SHADE INTENSITY AND ITS EFFECT IN MORPHOLOGY AND PHYSIOLOGY OF POINSETTIA (*Euphorbia pulcherrima* Willd.)

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SUMMARY

The effect of five shade levels (30, 48, 58, 78, and 92 %) imposed 15 days after transplant on stem elongation, mesophyll thickness, foliar area, bract area, shoot dry weight, CO_2 fixation rate and chlorophyll content of poinsettia (*Euphorbia pulcherrima* Will. ex Klotzsch) cv. Subjibi was studied under greenhouse conditions. Stem length and foliar area increased and reached in average 325.5 mm and 0.216 m^2, respectively, under 48, 58 and 78 % shade while bract area reached the maximum value under 48 % shade. Mesophyll thickness increased up to a value of 151.34 mm under 30 %, but it was significantly reduced at 92 % shade. Shoot dry weight increased under 48, 58 and 78 % shade but decreased significantly at 30 and 92 % shade. At 60 days after transplant the rate of CO_2 fixation increased steadily from 30 % shade reaching a maximum value under 58 % shade, from this point CO_2 fixation rate diminished and reached the lowest rate at 92 % shade. Chlorophyll content was determined twice, 90 and 128 days after transplant, in the first date the concentration of chlorophyll *a*, *b* and total raised steady as the shade intensity was increased and 92 % shade induced the maximum chlorophyll concentration; 128 days after transplant the absolute values of chlorophyll *a*, *b* and total were higher than 90 days after transplant, but the raise in chlorophyll content observed as the shade intensity increased was not significant. This study makes clear that as the shade intensity increased a delay in development of color in the bracts occured. Among the five shade treatments tested, 48 % shade produced the best quality plant.

ADDITIONAL KEY WORDS: CO_2 assimilation, chlorophyll content, mesophyll thickness, leaf area, shoot weight.

INTENSIDAD DE SOMBRA Y SU EFECTO EN LA MORFOLOGÍA Y FISIOLOGÍA DE NOCHEBUENA (*Euphorbia pulcherrima* Willd.)

RESUMEN

Se estudió el efecto de cinco niveles de sombra (30, 48, 58, 78, y 92 %) en el crecimiento del tallo, grosor del mesofilo, área foliar, área de bráctea, peso del vástago, tasa de fijación de CO_2, y contenido de clorofilas en plantas de nochebuena (*Euphorbia pulcherrima* Will. ex Klotzsch) cv. Subjibi, a partir de los 15 días posteriores al transplante en invernadero. La longitud del tallo y el área foliar incrementaron con 48, 58 y 78 % de sombra y alcanzaron 325.5 mm y 0.216 m^2 en promedio, respectivamente, mientras que el área de las brácteas alcanzó el valor máximo con 48 % de sombra. El grosor del mesófilo incrementó hasta 151.34 mm con 30 %, pero fue reducida significativamente con 92 % de sombra. El peso seco del vástago incrementó con 48, 58 y 78 % de sombra, pero disminuyó significativamente con 30 y 92 % de sombra. Sesenta días después del transplante la tasa de fijación de CO_2 incrementó gradualmente desde 30 % de sombra y alcanzó un máximo a 52 % de sombra. A partir de este punto la fijación de CO_2 disminuyó y alcanzó el valor más bajo en el tratamiento de 92 % de sombra. El contenido de clorofila fue determinado a los 90 y 128 días después del transplante, en la primera fecha el contenido de las clorofilas *a*, *b* y total aumentó con el sombreado, y 92 % de sombra indujo la máxima concentración de clorofila; 128 días después del transplante los valores absolutos de clorofila *a*, *b* y total fueron mayores que a los 90 días posteriores al transplante, pero el aumento del contenido de clorofila no fue significativo. El presente estudio mostró que el incremento del sombreado disminuyó la coloración de brácteas. Entre los cinco niveles de sombra evaluados, el 48 % produjo las plantas de mejor calidad.

PALABRAS CLAVE ADICIONALES: Asimilaión de CO_2, contenido de clorofila, grosor de mesófilo, área foliar, peso de vástago.

