Revista Brasil. Bot., V.25, n.2, p.229-235, jun. 2002

Effects of light stress on the growth of the epiphytic orchid *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe

GIULIO C. STANCATO[1,4], PAULO MAZZAFERA[2] and MARCOS S. BUCKERIDGE[3]

(received: August 8, 2001; accepted: March 27, 2002)

ABSTRACT – (Effects of light stress on the growth of the epiphytic orchid *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe). Considering the performance of CAM epiphytes under high levels of radiation or in shaded environments, with growth rate proportional to light intensity, the objective of this work was to evaluate the effects of long-term light stress on the growth of a Brazilian epiphytic orchid, *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe. Two groups of plants were used in the first experiment, one under 90% (≅ 1,650 $\mu mol.m^{-2}.s^{-1}$) of Photosynthetically Active Radiation (PAR) and the other maintained under 22.5% (≅ 400 $\mu mol.m^{-2}.s^{-1}$). In the second experiment the diffusive resistance, transpiration rate and fluorescence levels were monitored for plants that were under 22.5% of PAR, under 90% and plants transferred from 22.5 to 90%. Our results show that light intensity interfered with growth and development of this orchid. Data on the changes in pseudobulb volume throughout the time course of growth suggest that water and reserves stored in the back shoots are translocated to the current shoot. Regarding stomatal resistance, plants under 22.5% of PAR reached a largest stomatal aperture during the night, whereas those under 90% only after dawn. After transfer from 22.5% PAR to 90% PAR the ratio of F_v/F_m decreased from approximately 0.8 to 0.7. This suggests the limitation of photoprotection mechanisms in the leaf and the results observed after the transfer of plants from 22.5% to 90% reinforce the possibility that a photoinhibition is reflected in a decrease in growth rate.

RESUMO – (Efeitos do estresse luminoso sobre o crescimento da orquídea epífita *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe). Baseado no desempenho de plantas epífitas com metabolismo CAM, em ambientes sombreados ou sob alta irradiância, com taxa de crescimento proporcional à intensidade luminosa, o objetivo deste trabalho foi avaliar os efeitos do estresse luminoso prolongado sobre o crescimento de uma orquídea epífita brasileira, *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe. No primeiro experimento, dois grupos de plantas foram empregados, sendo que um grupo foi mantido sob 90% da radiação fotossinteticamente ativa (RFA) (≅ 1.650 $\mu mol.m^{-2}.s^{-1}$) e o outro sob 22,5% (≅ 400 $\mu mol.m^{-2}.s^{-1}$). No segundo experimento, a resistência difusiva, a taxa de transpiração e os níveis de fluorescência foram monitorados em plantas sob 90% da RFA, 22,5% da RFA e em plantas transferidas de 22,5 para 90% da RFA. Nossos resultados indicam que a alta intensidade luminosa reduziu o crescimento e o desenvolvimento desta orquídea. Dados da variação no volume dos pseudobulbos durante o crescimento sugerem que a água e as reservas armazenadas nos pseudobulbos mais velhos podem ter sido transferidas para o pseudobulbo mais novo. Considerando a resistência estomática, os estômatos das plantas submetidas à 22,5% da RFA atingiram a sua maior abertura durante à noite, enquanto que nas plantas sob 90% da RFA a maior abertura estomática ocorreu somente ao amanhecer. Após a transferência de 22,5% para 90% do RFA, a razão F_v/F_m diminuiu de aproximadamente 0,8 para 0,7. Isto sugere a restrição dos mecanismos de fotoproteção na folha e os resultados observados, após a transferência de plantas de 22,5% para 90% do RFA, reforçam a possibilidade de que a fotoinibição implicou numa diminuição da taxa de crescimento.

