

# Biomass Production in Ornamental Foliage Plants: Crop Productivity and Mechanisms Associated with Exogenous Cytokinin Supply

Adalberto Di Benedetto<sup>1,2\*</sup> • Jorge Tognetti<sup>2,3</sup> • Claudio R. Galmarini<sup>4</sup>

<sup>1</sup> Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

<sup>2</sup> Facultad de Ciencias Agrarias, Universidad de Mar del Plata, Ruta 226 Km. 73,5 Balcarce, Provincia de Buenos Aires, Argentina

<sup>3</sup> Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina

<sup>4</sup> CONICET, Facultad de Ciencias Agrarias, U.N.Cuyo, Almirante Brown 500, Chacras de Coria, Mendoza. INTA EEA La Consulta, CC 8, 5567, la Consulta San Carlos, Mendoza, Argentina

Corresponding author: \* [dibenede@agro.uba.ar](mailto:dibenede@agro.uba.ar)

## ABSTRACT

An important mediator of shoot physiological processes associated with ornamental biomass production can be the supply of signal molecules (other than water and nutrients) from the root system. Root-to-shoot signaling is often considered to be important in regulating shoot growth when soil conditions determine a change in shoot water or nutrient status. There are strong indications that cytokinins are root factors which are transported via the xylem to the shoot where they exert a major regulatory influence on growth and photosynthesis. Cytokinins play a critical role in many aspects of plant growth and development, including the regulation of the apical dominance, root growth and plastid differentiation. Although it has long been known that the cytokinin signal transduction pathway actively interacts with other pathways, particularly with light and auxin pathways, details of these interactions remain poorly understood. The possible technological use of this information on cultivation of ornamental shade plants is still unclear because knowledge about cause-effect relationships between exogenous cytokinin supply and plant response is still scarce. Regulatory processes conditioned the balanced growth of roots and shoots and the conclusion that root cytokinins are part of the signaling pathway can be drawn.

**Keywords:** foliage productivity, leaf production, leaf size, photosynthesis, photoassimilate partitioning

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## INTRODUCTION

Shaded plants have been an important research field for ecologists who describe plant adaptation under natural vegetation. Under this perspective, carbon gain is an important aspect of plant performance. There are strong links between resource availability, the ability of plants to use resources for carbon gain and productivity, and, at the population level, parameters such as size, survival, and reproductive output (Bazzaz *et al.* 1987). This environment is characterized by a low light intensity, both high temperature and high relative humidity, and low red/far red ratio, which determine low growth rate and resource availability (Givnish 1988). Although plant species may differ

considerably in biomass production, the ecological advantage of high growth rate may seem clear: fast growth results in the rapid occupation of a large space which is advantageous in competitive situations.

Foliage plants, which constitute an important crop production all over the world, comprise a group of plants about which only the vegetative growth and plant morphogenesis are of agricultural interest. Since flowering can be excluded, the success of producing commercial plants in a short time depends on the maintenance of high growth rates throughout the cultivation period. The demand for tropical foliage plants for its use in homes, apartments, hotels, business offices, airports and other public buildings continues to increase rapidly as individuals in modern society become

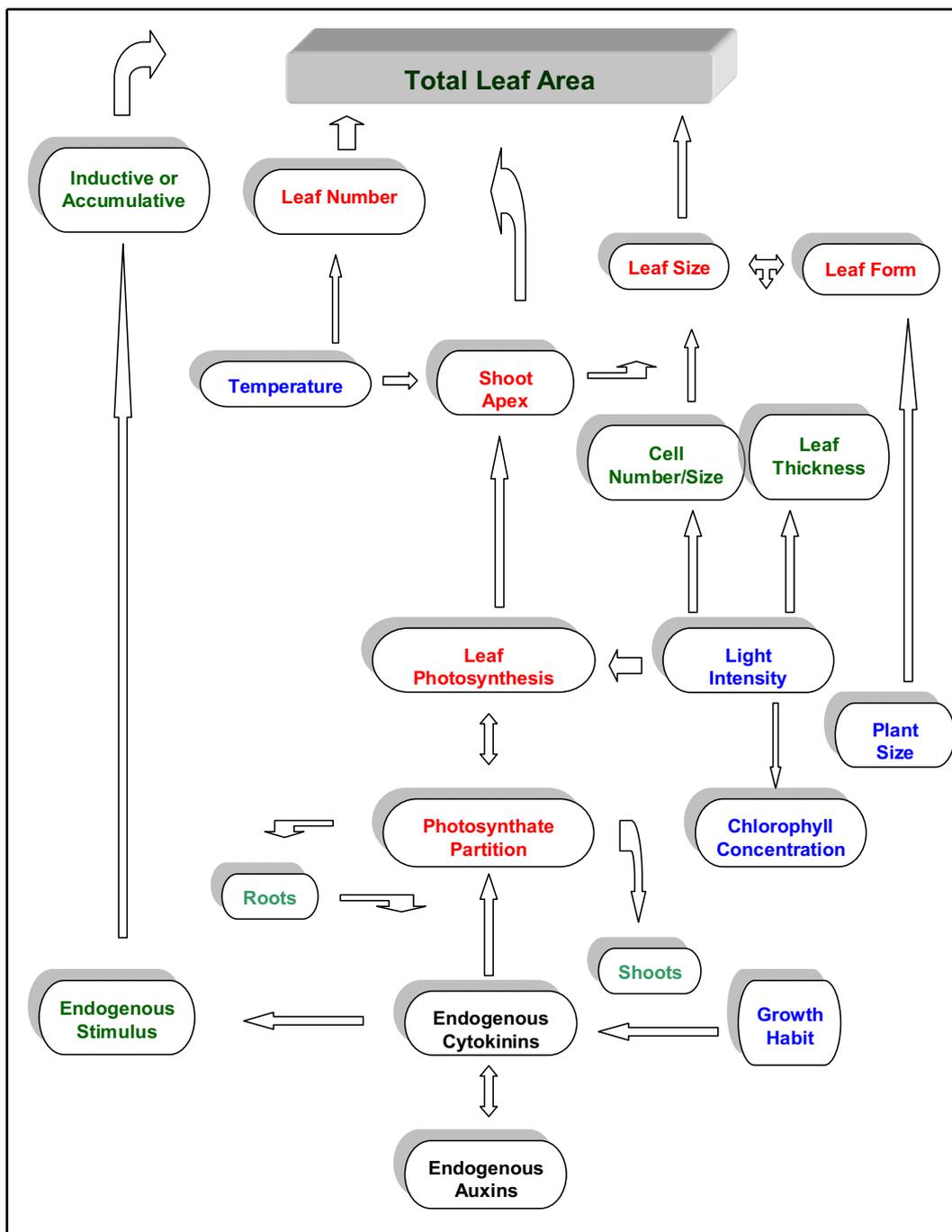


Fig. 1 Control set points for ornamental foliage plants leaf biomass accumulation and relationships between them.

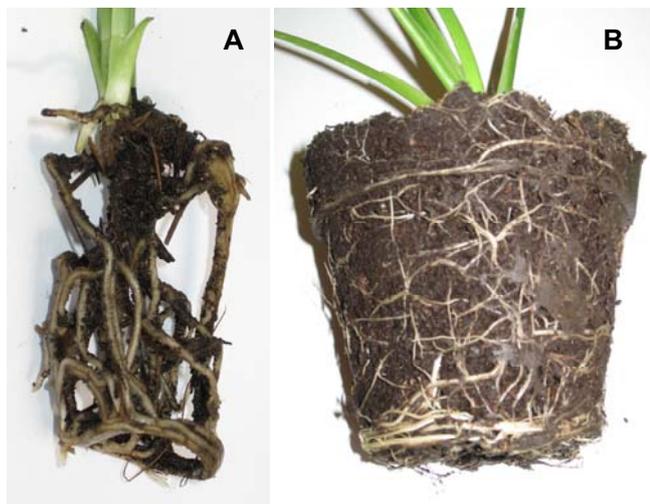
further removed from natural environments. Because of this demand, most of the production is done under complete environment greenhouse regulation, and the research is mainly aimed at studying adaptation to the final environment usually characterized by low irradiances but with a wide range for temperature, relative humidity and red/far red ratio, bearing no relationship to those occurring in their native environments (Blessington and Collins 1993).

There is abundant technological information on crop production under commercial facilities, but knowledge of the precise mechanisms and the cause-effects relationships between physiological traits and plant productivity of ornamental foliage plants under cultivation are mostly lacking. Some potential control points for biomass production in shade ornamental plants and the interactions between them are shown in Fig. 1.

Shade ornamental plants show a low both total leaf area and total leaf area expansion rate (Di Benedetto *et al.* 2006), although leaf size and plastochron index could increase as

plant size increases. Leaf photosynthetic capacity is age dependent and, as the plant grows, canopy structure and other such changes modify the environment of a particular leaf. Allocation of carbon and nutrients to new leaves is an especially important “reinvestment” because it drives growth in an exponential fashion. For a leaf to benefit a plant, the leaf cumulative carbon gain must be at least marginally greater than the carbon costs of its construction, maintenance, and protection. Leaf development and aging follows a predictable sequence of events involving changing patterns of costs and carbon gain (Pearcy *et al.* 1987).

During the initial phases of expansion, when photosynthetic capacity is low, leaves import much of its carbon for construction and the respiration rate is high. The maximum photosynthetic capacity is usually reached at about the time of full expansion. Finally during senescence, photosynthetic capacity declines as nitrogen is mobilized and exported before the leaf falls off the plant. Because total plant photosynthesis is increasing, relatively small changes in alloca-



**Fig. 2** Foliage pot plant root systems at the transplant stage (A) and at the sale stage (B).

tion to new leaves compensate for investment, leading to large changes in plant size.

It has been claimed that the close coordination between root and shoot growth is controlled by a signaling pathway which is largely hormonal in nature with a major site of control located in the root system. Increased root growth may lead to a corresponding increase in the synthesis of cytokinins (CKs) (O'Hare and Turnbull 2004). In plants grown in small pots, root growth restrictions often occur. At the transplant stage, ornamental plants grown in pots may show a well developed root system with white roots and without damage but with a horizontal root growth around the pot (Fig. 2A). The root restriction effects remain for the following weeks although a new root system may develop through the lateral roots that arise adventitiously. The same picture is repeated when root systems from transplanted seedlings which are grown in larger containers, reach to the pot base (Fig. 2B).

The availability of hormones synthesized in the root apex and reallocated to shoots would be reduced when the vertical root growth was impeded by the container base. There is strong evidence that CKs are root factors which are

transported via the xylem to the shoot, where they exert a major regulatory influence on growth and photosynthesis (Itai and Birnbaum 1996), but the response to exogenous CKs sprays on ornamental foliage plants is scarce (Nath and Pal 2007). The conclusion that root CKs are part of the signaling pathway by which the root/shoot ratio is regulated can be drawn from a number of studies (Mok and Mok 2001; Haberer and Kieber 2002; Howell *et al.* 2003; Dodd 2005; Rashotte *et al.* 2005; Glover *et al.* 2008). Exogenous CK supply favors the development of shoots (Maene and Debergh 1982; Wang and Boogher 1987), and specially tends to increase leaf biomass (Fig. 3) (Appendix 1, Experiment 1, unpublished data).

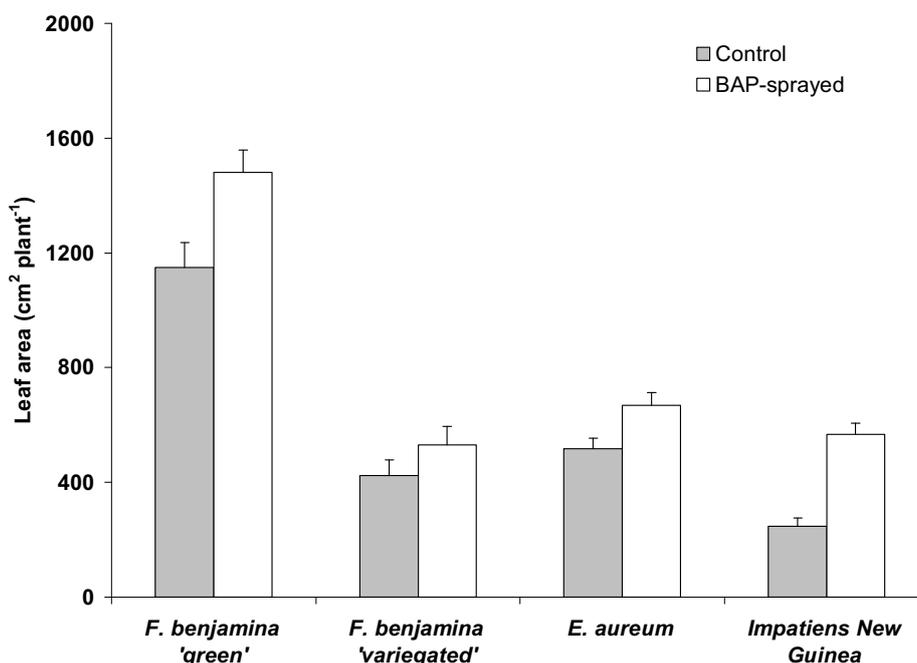
However, both clear studies on crop productivity in ornamental shade plants, as well as the mechanisms involved in plant response to exogenous CK supply under commercial facilities in this type of plants have not been well developed yet (Duan *et al.* 2006). Increase in leaf area of an ornamental shade plant can thus be divided into four morphological processes: (I) leaf-appearance rate, (II) leaf-elongation rate, (III) leaf-elongation duration and (IV) leaf shape. It has been suggested that cytokinins may directly affect all this processes (Tsukaya 2003; Bemis and Torii 2007).

### LEAF APPEARANCE

Leaves are initiated sequentially in precisely ordered patterns throughout the vegetative phase of shoot development by the apical meristem, which maintains itself in the process as an organized unit of cells whose fates within the shoot is not yet determined (Aida and Tasaka 2006). Leaves originate relatively uniformly as simple peg-like outgrowths, and subsequently exhibit divergent patterns of determinate growth. During the monopodial phase, as the leaf size increases from one leaf to the next, the apparent phyllochron decreases.

