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Irradiance, Temperature, and Carbon Dioxide Enrichment Affect Photosynthesis in *Phalaenopsis* Hybrids

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Abstract. **The short-term effects of photosynthetic photon flux (*PPF*), day/night temperatures and CO_2 concentration on CO_2 exchange were determined for two *Phalaenopsis* hybrids. At 20 °C, the saturating *PPF* for photosynthesis was 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At this *PPF* and ambient CO_2 level (380 $\mu L \cdot L^{-1}$), a day/night temperature of 20/15 °C resulted in the largest daily CO_2 uptake. Higher night temperatures probably increased the respiration rate and lowered daily CO_2 uptake in comparison with 20/15 °C. An increase in the CO_2 concentration from 380 to 950 $\mu L \cdot L^{-1}$ increased daily CO_2 uptake by 82%.**

Phalaenopsis hybrids are often cultivated at set temperatures without knowing their optimal temperature requirements. Several authors (Amberger and Fisher, 1995; Croci, 1988; Röber, 1994; Van Der Ende, 1985) recommended an optimal day temperature between 18 and 22 °C and night temperature between 21 and 25 °C. The suggested temperatures are the same for various photosynthetic photon flux (*PPF*) levels and CO_2 concentrations. Nevertheless, the optimum temperature for photosynthesis and plant growth can change with *PPF* and CO_2 concentration (Long, 1991).

Due to the presence of CAM in *Phalaenopsis* (McWilliams, 1970), light and dark reactions of carbon fixation are separated in time. To quantify the effect of different *PPF* on C_3 or C_4 plants, photosynthesis light response curves can be determined. This is not possible for CAM plants because most of the CO_2 is fixed at night. However, by determining the quantum efficiency of photosystem II photochemistry (Φ_{PSII}) at various *PPF*, a fluorescence light response curve can be defined. The quantum efficiency declines as the fraction of light utilized in photosynthesis increases, and Genty et al. (1990) showed that Φ_{PSII} is well correlated with the quantum yield of photosynthesis.

By measuring the CO_2 gas exchange at the leaf level at different environmental settings during day and night, an optimal environment for plant growth can be determined.

The objectives of this study were to determine the effects of *PPF*, air temperature, and CO_2 concentration on CO_2 exchange during vegetative growth for two *Phalaenopsis* hybrids.

Materials and Methods

Plant material. Sixty 3-month-old plants of two *Phalaenopsis* hybrids, '70' and 'L', purchased from a commercial grower were potted in 13-cm (0.86 L) plastic containers on 18 Mar. 1996. The substrate [initial pH = 5.9, EC = 0.154 µS (Bas van Buuren B.V., Maasland, The Netherlands)] consisted of 45% bark of *Pinus maritima* Lam. (medium fraction), 35% white peat (fraction 3, from German and Polish origin), 20% Oxygrow (coarse) (i.e., water-retaining mousse) (Agglorex, Lommel, Belgium), 2 kg slaked lime (Dolokal 10%; Ankerpoort, The Netherlands) and 0.75 kg PGmix (12N–14P–24K + micronutients; Hydroagri, Vlaardingen, The Netherlands) per m^3. Plants were grown in the greenhouse at a constant day/night temperature of 22 °C and a maximum *PPF* of 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Every 2 d, plants were watered (pH = 5.7) and fertilized with a 16N–10P–25K + micronutrients (EC = 0.9 µS) (Alkrisal Hydro, Schering, Germany). The first measurements started 3 June 1996, using the most recent mature leaves with a lamina length of 15 cm.

