak direct relationship between RGR and LAR (r^2= 0.093) (Figure 7B). Nevertheless, controls plants showed the lower NAR values.

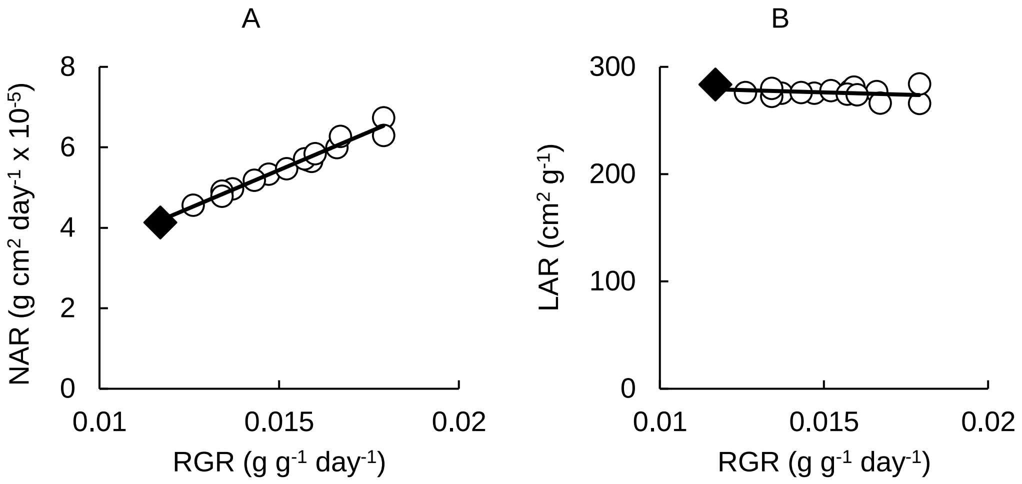


Fig. 7. The net assimilation rate (NAR) (A) and the leaf area ratio (LAR) (B) related to the relative growth rate (RGR). The straight-line regressions were NAR = 378.85 RGR - 0.25 (r^2= 0.977; P $\leq$ 0.001) and LAR = 857.45 RGR + 289.07 (r^2 = 0.093; P no significant). Full or empty symbols indicated both controls and IAA-BAP sprayed plants respectively.

Leaf anatomy

Increases in overall leaf thickness were observed in plants sprayed with different IAA-BAP combinations (Fig. 8A). A decrease in the contribution of the epidermal layer to overall leaf thickness was observed following either IAA or BAP treatment. Conversely, a significant increase in the proportion of intercellular spaces was observed, while only slight changes were seen in the proportion of the parenchymal layer (Fig. 8B).