Key words - Photoinhibition, storage, orchids, pseudobulb

Introduction

Studies involving comparison of light-saturation curves showed that some epiphytic orchids present characteristics of sun plants, or intermediate plants (Lüttge 1989). In general, CAM epiphytes show a better performance under high light intensity when compared to epiphytes with C_3 metabolism (Winter *et al*. 1983). However, few species of orchids resist direct exposure to sunlight for long periods, since photoinhibition may occur (Osmond 1978, Winter *et al*. 1983).

Even though the majority of CAM type epiphytes are found in sites with high light intensity, there are also CAM type plants adapted to shaded environments (Winter *et al*. 1983). Under natural conditions, high light intensity provokes an increase in leaf temperature, which intensifies deacidification and increases daytime CAM rhythm (Osmond 1978).

In practice, the evaluation of photosynthetic performance of CAM type plants is through

---

1. Instituto Agronômico, Centro de Horticultura, Caixa Postal 28, 13001-970 Campinas, SP, Brasil.
2. Universidade Estadual de Campinas, Instituto de Biologia, Caixa Postal 6l09, 13083-970 Campinas, SP, Brasil.
3. Instituto de Botânica, Seção de Fisiologia e Bioquímica de Plantas, Caixa Postal 4005, 01061-970 São Paulo, SP, Brasil.
4. Corresponding author: stancato@iac.br

fluorescence measurements, since it has been demonstrated experimentally that the F_v/F_m ratio is proportional to the photosynthetic rate of intact leaves, measured as CO_2 fixation or O_2 evolution (Björkmam & Demmig 1987). This ratio is affected by stress, particularly by photoinhibition.

Photoinhibition has been observed when plants are exposed to three different conditions: 1) when they are exposed to a level of irradiance greater than that under which they have been growing; 2) when they are temporarily subjected to a low level of CO_2 and 3) when maintained at temperatures below 10 °C, even under regular level of irradiance (Björkmam & Demmig 1987). The occurrence of photoinhibition may be promptly determined by measuring the photosynthetic transport of electrons, by the exchange of O_2 or through the levels of fluorescence of chlorophyll *a* (Winter & Gademann 1991, Keiller *et al.* 1994).

Recently, Stancato *et al.* (2001) found that pseudobulbs of the epiphytic orchid *Cattleya forbesii* X *Laelia tenebrosa* store glucomannan as a main storage compound. This polymer is mobilised as a result of several abiotic factors of stress and was shown to affect the photosynthetic system.

In the present work, we followed some growth and development parameters of the epiphytic orchid *C. forbesii* X *L. tenebrosa* under two different conditions of irradiance with the aim of finding long-term changes provoked by different light intensities. The results showed that high light intensities induced photoinhibition and the gas exchanges were significantly affected. These results are discussed regarding the possible effects of light regime on the incorporation and partitioning of dry mass among the parts of the plant.

Material and methods

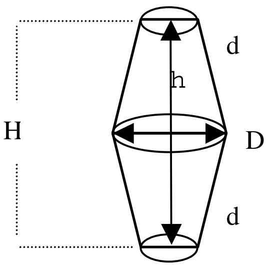
The trials were conducted with plants (F_1) of *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe, in São Paulo, Brazil (22°53' S and 47°05' W), which were obtained through artificial pollination and asymbiotic culture *in vitro* (Knudson 1946).

The plants were watered on alternate days, and once a month each one received 200 mL of Hoagland & Arnon (1950) solution soon after watering.

Two groups of plants were kept side by side on the top of benches in a greenhouse during approximately three years. One of the groups was covered with a 75% shade cloth whereas the other received no cover. The monitoring of incident solar radiation showed that, from the total of Photosynthetically Active Radiation (PAR), the incidence was 90% (≅ 1,650 µmol.m^{-2}.s^{-1}) in the greenhouse whereas plants maintained under a 75% shade cloth received only 22.5% (≅ 400 µmol.m^{-2}.s^{-1}) of PAR.