PPF response. Leaf chlorophyll fluorescence measurements were made with a field-portable fluorometer (PAM-2000; Walz, Effeltrich, Germany). For the determination of the fluorescence light response curves, plants were placed in growth chambers with a relative humidity of 65% and a CO_2 concentration at 380 $\mu L \cdot L^{-1}$. Temperature was set at 20, 25, or 30 °C. Plants were kept in darkness for 12 h to equilibrate prior to measurement. Several papers (Adams et al., 1989; Winter and Awender, 1989; Winter and Demmig, 1987) showed that treatments before measuring fluorescence of CAM plants were analogous with those for C_3 and C_4 plants. The determination of a fluorescence light response curve required 3.5 h. During the first half hour of the measurement, plants were kept in darkness. At the end of the dark period, minimal fluorescence (F_o) was determined, while maximal fluorescence (F_m) was measured after a saturation pulse of about 2300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a halogen lamp (8V/20W; Bellaphot, Osram, The Netherlands). The fluorescence ratio F_v/F_m (Y), with $F_v = F_m - F_o$, was then calculated. Afterwards, *PPF* was increased at a constant rate of 1.66 $\mu mol \cdot m^{-2} \cdot s^{-1} \cdot min^{-1}$ up to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During this period, the fluorescence intensity at steady state (F_s) and the fluorescence intensity with all PSII reaction centers closed in a light adapted state (F_m') were measured every 5 min. Quantum efficiency of PSII photochemistry ($\Phi_{PSII} = \Delta F/F_m'$, with $\Delta F = F_m' - F_s$) was calculated as in Genty et al. (1989). During the 5 min between each measurement, *PPF* changed only 8.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. As this was only a minor change, we assumed the *PPF* to be constant.

For each cultivar and temperature treatment, eight mature leaves from each of eight different plants were used. The measurements were done on the middle of the upper side of the leaf, 1 cm away from the vein.

Temperature response. To estimate the effect of air temperature on growth, CO_2 exchange of single leaves was measured. The integrated CO_2 exchange at the leaf level is an indication of the growth rate of the plant under environmental conditions.

The leaf gas exchange system, operating in open differential mode, consisted of ten leaf cuvettes (PP-systems, Hitchin, England). The opening in each cuvette was 4.9 cm^2, and the volume was 41.5 cm^3. The air flow entering each cuvette was about 200 $mL \cdot min^{-1}$. The CO_2 concentration of incoming and outgoing air was monitored continuously by using an infrared gas analyzer (IRGA, MK3, Analytical Development Co., Hoddesdon, England). Data were stored on a data logger (DL2, Delta-T, Cambridge, England). The cuvette was placed at the middle of the leaf next to the vein. Mature leaves were measured during two successive days, but data for the second day were used for the calculations of the accumulated net photosynthesis (ΣP_n) to allow adaptation of the plants to the measuring environment. During the measurements, *PPF* was set at 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 12-h photoperiod was used. The following day/night temperatures were tested: 20/15 °C, 20/20 °C, 25/20 °C, and 25/25 °C. Lower and higher day air temperatures were not tested because <20 °C is too cold for the tropical *Phalaenopsis*, and energy costs are high at temperatures >25 °C. Combinations with a difference between day and night temperature of 10 °C can induce flowering

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(Röber, 1994). In the current assays, temperature differences of more than 5 °C were not examined, since our aim was to determine the optimal conditions for vegetative growth.

CO_2 concentration. For measuring the effect of elevated CO_2 concentration, a day/night temperature of 25/20 °C and a *PPF* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were used. The CO_2 concentrations tested were 380 and 950 $\mu L \cdot L^{-1}$. Ten leaves of each of 10 different plants were continuously measured during 1 week at each CO_2 concentration.

Statistical analysis. The CO_2 gas exchange data were analyzed as a two-factor model, using PROC ANOVA (SAS Institute, 1988). The factors were cultivar and temperature regime. Treatment means for temperature regimes were separated by Duncan's multiple range test. By using paired comparisons, the effect of the difference between day/night temperature (DIF) was demonstrated.

Results and Discussion

Effect of PPF. The evolution of Φ_{PSII} at 20 °C decreased to 0.4 for both cultivars at a *PPF* of 160–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 1). At higher *PPF* no further changes were observed. We conclude that at this temperature and CO_2 concentration a saturating *PPF* was around 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This is higher than the 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ found by Ota et al. (1991) for comparable environmental conditions.

At higher temperatures (25 and 30 °C), Φ_{PSII} for both cultivars remained higher and decreased slowly at a constant rate. The higher the temperature, the higher Φ_{PSII} was at a given *PPF*. No saturating *PPF* was found at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when measured at 25 or 30 °C.