Application of CKs can promote leaf unfolding and expansion in intact plants in a range of species (Hayes 1978; Goodwin 1978; Doerner 2007). Fig. 4 (Appendix 1, Experiment 1, unpublished data) indicates that exogenous applied CKs significantly increase the number of leaves initiated by the apex in *E. aureum*, *Impatiens* New Guinea, *F. benjamina* both 'green' and 'variegated' clones related to the control plants during the same cultivation period.

*Schefflera arboricola* is a plant which contains leaflets



**Fig. 3** Effects of a 5 mg L<sup>-1</sup> 6-benzylaminopurine solution on total leaf area (cm<sup>2</sup> plant<sup>-1</sup>) on ornamental shade plants (Appendix 1, Experiment 1, unpublished data). Means of the replicates and standard errors are given.

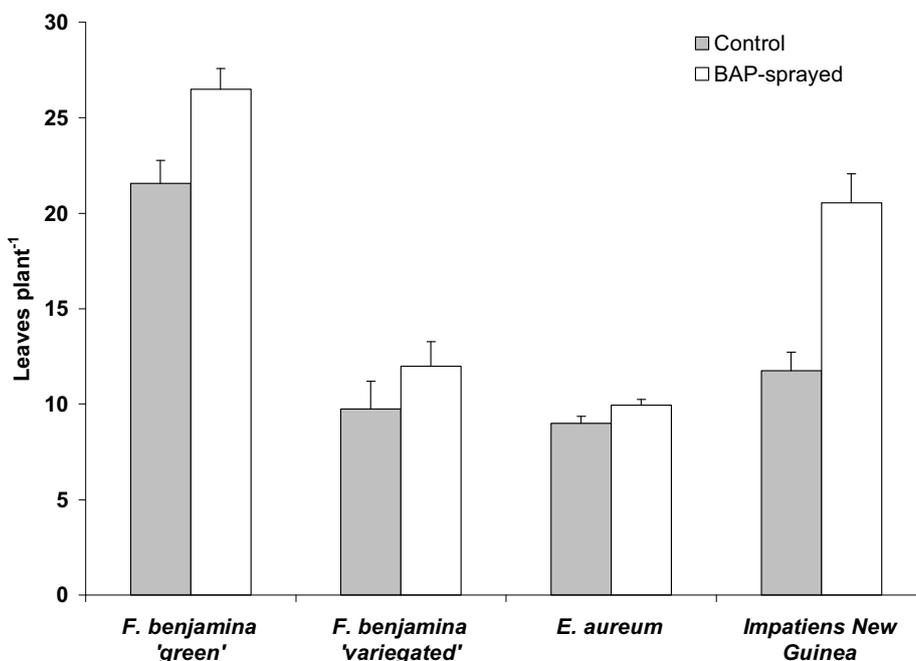


Fig. 4 Changes in leaves developed by plants sprayed with and without a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (Appendix 1, Experiment 1, unpublished data). Means of the replicates and standard errors are given.

that are either completely green or yellow or may show varying degrees of variegation. It has been reported that CK movement into yellow leaflets or leaf parts is slower than into green leaflets (van Staden *et al.* 1994). In this work, CKs transported into the yellow parts were metabolized at a much faster rate. These data, obtained with applied radio-labelled CKs, suggest that within the plant, yellow leaves contain less endogenous CKs than green leaves. Analysis of green and yellow leaflets of *S. arboricola* indicated that the green leaflets contained ten times more endogenous CKs than the yellow ones. Leaves have been shown to synthesize CKs, but this occurs predominantly in upper or younger leaves. To what extent such biosynthesis occurs in plants with variegated leaves is not known. The lower level of endogenous CKs found to be present in the yellow leaflets correlates with the fact that *trans*-zeatin was imported into yellow leaflets at a slower rate, and that whatever CKs were imported into the leaf was metabolized at a faster rate. This indicates a differential supply of xylem CKs and differences in the degree of metabolism in the yellow leaflets (van Staden *et al.* 1994). These results would be in agreement with the lower effect of the same 6-benzylaminopurine (BAP) solution on leaves expanded by the variegated *F. benjamina* clone (Fig. 4; Appendix 1, Experiment 1, unpublished data).

CKs have been shown to promote lateral branching in a variety of plants (Dun *et al.* 2006; Bessonov 2008; Ongaro and Leyser 2008). Foliar sprays of BAP increased the number of lateral branches in *Peperomia obtusifolia*, resulting in fuller and shorter plants (Henny 1985); similar effects of CKs were also observed in *Cordyline terminalis* (Maene and Debergh 1982), *Dieffenbachia* sp. (Wilson and Nell 1983), *Pilea cadieriei* and *Dracaena marginata* (Henley and Poole 1974) and *Anthurium andreaeanum* (Higaki and Rasmussen 1979). Conversely, 4 weekly foliar applications of BAP did not induce the development of lateral shoots in *Epipremnum aureum*, and only enlarged lateral buds were observed (McConnell and Poole 1972).

## LEAF EXPANSION

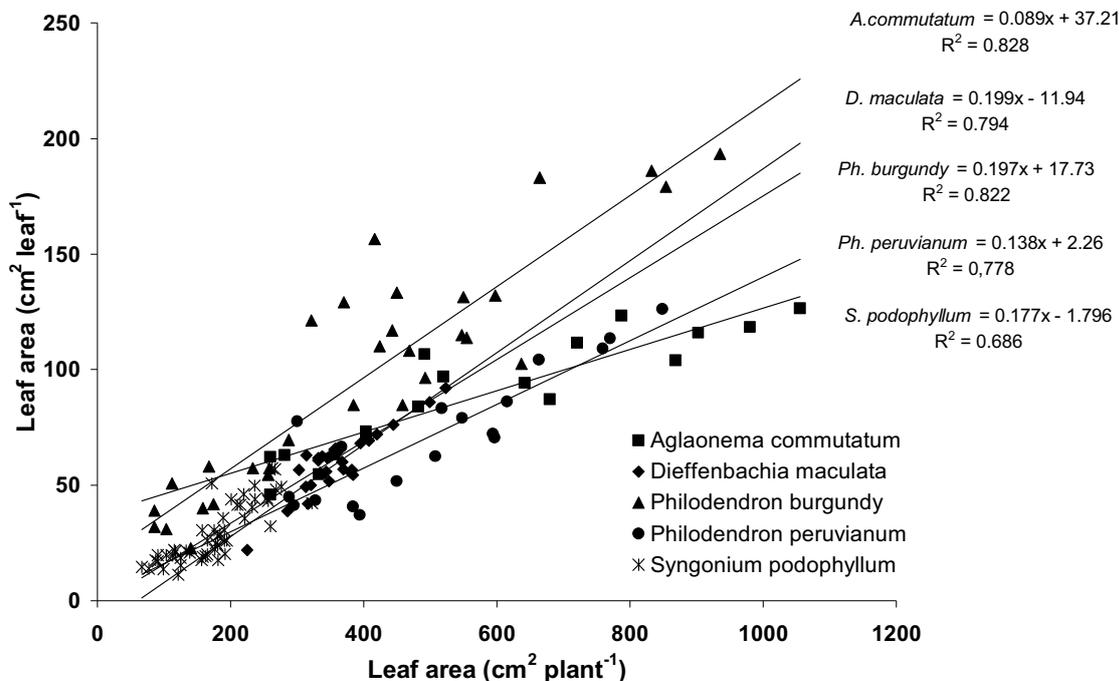
The photosynthetic capacity in the mature leaf is determined by both the size and metabolic activity of the leaf. Leaf expansion and the development of leaf metabolism are influenced by a number of factors including environmental factors such as temperature, light intensity and quality, and

endogenous factors such as 'shoot age' and assimilate supply. However, little is known about modulations of leaf development, and more information is needed to understand the physiology of leaf development in ornamental shade plants. There are many well documented examples of size-related physiological changes in animals, but only contradictory results for plants (Di Benedetto and Cogliatti 1990b; Donovan and Ehleringer 1991, 1992; Soyza *et al.* 1996; Di Benedetto *et al.* 2005). Because total plant photosynthesis is increasing, relatively small changes in allocation to new leaves lead, and large changes in plant size; large differences in growth rates can be attributed to allocation pattern differences (Pearcy *et al.* 1987).

The influence of plant size on physiological responses on ornamental foliage has been addressed under both constant and varying environmental conditions (Di Benedetto and Cogliatti 1990a; Di Benedetto 1991; Gerry and Wilson 1995). Differences in leaf size that correlated with plant size also influenced physiological performance of the plant (Mendez and Obeso 1993; Zotz 1997). Regression lines for single leaf area (cm<sup>2</sup> leaf<sup>-1</sup>) vs. total leaf area (cm<sup>2</sup> plant<sup>-1</sup>) for six ornamental aroids plants (Fig. 5) show that previous leaf area modulates single leaf size (Di Benedetto *et al.* 2006).

It is well known that carbon partitioning between leaf area expansion and growth of non-photosynthesizing tissues generally increase with the age of the individual (Schulze 1982). The suggestion that plant 'size' would be associated to photoassimilate allocations is supported by the fact that allocation to resource-capturing organs has also been indicated to be correlated with competitive ability to clonal dispersal (Abrahamson 1975; Hartnett and Bazzaz 1983; Brewer *et al.* 1998).

Golden photos (*E. aureum*) are among the most important foliage plants produced commercially. Growers cut the long vines into single node, leaf-bud cuttings for propagation. Non-uniform growth of axillary shoots has been observed among cuttings of axillary pothos. The most obvious effect of internode length of cuttings on plant growth was on leaf number and stem length. Cuttings with a 3-cm or longer internode below the node produced leaves faster and had longer axillary shoots than those with shorter stems; increases in stored nutrients in the longer internodes might have triggered faster shoot growth (Wang and Boogher 1988). On the other hand, Steinitz *et al.* (1992) suggested that young *E. aureum* plants with soft stems will grow in a



**Fig. 5** Changes in leaf area ( $\text{cm}^2 \text{leaf}^{-1}$ ) related to total leaf area ( $\text{cm}^2 \text{plant}^{-1}$ ) for five ornamental Araceae plants. Reprinted from Di Benedetto A, Molinari J, Boschi C, Benedico D, Cerrotta M, Cerrotta G (2006) Estimating crop productivity for three ornamental foliage plants. *International Journal of Agricultural Research* 1, 522-533, ©2006, with kind permission from Academic Journals Inc., USA.



**Fig. 6** Climbing photos plant stuck to the bench bracket showed an increasing leaf size.

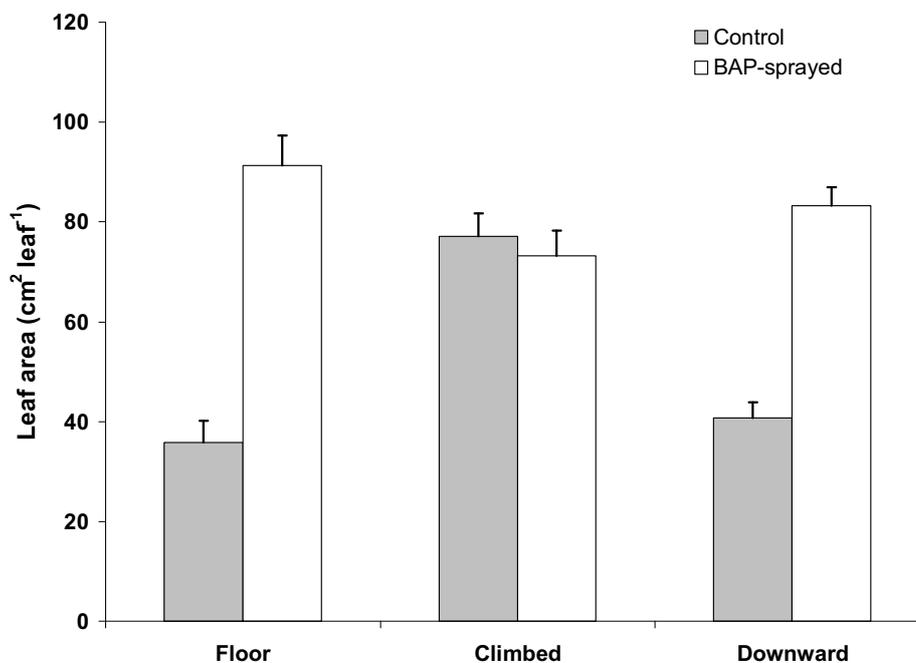
vertical downwards hanging manner unless they encounter a surface on which they can climb and grow in a direction deviating from the direction of the gravitational vector. The climbing plant finds anchorage with adventitious aerial clasp roots appraised to an adjacent surface of host plants, rocks or walls (Fig. 6). Steinitz and Hagiladi (1987, 1992) found that climbing *E. aureum* plants had larger

leaves, shorter internodes and a better developed aerial root system than unsupported plants and suggested that the morphological differences between climbing and free hanging plants represent thigmomorphogenic responses of climbing plants to a mechanical stimulus generated by the contact of the stem with the support's surface. A marked increase in leaf area was found only when the 'ventral' stem flank came in contact with the support's surface which indicating that the thigmomorphogenic touch sensitivity with regard to stem circumference is restricted to the 'ventral' side. Thigmostimulation started after the plants developed three internodes.

Data from Fig. 7 (Appendix 1, Experiment 2, unpublished data) are partially in agreement with Steinitz and coworkers in the sense that climbed plants increase leaf size in pothos; however, the response would not be associated with a thigmomorphogenic response of climbing plants to a mechanical stimulus. Pothos plants grown at a horizontal or downward position gave smaller leaves size on the axillary shoots. Interestingly, in these plants BAP spraying led to a significant increase in leaf size, thus suggesting that smaller leaves in horizontal or downward growing plants may be associated with lower CK levels than in those growing upwardly.

Leaves of ornamental plants have a large variety of shapes and sizes. One of the commoner variants in shape is the dissected or compound leaf. The blades of these leaves are cut into segments of leaflets. In terms of comparative development dissected leaves are of particular interest because they so clearly illustrate how different paths of development can lead to leaves that are closely similar in appearance.