Growth of plants under 90% and 22.5% of PAR - To evaluate the effects of light intensity upon growth and development, ten plants were used, drawn at random from the pool of plants. The experiment was followed for 173 days, along which data collection was performed nine times.

For determination of the pseudobulb volume, a correlation with the volume of a conic section was used, since a pseudobulb has the approximate form of two conic sections juxtaposed through their base. Thus, the volume of one pseudobulb was calculated as follows:

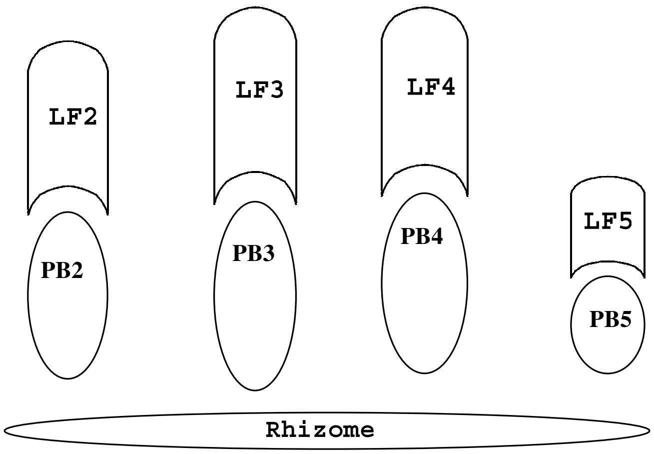


V_{pb}= 2.{1/3.p.h.[$(d/2)^2$ + d/2.D/2 + $(D/2)^2$]}, where
V_{pb} = volume of the pseudobulb,
D/2 = larger semidiameter and
d/2 = smaller semidiameter
h = height and H = 2h

Leaf area was determined by measurements of length and width of leaves by use of a correlation factor (0.798), determined by linear regression between the actual area and the respective length and width measurements.

The experiment was outlined with two treatments, nine samplings with five replicates and the statistical analyses were F test and Tukey's test, $p < 0.05$.

Photosynthetic performance and gaseous exchange under 90% and 22.5% of PAR - In this experiment, nine plants were used, six of which were under 22.5% and three under 90% of PAR. Measurements of fluorescence, transpiration and diffusive resistance were performed in leaves 3 and 5. A diagram of the sympodial orchid used in this work is shown below, with the key numbers for leaves (LF) and pseudobulbs (PB). As higher the number, younger is the shoot:



Seven measurements were performed throughout one night and one day (at 173 days), at the following times: 1 h dark, 6 h dark, 14 h dark (0 h light), 1 h light, 2 h light, 4 h light and 8 h light.

Chlorophyll fluorescence was measured with a fluorometer (Hansatech, PEA), and a Li-Cor steady-state porometer (LI-1600) was used to measure diffusive resistance and transpiration. The measurements were non-destructive and made upon the same leaves, each time. The diffusive resistance was measured at all sampling times while chlorophyll fluorescence measurements were made on the 4th, 5th, 6th, and 7th sampling, i.e. from 1 h of light onwards.

At the 5th sampling, three plants that had grown under 22.5% of PAR were transferred, immediately after the measurements, to 90% of PAR. From then on these plants were monitored under those light conditions until the end of the experiment.

The three treatments consisted of plants kept under 22.5% of PAR, plants kept under 90% of PAR and plants transferred from 22.5% to 90% of PAR. Measurements were performed in three replicates at seven and four time intervals (two leaves per plant), and data were subjected to variance analysis and Tukey's test ($p < 0.05$).

Results and Discussion

Daily performance of plants under different levels of irradiance - Data on figure 1 (A, B, C and D) show that plants under 22.5% of PAR presented the largest stomatal aperture after six hours in the dark. However, in plants under 90% of PAR, the maximum stomatal aperture was reached only after fourteen hours in the dark. At the peak, the levels of transpiration (figure 1A and C) were higher and the levels of diffusive resistance (figure 1B and D) were lower in plants under 22.5% of PAR than those plants under 90% of PAR, showing a variation of 75% and 70%, respectively.