Effect of temperature. Figure 2 shows a typical CO_2 exchange pattern of *Phalaenopsis* during a 24-h period. Gas exchange was limited during the day. Only 4 h before dark, which corresponds with the moment that all captured CO_2, as malate, was used, the stomata re-opened. At the end of the night, CO_2 uptake stopped when stomata closed. Cultivar '70' took up 46% more CO_2 than did 'L' (Table 1) and the difference was highly significant. This corresponds with the experience of growers: '70' grows more rapidly than does 'L'. At a *PPF* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, leaves took up significantly more CO_2 at 20/15 °C than at 20/20, 25/20, or 25/25 °C. The maximum relative difference was 21%. The decreased CO_2 uptake with increasing night temperatures might be caused by a higher respiration rate. Jiao et al. (1997) also stated that the dark respiration rate was more sensitive to changes in temperature than was leaf photosynthesis. No significant interaction of cultivar × temperature regime was found.

When the data were analyzed for cultivar and DIF effects, highly significant ($P < 0.001$) differences were found for both factors. The interaction cultivar × DIF was nonsignificant. This indicated that +5 DIF resulted in a higher CO_2 uptake for both cultivars in comparison with 0 DIF, in accordance with the results found for *Dendranthema ×grandiflora* Kitam. 'Bright Golden Anne' (Karlsson et al., 1989)

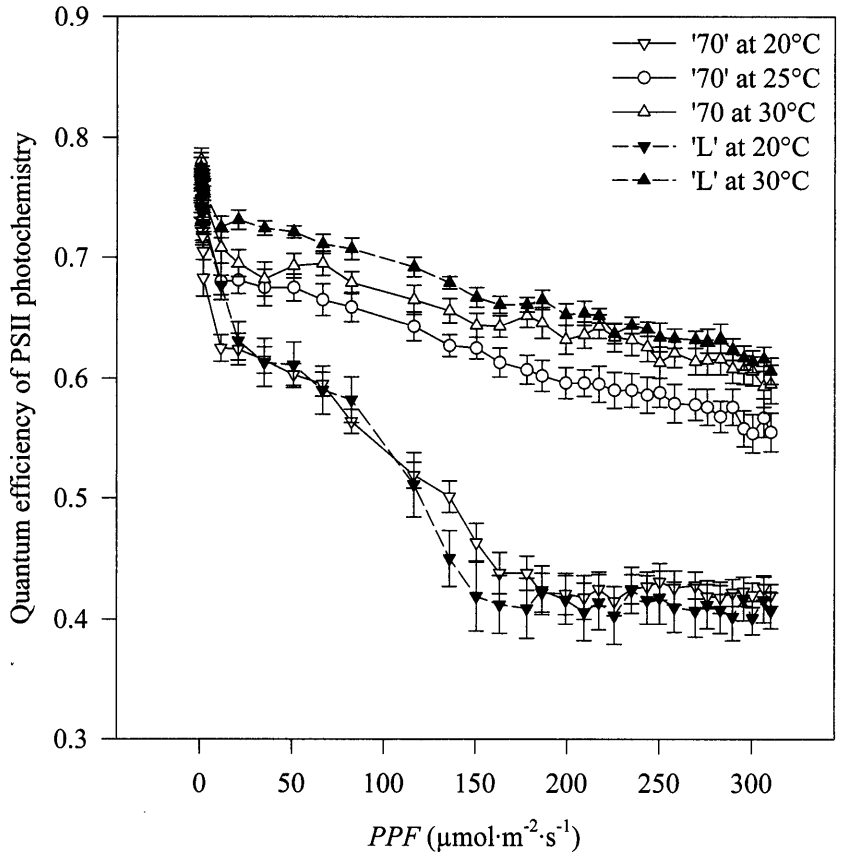

Fig. 1. Response curves of quantum efficiency of photosystem II photochemistry (Φ_{PSII}, –) in *Phalaenopsis* '70' and 'L' mature leaves to light (*PPF*, $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20, 25, or 30 °C. Each data point is an average for eight leaves from each of eight different plants ± SE.