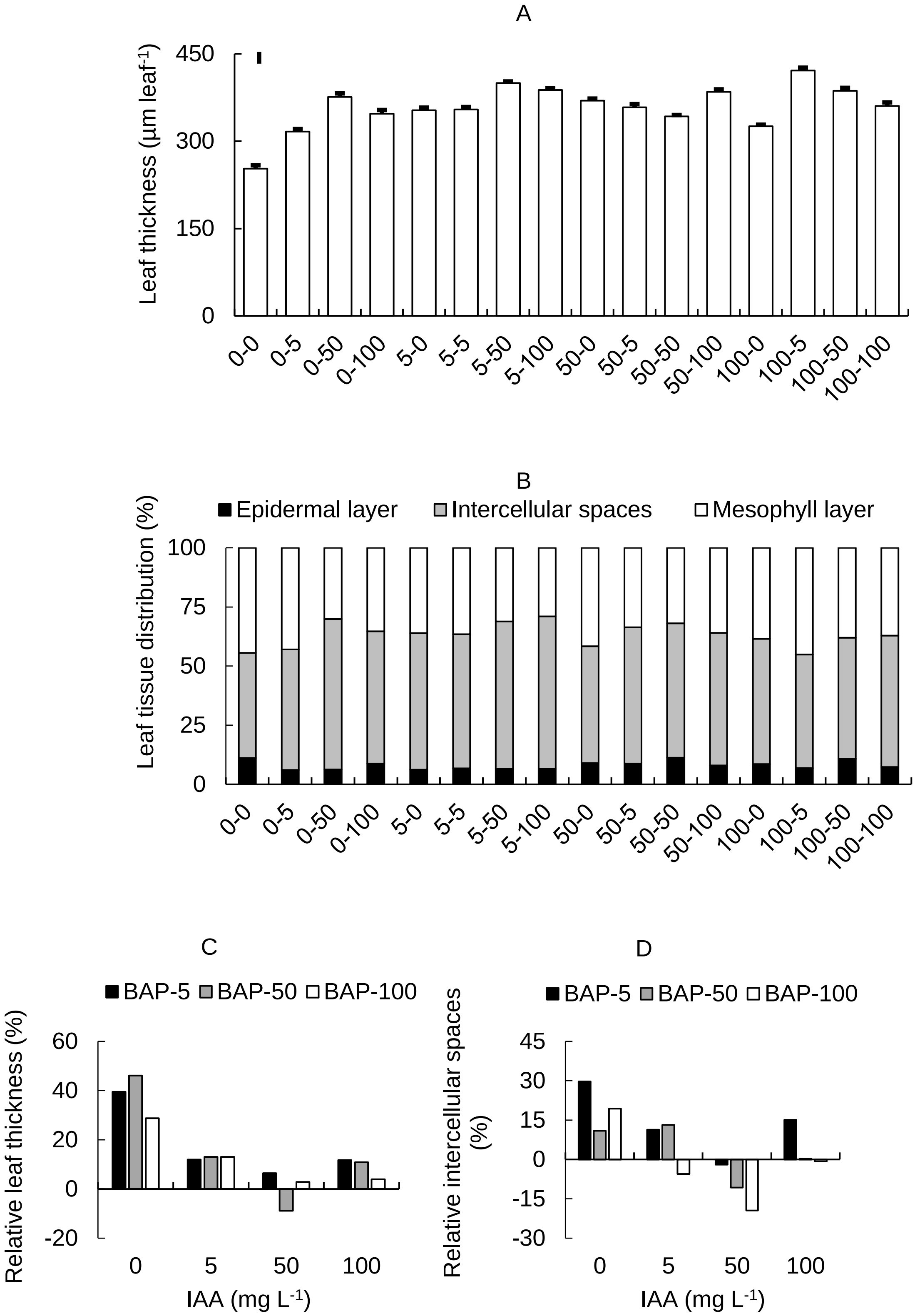


Fig. 8: Changes in leaf thickness (A) and contributions (expressed as percentages) of the epidermal layer, parenchymal layer, and intercellular spaces respectively to the overall thickness (B) of leaves on *I. hawkeri* plants sprayed with 0, 5, 50, or 100 mg L^{-1} IAA or BAP as a single or combined hormone application. Vertical lines indicate least significant differences (LSD). In panel C and D plants were sprayed with IAA at 0, 5, 50 or 100 mg

L^{-1}. data are expressed as the percentage change observed following BAP application of 5, 50 or 100 mg L^{-1} relative to plants sprayed with 0 mg L^{-1} BAP.

In plants treated with 5 - 100 mg L^{-1} IAA, a later application of BAP had no consistent effect on these variables (Fig. 8C), although, in general, BAP contributed to a decrease the proportion of the epidermal layer, increases in the percentage of intercellular spaces were lower as the BAP concentration increased (Fig. 8D).

Positive relationships between NAR related to both leaf thickness ($r^2 = 0.641$) (Fig. 9A) and intercellular spaces ($r^2 = 0.708$) (Fig. 9B) when all data were plotted together. In both cases, control plans showed the lower values.