The transpiration may be considered as a suitable parameter to evaluate the performance of this epiphytic orchid in relation to irradiance levels, since plants under 22.5% of PAR exhibited a behaviour characteristic of the epiphytic habitat, opening the stomata during the most humid period, that is, at night. Plants under 90% of PAR showed less sensitivity to variations in relative humidity, presenting greater capacity for gaseous exchange with the atmosphere, right at dawn.

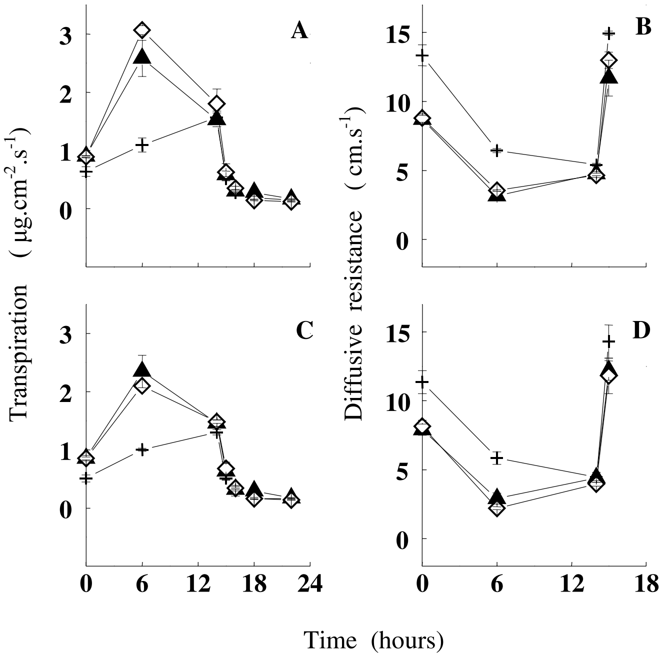


Figure 1. Transpiration (A, C) and diffusive resistance (B, D) in leaf 5 (A, B) and 3 (C, D) of the epiphytic orchid *C. forbesii* X *L. tenebrosa* kept under 90% (+), under 22.5% (▲) and plants transferred from 22.5 to 90% (◇) of PAR after two hours of light. The measurements were initiated during the dark period and continued in the subsequent light period. The points represent averages (three replicates) and bars standard errors.

Although in a plant with CAM type metabolism, stomatal aperture can not be used directly to deduce quantitatively CO_2 assimilation, our data afford the speculation that the characteristics described probably mean that plants subjected to 22.5% of PAR show higher rates of atmospheric CO_2 assimilation, as well as a greater flow of water towards the leaves, since they maintain their stomata open for a longer period of time during the night. Indeed, CAM plants from other families can present morphological and physiological differences when cultivated under high light intensity and in the shade (Boardman 1977, Medina 1987, Osmond & Chow 1988, Lüttge *et al.* 1991a, Lüttge *et al.* 1991b, Medina *et al.* 1991).

Light intensity also affected the efficiency of the photosynthetic process. The F_v/F_m ratio is proportional to the rate of photosynthesis of intact leaves (Björkman & Demmig 1987) and was higher in plants under 22.5% of PAR (figure 2A and B). In plants under 90% of PAR, the F_v/F_m ratio clearly shows some degree of photoinhibition (figure 2A and B). This phenomenon can be quickly induced by transference from low to high light intensity, as has been demonstrated by the gradual decrease in F_v/F_m ratios of LF 5 and LF 3 from 0.84 to 0.73 and 0.69 respectively. In addition, these results indicate that LF 3, an older leaf, is relatively more sensitive to light stress. Through visual inspection, it could be inferred that plants grown under 90% of

PAR displayed a chlorotic aspect. This suggests the limitation of photoprotection mechanisms, including the imbalance between the biosynthesis and degradation of accessory and heat dissipating photosynthetic pigments in the leaf.