The growth of different tissues at different rates soon gives the leaf's future leaflets the appearance of bump like protuberances along the leaf's two free edges. The older and slightly larger bumps appear toward the tip of the leaf; the younger and smaller ones arise progressively neared the leaf base. Once a complement of four or five leaflet pairs has appeared, enlargement continues and the bumps soon assume the shape of mature leaflets. This mode of leaflet initiation from the free margins of a leaf is the most common mechanism of development among higher plants with dissected leaves, whether they are simple vascular plants such as ferns, gymnosperms (cycads) or the higher flower-



**Fig. 7** Leaf area ( $\text{cm}^2 \text{leaf}^{-1}$ ) for pothos plants grown in a different vertical gradient and sprayed with a  $5 \text{ mg L}^{-1}$  6-benzylaminopurine (BAP) solution (Appendix 1, Experiment 2, unpublished data). Means of the replicates and standard errors are given.

ing plants. Through progressive degrees of elaboration it is possible for compound leaves to be twice, three times or even many times dissected. Indeed, in principle there is no limit to the degree of elaboration that can be found in a dissected leaf (Kaplan 1983).

The arum that shows a sharply different mode of leaf dissection is popularly known as the split-leaf philodendron, one of the climbing *Monstera* genus. These plants are noted for the distinctive holes in the blades of their leaves and are widely grown as ornamental plants for that reason. The size and shape of the holes vary in different parts of the blade have different rates of growth. For example, the first holes to appear lie close to the edge of the blade; at maturity they are large and elliptical in outline because that part of the blade undergoes the greatest amount of lateral expansion. The holes that develop later are found closer to the leaf midrib. They tend to be smaller and to retain their original rounder outline because the tissue surrounding them does not undergo as much lateral growth (Kaplan 1983).

In many species of the genus *Monstera* the marginal strip of blade tissue adjacent to the most peripheral hole will not grow in width at a rate that matches the growth of the rest of the blade. As a result, thin bridge of marginal tissue usually breaks, converting what had been a perforated blade surface into a marginally lobed one. Usually the development of four to six leaves after transplant are required to show the first holes. This requirement has been found to be unmodified by exogenous applications of BAP, even when they reach faster at this stage because an accelerated leaf expansion rate (Di Benedetto, unpublished data).

Plant morphogenesis is controlled by the integration of endogenous genetic programs and responses to exogenous signals. The leaf is a good subject for studying plant morphogenesis, the diversity of which is reflected in leaf shape. Early control of leaf shape relies on controlling leaf initiation at the shoot apical meristem, the rates and planes of cell division, and the polarity-dependent differentiation of leaf cells. Final leaf form involves coordination of the rates of division, enlargement, and differentiation of leaf cells. In addition, recent genetic studies have revealed a different mechanism that plays an important role in regulating leaf shape: the control of spatial and temporal expression by micro RNA and programmed cell death (Kim 2006).

Some connections has been described between auxin and CK function and transcription factor patterning systems

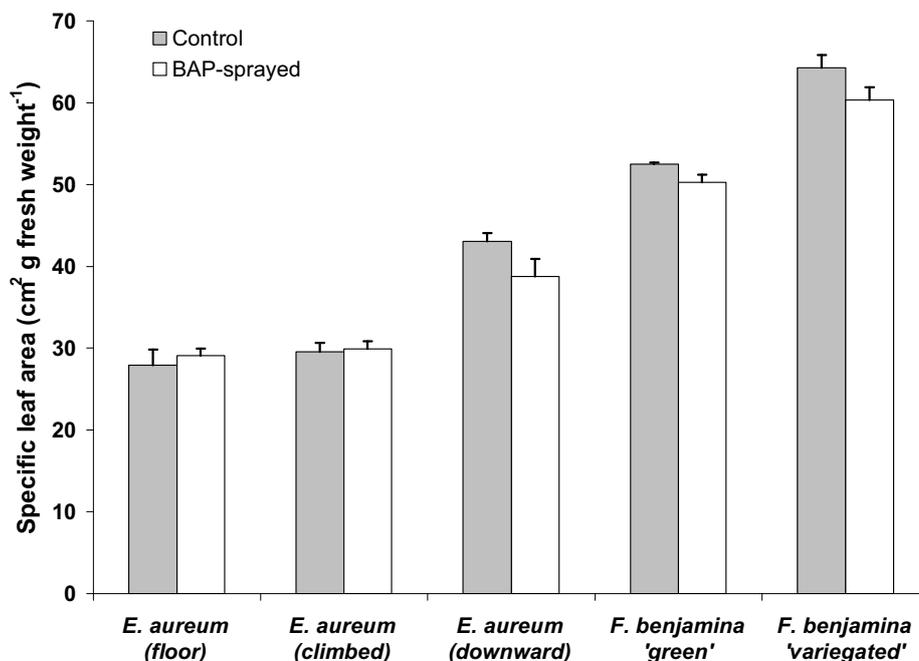
involved in both leaf initiation and elaboration of leaf axial patterning. A cascade of small RNA-based regulatory steps was suggested to facilitate delimitation of cell types comprising the upper versus lower parts of the leaf. Developmental regulation of cellular growth emerged as a crucial component in regulation of leaf form with transcription factors playing a key role in this process. The *cis*-regulatory evolution of developmental genes emerged as a process that likely contributed to diversification of leaf form (Barkoulas *et al.* 2007). Morphogenesis of leaf shape and formation of the major elements of leaf vasculature are temporally coordinated during leaf development. Current analyses of mutant phenotypes provide strong support for the role of auxin signaling in vascular pattern formation and indicate that leaf shape and vasculature are developmentally coupled. Two other mechanisms that may contribute to the regulation of these processes are a diffusion–reaction system and long-distance signaling of informational macromolecules such as CKs (Dengler and Kang 2001).

Leaf development is characterized by substantial changes in morphology and anatomy that are the product of differential cell/tissue expansion and growth. Such growth in developing leaf tissue results in the enlargement of specific tissues and the separation of cells that were closely associated during early stages of development. The latter process results in the development of air spaces within the leaf. These changes presumably facilitate the physiological function of the leaf and represent an important aspect of leaf function (Maksymowych 1973).

Plants acclimated under high-light environments and transferred to low-light ones showed anatomical changes that provide better use of low light conditions (Perry *et al.* 1986; Vidal *et al.* 1990). Changes in leaf size under limited light were related to a decrease in total mesophyll thickness and an increase in air space between cells.

Leaf thickness is the result of changes in both number of cell layers and cell size. Major differences may occur in leaf thickness between species of the same genus, as shown in aroids (Di Benedetto and García 1992).

Hormones affect plant growth and developmental processes (Chuan *et al.* 2007). We are just beginning to learn that part of their effect is a consequence of the modulation of the cell proliferation potential in a spatial- and temporal-specific manner (Pozo *et al.* 2005; Guimil and Dunand 2007; Tucker and Laux 2007). Understanding the intimate



**Fig. 8** Specific Leaf Area for ornamental shaded plants with different growth habits under greenhouse facilities. Plants were sprayed with either water (controls) or with a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (**Appendix 1, Experiments 1 and 2**, unpublished data). Means of the replicates and standard errors are given.

**Table 1** Changes in anatomical attributes for three ornamental foliage plants sprayed with a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (**Appendix 1, Experiment 1**, unpublished data).

	<i>Epipremnum aureum</i>		<i>Ficus benjamina</i> 'green'		<i>Ficus benjamina</i> 'variegated'	
	Control	BAP-sprayed	Control	BAP-sprayed	Control	BAP-sprayed
Leaf thickness (µm)	312.12 a	403.03 b	206.06 a	212.12 a	193.33 a	170.67 a
Stomata density (mm <sup>-2</sup> )	39.81 a	39.78 a	167.20 a	191.08 a	143.31 a	127.39 a
Cell density (mm <sup>-2</sup> )	437.90 a	310.51 b	931.53 b	1313.69 a	1397.29 b	3065.29 a

Different lower-case letters indicate statistically differences ( $p \leq 0.05$ ) from Tukey's test between control and BAP-sprayed plants.

coupling of cell proliferation with growth and development in plants in response to hormones is one of the major challenges ahead. However, an extra layer of complexity comes from the fact that, frequently, the same hormone impinges on cell cycle control at different stages, and several hormonal pathways converge at a given cell cycle transition. Furthermore, it should be kept in mind that most of the results available are derived from studies using the exogenous application of hormones that may alter the levels of other hormones.

Leaf thickness and specific leaf area, which is often correlated with the former, may be modified by exogenous CK supply. It has been proposed that CKs promotes the rate of parenchyma cell expansion, and prolong the duration of cell enlargement and promote the rate of the cell enlargement (Kyojuka 2007; Hamada *et al.* 2008). In pothos plants growing downwards, BAP spray induces a significant decrease in specific leaf area (suggesting thicker leaves) while little or no changes were observed in plants growing on the floor or climbed **Fig. 8** (**Appendix 1, Experiments 1 and 2**, unpublished data). Direct microscopy thickness observations (**Table 1; Appendix 1, Experiment 1**, unpublished data) are in agreement with these suggestions. On the other hand there are little changes in both green and variegated *F. benjamina* plants. BAP-sprayed pothos plants increased leaf thickness but in *F. benjamina* green or variegated clones there is no significant differences related to controls (**Table 1; Appendix 1**, unpublished data).

Our results (**Table 1; Appendix 1, Experiment 1**, unpublished data) showed there are no change in stomata density for *E. aureum* and *F. benjamina* clones when plants were sprayed with a 5 mg L<sup>-1</sup> BAP solution. Cell density increased in pothos BAP-sprayed plants but decreased for both clones of *F. benjamina* (**Table 1; Appendix 1, Experi-**

**ment 1**, unpublished data). Previously it has been demonstrated that, under similar experimental conditions, BAP promoted the expansion of mesophyll cells (Ron'zhina 2003). In the juvenile leaves, BAP promoted cell divisions, and in the expanding and mature leaves, cell expansion. During leaf formation, cytokinin is required to drive the cell division cycle at a normal speed and to obtain the required number of cell divisions to reach a normal leaf size. Goldstein and Chory (2008) suggested that cells in the epidermis both promote and restrict growth of the entire shoot by sending growth signals – either physical or chemical – to the inner layers.

The shoot apical meristem functions to generate external architecture and internal tissue pattern as well as to maintain a self-perpetuating population of stem-cell-like cells. The internal three-dimensional architecture of the vascular system corresponds closely to the external arrangement of lateral organs, or phyllotaxis. The close correspondence between phyllotaxis and vascular pattern present in mature tissues arises at early stages of development, at least by the first plastochron of leaf primordium outgrowth (Dengler 2006). CKs induce de novo formation of phloem elements (Mahonen *et al.* 2006). Experiments performed in isolated cotyledons of pumpkin (*Cucurbita pepo* L.) demonstrated that BAP may induce the formation of new veins. In the leaves of flat bean (*Phaseolus* sp. L.), BAP treatment increased the amount of mesophyll tissues by extending mesophyll cells; exogenous CK contributed to enlargement of mesophyll cells and lignification of mechanical leaf tissues. The number of vascular bundles, xylem vessels and phloem elements increased. These results were in agreement with those in the leaves of wheat, sugar beet, and perennial cereal herbs (Chernyad'ev 2005). Data from our laboratory on pothos plants are in agreement with these

previous reports (Di Benedetto, Tognetti, Galmarini data not shown).

## PHOTOASSIMILATE PRODUCTION

The value of a leaf, to the plant that bears it, is the contribution that it makes to the fitness of the plant: the contribution that descendants of the plant (or its genes) make to future populations. The cost of producing a leaf is the investment of resources of carbon, nitrogen, etc.; the interest that is gained from this investment is the carbon fixed by the leaf in photosynthesis (Noguchi *et al.* 2001). Some of the fixed carbon may go to stems and roots, organs of clonal growth and perennation in ornamental shade plants that may contribute to subsequent generations. An increase in total leaf area for ornamental foliage plants would be associated as higher leaves number as higher leaf size.

Plant growth and development is sustained through photosynthetic carbon fixation and the subsequent translocation of photoassimilate to growing tissues or storage organs. Mature leaves are a source tissue because there is a net export of photoassimilate, generally sucrose, from them to the sink organs, which include roots and actively growing meristems. However, Di Benedetto *et al.* (2006) have proposed that the returns for *Ph. burgundy* would be greater than for *A. commutatum*, *D. maculata*, *S. podophyllum* and its related species *Ph. peruvianum* according as plant size increased. The reasons for such responses are yet unclear.

It has been suggested that leaves with a longer life-span, such as those of the ornamental shade plants have a higher leaf construction cost, because of a larger investment in structural (Oikawa *et al.* 2004) and defensive compounds (Seilaniantz *et al.* 2007) to live for a longer period. Some studies support this hypothesis (Merino 1987) but others do not (Merino *et al.* 1987). Williams *et al.* (1989) showed that leaf life-span did not correlate with the construction cost, but was positively correlated with the ratio of leaf construction cost to daily carbon gain. This ratio was assumed to be indicative of the time the plant spends paying back the investment in constructing leaves. Leaf cost per unit of mass was inversely correlated with leaf size, perhaps as a result of the greater proportional contribution of expensive cuticle to leaf mass in small leaves. It would be possible that plants sprayed with CKs change their leaf construction cost; but although there is no report available, critical research are under progress.

The photosynthetic activity of a leaf changes with its age, typically rising to a peak or plateau and then declining. At first sight it might appear that the value of the leaf to the plant is simply the sum of assimilates that it exports. This assumes that carbon exported from a leaf early in its life has the same value to the plant as carbon exported later. In the case of a plant growing vegetatively this is unlikely to be true. The exported assimilates that find their way into new leaves represent reinvestment that then generates new interest. The early exports from a leaf are therefore of potentially greater value to the future growth of the plant than the same material exported from the same leaf later in its life.

Several attempts have been made to relate net photosynthetic rate ( $P_n$ ) to plant productivity in ornamentals, although it has been pointed out the difficulty of relating leaf  $P_n$  rates to yield parameters (Ottosen 1990; Vidal *et al.* 1990; Ottosen 1994). Measurements of  $P_n$  rates of only parts of plants under laboratory conditions may not fully reflect conditions encountered in commercial production.