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INTRODUCTION

The production of high quality ornamental plants involves knowledge of proper cultural procedures and the specific environmental conditions where these plants can reach the best morphological characteristics desired for the market. Light intensity is one of the main environmental factors determining the morphology and physiology of plants. However, morphological and physiological plasticity can differ among species, and different species might exhibit different growth responses to similar environmental conditions (Körner, 1991). A typical morphological response under conditions of low photosynthetic photon flux density (PPFD) is an increase in stem elongation and a reduction in leaf dry mass per area (Warrington *et al*., 1988; Niinemetes, 1999). Physiological responses involve changes in foliar chemistry at the levels of carbon fixation and reduction, photosynthetic electron transport components, and protein and pigments associated with the light harvesting complex.

Several reports have shown consistently that plant quality of floral crops is affected by the level of PPFD where they are grown. In *Begonia* x *semperflorens-cultorum*, shoot dry weight, lateral shoot leaf area, total leaf area, and node number decreased over time under increasing shade (Kessler and Armitage, 1992).

Poinsettia is a species native to Mexico and is used world-wide as an ornamental plant during Christmas. In recent time the production of this plant has been increased in many States of México and therefore knowledge about the best environmental conditions required to produce high quality plant for the market demand are urgent.

In the present study, the effect of five different shade levels in morphology and physiology of *Euphorbia pulcherrima* was investigated.

MATERIALS AND METHODS

Rooted cuttings of *Euphorbia pulcherrima* Willd ex. Klotzsch cv. Supjibi were obtained from PLANTEC, Morelos, Mexico. Cuttings were transplanted into 0.15 m diameter pots filled with 30 % forest soil, 50 % coir dust and 20 % “tepojal” (an inert volcanic substrate). The substrate was previously sterilized with steam and then amended with a commercial fertilizer (osmocote, 14-14-14) was added and also some minor elements (zinc, iron, magnesium, cooper and manganese) to a dose of 2.0 $kg{\cdot}m^{-3}$. The pots were watered to field capacity and left in a greenhouse with 60 % artificial shade maintaining a high relative humidity (75 to 80 %) for a week (according to the commercial propagator instructions). Fifteen days after transplanting (DAT) the plants were pinched, divided in groups and assigned randomly to five different shade levels: 30, 48, 58, 78 and 92 %, these values were calculated assuming 100 % maximum radiation received outside the greenhouse in a clear day. The different shade levels were achieved with shading nets of different nominal transmission values and measuring the photosynthetic photon flux density (PPFD) every week in each treatment at the top of the plants with a light quantum meter sensor (Licor, model LI-185A). The plants were maintained under these conditions until 60 DAT, when flower initiation was induced by reducing the photoperiod to nine hours (the frames were covered with black plastic every day from 17:00 to 8:00 h during eight weeks). The plants were watered every day to field capacity and fertilised once per week with a modified Hogland´s nutrient solution (Epstein, 1972). The average maximum and minimum temperatures registered inside the greenhouse were 35/10 ºC.

Once the shade treatments started (15 DAT) the elongation of one branch from ten different plants on each shade treatment was measured using a flexible plastic rule every week for 15 weeks.

Sixty DAT CO_2 assimilation was evaluated in the youngest full expanded leaf from ten different plants for each treatment; for this evaluation a portable open infrared gas exchange system was used (CIRAS1, PPSystems).

Chlorophyll content was quantified twice, during vegetative stage, 90 DAT, and during reproductive stage, 128 DAT, using two 20 mm diameter leaf discs from five different plants. Discs were incubated in 5 ml N,N-dimethylformamide at 4.0 ºC for 48 hours and then the absorbance of 1.0 ml sample was determined at 664.5 nm for chlorophyll *a* and at 647 nm for chlorophyll *b* using a spectrophotometer (Spectronic 21D, Milton Roy). Extinction coefficients developed by Inskeep and Bloom (1985) were used to estimate chlorophyll concentration.

Mesophyll thickness was measured in 100 mm^2 in 127 DAT leaf tissue. In this case, the leaf of the third node from the base to the apex from five different plants of each treatment was used. Tissue was fixed in formaldehyde-acetic acid-ethanol (Berlyn and Miksche, 1976) for 24 h and then a butyl alcohol series was used for dehydration followed by embedding with paraffin, sectioning into 10 mm ribbons and mounting on glass slides with chrome adhesive. Staining was accomplished using a safranin-fast-green system.