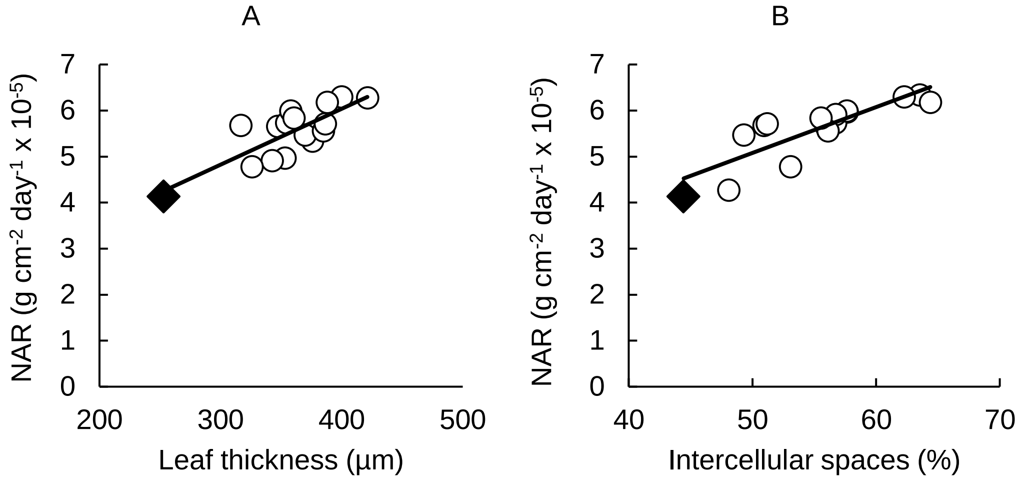
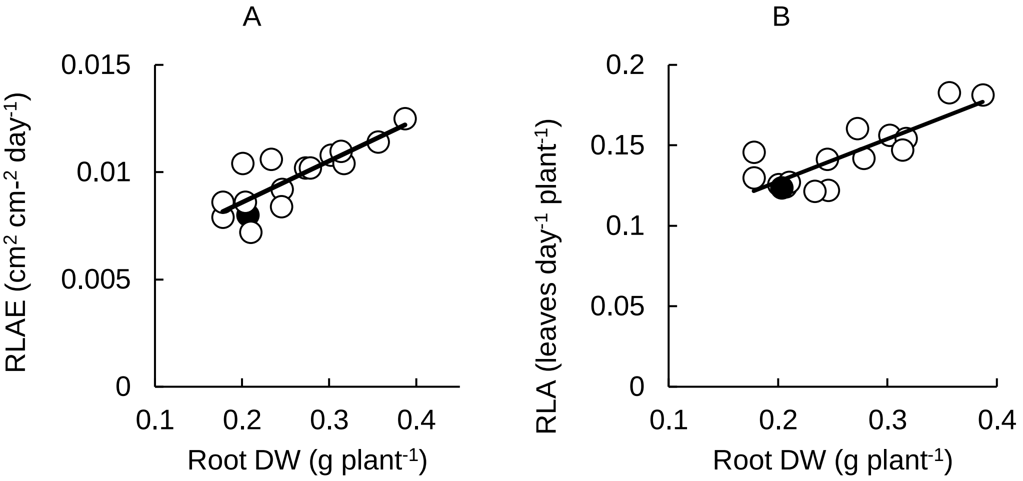


Fig. 9: The net assimilation rate (NAR) related to leaf thickness (A) or the intercellular spaces (B). The straight-line regressions were NAR = 0.012 leaf thickness + 1.19 (r^2= 0.641; $P \leq 0.05$) and NAR = 0.100 intercellular spaces + 0.098 ($r^2 = 0.708$; $P \leq 0.001$). Full or empty symbols indicated both controls and IAA-BAP sprayed plants respectively.

Growth parameters relationships

Positive relationships between RLAE (Fig. 10A), RLA (Fig. 10B), RGR (Fig. 10C), NAR (Fig. 10D) and root DW (r^2 = 0.691, 0.700, 0.644 and 0.638 respectively) were found at the end of the experiment with the lower values belonged to control plants always. On the other hand, a negative relationship between the β partition coefficient (Fig. 10E) and root DW ($r^2 = 0.546$) was found. In this case, control plants showed the higher β values.