As many of the examined species have been grown under greenhouse conditions, growth rate determinations based on short term measurements of photosynthesis have proved difficult (Zelitch 1982). Leaf photosynthetic capacity is age dependent and besides, as the plant grows, canopy structure and other such changes modify the environment of a particular leaf. Allocation of carbon and nutrients to new leaves is an especially important "reinvestment" because it drives growth in an exponential fashion.

One of the problems inherent in making photosynthetic comparisons, which are concurrent with changes in leaf morphology, is in determining a valid basis of expression of photosynthetic rate. Araus *et al.* (1986) found that differences in maximum  $P_n$  between species are even more pronounced when they are expressed on the basis of chlorophyll rather than leaf area for different sun and shade species. Positive correlation between maximum  $P_n$  (per unit leaf area) determined at normal  $CO_2$  pressure, and leaf thickness is often found among leaves of the same species. However, results from Di Benedetto *et al.* 2006 showed a poor correlation between photosynthetic rate for a single leaf area-, chlorophyll content- and leaf thickness-base for *A. commutatum*, *Ph. 'Burgundy'* and *Ph. peruvianum* grown under optimal (summer) or suboptimal (winter) environments. One possible explanation of these relationships between leaf size and photosynthetic capacity (both leaf area- and chlorophyll content-base) is that genotypes with large leaves, such as the ornamental aroids tested, may have more severe mutual shading which would reduce their photosynthetic capacity.

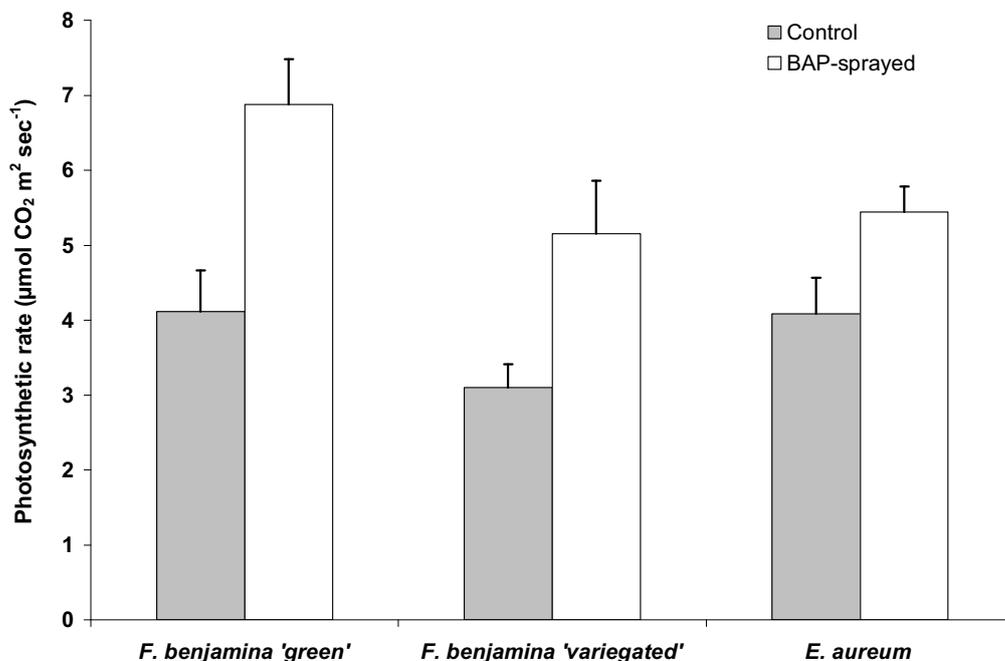
Only few studies have focused on the relationship between photosynthetic rates ( $P_n$ ) and plant productivity or growth of ornamentals (Zelitch 1982; Ottosen 1990). Results from Di Benedetto *et al.* (2006) are in agreement with the apparent paradox in lack of correlation between measurement of single-leaf photosynthesis and plant growth rate. Short-term measurements of photosynthesis may not be a good indicator of the long-term growth effects. Over time, acclimation to a changing environment takes place in plants, and short-term measurements of photosynthesis will often overestimate the effect of, for example, increasing light levels during summer. Besides, when growth respiration for shoot maintenance and leaf growth is increased plant investment in root growth increase. Photosynthetic measurements as a means of gaining insight into the optimal growing conditions for plants should therefore be used with care.

CKs are known to play an important part in the regulation of photosynthetic pigments (Chernyad'ev 2005) but there are no previous reports on a direct effect of exogenous CK supply on photosynthetic rate for ornamental foliage plants. Our results on *E. aureum* and two *Ficus benjamina* clones sprayed with 6-benzylaminopurine showed an increase in photosynthetic rate related to controls grown under the same greenhouse environment (**Fig. 9; Appendix 1, Experiment 1**, unpublished data). However, higher photosynthetic rates would not be related to a decrease in stomata density (**Table 1; Appendix 1, Experiment 1**, unpublished data).

Boonman *et al.* (2007, 2009) showed that a lowering light gradient over the foliage of a plant result in reduced CK activity in shaded leaves as a consequence of reduced import through the xylem and that CKs are involved in the regulation of whole-plant photosynthetic acclimation to light gradients in canopies; external application of CKs to shaded leaves could rescue multiple shade effects.

Growth rate is always controlled by the source-sink balance within the plant. The source consists of photosynthetically active leaves and stored reserves and the sink is made of growing organs. The decrease in the apparent phyllochron means that the increase in assimilate source is greater than the increase in sink (Dufour and Guerin 2003). The leaf expansion rates for all ornamental Araceae plants (*A. commutatum*, *D. maculata*, *Ph. 'Burgundy'*, *Ph. peruvianum* and *S. podophyllum*) tested by Di Benedetto *et al.* (2006) are in agreement with previous reports, but the response are related to plant material used (*Ph. 'Burgundy'* showed the higher increased in reinvestments) and season.

The efficiency by which light may be absorbed by a leaf is directly related to chlorophyll per unit leaf area. Obligate shade plants grown under deep shade often have at least as high chlorophyll content as do sun plants grown at high light levels; high chlorophyll content appears to be especially frequent among evergreen shade plants native to tro-



**Fig. 9** Photosynthetic rate changes related to the 6-benzylaminopurine (BAP) sprays (5 mg L<sup>-1</sup>) on three ornamental foliage plants (**Appendix 1, Experiment 1**, unpublished data).

**Table 2** Chlorophyll/Protein ratios from thylakoid preparations in 80% acetone for leaves of six aroid species grown under a commercial greenhouse environment (Di Benedetto, unpublished data).

Ornamental foliage plants	Chlorophyll/Protein ratio
<i>Aglaonema commutatum</i>	64.6
<i>Epipremnum aureum</i>	29.8
<i>Philodendron</i> 'Burgundy'	23.1
<i>Philodendron cordatum</i>	54.7
<i>Philodendron peruvianum</i>	167.7
<i>Syngonium podophyllum</i>	73.8

pical forest (Wang *et al.* 2005). Di Benedetto (1991) has showed that although chlorophyll was present in *A. commutatum* leaf primordia of 0.2 mm long, its accumulation was low (about 24% of maximum) until leaves had reached approximately half their final leaf area; after this growth stage there was a steady increase until fully expansion.

Based on leaf fresh weight, the shade plants generally have higher chlorophyll content but a lower enzyme activity (i.e. Rubisco) than the sun plants. This is of a particular interest from an ecological viewpoint since it lends support to the hypothesis that, in shade plants, the investment on light harvesting structures is higher than investment on carbon fixation and mobilization.

Leaves of shade plants have considerably less total protein per total chlorophyll (and per leaf area) than do sun leaves of sun plants (Di Benedetto *et al.* 2003). Since it is generally considered that roughly one-half of the total chloroplast protein is associated with carbon fixation (mostly Rubisco), significant nitrogen take place in shade plants (**Table 2**) (Di Benedetto, unpublished data).

When the absorption spectrum in 80% acetone of thylakoid preparations for ornamental aroids was analyzed by Di Benedetto and García (1992), they would be seen that leaves expanded in the greenhouse light environment showed a characteristic absorbance spectrum with a peak at 600-700 nm, a lower absorption between 600-500 nm and the Soret band below 500 nm. However, when light intensity was reduced and the light quality changed, the absorption spectra differed. Changes in light absorption properties of leaves with light intensity seem to be related to CK contents. CKs are involved in synthesis of light harvesting chlorophyll polypeptides (LHC) (Flores and Tobin 1988); a reduced supply of CKs would thus rather reduce LHC (Pons and



**Fig. 10** Cross-section electron micrographs of the ultrastructure of greenhouse grown *Epipremnum aureum* (A), *Philodendron* 'Burgundy' (B), *Philodendron peruvianum* (C) and *Syngonium podophyllum* (D) aroids (magnification 20,000 x). Adapted from Di Benedetto A, García AF Adaptation of ornamental aroids to their light environments I. Spectral and anatomical characteristics. *Journal of Horticultural Science* 67, 179-188 ©1992.

Bergkotte 1996). However, chlorophyll concentration under greenhouse environment would not be a limiting factor for

**Table 3** Chlorophyll concentration for five ornamental foliage plants sprayed with a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (**Appendix 1, Experiment 1**, unpublished data).

Ornamental foliage plants	Chlorophyll content (mg m <sup>-2</sup> )	
	Control	BAP-sprayed
<i>Epipremnum aureum</i>	335.28 ± 20.64	362.29 ± 26.27
<i>Ficus benjamina</i> 'green'	417.02 ± 43.56	467.04 ± 22.36
<i>Ficus benjamina</i> 'variegated'	391.72 ± 39.51	422.74 ± 36.64
<i>Philodendron</i> 'Wang B'	761.63 ± 35.43	757.50 ± 17.99

Means of the replicates and standard errors are given.

photosynthesis in ornamental shade plants.

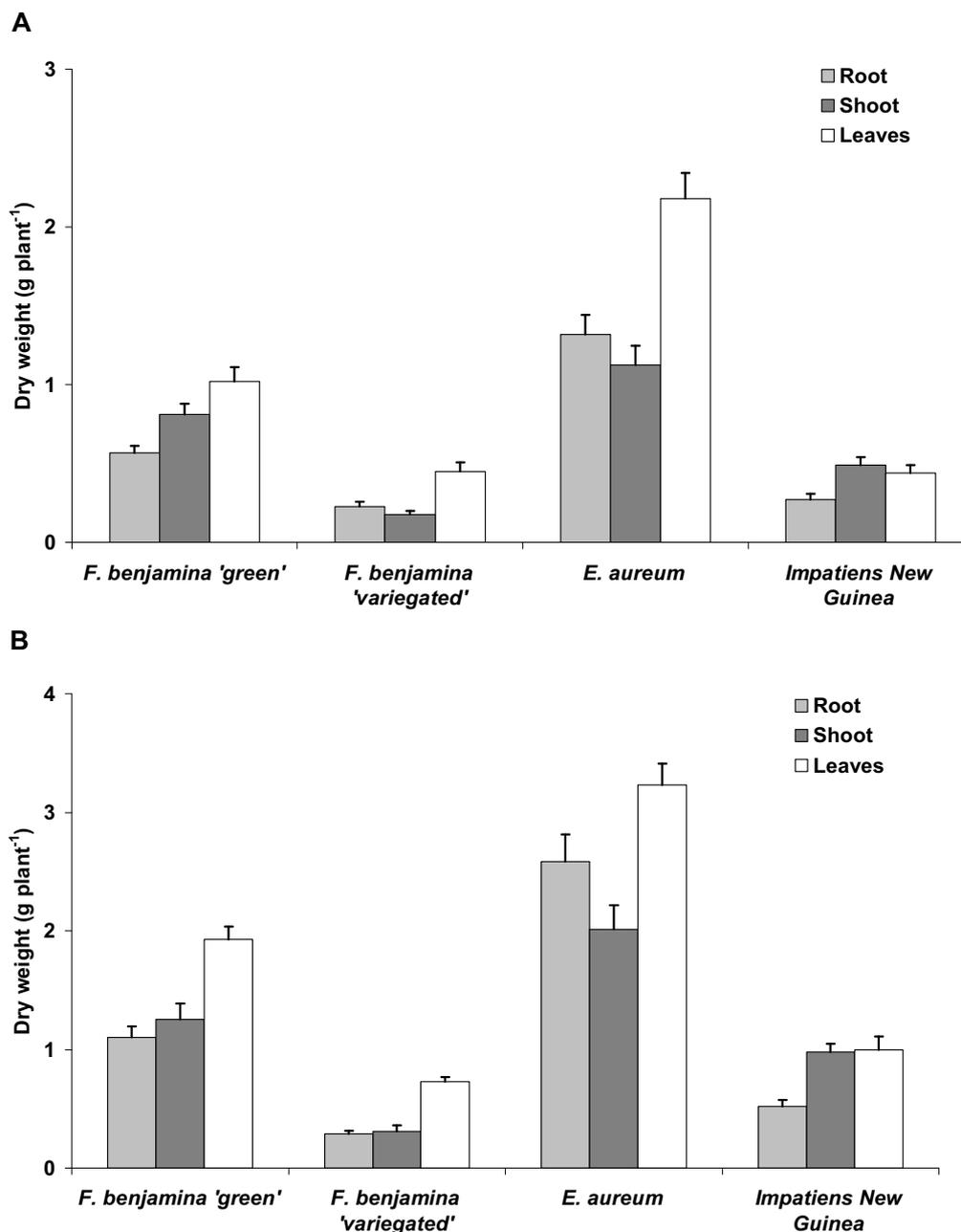
Electron micrographs of chloroplasts from leaves grown under the greenhouse environment showed typical high-light chloroplasts, characterized by grana stacks with few thylakoids per granum, little lamellar material in the starch-free stroma part of the chloroplast sections, a low degree of thylakoid stacking and large starch grains, specially in *E. aureum* and *Ph. peruvianum* (**Figs. 10A, 10B**).

Although it is generally accepted that root-produced

CKs play a role in both the synthesis and maintenance of chlorophyll and that they affect chloroplast development and metabolism (van Staden *et al.* 1994) and that CKs are involved in regulation of cell division and differentiation of chloroplasts in sun plants (Kulaeva and Prokoptseva 2004), CK addition to shade plants (which naturally bear high chlorophyll contents) may not enhance total chlorophyll. For instance, when plants of four ornamental shade species mentioned above, grown at 70% full-sun greenhouse environment were sprayed with BAP, no changes in chlorophyll content were found (**Table 3; Appendix 1, Experiment 1**, unpublished data).