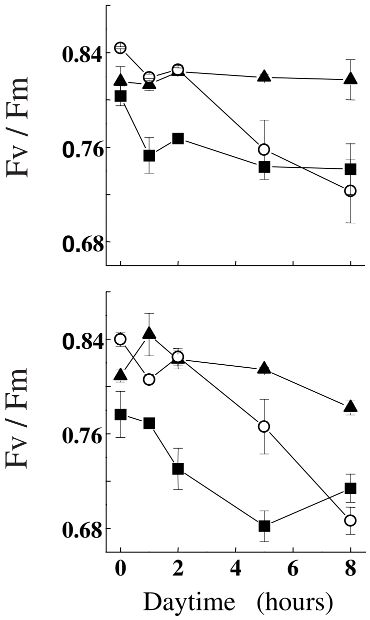


Figure 2. Fluorescence measurements obtained for leaves 5 (A) and 3 (B) in the epiphytic orchid *C. forbesii* X *L. tenebrosa* during daytime, with plants under 90% (+), under 22.5% (▲) and plants transferred from 22.5% to 90% (◇) of PAR after two hours of light. The zero hour corresponds to 8 am. Points represent averages (three replicates) and bars standard errors.

The transfer of some plants from 22.5% to 90% of PAR has shown how this orchid responds to a sudden exposure to high levels of irradiance, tending gradually to behave similarly to those maintained for longer periods under this condition. After the transfer, a rapid reduction in the photosynthetic efficiency of these plants might have occurred, reinforcing the possibility of the occurrence of photoinhibition (Björkman & Demmig 1987). In fact, it has been proposed that CAM type plants grown in the shade may be sensitive to photoinhibition (Adams & Osmond 1988).

It is probable that under 90% of PAR plants were also exposed to high temperatures, which might have induced injury to the photosynthetic apparatus, mimicking the effects of photoinhibition. This fact agrees with the findings of Medina *et al.* (1986) and Medina (1987) who demonstrated that some epiphytic orchids native to tropical and subtropical forests incorporate atmospheric CO_2 at night, and show photoinhibition when exposed to higher levels of irradiance.

The behaviour of the stomata of plants under 90% of PAR (figure 1) could be contributing towards an increase in the leaf internal temperature, avoiding thermal exchanges with atmosphere at night.

Long-term performance of plants under different conditions of irradiance - The changes in leaf area of plants of *Cattleya forbesii* X *Laelia tenebrosa* growing under two levels of light intensity are presented in figure 3. These results show that light intensity interfered with growth and development of this orchid. From the beginning of the experiment, the plants cultivated under 22.5% of PAR exhibited a larger leaf area than plants cultivated under 90% of PAR, the difference being related to LF 4 and LF 3, but not LF 2.