At the end of the experiment (170 DAT) foliar and bracts areas were determined in a leaf area meter (LI-3100 area meter, LI-COR, Inc. Lincoln, Nebraska) and the dry weight of aerial biomass from ten different plants was quantified on each treatment.

The experiment was arranged and analysed as a completely randomised design. The statistical procedures included analysis of variance and multiple comparison of means by Tukey's test. All analyses were carried out using the SAS statistical software (SAS, 1989) package for personal computer.

RESULTS

In July when the experiment started, the maximum light intensity was received and it was proportional for each of the shade levels (Table 1). As time went on a gradual reduction in PPFD was recorded and in November, when plants were ready for sale only about half of the initial PPFD value was obtained.

TABLE 1. Photosynthetic photon flux density (PPFD) received inside the greenhouse during five months under five shade levels, which were calculated assuming 100 % maximum radiation outside the greenhouse in a clear day.

	PPFD ($mmol{\cdot}m^{-2}{\cdot}s^{-1}$)				
Shade (%)	July	August	September	October	November
30	1220	944	757	738	720
48	858	715	605	565	546
58	720	651	485	436	428
78	374	224	322	202	192
92	147	116	92	77	73

The different shade treatments had strong effects in the morphological and physiological traits. Growth, measured as stem elongation was strongly inhibited under 30 and 92 % shade while 48, 58 and 78 % shade treatments produced the tallest plants though non statistical difference among these three treatments was found (Figure 1).

The CO_2 assimilation rate evaluated 60 DAT increased non-significantly from 5.8 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$ under 30 % shade to 7.9 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$ at 58 % shade, from this point CO_2 assimilation was drastically diminished to values of 3.3 and 2.6 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$ for 78 and 92 % shade, respectively (Figure 2).

Chlorophyll *a* content at 90 DAT (vegetative stage) increased gradually as shade increased and at 92 % level the concentration was highest. In contrast, chlorophyll *b* content was constant from 30 to 78 % shade (Figure 3). At 128 DAT (reproductive stage) the absolute values of chlorophyll *a*, *b* and total were higher than in the vegetative state and in inspite a tendency to increase chlorophyll content as shade increased it was not significant (Figure 4). Contrasting effects of different shade levels in chlorophyll *a* and *b* during vegetative stage are clearly observed when *a/b* chlorophyll ratio is calculated from Figure 3.

Shade increase had an inverse effect in mesophyll thickness (Figure 5). Thirty percentage shade produced the thicker leaves; 48 and 58 % shade had the middle values in this variable without statistic difference between them and 78 and 92 % treatments presented the thinner mesophyll with values 37 % lower than that reached under 30 % shade.

Foliar area had the higher values at 48, 58 and 78 % shade with an average of 0.20 m^2 per plant. Thirty and 92 % induced the lower values in this variable (Figure 6a). Bract area was reduced under 30 % shade, thereafter a maximum value of 0.0943 m^2 was obtained under 48 % and then a progressive reduction was observed as the shade level increased (Figure 6b). It is important to mention that 48 % shade was the first treatment to induce the development of color in the bracts starting 120 day after transplant, followed by 58 % shade 125 day after transplant, then 30 % shade 136 days after transplant and 78 and 92 % shade were the latest treatments to induce color in the bracts 144 and 155 days after transplant, respectively.

It is clear that the lower and higher shade levels induced a reduction in dry weight with average values of 10.5 and 7.0 g, respectively (Figure 7). Level of 48 % shade caused a major accumulation of dry weight with an average value of 24 g, while 58 and 78 % produced in average the same amount of dry weight with medium values compared with the other treatments (17 g).