## PHOTOASSIMILATE PARTITIONING

The distribution of biomass among plant organs is not fixed (Gerry and Wilson 1995) but, instead, it is affected by the environment, habit of the plant, life span of the plant and, competitive interactions. Brewer *et al.* (1998) have been suggested that the best belowground competitors should allocate the greatest fraction of resources to roots. On the other hand, Harnett and Bazzaz (1983) have suggested that



**Fig. 11** Dry weight accumulation through plant organs for four ornamental shade plants grown under greenhouse environment and sprayed with (B) or without (A) a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (**Appendix 1, Experiment 1**, unpublished data). Means of the replicates and standard errors are given.

**Table 4** Relative growth rate ( $\text{day}^{-1}$ ) for four ornamental shade plants (A), (B), (C) and (D) grown under greenhouse facilities with and without the application of  $5 \text{ mg L}^{-1}$  6-benzylaminopurine solution as a spray (**Appendix 1, Experiment 1**, unpublished data).

Ornamental foliage plants	Relative growth rate ( $\text{day}^{-1}$ )	
	Control	BAP-sprayed
<i>E. aureum</i>	0.0180	0.0210
<i>F. benjamina</i> 'green'	0.0120	0.0140
<i>F. benjamina</i> 'variegated'	0.0095	0.0102
<i>Impatiens</i> 'New Guinea'	0.0137	0.0154

if the expansion of shade leaves is supported by assimilate import, there should be an increase in growth, reproduction, and survival under field conditions. While the acquisition of resources by a plant provides the necessary substrates for growth (primarily C and N), it is the allocation of these resources at the whole-plant level that ultimately determines growth rate and final size. Work from our laboratory showed that, in ornamental shade plants grown under greenhouse facilities, although investment in root system is low: 11.3, 12.5, 17.5 and 13.2% of the total dry weight for *A. commutatum*, *D. maculata*, *Ph. Burgundy* and *S. podophyllum* respectively, root: shoot ratio may show an erratic response as plant size (estimated as total dry weight) increases (Di Benedetto *et al.* 2006).

CKs may change photoassimilate partitioning but, carbohydrates affect plant growth regulation too (Liscova *et al.* 2006). Although dry weight accumulation between shade ornamental species is quite different (**Fig. 11A; Appendix 1, Experiment 1**, unpublished data), the result of one spray application of BAP significantly increased it for *F. benjamina* (with a lesser extension for the variegated than for the green clone), *E. aureum* and *Impatiens* New Guinea (**Fig. 11B; Appendix 1, Experiment 1**, unpublished data).

The ecological advantage of a high relative growth rate (RGR) may seem clear: fast growth resulted in the rapid occupation of a large space which is advantageous in competitive situations. A high RGR may also facilitate a rapid completion of the life cycle of a plant (Poorter 1989). There are quite large differences in biomass production (**Fig. 11; Appendix 1, Experiment 1**, unpublished data) among typical RGRs of ornamental shade plants and usually RGR values would be slightly increased by cytokinin sprays (**Table 4; Appendix 1**, unpublished data); this result might be achieved by different ways but differences in the carbon economy, i.e., the balance of photosynthesis and respiration expressed per unit leaf area, would be of overriding importance in explaining variation in RGR. The more a plant invests in leaf area, the higher the total carbon gain and the faster dry weight accumulation will be. Reich *et al.* (1998) suggested that specific leaf area (SLA), net assimilation rate (NAR) and RGR are closely associated with variation in life-history traits and that variation in leaf and root structure more strongly influences patterns of RGR among species and light environments than does biomass partitioning. Data from Osone *et al.* (2008) are in agreement with these previous findings.

Veneklaas *et al.* (2002) have shown that differences in RGR of *F. benjamina* clones could be attributed to differences in leaf assimilation rate. One strategy for obtaining increased growth rates and high-quality *F. benjamina* plants of good size in a reasonably short period would be work in the selection for high specific leaf area. The use of 6-benzylaminopurine sprays would be another way (**Table 4; Appendix 1, Experiment 1**, unpublished data).

Differences in competitive abilities may be due to traits such as leaf area, plant height, and mass. In particular, initial plant size, expressed as total plant mass, may influence competitive ability (Di Benedetto *et al.* 2005). According to the model of Samson and Werk (1986), reproductive allocation is expected to increase allometrically with increasing plant size (Mendez and Obeso 1993). Although it is expected that plastic responses in resource allocation should be

common in species inhabiting heterogeneous environments, the arum species tested by Di Benedetto *et al.* (2006) are shade plants which have evolved under homogeneous tropical and subtropical rainforests. Another factor that may contribute to variation in competitive ability is growth rate (Gerry and Wilson 1995).

The distribution of photoassimilates within the plant is regulated by source-sink interactions. Sources are exporters of photoassimilates and sinks are net importers of these photoassimilates. The major sources in vegetative plants are the mature leaves but other photosynthetic tissues that fix carbon produce more photoassimilate than that required for their own growth and maintenance. Mature leaves fix carbon dioxide and export photoassimilates, primarily sucrose, to other parts of the plant. Stem and root storage tissue may also act as either source or sink (net importers of photoassimilate), depending on needs of the rest of the plant. Young, developing leaves have high metabolic rates and expansions rates and thus are strong sinks. These developing leaves compete with other sinks of the plant and strongly influence allocation patterns. The conversion of developing leaves from sinks to sources involves profound structured and physiological changes.

The allocation of photoassimilate to different sinks is largely independent of photoassimilate production but is related to relative sink strength (Cline *et al.* 1997). Sink strength is the ability of the sink organ to import photoassimilates relative to other sinks in the plant and is related to sink size, growth rate, metabolic activity, and respiration rate. Developing sun leaves are relatively strong sinks while stem and root storage tissue are weak sinks. However, an inverse relationship would be found for shade plants.

From its inception as a primordium, until some time during that phase of lamina expansion, the developing leaf is dependent on import of carbon from other parts of the plant. Even after photosynthetic activity and carbon assimilation have commenced import occurs concurrently and only falls to low and insignificant amounts once lamina expansion is well advanced. An expanding leaf may be supplied from several older leaves in a complex pattern that changes with time. From primordial initiation until well into the unfolding phase the leaf is dependent on supply of metabolites from other parts of the plant. Although the significant developmental changes that occur during leaf expansion with respect to carbon relations can be identified, there remain major uncertainties about the nature of the controlling mechanisms.

One of the few integrating principles related to allocation is the hypothesis of balanced root and shoot activity. It has been reported (Poorter and Remkes 1990) that fast-growing species are more oriented to maximize shoot functioning, whereas the slow-growing them tend to maximize root functioning. However, water and nutrient supply would not be a limiting growth factor for the ornamental foliage plants grown under greenhouse commercial facilities such as happened in the primitive wild environment.

There are strong links between resource availability, the ability of plants to use resources for carbon gain and productivity, and, at the population level, parameters such as size, survival, and reproductive output (Bazzaz *et al.* 1987; Pearcy *et al.* 1987). In many species, the rate of export of photoassimilates from leaves and leaf photosynthesis rate are influenced by the assimilate demand from other parts of the plants (Mendez and Obeso 1993).

It has been suggested that leaf development may be affected through endogenous plant hormones, and based on early findings that CKs modify the distribution of metabolites in detached leaves the idea of a general promotive effect of CKs on sink strength has emerged. However, the specific mechanism by which CKs regulate the physiology of the plant cell is still not well-known (Nielsen and Ulskov 1992).

For addressing the question of CK effects on sink strength, those enzymes which contribute to the ability of the tissue to metabolize imported photoassimilates must be

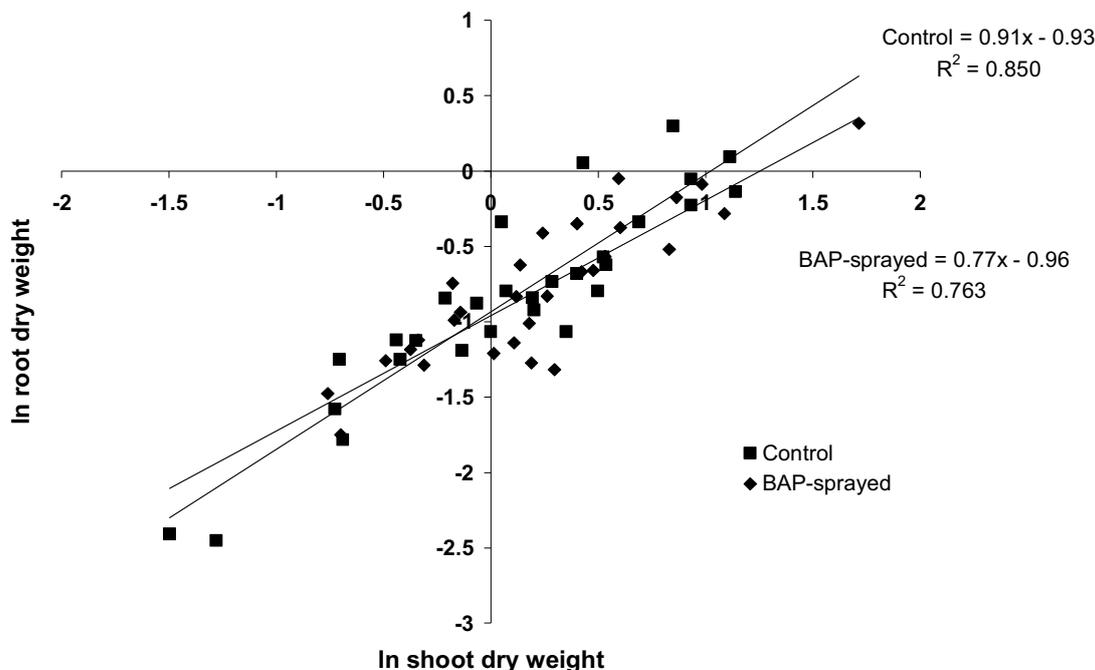


Fig. 12 Root-shoot partition from *E. aureum* plants sprayed with and without a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution (Appendix 1, Experiment 1, unpublished data).

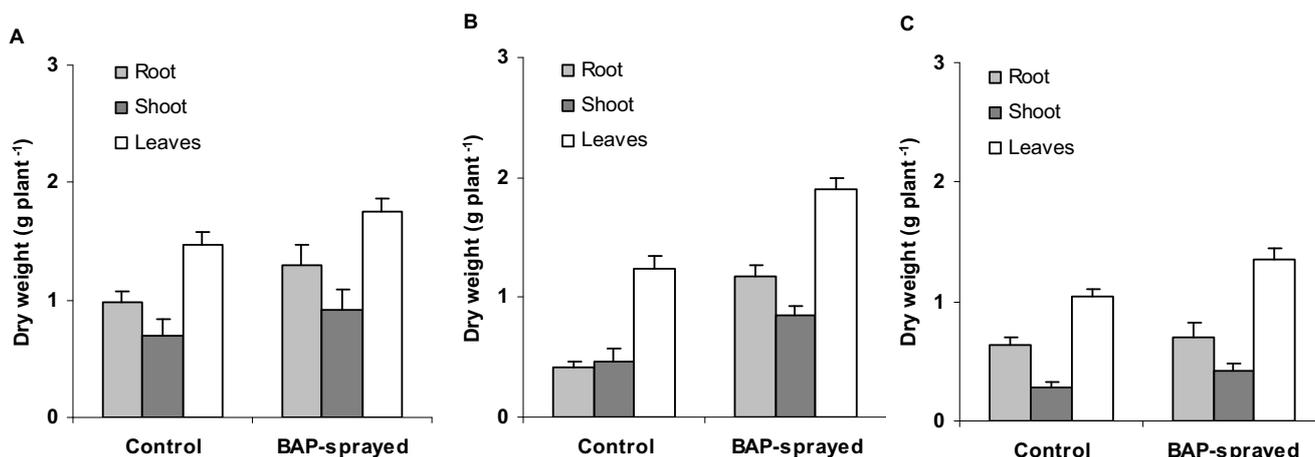


Fig. 13 Total dry weight for *E. aureum* plants sprayed with and without a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution grown at three light environments (70, 50 and 30% full sun) from *Scindapsus aureum* (A) and both *Ficus benjamina* ‘green’ (B) and ‘variegated’ (C) (Appendix 1, Experiment 3, unpublished data). Means of the replicates and standard errors are given.

monitored. Another approach would be to measure metabolism itself. If the action of CKs on leaf development were mediated through a change in carbon allocation in the plant, it should be possible to observe a changed import of sugars into a CK-responding sink leaf. This could result in increased biosynthesis of cell components required for the division and expansion of the cells. Alternatively, increased import of solutes could be the basis for decreasing water potential. This would lead to increased turgor pressure, which potentially could be the driving force for cell enlargement. Any of these possibilities should be revealed previously by studying carbon uptake and partitioning in CK-responsive tissue (Nielsen and Ulvskov 1992). These authors suggested that the action of CK on sink strength may rely on different mechanisms. Either the transport processes during unloading or the intracellular compartmentation and utilization of photoassimilates could be influenced in order to increase the ability of the tissue to take up photoassimilates. Alternatively, the carbon utilization in the tissue could be influenced in a manner that would specifically favor processes leading to increased expansion rate. These two possibilities are not mutually exclusive. The relevance of assimilate uptake into the leaf discs was tested by

changing the supply of sucrose and an increase the sucrose concentration to levels similar to *in-situ* levels in photosynthetic tissue, and this resulted in elevated expansion rates of the discs. This demonstrates that photoassimilate supply and expansion rate may be closely linked. However, the effect of hormone treatment was not dependent on the sucrose concentration which indicates that the CK-promoted area expansion is not linked to sucrose uptake (Nielsen and Ulvskov 1992).

Although the results presented by these authors suggest that BA-promoted expansion of the experimental leaf discs was not mediated through changes in net uptake or utilization of carbohydrates (Nielsen and Ulvskov 1992), in our laboratory, when *E. aureum* pot plants were sprayed with a BAP solution a significant change in assimilate partitioning was observed (Fig. 12; Appendix 1, Experiment 1, unpublished data).