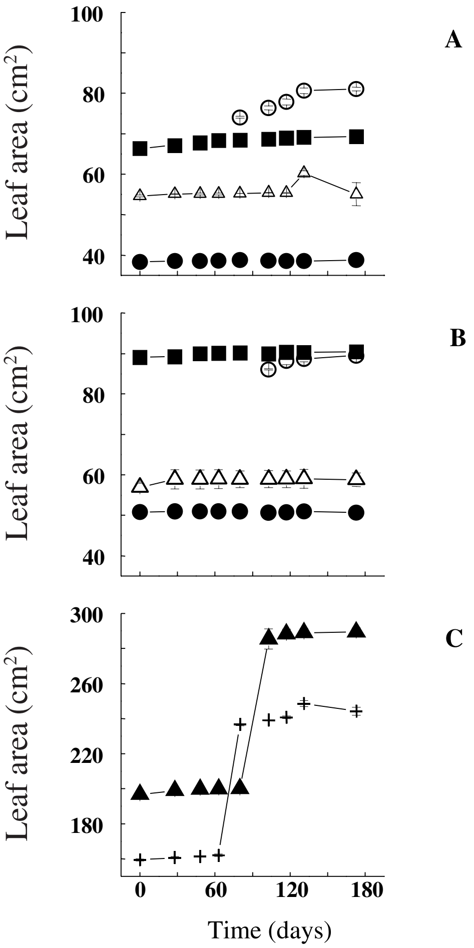


Figure 3. Leaf area measurements of the epiphytic orchid *C. forbesii* X *L. tenebrosa* cultivated in a greenhouse under 90 or 22.5% of PAR, for 173 days. (A) (90%) and (B) (22.5%) show the variation on each leaf in the plants. (C) total leaf area per plant under 22.5 (%) or 90% (+) of PAR. Points represents the averages (five replicates) and bars mean standard errors. [○ leaf 5, ■ leaf 4, ● leaf 3, △ leaf 2].

During the period of observation, both groups of plants developed new leaves (figure 3). Plants maintained under 90% of PAR started to develop a new leaf (LF 5) after 80 days (figure 3A), whereas under 22.5% of PAR the appearance of LF 5 occurred only after 103 days from the start of the experiment (figure 3B). With the development of LF 5, the total leaf area increased by ca 40% and 50% for 22.5% and 90% of PAR respectively (figure 3C).

The data on figures 4A and 4B show that all pseudobulbs underwent reversible changes in their volumes. These organs are chronologically related to the appearance of the youngest shoot, and their development was independent of light intensity. Under 90% of PAR, the volume of PB 4 decreased linearly from 30 to 100 days and after 180 days increased to a level similar to the initial. This suggests that part of storage compounds of this pseudobulb might have been transferred to the current shoot (LF 5 and PB 5), as previously observed by Stancato *et al.* (2001). The present observation can be compared with the changes in the plants maintained under 22.5% of PAR in which a decrease in volume was observed from 60 to 90 days with a relatively quick recovery to higher levels at 120 days. All the back shoots apparently mobilised some of their reserves (glucomannan, according to Stancato *et al.* 2001) transiently with faster recovery under 22.5% than under 90% of PAR (figure 4). This coincided with development of LF 5 and PB 5, suggesting that the current shoot is a sink and PB 4 is possibly the main source. Concerning this source-sink relationship, a distinction can also be made between plants growing under different light intensities. Whereas under 90% of PAR PB 4 and PB 3 present transient mobilisation, under 22.5% of PAR PB 5 seems to be a stronger sink.

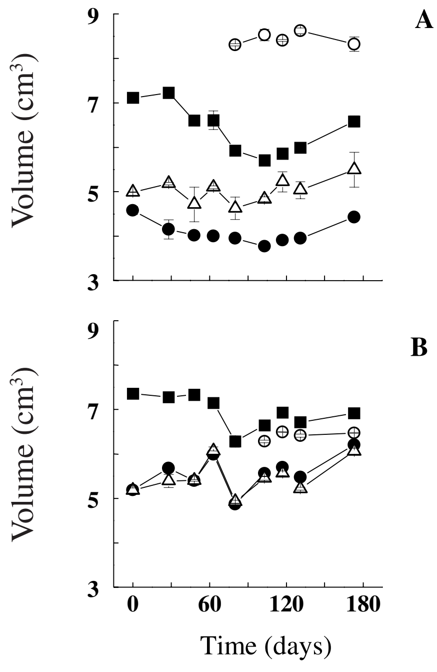


Figure 4. Variation of pseudobulb volume in plants of the epiphytic orchid *C. forbesii* X *L. tenebrosa* maintained under greenhouse conditions (22.5% or 90% of PAR) for 173 days. (A) plants subjected to 90%, and in (B) subjected to 22.5% of PAR. Point represent averages (five replicates) and bars mean standard errors. [○ pseudobulb 5, ■ pseudobulb 4, ● pseudobulb 3, △ pseudobulb 2].