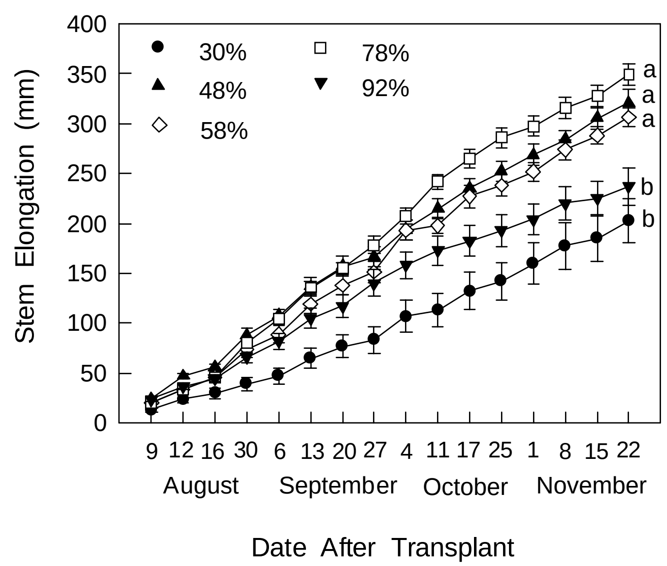

Figure 1. Effect of five shade levels on stem elongation of *Euphorbia pulcherrima* Willd. cv. Subjibi growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

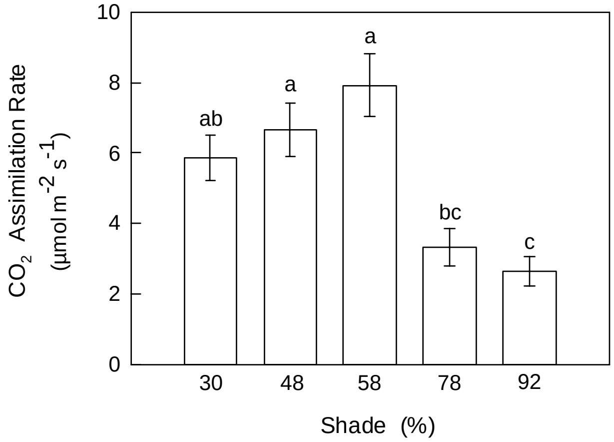

Figure 2. Effect of five shade levels on CO_2 assimilation of *Euphorbia pulcherrima* Willd. cv. Subjibi growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

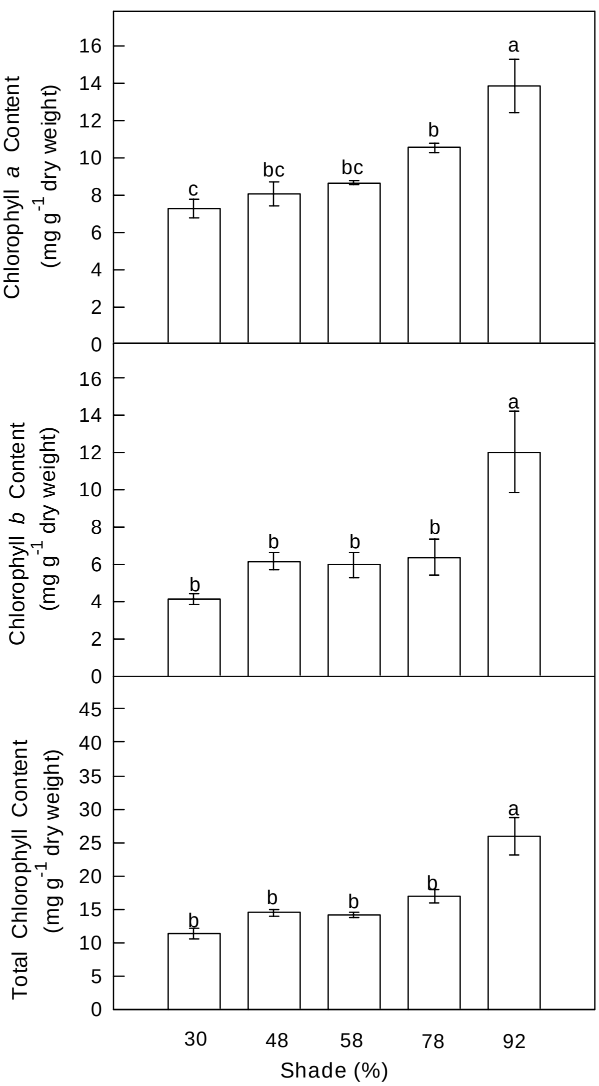

Figure 3. Effect of five shade levels on chlorophyll *a*, *b* and total content of *Euphorbia pulcherrima* Willd. cv. Subjibi 90 days after transplant (vegetative stage) growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