The hypothesis that leaf growth is limited by photoassimilate availability included ornamental shade plants (Di Benedetto and Cogliatti 1990a) although the response of the leaves is generally slow; ten to twelve months and three to five leaves for *A. commutatum* plants were required to show changes in leaf size when plants were transferred from high

to low light intensities. The response to pothos plants to a BAP spray would be light-dependent (**Fig. 13; Appendix 1, Experiment 3**, unpublished data).

CKs are predominantly produced in the roots and transported in the xylem (Itai and Birnbaum 1996; Kudoyarova *et al.* 2007). Shaded leaves lose less water by transpiration than unshaded leaves and should thus receive less CKs, which would, in turn, decrease protein synthesis and, consequently, reduce the sink strength of the leaf for amino acids relative to other leaves. This would then result in net export of amino acids from fully expanded leaves due to reduced protein synthesis and ongoing protein turnover. For the same reason, shaded young growth in leaves would accumulate less protein than unshaded ones. Leaves receiving more CKs in the transpiration stream would act as stronger sinks (Pons and Bergkotte 1996).

Plant tissues and organs rich in CKs are known to attract the photoassimilate translocation. Such enhancement of the sink capacity is among the most characteristic and yet the least understood physiological outcomes of these phytohormones (Ron'zhina 2004). The basis of this phenomenon has not been as yet clarified, apparently because the integral and sophisticated processes of photoassimilate translocation and distribution comprise the numerous sinks and sources competing for photoassimilates.

Having considered the current concepts of the regulation of the photoassimilate translocation in plants it has been presumed that CKs could evoke such structural and metabolic rearrangements in the mesophyll cells that would change the metabolite concentrations and develop the corresponding concentration gradients between the sources and sink regions of leaves. In their turn, these changes in metabolite concentration would direct the photoassimilate translocation towards the CK-treated leaf region.

The sink-promoting effect of CKs in detached leaves is related to the source-to-sink transformation of the portion of the mature leaf and manifests itself by changing the direction of the phloem photoassimilate translocation. Ron'zhina (2004) suggested that between plant organs producing and consuming photoassimilates there is a driving force of the phloem transport. In detached leaves, the source and sink tissues would be represented by the mesophyll of the control and BAP-treated leaf halves, respectively. The radiochemical analysis of the composition of  $^{14}\text{C}$ -products of photosynthesis and electron-microscopic studies demonstrated that the CK-enhanced leaf growth was accompanied by redirecting the carbon metabolism, including the superior synthesis of the cell-wall, protein, and lipid components essential for cell growth and the promotion of starch synthesis. In addition to the mesophyll, CKs redirect metabolism in other leaf tissue as well. Thus, the study of the sink-promoting BA effects in detached sugar beet leaves (Paramonova *et al.* 2002) demonstrated starch and lipid accumulation in chloroplasts of the companion cells in the terminal phloem.

The effect of CKs on translocation processes in leaves as related to plant species and leaf developmental characteristics. Thus, in broad beans, the growth response to CKs was lower than in pumpkin plants, however, the former manifested greater changes in starch synthesis (Ron'zhina 2004). In the juvenile leaves, BAP promoted cell divisions in the mesophyll and did not notably affect cell expansion (Ron'zhina 2003). Exogenous supply of sucrose to the roots of girdled plants stored root CK export, suggesting that root sucrose levels regulate root CK export (Dodd 2005).

## ENVIRONMENTAL INFLUENCES

It is well known that most environmental factors can largely affect biomass production in plants. In several cases, at least part of these effects are mediated by CKs. For instance, decreased water availability, which severely affects leaf expansion, has been found to decrease leaf CK activity (Havlova *et al.* 2008). In this work, during drought, significant accumulation of CKs occurred in roots, while simultane-

ously, auxin increased in roots and lower leaves. This suggests that both CKs and auxin play a role in root response to severe drought, which involves the stimulation of primary root growth and branching inhibition. The possibility of alleviation drought stress by CK has also been suggested (Rivero *et al.* 2009). Similarly, nutritional status (especially nitrogen levels) may strongly affect leaf growth, and CKs may be involved in this response. It has been reported that, leaf expansion and xylem CK concentration decrease in response to nitrogen (N) deprivation (Dodd and Beveridge 2006; Hirose *et al.* 2008; Schachtman and Goodger 2008). Inorganic nitrogen sources, such as nitrate, are a major factor regulating gene expression of adenosine phosphate-isopentenyltransferase, a key enzyme of CK biosynthesis. Nevertheless, both water and mineral nutrition stresses are normally avoided in cultivation of ornamental foliage plants simply by giving plants appropriate supply of these factors. On the other hand, it is light and temperature stresses those most frequently encountered in these species due to technological difficulties with providing plants optimum levels of these factors under commercial facilities. We will subsequently deal with them in more detail.

## Temperature

In modern glasshouse cultivation, climate computers are used to monitor the plant environment. Ambient temperature must be controlled to optimize plant growth and yield. For most ornamentals foliage crops, however, detailed information on optimum temperature data is not yet available.

Most foliage plants have their origins in the tropics and require high temperatures to sustain rapid growth (Poole and Conover 1981). Other results showed that many foliage plants can benefit greatly if grown at significantly higher temperatures than usual at the commercial greenhouse facilities (Mortensen and Larsen 1989; Mortensen 1991). It is important to address the question of how temperature interacts with light to maximize growth during winter as well as to decide how much shading the plants should be given during summer.

For many crops, leaf primordia and leaf appearance rate increase near linearly with accumulated average temperature between the base temperature and an optimum value (Faust and Hein 1993). The fastest developmental rate under any average daily temperature occurs at a constant day and night temperature (Erwin and Heins 1990). Karlsson *et al.* (1991) have reported that a decrease in leaf unfolding rate is expected in all plants as temperature increases above the optimum temperature for maximum leaf growth. It must also be noted that significant errors in predicting leaf unfolding are expected if temperatures which fall below the base temperature for growth.

Base temperature values of ornamental crops ranged from 5.2°C for rose (Pasian and Lieth 1994) to 11.5°C for shade ornamental aroids (Boschi *et al.* 2004). Development of plants (including ornamentals) requires the accumulation a fixed amount of thermal units (Liu and Heins 1997, 1998). Different developmental processes can have different base temperature and thermal unit accumulations. For example, *Spathiphyllum floribundum* 'Petite' is quite a temperature-sensitive aroid crop compared with other foliage plants (Vogelezang 1991): information about optimal temperatures under greenhouse facilities indicates that temperatures should be between 18-20°C (Vogelezang 1992). However, although an 11.55°C base temperature for leaf expansion on main stem has been reported (Boschi *et al.* 2004), the developmental events between transplant, the first, second and third tiller emergence require 13.8, 8.25 and 10.27°C, respectively. Plant needs 108.0-306.5-469.5 and 642.9°C day<sup>-1</sup> for leaf expansion, first, second and third tiller emergence, respectively.

There are no reports on the effect of CKs in ornamental foliage plants temperature response but a previous work from Araki *et al.* (2007) showed that the use of BAP pre-transplant sprays on foliage vegetables, such as lettuce and

celery, would change the temperature base. Hye *et al.* (2007) showed that CK concentration decreased in leaves of cucumber plants grown at 15°C and especially at 35°C than in those of plants grown at 20°C root-zone temperature. These results suggest that growth inhibition at 15 and 35°C root-zone temperature was affected by low water status and CK concentration in cucumber leaves.

A decrease in temperature produces significant morphological and anatomical changes; leaves tend to become wider and shorter, and the length of the petiole and midrib may be reduced; a lower temperature increased leaf thickness and the amount of leaf tissue per unit area due to a greater mesophyll cell size. Many plant species used in outdoor gardening are limited in their growth and use because of changing temperature regimes; low temperature are especially important. Ornamental plants, usually tropical species, are often damaged by chilling in the range of 0 to 10°C (McConnell *et al.* 1978). Plants exhibit increased visible injury with decreased night temperatures or as exposure at a chilling temperature is prolonged. Sections of fresh tissue of chill-damaged leaves showed that the dark, greasy-appearing patches on the upper leaf surfaces occurred due to a collapse of epidermal cells. Hummel and Henny (1986), using detached leaves of *A. commutatum* 'Silver Queen', reported chilling injury was more severe on old leaves than on young ones. Injury was observed on leaves below the fourth node from the top of the plant (Fooshee and McConnell 1987). Chilling injury in *Phalaenopsis* sp. and *Fittonia verschaffeltii* were confined to immature leaves (McConnell and Sheehan 1978).

There are no reports on the effects of exogenous CK supply on chilling acclimation in ornamental shaded plants, but a possible role should not be ruled out since Savé *et al.* (1995) have shown that CK levels decreased in leaves and roots with decreasing temperatures, and leaf CKs retained the initial values in the recovered plants. Reduction in CK content would be caused by a chilling-related reduction in *de novo* synthesis and transport from the roots to the leaves.

### Light intensity

Radiant energy drives plant photosynthesis and, consequently, plant biomass production. Plants are classified into sun and shade plants depending on their adaptability to a selected light intensity. This adaptability is inherited; it is determined by the genotype and results from genetic adaptation to the light environment prevailing in the native habitat.

There are factors which are known to be capable of influencing the photosynthetic rate of an intact plant, and attempts have been made to assess their relative importance in determining the differences in photosynthetic characteristics of sun and shade plants. The capacity of light-saturated photosynthesis is expected to be independent of the efficiency of light absorption and the primary photochemistry. It will be influenced by one or more of the dark steps of photosynthesis: the resistance to CO<sub>2</sub> diffusion at the stomata, the rate of diffusion of carbon dioxide from the cell wall to the chloroplast, the carboxylation reaction and possibly other reactions of the carbon reduction cycle, and the rate of photosynthetic electron transport and photophosphorylation (Boardman 1977).

The potential range of individual adjustment of photosynthetic characteristics to light intensity differs considerably between sun and shade populations even within species of the same botanical genus (Table 5) (Di Benedetto, unpublished data). Foliage plants growers require a high productivity for these plants; for this objective, plants are grown in a light environment near photosynthetic light saturation point. Kinetic properties of shade-adapted plants indicate that their low capacity of light-saturated photosynthesis is caused by a low capacity of enzymatic steps.

It seems probable that the major factor contributing to an increased specific leaf area in response to shading is a reduction in several components of the photosynthetic sys-

**Table 5** Light-saturated photosynthetic rate for ornamental shade plants grown under greenhouse facilities (Di Benedetto, unpublished data).

Ornamental foliage plants	Light-saturated photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	
	Summer	Winter
<i>Aglaonema commutatum</i>	2.57	1.32
<i>Epipremnum aureum</i>	4.08	2.86
<i>Ficus benjamina</i>	4.11	2.77
<i>Philodendron</i> 'Burgundy'	2.61	2.43
<i>Philodendron peruvianum</i>	1.94	1.35

tem which governs the capacity at high quantum flux densities. However, it should be emphasized that changes in specific leaf area also likely to involve changes in the proportion of photosynthetically inactive to photosynthetically active leaf material. Although little quantitative information exists on this subject, rough estimates indicate that a reduction in the pool of photoassimilate, mainly sugars and starch, could perhaps account for up to a 20% increase in specific leaf area. A reduction in other photosynthetically active components such as epidermal tissue, cell walls, and vascular tissue could perhaps cause a similar increase.

There are conflicting reports on leaf anatomical changes in shade species in response to light. An increase in leaf thickness under low light was found in *Aphelandra squarrosa* (obligate shade plant) (McConnell *et al.* 1984) and *Fatsia japonica* (relatively light-tolerant shade plant) (Vidal *et al.* 1990). In contrast, Araus *et al.* (1986) reported a low anatomical adaptation pattern in the obligate shade plant *Philodendron scandens*, while Di Benedetto and Garcia (1992) showed an increase in leaf thickness related to high light intensities for three arum species.

It seems evident that a prerequisite for success in a low light environment is that the leaves must be able to trap the available light and to convert it into chemical energy with the highest possible efficiency. At the same time, respiratory losses and the cost of producing and maintaining the photosynthetic systems relative to the gain in photoassimilate production must be kept as low as possible owing to the fact that the highest possible fraction of the photoassimilate must be reinvested into photosynthetic tissue, i.e., that allocation to non-photosynthetic tissue be kept as low as possible. For instance, in some ornamental aroids grown in pots, dry mass allocated to the root system can be as low as less than 15% of total plant dry weight (Di Benedetto *et al.* 2006).

Shade plants often have a very low rate of dark respiration. This may be caused by regulation of respiration (primarily determined by the demand for ATP required for heterotrophic biosynthesis), but also with a lower content of respiratory machinery than in sun plants. In the latter case, shade plants would presumably incur a significantly lower cost also in the production and maintenance of the respiratory system (Bjorkman 1981).

The response to a BAP exogenous supply appears to be light-dependent too; Fig. 13 (Appendix 1, Experiment 3, unpublished data) shows that dry weight accumulation was higher at higher light irradiances for *E. aureum* plants grown under greenhouse facilities.

It is widely recognized that obligate shade plants may suffer from severe damage to their leaves, grow poorly, and even die when they are exposed to light intensities in excess of those normally encountered in their natural environments. This is a consequence of their inherent low ability to increase their capacity for effective utilization of high quantum flux densities for photosynthesis. The exposure of leaves to excess light could also cause an inhibition of starch formation and even disappearance of starch from the leaves. This phenomenon (photoinhibition) (Anderson and Osmond 1987) results from a direct inhibitory effect of excess light energy on photosynthesis, mostly observed as bleaching of the chlorophyll from the exposed leaf tissue. However, usually there are no troubles under greenhouse

facilities due to the use of plastic screens. Up to our knowledge, there are no reports on the possible photoinhibition alleviation with exogenous CK supply.