Zimmerman & Whigham (1992) found that the water-soluble carbohydrates present in the underground corm of the orchid *Tipularia discolor* were hydrolysed and were important as a support for the beginning of the new season of growth. On other hand, studies on translocation in the Liliaceae indicated that the beginning of growth is accomplished at the expense of storage carbohydrates, but the subsequent growth and blooming are supported by photosynthesis (Ho & Rees 1976, 1977).

Stancato *et al.* (2001) observed that water-soluble carbohydrates (predominantly glucomannan) are present in pseudobulbs (10-30% of the dry mass, depending on the age) and leaves (3-5% of the dry mass) of plants of *Cattleya forbesii* X *Laelia tenebrosa*.

Altogether, our results indicate that these carbohydrates might be involved in the transfer of storage compounds from the older to the youngest pseudobulb and to the developing leaf, to be used for growth and development. It is likely that after the initial growth, the reserves in older pseudobulbs would be replaced by the newly produced photoassimilates. Such features were described for the terrestrial orchids *Orchis morio* by Franz & Meier (1971) and for *Tipularia discolor* by Zimmerman & Whigham (1992) and for epiphytic orchids such as *Catasetum viridiflavum* (Zimmerman 1990), *Dendrobium* (Wadasinghe & Hew 1995) and *Spatoglottis unguiculata* and *Bromheadia finlaysoniana* (Hew *et al.* 1998).

Our daily evaluations of the photosynthetic apparatus showed that plants under 90% of PAR presented photoinhibition and therefore plants under 22.5% of PAR displayed a more efficient carbon assimilation system. If this situation persisted during 173 days of the long-term experiment, it is possible that the occurrence of photoinhibition might be related to the long-term reserve mobilisation in the pseudobulbs. This might even have been reflected into the source-sink transition of the newly produced leaves of the plants cultivated under the two light intensities used. Indeed, LF 5 of plants under 90% of PAR appeared before the recovery of the levels of reserve in PB 4 which started after 80 days. This suggests that LF 5 might have contributed for its recuperation. On the other hand, LF 5 of plants under 22.5% of PAR appeared 103 days after reserve degradation was completed. This probably occurred because leaves 1-4 were capable to assimilate carbon more efficiently, resulting in a shorter reserve mobilisation period. In this case, the other leaves probably supported recovery.

Our results indicated that dry mass partitioning of plants of the hybrid *C. forbesii* X *L. tenebrosa* was affected by the light conditions in which they were cultivated. In the case of plants that grew under higher light intensity, development was relatively more dependent on the storage compounds of pseudobulbs. On the other hand, for plants cultivated under low light intensity, the development of the new shoot was dependent on both PB storage compounds and the actual photosynthetic capacity. Thus, for the hybrid studied in this work, an appropriate level of light favours a more efficient resource management, so that development of the new shoots and flowers are assured.

References

ADAMS, W.W. & OSMOND, C.B. 1988. Internal CO_2 supply during photosynthesis of sun and shade grown CAM plants in relation to photoinhibition. Plant Physiology 86:117-123.

BJÖRKMAN, O. & DEMMIG, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origin. Planta 170:489-504.

BOARDMAN, N.K. 1977. Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology 28:355-377.

FRANZ, G. & MEIER, H. 1971. Bildung und Abbau des Schelimpolysaccharids (salepmannan) vos Orchideenknollen. Planta Medica 19:326-332.

HEW, C.S., KOH, K.T. & KHOO, G.H. 1998. Pattern of photoassimilate partitioning in pseudobulbous and rhizomatous terrestrial orchids. Environmental and Experimental Botany 40:93-104.

HO, L.C. & REES, A.R. 1976. Re-mobilization and redistribution of reserves in the tulip bulb in relation to new growth until anthesis. New Phytologist 76:59-68.