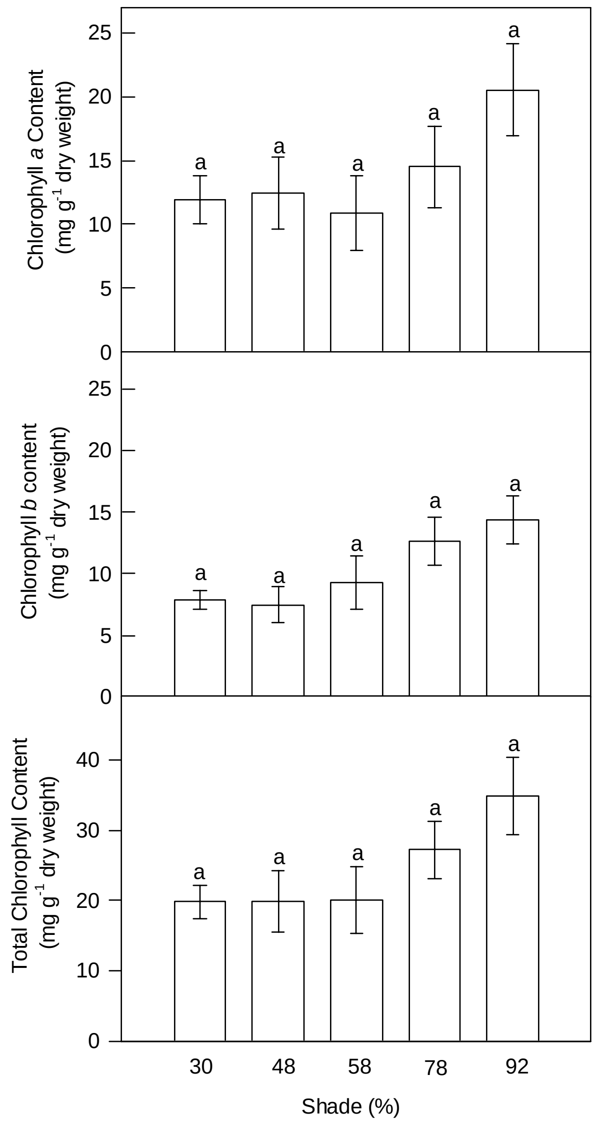

Figure 4. Effect of five shade levels on chlorophyll *a*, *b* and total content of *Euphorbia pulcherrima* Willd. cv. Subjibi 128 days after transplant (reproductive stage) growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

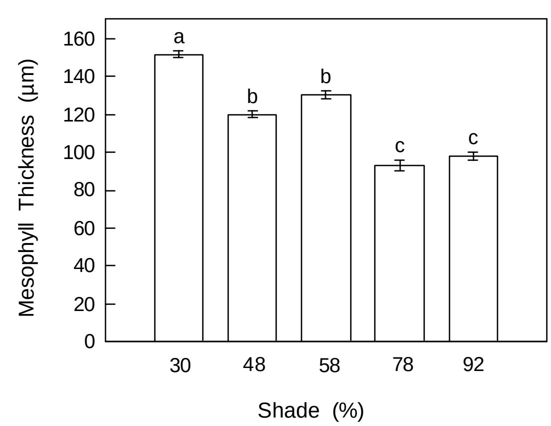

Figure 5. Effect of five shade levels on mesophyll thickness of *Euphorbia pulcherrima* Willd. cv. Subjibi growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