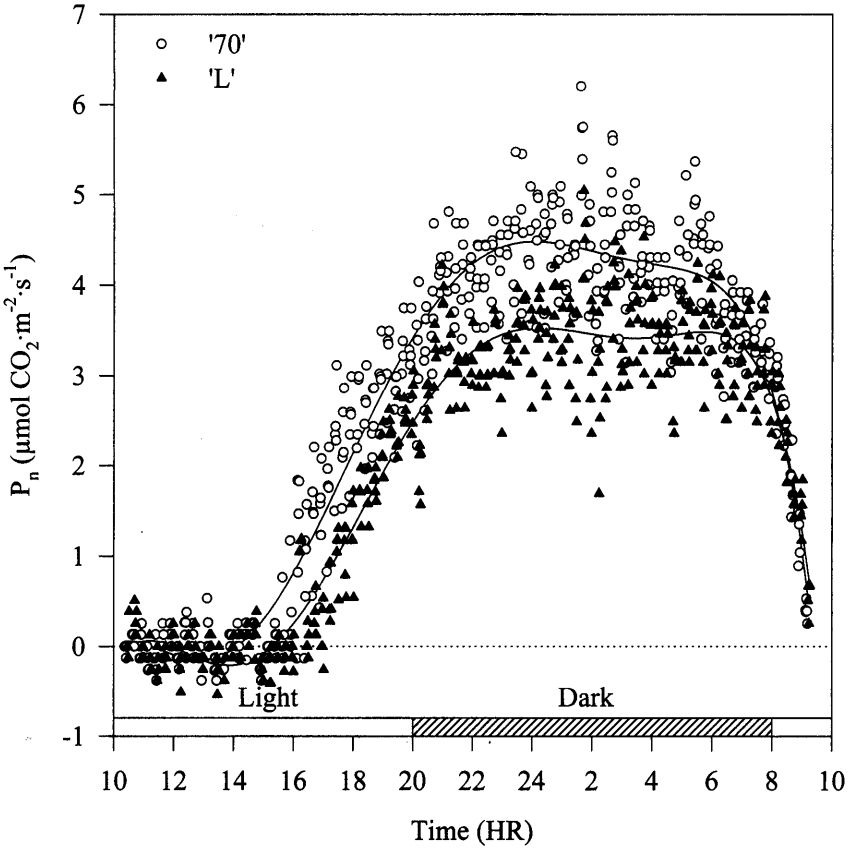

Fig. 2. Carbon dioxide exchange (P_n, $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$) of leaves of *Phalaenopsis* '70' and 'L' during a 24-h period. Data for five mature leaves from each of five different plants.

and *Cucumis sativum* L. 'Ashley' and 'Poinsett' (Agrawal et al., 1993).

The effect of each DIF was analyzed using paired comparison (Table 2). For 0 DIF the differences between 20/20 and 25/25 °C were nonsignificant, but for +5 DIF the difference between 20/15 and 25/20 °C was significant, with higher CO_2 uptake at the lower temperatures.

Effect of CO_2 concentration. The cumulative CO_2 uptake increased significantly from 126 mmol $CO_2 \cdot m^{-2}$ at 380 $\mu L \cdot L^{-1}$ to 228 mmol $CO_2 \cdot m^{-2}$ at 950 $\mu L \cdot L^{-1}$. This 82% increase occurred at the same period during the night as the lower CO_2 level. The increase of CO_2 uptake with higher CO_2 levels for *Phalaenopsis* contradicts the results of Matschke et al. (1998). The latter found that an increase of the CO_2 concentration from 340 to 600 $\mu L \cdot L^{-1}$ reduced the 24-h CO_2 uptake 28% at 25/15 °C and a *PPF* of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, suggesting that higher atmospheric CO_2 concentrations do not always increase CO_2 uptake in CAM plants. In *Kalanchoë pinnata* (Lam.) Pers. (Winter and Engelbrecht, 1994) a higher ambient CO_2 level (680 $\mu L \cdot L^{-1}$) did not increase CO_2 uptake, whereas night-time assimilation of *Agave deserti* Engelm. and *Ferocactus cylindraceus* (Engelm.) Orcutt doubled (Nobel and Hartsock, 1986).