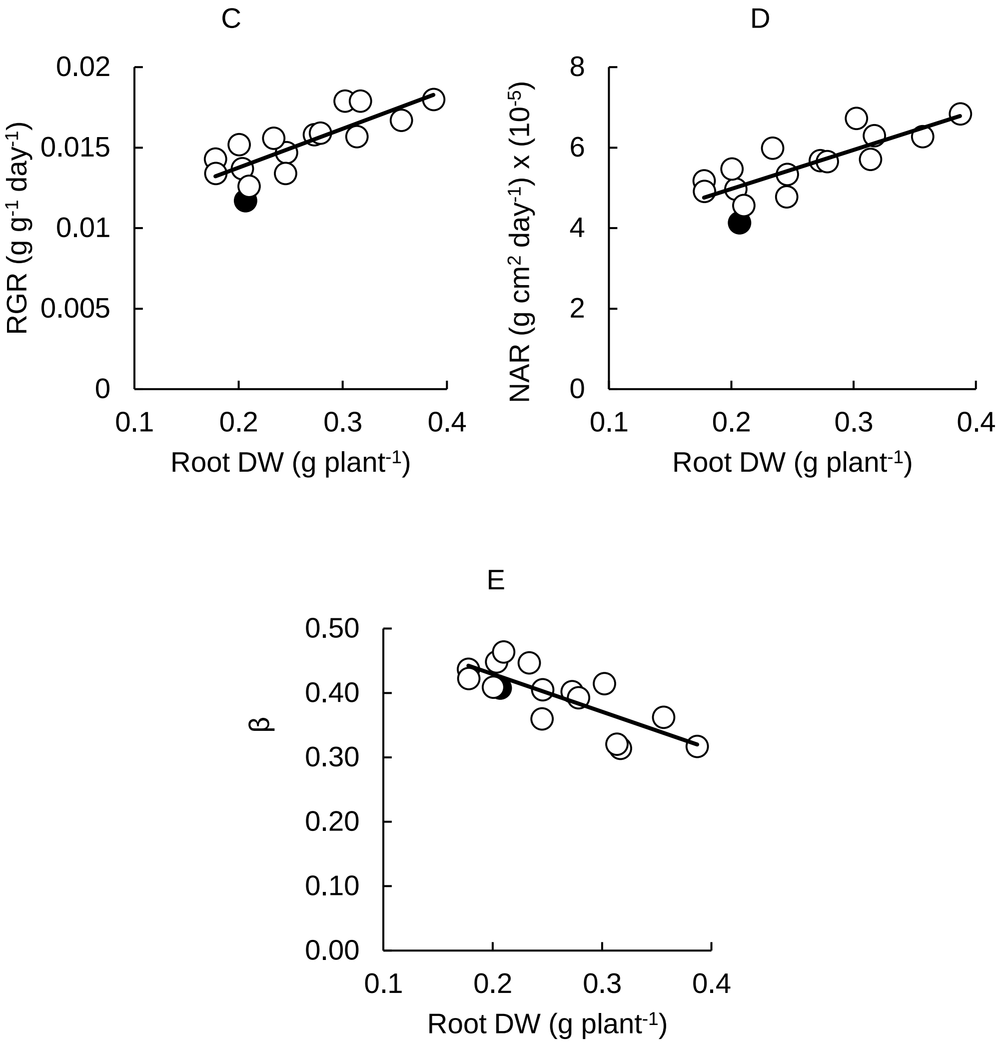


Fig. 10. The relative leaf area expansion rate (RLAE) (A), the rate of leaf appearance (RLA) (B), the relative growth rate (RGR) (C), the net assimilation rate (D) and the partition coefficient β (E) related to the relative root DW (RDW). The straight-line regressions were RLAE = 0.019 RDW - 0.005 ($r^2 = 0.691$; $P \leq 0.001$), RLA = 0.265 RDW - 0.075 ($r^2 = 0.700$; $P \leq 0.001$), RGR = 0.024 RDW + 0.009 ($r^2 = 0.644$; $P \leq 0.05$), NAR = 9.696 x 10^{-5} RDW + 3.03 ($r^2 = 0.638$; $P \leq 0.05$), β = -0.585 RDW + 0.546 ($r^2 = 0.606$; $P \leq 0.05$). Full or empty symbols indicated both controls and IAA-BAP sprayed plants respectively.

Discussion

The productivity of ornamental plants is closely associated with an increase in their total leaf area over time, but most ornamental plants show low rates of leaf expansion (Di Benedetto *et al.*, 2006, 2010). Although biomass accumulation on a DW base appears to be the best leaf trait to be quantified for plant functional screenings (Poorter *et al.*, 2012), the quality of ornamental plants can be appraised with other types of criteria, such as tolerance to biotic and abiotic stresses, development potentialities and aesthetics (Santagostini *et al.*, 2014).