## HORMONAL INFLUENCES

### Endogenous stimulus

If we are to understand the control of leaf growth it seems essential to understand the ways in which the growth and activity of leaves related to the growth and activity of other plant organs (Horvath *et al.* 2003) including horticultural crops (Malladi and Burns 2007). The interplay between leaf and root growth is particularly important for two reasons. First, the roots provide the water needed to maintain turgor in the leaf and the mineral nutrients needed for growth. There is sound evidence that a 'functional equilibrium' exists between roots and shoots growth. The second reason why leaf-root interrelations are important involves the capacity of roots for hormone synthesis. If root growth is restricted in some way, shoot and leaf growth is also likely to be decreased. It has been suggested that the coordinated response was mediated via changes in the synthesis of CKs in the roots.

Hormones are one class of compounds to regulate developmental processes in plants; however, they are probably the most important mediators in signal transduction. The classic concept of a plant hormone as a substance being produced in one part of the organism and then transferred to another part to influence a specific physiological process is, as expected after more than 70 years of plant hormone research, inadequate to explain hormonal regulation of development in plants. The lack of exclusive control of any one step of development by any one of the known hormones as well as the lack of correlations between hormone concentrations and changes in developmental processes has led to doubts as to whether the plant hormones are indeed the regulators of development (Barendse and Peeters 1995).

An important aspect of hormone regulation is signal perception followed by transduction (Aoyama and Oka 2003; Muller and Sheen 2007). The transduction of signals involves the activation of a major part of the plant cell proteins, since cells have to perceive a variety of external signals, e.g. light, temperature, nutrients, several forms of stress and so on. This perception is transduced through internal signals or messengers into an appropriate response by activating receptors through binding. Signal transduction sequences start with signal receptors (Argyros *et al.* 2008). Variation in the number of receptors and their distribution can alter the sensitivity of cells to hormones or other signals as well as sensitivity changes during development. The site of perception of hormones is a hardly explored area in plant physiology, but for CKs the situation is not clear. Although some CK binding proteins have been reported (Binns 1994), none of these were proven to be CK receptors.

A further complication of the hormone concept is that a hormone produced at one site is directionally transported to exert its action at the target site, and that the compound produced may not be the same as the one acting at the target site. An individual hormone system may have numerous sites for control, and regulation may be accomplished by more than one step. Multiple hormonal controls may be based on different types of interaction between hormones; regulation may be achieved by a balance or ratio between hormones, by opposing effects between hormones, by alterations of the effective concentrations of one hormone by another, by alterations in the sensitivity to one hormone by another hormone, and by sequential actions of different hormones. Further complications arise from compartmentation; developing organs and tissues consist of different cell types, which mean that several hormone control systems could be operating within a tissue or organ. In addition, subcellular compartmentation of hormones does exist. Thus, the complexity of hormonal regulation of specific processes by several hormones has probably so far precluded substantial

progress in our understanding of multiple regulatory controls. Hypotheses on hormone action have generally failed to present a comprehensive description of how they induce their many effects. Moreover, most studies are confined to the action of one but rarely more than two hormones.

Finally, it has become clear that plant hormones not necessarily influence plant responses exclusively via changes in their concentration, but that hormonal regulation may also be exercised via changes in sensitivity of the target cells. Thus, interaction of two hormones may also mean that one hormone may change the sensitivity, and thereby the responsiveness, of tissue to another hormone bringing about the response. Another aspect of hormone interaction is that it may occur at different levels in the sequential cascade of processes initiated by the hormone, which includes interaction at distance (spatial) or interaction in time (temporal).

Traditionally, the root system has been perceived as providing essential resources (water and nutrients) to the shoot and in many circumstances reductions in shoot growth can be explained by the inability of the root system to adequately supply these resources. By adjusting the rate of nitrogen (N) supply to the roots, it has been possible to precisely control shoot N concentration and plant relative growth rate (Rubio *et al.* 2009). When water is withheld from the roots, leaf water status can decline in parallel with leaf gas exchange and leaf growth; however, shoot physiology cannot always be explained only by the availability of resources in the shoot (Dodd 2005).

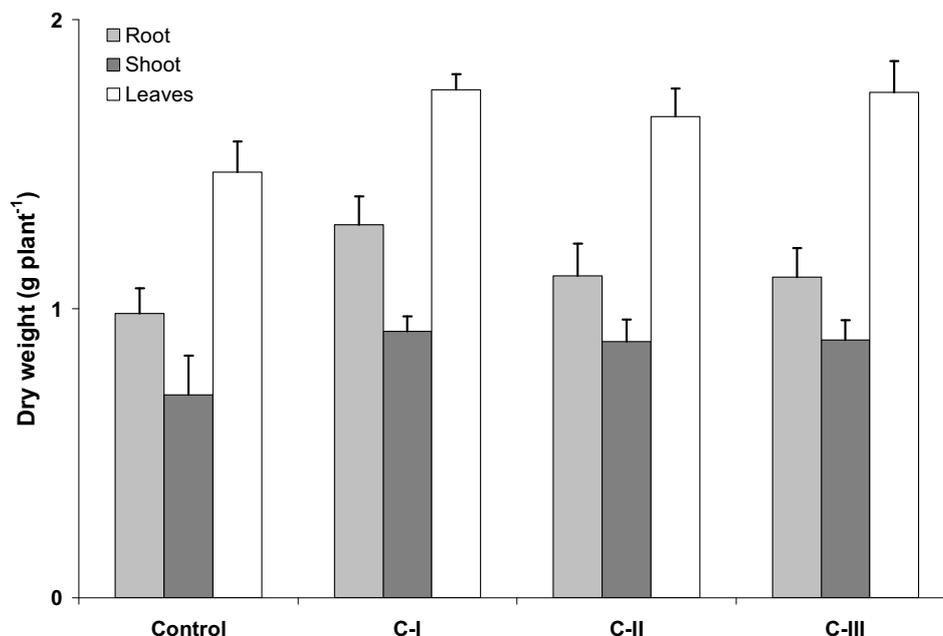
Thus, consideration of recent data suggests the existence of alternative mechanisms of CK signaling in the cell (Miyawaki *et al.* 2004; To and Kieber 2008). The membrane receptors of CKs considered in the previous section accept signals from exogenous CKs (López-Bucio *et al.* 2007). However, there are also intracellular CKs (Kulaeva and Prokoptseva 2004).

There is also evidence that long-distance signaling can regulate shoot gene expression following changes in nutrient supply to the root system, independently of changes in nutrient delivery to the shoot. Re-supply of N to N-starved maize (*Zea mays*) plant increased delivery of both N and CKs to the shoot and promoted shoot expression of gene (*ZmCip*) encoding a CK-inducible protein (Takei *et al.* 2002; Dodd 2005; Sakakibara 2006).

To fulfill the criteria of a root-to-shoot signal, a compound must:

- move acropetally in the plant via apoplastic (predominantly the xylem) or simplastic pathways;
- influence physiological processes in a target organ (such as leaves) that is remote from the putative site of synthesis (the root).

Although the detailed mechanisms remain to be worked out, in many cases it seems that changes in the root environment modify the hormone production in the root, change the export of hormone via the xylem sap to the shoot, and thereby elicit changes in the shoot. A coordinated phytochrome-CK growth regulation system responsive to a variable supply of nutrients and light has been proposed. In this model, mineral sufficiencies promote root growth; subsequent CK synthesis in roots promotes protein and chlorophyll synthesis in shoot. Phytochrome induction of nitrate reductase and mediation of nitrate transport from storage to metabolic pools provides a mechanism for feedback signals regulating nutrient cycling and metabolism. Conclusive evidence of hormone regulation of N-mediated growth responses requires further investigation to identify factors regulating N cycling between root and shoot under N-limited growth. The role of CKs in establishing sink strength and the effects of root synthesis of CKs on shoot synthesis and subsequent responses can only be understood in the context of signal transduction within whole plants (Aiken and Smucker 1996). Data from Fig. 12 (Appendix 1, Experiment 1, unpublished data) and Fig. 13 (Appendix 1, Experiment 3, unpublished data) suggest a feedback regulation associated to photoassimilate availability and partitioning between roots and shoots when exogenous BAP supply was involved.



**Fig. 14** Total dry weight for *E. aureum* plants sprayed with and without a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution at different times (I-II-III: one, two or three monthly sprayed) (Appendix 1, Experiment 4, unpublished data). Means of the replicates and standard errors are given.

CK signals are perceived by trans-membrane histidine kinase receptors in the control of phyllotaxy and regulate gene expression through transcription factors known as response regulators; the molecular details of this pathway have been described in detail but the precise biological functions of the response regulators are unknown (Giulini *et al.* 2004; Lee *et al.* 2009). Although 40 years have passed since the first discovery of a naturally occurring Cks in plants, the biosynthetic pathway still remains unresolved. Numerous factors have contributed to the lack of success in this area, but most alarming has been the reliance upon unproven hypotheses and preconceived ideas. The greatest obstacle has been the identification and isolation of CK biosynthetic enzymes in plants, although the classical isoprenoid CKs biosynthetic pathway has been partly challenged and it has been suggested that a multiple pathways exist in plants (Taylor *et al.* 2003).

The question if endogenous stimulus is inductive (i.e. one simple event or CK application is enough for eliciting response) or accumulative (prolonged or repeated exposure to stimulus) remain unresolved. Data from our laboratory about total dry weight on pothos response to BAP (Fig. 14; Appendix 1, Experiment 4, unpublished data) suggest that the other responses (i.e. total leaf area, leaf appearance, single leaf area, specific leaf area, photosynthetic rate, relative growth rate, leaf thickness and both cell and stomata densities) would be associated to an inductive mechanism from a single low-concentration BAP application, but, nevertheless a second spray one month later increase pothos dry weight accumulation to BAP.

## AUXIN-CK INTERACTIONS

The initiation and development of adventitious roots in cuttings are highly complex processes, influenced by both endogenous and exogenous factors (Ashvarya *et al.* 2006). Evidence has been provided to show that in the root meristem CK acts in defined developmental domains to control cell differentiation rate, thus controlling root meristem size (Ioio *et al.* 2008). Pernisova *et al.* (2009) proposed that an important mechanism of this CK action is its effect on auxin distribution via regulation of expression of auxin efflux carriers; while, Ruzicka *et al.* (2009) has suggested that CKs influence cell-to-cell auxin transport by modification of expression of several auxin transport components and thus modulates auxin distribution important for regulation of

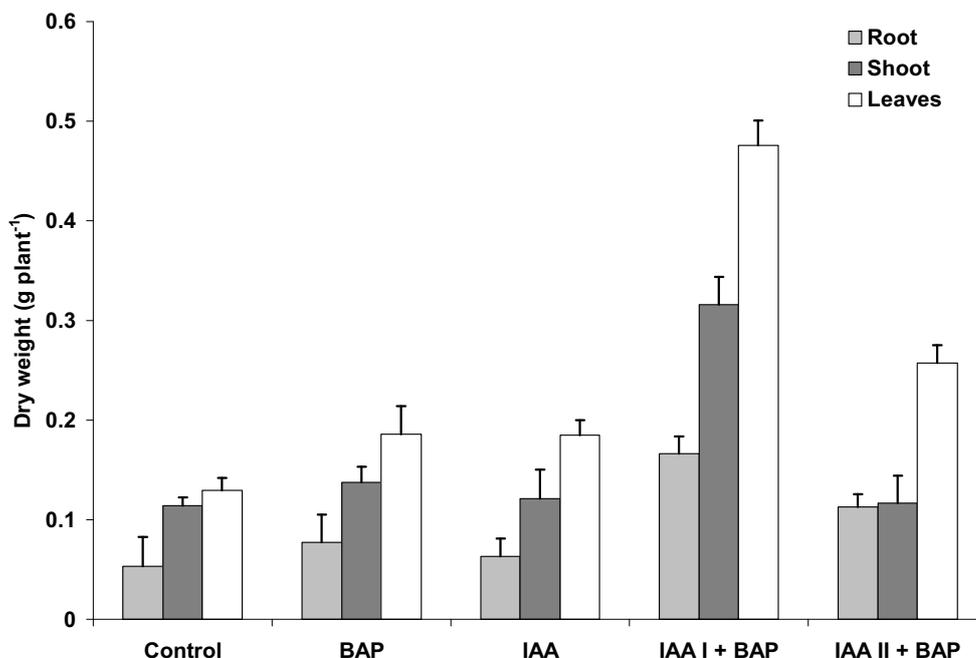
activity and size of the root meristem.

As opposed to auxins, CKs are considered to be inhibitors of adventitious root formation (Bollmark and Eliasson 1988; Lohar *et al.* 2004). CKs have been known to inhibit primary root elongation and suggested to act as an auxin antagonist in the regulation of lateral root formation. While the role of auxin in root development has been thoroughly studied, the detailed and overall description of CK effects on root system morphology, particularly that of developing lateral root primordia, and hence its role in organogenesis is still in progress (Kuderova *et al.* 2008).

Adventitious root formation and development can be divided into three phases (Wiesman and Riov 1994). The first phase persists until the first cell divisions. During this phase there is increased sensitivity to IAA. The second stage is the period of radicle induction, and is characterized by active cell division, increased sensitivity to metabolic inhibitors, and a lack of sensitivity to IAA. The final phase, resulting in the formation of a root, is characterized by the elongation of the root, diminished sensitivity to inhibitors, and a lack of response to IAA (Taylor and van Staden 1997).

The application of CKs to stem cuttings generally inhibits adventitious root formation, the early stages of the process being the most sensitive (Kuroha and Satoh 2007). Plants alter the architecture of their root systems to adapt to the environment by modulating post-embryonic (lateral and adventitious) root formation and growth. An example where the application of CKs has promoted adventitious root formation is rare because usually CKs affect lateral root founder cells and inhibited root initiation (Li *et al.* 2006; Laplaze *et al.* 2007), but Fig. 15 (Appendix 1, Experiment 5, unpublished data) shows that it is possible to stimulate both root and shoot growth with low concentration solutions of BAP sprayed with and without a previous indole-3-acetic acid (IAA) application in *Impatiens* New Guinea cuttings.