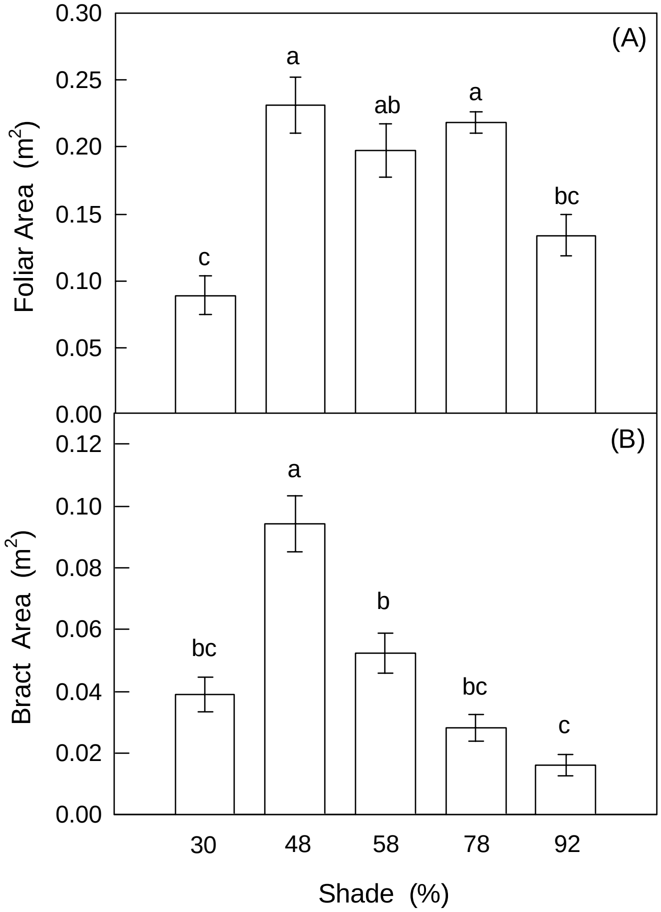

Figure 6. Effect of five shade levels on foliar (A) and bract (B) area of *Euphorbia pulcherrima* Willd. cv. Subjibi growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

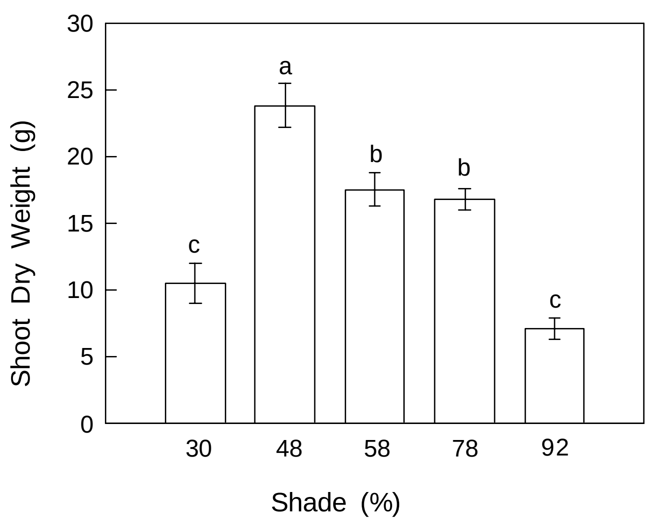

Figure 7. Effect of five shade levels on shoot dry weight of *Euphorbia pulcherrima* Willd. cv. Subjibi growing under greenhouse conditions. Each point represents the average of ten different plants ± standard error. Same letters have no statistical differences (Tukey, *P*≤0.05).

DISCUSSION

The results show an obvious effect of shade on the different variables measured in *Euphorbia pulcherrima* cv. Subjibi. Stem elongation, foliar area and shoot dry weight were negatively affected under high (896 mmol·m^{-2}·s^{-1}) or low (101 mmol·m^{-2}·s^{-1}) light intensity (30 and 92 % shade, respectively) while medium shade levels (78, 58 and 48 %) stimulated these growth variables. Experimental evidences in the literature show that growth responses to changes in light conditions are variable (Hunt and Cornelissen, 1997). Raveh *et al.* (1998) found that under 30 % shade *Selenicereus megalanthus* had the maximum stem elongation while *Hylocereus polyrhizum* had the maximum at 60 % shade. Higher levels of shade (90 %) had also a differential inhibition on stem length in these two species. It has been observed in different studies that species grown at low or high PPFD show a shift in the allocation of biomass from roots and stems to leaves, whereas in other species may show an opposite behaviour (Sims and Pearcy, 1994).