HO, L.C. & REES, A.R. 1977. The contribution of current photosynthesis to growth and development in the tulip during flowering. New Phytologist 78:65-70.

HOAGLAND, D.R. & ARNON, D.I. 1950. The water culture method for growing plants without soil. California Agricultural Experimental Station, Circular n. 347. California.

KEILLER, D.R., SLOCOMBE, S.P. & COCKBURN, W. 1994. Analysis of chlorophyll *a* fluorescence in C_3 and CAM forms of *Mesembryanthemum crystallinum*. Journal of Experimental Botany 45:325-334.

KNUDSON, L. 1946. A new nutrient solution for germination of orchid seed. American Orchid Society Bulletin 15:214-217.

LÜTTGE, U. 1989**.** Vascular plants as epiphytes: Evolution and Ecophysiology (U. Lüttge, ed.). Spring-Verlag, Heidelberg.

LÜTTGE, U., BALL, E., FETENE, M. & MEDINA, E. 1991a. Flexibility of crassulacean acid metabolism in *Kalanchoe pinnata* (Lam.) Pers. I. Response to irradiance and supply of nitrogen and water. Journal of Plant Physiology 137:259-267.

LÜTTGE, U., BALL, E. & FETENE, M. 1991b. Flexibility of crassulacean acid metabolism in *Kalanchoe pinnata* (Lam.) Pers. II. Light-use characteristics of plants grown in low or high light. Journal of Plant Physiology 137:268-272.

MEDINA, E. 1987. Aspectos ecofisiológicos de plantas CAM en los trópicos. Revista de Biologia Tropical 35(supl. 1):55-70.

MEDINA, E., OLIVARES, E. & DIAZ, M. 1986. Water stress and light intensity effects on growth and nocturnal acid accumulation in a terrestrial CAM bromeliad (*Bromelia humilis*) under natural conditions. Oecologia 70: 441-446.

MEDINA, E., POPP, M., LÜTTGE, U. & BALL, E. 1991. Gas exchange and acid accumulation in high and low irradiance grown pineapple cultivars. Photosynthetica 254:489-498.

OSMOND, C.B. 1978. Crassulacean acid metabolism: a curiosity in context. Annual Review of Plant Physiology 29:379-414.

OSMOND, C.B. & CHOW, W.S. 1988. Ecology of photosynthesis in the sun and shade: summary and prognostications. Australian Journal of Plant Physiology 15:1-9.

STANCATO, G.C., MAZZAFERA, P. & BUCKERIDGE, M.S. 2001. Effect of a drought period on the mobilisation of non structural carbohydrates, photosynthetic efficiency and water status in an epiphytic orchid. Plant Physiology and Biochemistry 39:1009-1016.

WADASINGHE, G. & HEW, C.S. 1995. The importance of back shoots as a source of photoassimilates for growth and flower production in *Dendrobium* cv. Jashika Pink (Orchidaceae). Journal of Horticultural Science 70:207-214.

WINTER, K., WALLACE, B.J., STOCKER, G.C. & ROKSANDIC, Z. 1983. Crassulacean acid metabolism in Australian vascular epiphytes and some related species. Oecologia 57:129-141.

WINTER, K. & GADEMANN, R. 1991. Daily changes in CO_2 and water vapour exchange, chlorophyll fluorescence and leaf water relations in the halophyte *Mesembryanthemum crystallinum* during the induction of Crassulacean Acid Metabolism in response to high salinity. Plant Physiology 95:768-776.

ZIMMERMAN, J.K. 1990. Role of pseudobulbs in growth and flowering of *Catasetum viridiflavum* (Orchidaceae). American Journal of Botany 77:533-542.

ZIMMERMAN, J.K. & WHIGHAM, D.F. 1992. Ecological functions of carbohydrates stored in corms of *Tipularia discolor* (Orchidaceae). Functional Ecology 6:575-581.