Results obtained by Ericksen (1974) seem to indicate that the influence of CK changes with the stage of root development. There is possible an interaction between CKs and other growth hormones, specifically auxin (Kotoba *et al.* 2004); CKs in high concentrations may have an inhibitory effect on an early stage in rooting by blocking the activity of auxin. The loss of the inhibitory effect of CKs during the latter phase of initiation suggests that at this stage, developing root primordia are capable of controlling the level of active CKs and thus do not react to the exogenous application of this hormone. This then implies that the control of



**Fig. 15** Total dry weight for *Impatiens* New Guinea plants sprayed with a 5 mg L<sup>-1</sup> indole-3-acetic acid (IAA) seven (I) or 15 (II) days after cutting followed by a 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution seven days later (**Appendix 1, Experiment 5**, unpublished data). Means of the replicates and standard errors are given.

CK levels during the early phases of root initiation lies elsewhere. The two possibilities that arise are the ability of the basal stem tissue to metabolize CKs, and to influence their transport to and from the leaves. It was suggested that CKs are essential growth substances for the latter part of the initiation phase (i.e. when root primordia develop into roots), and that partially initiated root primordia can synthesize CKs, being self-sufficient for these hormones. The results from Muller and Sheen (2008) provided a molecular model of transient and antagonistic interaction between auxin and CK critical for specifying the first root stem-cell niche.

Morphological and environmental factors can affect the ease of rooting of cuttings, but this may or may not be affected through a change in CK levels. CK concentrations differ markedly between juvenile and adult plants with differing rooting ability. It has been found that levels of polar CKs in juvenile plants were higher than in adult plants at the stage of bud activation, just prior to the breaking of dormancy (Taylor and van Staden 1997). Another factor that may affect the rooting capacity of the cutting at these stages is the qualitative and quantitative differences in the CKs present (Taylor and van Staden 1997).

Immediately following adventitious root induction there is a substantial increase in the non-polar compounds exhibiting CK-like activity. These active forms of CKs show their highest levels once the roots have emerged from the epidermis and have begun to elongate. This implies differing roles for the different CK types at different developmental stages, and starts to explain the differing sensitivities shown by cuttings to the application of CKs at various developmental stages of root formation (Taylor and van Staden 1997).

It is also evident from these results that this easy-to-root plant undergoes significant changes in both the levels and types of the CKs present in the lower portion of the stem cutting during the course of root initiation and development. Differences in levels and activity of these enzymes could thus play a role in the stems ability to initiate adventitious roots. This lends support to the theory that it is not so much the initial levels and/or CK complement that is important in the ability of the cutting to initiate and develop adventitious roots, but the ability of the stem tissues to change these levels through metabolism, allowing inactivation and/or transport away from the site of root initiation (Taylor and van Staden 1997). This would imply that difficult-to-root

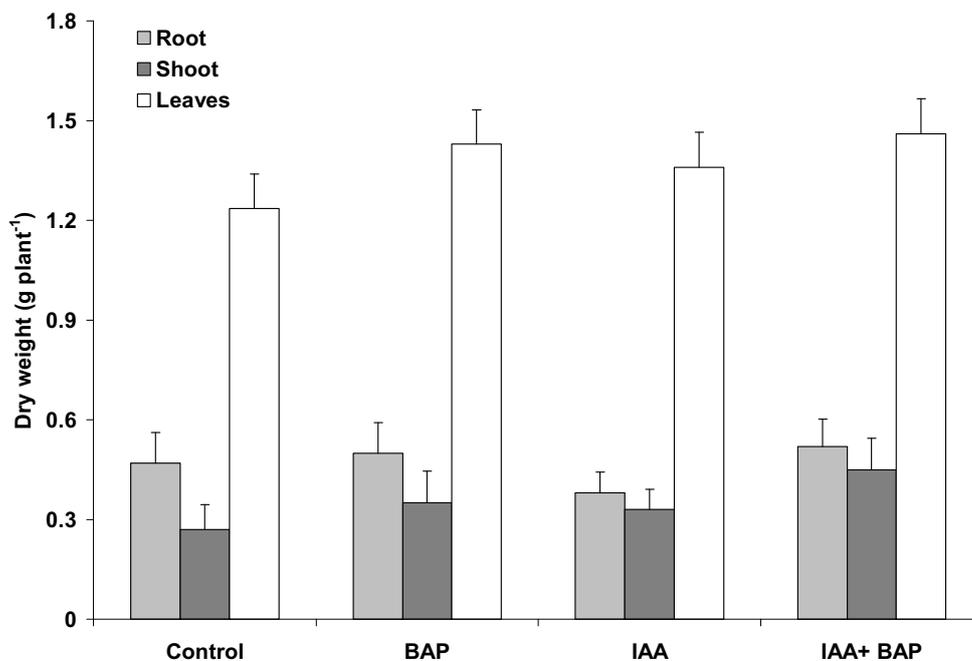
species lack this ability. Unpublished data (Di Benedetto, Tognetti and Galmarini) on *Ficus benjamina* 'green' and 'variegated' clones are in agreement with this possibility.

Synthesis and transport of growth regulators and phytohormones is one of the mechanisms for integration of growth of plant parts during development. The auxin-CK interactions and the balance of these hormones play a key role in this integration (Cline 1997; Coenen and Lomax 1997; Kotova *et al.* 2004).

There has been considerable interest in the hypothesis that the ratio of CKs to auxin controls apical dominance (Klee and Romano 1994, McSteen 2009); recent findings about the participation of both hormones in this process have been reviewed by Chao *et al.* (2007) for dicotyledons and by Assuero and Tognetti (2010) for grasses. Over the years it has been demonstrated in most species that exogenous auxin applied to the stump of a decapitated shoot will repress outgrowth of one or more of the lower axillary buds (Cline 1996; Tworowski and Miller 2007) and that the direct application of CKs to the axillary bud of an intact shoot will often promote the initiation or outgrowth of this bud.

It may be that CKs directly enter the bud and initiates outgrowth whereas auxins influence is indirect via its effect on CK production and/or transport. That being the case, it would seem likely that CKs antagonists would repress bud outgrowth. Although little is known concerning the effects of these compounds on bud growth, Valdes *et al.* (2007) have reported that the enzyme activity is affected by the endogenous supply of CKs but CK affinity for the ribosylated form of isopentenyladenine was dependent on the developmental stage, being higher in seeds than in seedlings, and not detectable in plantlets. The results are indicative of the presence of different isoenzymes throughout the development.

The fact that alterations in auxin and CK contents in shoots (either in exogenous treatments or by overproduction in transgenic plants) can significantly affect lateral bud outgrowth together with the fact that these hormones are naturally present in plant tissue is suggestive that apical dominance may be strongly influenced by the interaction between these two growth substances. Although the precise mechanism of action of these antagonists is not fully understood, the evidence of various workers demonstrating the important role of CKs in the promotion of bud formation and its outgrowth is convincing (Cline 1994).



**Fig. 16** The effect of 5 mg L<sup>-1</sup> indole-3-acetic acid (IAA) and 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution on total dry weight for *E. aureum* plants (Appendix 1, Experiment 5, unpublished data). Means of the replicates and standard errors are given.

The presence of CKs in the lateral bud would be required for its outgrowth. The major inhibitory influence of auxin on bud outgrowth would be indirect via inhibition of CK production in the roots. If auxin must move to the roots before it can inhibit CK production, then it could be presumed that the closer are the auxin treatments to the roots (assuming auxin penetration into the stem and movement to the roots), the more effective would be their inhibition of CK production and the more complete would be their repression of bud outgrowth. Hence, the critical factor would not be the distance between the locations of the auxin treatment on the stem to the repressible lateral bud but rather it would be the distance to the roots.

The auxin-CKs interactions on photoassimilate production and partition have been unexplored. Data for our laboratory on rooted plants sprayed with BAP following to an IAA application showed not significant differences related to a separated spray with these two hormonal regulators although both increased dry weight in relation to the control potted plants (Fig. 16; Appendix 1, Experiment 5, unpublished data).

## CONCLUDING REMARKS

Plant hormones are tightly regulated in response to environmental and developmental signals. It has long been speculated that biosynthesis of hormones occurs broadly in plant organs and that intricate, spatiotemporal regulation of hormones in developing organ primordia is achieved through transport and signal perception. However, recent identification of genes crucial for biosynthesis of auxin and CK reveals that localized hormone biosynthesis also plays an important role in organ growth and patterning (Zhao 2008).

CKs play a major role in many different developmental and physiological processes in plants, such as cell division, regulation of root and shoot growth and branching, chloroplast development, leaf senescence, stress response and pathogen resistance (Heyl and Schmülling 2003; Seilaniantz *et al.* 2007). Chernyad'ev (2005) showed that a treatment with CK-type phytohormones is a means of increasing the stability of the photosynthetic apparatus in agricultural plants exposed to unfavorable environmental factors, because CKs are known to play an important part in the regulation of photosynthesis and other physiological processes. He suggested that the protective effects of CKs,

exerted on the photosynthetic apparatus under the conditions of stress, may be subdivided into three types: (1) formation and renewal of cellular structures; (2) regulation of the resistance of stomata; and (3) induction and activation of protein synthesis.

It has been proposed earlier that CKs have a role in coordinating root and shoot development by carrying information about the nutritional status of the root to the shoot. It was recently shown that nitrogen treatment of nitrogen-starved plants leads to increased CK export from the root. CKs are then transported via the xylem to the leaves, where they cause enhanced expression of type-A response regulator genes (Sakakibara *et al.* 1998, Taniguchi *et al.* 1998, Takei *et al.* 2002). Thus CKs may represent a long-distance signal for the relief of nitrogen starvation that is able to trigger downstream events in shoot tissues. A similar CK-light interaction has been described (Fankhauser 2002). The results from Werner *et al.* (2008) are consistent with the hypothesis that CKs have central, but opposite, regulatory functions in root and shoot meristems and indicate that a fine-tuned control of catabolism plays an important role in ensuring the proper regulation of CK functions.

Introductory experiments with primary leaves of the C<sub>4</sub> plant *Zea mays* L. revealed that benzyladenine supplied exogenously stimulates photosynthetic activity, chlorophyll accumulation, enzyme activity and development of mesophyll and bundle sheath chloroplasts under certain circumstances. But it is not sure whether endogenous CKs fulfil the same regulatory role (Chernyad'ev 2005).

The import of CKs could not be correlated with plant development, as it increased linearly with time. The estimated relative degradation rate of CKs in the shoot decreased as the plants matured. The half-life of CKs in the shoot was found to be approximately 1 day, indicating that CKs are rapidly metabolized in the shoot (Dieleman *et al.* 1997).

CK-modulated resource allocation has received little attention regarding competition within a single organ. Such competition for photoassimilates within a single organ may exist in rapidly expanding leaves. This gradient is strongly correlated to carbon partitioning of the tissue which is further underlined by the studies of the regulation of the carbohydrate metabolism (Ulvskov *et al.* 1992). They often strongly influence the number of leaves that a shoot will produce, the appearance rate of leaves and final leaf size. It

seems important that the effects of such early influences on the rates of leaf initiation and appearance are clearly understood if subsequent patterns of leaf expansion are to be correctly interpreted.

In summary, saleability of tropical ornamental foliage plants and grower's profit demands high plant quality and productivity. The previous and unpublished results included here showed that the more a plant invests in leaf area, the higher the total carbon gain and the faster growth will be. Most foliage plants have their origins in the tropics and require relatively high night temperatures to sustain rapid growth (Poole and Conover 1981); a significant change in growth rates would be associated to environmental seasonal influences. Despite the high climate environmental control and technology facilities, pot ornamental plants support several stresses related to pot size (Di Benedetto and Klasman 2004, 2007; Di Benedetto and Rattin 2008) and growing media quality (Di Benedetto 2007) for which the knowledge of the whole plant physiology is necessary in order to devise a greenhouse management program which is close to optimal with respect to growth. In this context the use of BAP spray solutions would be a new tool to increase ornamental shade plant productivity but the precise dose-effect relationships would be previously adjusted by each ornamental plant.

## ACKNOWLEDGEMENTS

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## APPENDIX 1

Ornamental foliage plants were collected from a commercial source and all the experiments were carried out at the greenhouse facilities from Buenos Aires campus, Argentina (34°28'S) from the beginning autumn to the end of summer. The greenhouse was covered with black shade cloth (30% sunlight) during summer. Plants were harvested at the end of summer and winter season respectively, dried at 80°C for 48 h and weighed to obtain the dry aerial and root biomass weight. Leaf area was determined with a LI-COR 3000A automatic planimeter. Data were subjected to one-way ANOVA for a completely design analysis and means were separated by the Tukey's test ( $p \leq 0.05$ ) or changes were estimated through a straight-line regression too; the significance of differences was determined through the test for zero slopes and test for zero intercept.

**Experiment 1:** plants of *Epipremnum aureum*, *Impatiens* 'New Guinea', and *Ficus benjamina* ('green' and 'variegated' clones) were grown from 2007 to 2008 on the greenhouse floor. Net rate of CO<sub>2</sub> uptake was measured at ambient O<sub>2</sub> and CO<sub>2</sub> concentrations, on the last fully expanded leaf, using a portable LICOR LI-6200 system. Leaves were sprayed with 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) solution seven day after transplant; a control was included for each ornamental shade plant tested. Individual and total leaf area and dry weight were determined and samples for anatomical attributes (leaf thickness, cell density and stomata density) and total chlorophyll were collected.

**Experiment 2:** plants of *E. aureum* with three growth habits (floor, climbing and downward-oriented) were grown between 2007 and 2008 under the same greenhouse facilities as experiment 1 and sprayed with a BAP solution seven days after transplant. Individual leaf area and dry weight were determined.

**Experiment 3:** plants of *E. aureum* sprayed with and without a 5 mg L<sup>-1</sup> BAP solution grown at three light environments (70, 50 and 30% full sun) between 2008 and 2009. Dry weight was determined.

**Experiment 4:** plants of *E. aureum* were grown from 2008 to 2009 under the same greenhouse facilities as experiment 1 but BAP sprays were used at different times (I-II-III: sprayed once, twice or three times monthly). Dry weight was determined.

**Experiment 5:** plants of *E. aureum* and *Impatiens* 'New Guinea' were grown from 2008 to 2009 under the same greenhouse facilities as experiment 1. They were sprayed with a 5 mg L<sup>-1</sup> indole-3-acetic acid (IAA) seven (I) or 15 (II) days after cutting followed by a 5 mg L<sup>-1</sup> BAP solution seven days later. Dry weight was determined.