During the last years, strong suggestions from our laboratory on the effect of a single BAP spray to increase vegetable (Pagani *et al.*, 2013; Coro *et al.*, 2014; Di Matteo *et al.*, 2015; Della Gaspera *et al.*, 2016; Rattin *et al.*, 2017, 2018; Geraci *et al.*, 2018) and ornamental (Di Benedetto and Pagani, 2013; De Lojo and Di Benedetto, 2014; Gandolfo *et al.*, 2014; Di Benedetto *et al.*, 2015a; De Lojo *et al.*, 2017; Piotti *et al.*, 2018) growth rate has been collected. Data from Figs. 1 and 3A are in agreement with these previous reports. However, only recently, it has been shown that a successive IAA and BAP spray increased pre- (Molinari *et al.*, 2018) and post-fresh weight in foliage ornamental plants (Di Benedetto *et al.*, 2015b, 2018). On the other hand, under a non-limited light environment and optimal growth facilities, Fig. 3B showed that a single exogenous BAP seven days after an IAA spray increased New Guinea *Impatiens* fresh weight. These results are in agreement with previous reports, which indicated that the activity of a given hormone is also dependent on its interaction with other hormones (Garay-Arroyo *et al.*, 2012).

Aesthetically, total leaf area is the main trait related to plant quality of ornamentals and it determines the time of plant sale. In physiological terms, it implies to expand leaf area at the higher growth rate which included both individual leaf size and leaf number. The primary shoot apical meristem is responsible for generating all above ground organs (Perilli *et al.*, 2010) and is controlled by hormones, which regulate biosynthesis and transport of other hormones and by hormone interactions. They included auxins, cytokinins and gibberellins, which act both independently and in combination to regulate meristem function (Durbak *et al.*, 2012, Di Benedetto *et al.*, 2013, 2015a, b; 2018).

Leaves are formed into an initial group of cells within the meristem; one of the earliest markers for leaf initiation is the down regulation of *KNOTTED* and *WUSCHEL* genes in these cells (Moon and Hake, 2011; Holt *et al.*, 2014). Genetic analyses have demonstrated that a high cytokinin: low gibberellin ratio is important for *KNOX* gene function (Hake *et al.*, 2004; Hay and Tsiantis, 2010). Cytokinins are mainly synthesized in the roots (Kieber and Schaller, 2004) and move through the stem xylem to the shoot apical meristem, although the effective cytokinin concentration is the result of endogenous and environmental signals (Kudo *et al.*, 2010).

The main function of endogenous cytokinins are to control the cell cycle and shoot apical meristem growth (Bögre *et al.*, 2008; Schaller *et al.*, 2014; Brenner and Schmulling, 2015). Cytokinin-rich tissues, such as the shoot apical meristem, are photo assimilates sinks (Francis and Halford, 2006). In agreement with these reports, our results showed that a single BAP spray (Table 1) or a combined IAA-BAP spray (Table 3) increased total leaf area and RLAE with a strong effect on RLA. An additive weak effect of IAA-BAP combinations in total leaf area, individual leaf area and RLAE was found. However, RLA showed a higher complementary effect.

A higher individual leaf area would be explained by the common effect of cytokinins on leaf expansion (Shani *et al.*, 2010; Gonzalez *et al.*, 2010; Hepworth and Lenhard, 2014). Since *I. hawkeri* leaves appeared on a single shoot without branches, the increase in RLA would indicate a shorter plastochron. RLA increases are related to plastochron decrease, which require a shoot apical meristem increase as well (Skylar and Wu, 2011) and the non-limiting photo assimilate supply to hold vegetative plant growth (Demura and Ye, 2010; Hao and Cui, 2012). The last, needs for a photosynthetic capacity increase and/or a change in photo assimilates partitioning (Feller *et al.*, 2015). Tables 2 and 4 showed that single or combined IAA and BAP sprays increased NAR (a photosynthetic estimator). In the same way, as root: shoot as photo assimilates partitioning increase to shoots would justify RLA changes through to a plastochron decrease. Although Shani *et al.* (2010) have indicated that cytokinins can affect foliar primordium insertion in the shoot apical meristem, the change in plastochron in response to an exogenous BAP spray has only recently suggested in other vegetable and ornamental plants (Di Benedetto *et al.*, 2013, 2018; Pagani *et al.*, 2013; De Lojo and Di Benedetto, 2014; Coro *et al.*, 2014; Di Matteo *et al.*, 2015; Della Gaspera *et al.*, 2016; De Lojo *et al.*, 2017; Rattin *et al.*, 2017, 2018; Geraci *et al.*, 2018; Piotti *et al.*, 2018; Molinari *et al.*, 2018).