Bract area behaved something different to the variables mentioned above. Only 48 % shade produced significantly the largest bract area among the shade levels. However, large bracts and colorful bracts are important characteristics from the commercial point of view. In this study it was evident that high and low light intensities delayed the development of color in the bracts. Thirty percent shade induced a delayed of 16 days compared with 48 % shade which was the first level to develop color in the bracts; 58 % shade delay only five days while 78 and 92 % shade delay 24 and 35 days, respectively. Flower initiation and characteristics with commercial value in ornamental plants are influenced mainly by temperature and light intensity. The optimum values of these environmental variables to produce high quality plant will depend of the species. De Jong (1989) showed that in *Chrysanthemum* low light intensity delayed flowering but, in other species low light intensities promote early flowering (Moe *et al*., 1992; Welander and Hellgren, 1988; Gislerød and Mortengen, 1997; Bredmose, 1997).

High levels of shade (78 and 92 %) significantly reduced CO_2 assimilation in poinsettia but, it was stimulated gradually from 30 % shade up to a maximum at 58 % shade. It has been observed in other species like cotton that as the light intensity is reduced up to a value of 63 %, CO_2 fixation is significantly reduced about 43 and 50 % in relation to plants exposed to full light (Zhao and Oosterhuis, 1998). CO_2 fixation as a function of light intensity is not constant and is determined for the adaptation of the species to specific light conditions (sun or shade plants). For example four different species of *Shorea* were grown under four light levels (50, 350, 800 y 1600 mmol·m^{-2}·s^{-1}) and the response in terms of CO_2 fixation was quite different among the species (Ashton and Berlyn, 1992). Similar results were obtained in three species of *Quercus* grown under the same light intensities mentioned before, the maximum CO_2 fixation found among the species also was different reaching the maximum CO_2 fixation at different light intensities (Ashton and Berlyn, 1994).

In general chlorophyll *a*, *b* and total concentration expressed in dry weight basis increased as the shade level increased; 92 % shade had the maximum value in vegetative stage and it was statistically different. The same trend was found in reproductive statge and again 92 % had the maximum concentration though it was not significant. This kind of increments in chlorophyll concentration as a response to shading have been observed in different species. In *Aglaonema commutatum* it was found a high chlorophyll content at low light intensities having chlorophyll *a* the major increment (Benedetto, 1991). In another study with three deciduous species of trees (*Populus tremula* L., *Fraxinus excelsior* L. and *Tilia cordata* Mill.) chlorophyll *a+b* concentration showed an hyperbolic increment as the light intensity decreased (Ninemetes *et al*., 1998). Lee *et al.* (2000) also found in *Hopea helfery* and *H. odorata* increments of chlorophyll under conditions of low light intensities. These increments in chlorophyll concentration are one of most evident responses of plants when they are grown under low light intensities. This response has been interpreted as a mechanism used by the plants to optimise quantum harvesting when irradiance is reduced (Ninemetes *et al*., 1998).

Leaves of shade species are frequently thinner than those of sun species (Boardam, 1977). The results found in this study are in agreement with this assertion since 30 % shade produced thicker leaves (151.34 mm) whereas 78 and 92 % shade had thinner leaves (99.81 mm). Thicker leaves were the result of an increment in the size of palisade cells and also due to a major number of spongy parenchyma layers (data not shown). The opposite behaviour was the result of thinner leaves. In a study where *Hopea helferi* and *H. odorata* were grown under different light levels produced an increment in mesophyll thickness at higher light intensities and this was highly correlated with and increment in the length of palisade cells for *H. helfer* while in *H. odorata* the spongy parenchyma layers were thicker (Lee *et al*., 2000). Changes in mesophyll thickness as a result of alteration of palisade/spongy parenchyma cells ratio were also observed in three ornamental species (*Fatsia japonica*, *Cissus rhombifolia* and *Philodendrum scandens*) growing under different light intensities (Araus *et al*., 1986).

In conclusion the results of this study indicate that there was a marked effect in the morphology and physiology among the shade levels. It was clear that 48 and 58 % shade produced plants with best characteristics for the market besides high levels of shade increased time to reproductive maturity.

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