In the same way than for FW, changes in DW were found (Fig. 5A). Once again, most IAA-BAP combinations led a DW increase, although the relative effect of a second hormone was low (no more than 30%). Anyway, a close direct relationships between shoot DW and root DW was found (Fig. 5c), in agreement with previous reports. Changes in DW would be explained through higher RGR and NAR (Tables 2 and 4) with lower effects of a second hormone spray (Figs. 6A and B). When RGR was disagreed, close direct relationships between RGR and NAR for *I. hawkeri* plants sprayed with different IAA-BAP combinations (r^2 = 0.977) (Fig. 7A) and an inverse relationships between RGR and LAR (r^2 = 0.093) (Fig. 7B) were found in agreement with previous reports (Gandolfo *et al.*, 2014; Di Benedetto *et al.*, 2015a; Di Matteo *et al.*, 2015; Geraci *et al.*, 2018), including *I hawkeri* (Molinari *et al.*, 2018).

Because Boonman *et al.* (2007) have indicated that cytokinins stimulate photosynthetic enzymatic expression, a relationship between photosynthetic rates and leaf anatomy has been indicated (Oguchi *et al.*, 2003). Light-saturated rates of photosynthesis on a leaf area basis depend not only on photosynthetic biochemistry but also on mesophyll structure. Because resistance to CO_2 diffusion from the sub stomatal cavity to the stroma is substantial, it is likely that mesophyll structure affects the photosynthetic rate by affecting CO_2 diffusion in the leaf (Niinemets *et al.*, 2009, Tholen *et al.*, 2012). Our data showed significant changes in leaf tissue distribution when sprayed with different concentrations of IAA and/or BAP prior to transplanting (Fig. 8).

In this way, Gandolfo *et al.* (2014) found positive relationships between leaf thickness, intercellular spaces and NAR, similar to those showed in Fig. 9A. When the mesophyll thickness of the leaf is increased, the maximum photosynthetic rate increased as well. This probably explains the strong relationship between NAR and mesophyll thickness. On the other hand, in agreement with that found by Tosens *et al.* (2011), we found a close positive correlation between NAR and the proportion of intercellular spaces (Fig. 9B).

Previous and present results showed that cytokinins applied to the pre-transplant stage can influence the leaf structure (Magyar-Tabori *et al.*, 2010, Fig. 8). At the early stages of leaf development, treatment with exogenous BAP accelerates division of mesophyll cells, whereas at the later stages of development, BAP treatment activates expansion of growing cells and those, which have just accomplished their growth (Ron’zhina 2003a, b). However, the additive effect of a BAP spray after an IAA treatment would be little effect on leaf thickness (Fig. 8C) and intercellular spaces (Fig. 8D).

RGR changes would be associated with photo assimilates partitioning as well. In this way, a single BAP spray (Table 2) or a combined IAA-BAP spray (Table 4) partitioned a higher photo assimilates into shoots as was indicated by the β coefficient decrease, with a low effect of the second hormone spray in the experiment 2 (Fig. 6).

Roots are able to detect the presence of their neighbor’s below-ground (Puig *et al.*, 2012; Chen *et al.*, 2015) and respond accordingly. Cytokinins are root-synthesized, which are transported via the xylem to the shoot (Ghanem *et al.*, 2011; Brenner and Schmülling, 2015; Hwang *et al.*, 2012). While O’Hare and Turnbull (2004) showed that the higher the root system the higher the zeatin ribosides, it is not easy to show quantitative changes in endogenous cytokinin concentration (van Staden *et al.*, 2008) because plants synthesize different cytokinin-ribosides and not all have biological activity. Nevertheless, in the present study, when the root system increased, positive relationships with RLAE (Figs. 3A and 10A), RLA (Figs. 3B and 10B), RGR (Figs. 3C and 10C), NAR (Figs. 3D and 10D), but a negative relationship with the β partition coefficient (Figs. 3E and 10E).

In agreement with Di Benedetto *et al.* (2015b) it is possible to suggest a direct effect of the exogenous cytokinins (BAP) on *I. hawkeri* biomass accumulation associated with a higher both primary shoot apical meristem growth and auxins synthesis. On the other hand, an exogenous auxin spray (IAA) would be an indirect effect on biomass accumulation related to a higher root meristem number and cytokinins synthesis.

Conclusions

In summary, an increase in biomass accumulation in whole-plants of *I. hawkeri* was observed as a consequence of either a single IAA or BAP spray. This was associated with an increased NAR and photo assimilates partitioning to shoots. Changes in leaf anatomy induced by either hormone (including increased 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 this ornamental plant by using early foliar sprays.

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