During the week when *Phalaenopsis* plants were supplied with a higher CO_2 concentration, no significant difference in ΣP_n was observed between the first and the last day. This indicates that no adaptation of the photosynthetic apparatus to higher CO_2 levels was activated.

Our findings indicate that at a *PPF* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ the optimal day/night temperature for *Phalaenopsis* cultivars '70' and 'L' was 20/15 °C. A difference of 5 °C between day and night temperature during the vegetative growth period probably does not induce flowering (Röber, 1994); however, this should be tested in detail. An increased CO_2 concentration can enhance the short-term photosynthetic rate, and, presumably, growth.

Table 1. Main effects of cultivar and day/night temperature regime on cumulative CO_2 uptake during 24-h (ΣP_n) by leaves of two *Phalaenopsis* cultivars.

Factor	ΣP_n[z] (mmol $CO_2 \cdot m^{-2}$)
Cultivar	
70	167 b
L	114 a
Temperature regime	
20/15 °C	159 m[y]
20/20 °C	133 n
25/20 °C	139 n
25/25 °C	131 n
Significance	
Cultivar (CV)	***
Temperature regime (TR)	***
CV × TR	NS

[z]Determined at a *PPF* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 12-h photoperiod, and a relative humidity of 65%.
[y]Mean separation among cultivars by ANOVA and among temperature regimes by Duncan's multiple range test at $P \leq 0.05$ (m, n).
NS, ***Nonsignificant or significant at $P < 0.001$, respectively.

Table 2. ANOVA for main effects of cultivar and temperature on cumulative CO_2 uptake during 24 h (ΣP_n) by leaves of two *Phalaenopsis* cultivars.[z]

Factor	Significance	
	Expt. 1 (0 DIF)[y]	Expt. 2 (+5 DIF)[x]
Cultivar	***	***
Temperature	NS	**
Cultivar × temperature	NS	NS

[z]Determined at a *PPF* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 12-h photoperiod, and a relative humidity of 65%.
[y]Temperature regimes were 20/20 °C and 25/25 °C.
[x]Temperature regimes were 20/15 °C and 25/20 °C.
NS, **, ***Nonsignificant or significant at $P < 0.01$ or 0.001, respectively.

Literature Cited

Adams, W.W., III, M. Diaz, and K. Winter. 1989. Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy dissipation, and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. Oecologia 80:553–561.

Agrawal, M., D.T. Krizek, S.B. Agrawal, G.F. Kramer, E.H. Lee, R.M. Mirecki, and R.A. Rowland. 1993. Influence of inverse day/night temperature on ozone sensitivity and selected morphological and physiological responses of cucumber. J. Amer. Soc. Hort. Sci. 118:649–654.

Amberger, S. and P. Fisher. 1995. Nährstoffbedarf von Topf-*Phalaenopsis*. Deutscher Gartenbau 49:2817–2823.

Croci, D. 1988. *Phalaenopsis*: Culture et commercialisation. Lien horticole, 17 Mar. 1988, p. 17.

Genty, B., J.-M. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990:87–92.

Genty, B., J. Harbinson, and N.R. Baker. 1990. Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-respiratory conditions. Plant Physiol. Biochem. 28:1–10.

Jiao, J., E.D. Leonardos, and B. Grodzinski. 1997. Approaches to measuring plant bioproductivity and growth, p. 699–716. In: M. Pessarakli (ed.). Handbook of photosynthesis. Marcel Dekker, New York.

Karlsson, M.G., R.D. Heins, J.E. Erwin, R.D. Berghage, W.H. Carlson, and J.A. Biernbaum. 1989. Temperature and photosynthetic photon flux influence chrysanthemum shoot development and flower initiation under short-day conditions. J. Amer. Soc. Hort. Sci. 114:158–163.

Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant Cell Environ. 14:729–739.

Matschke, J., B. Orthen, and D.J. von Willert. 1998. *Phalaenopsis*, eine CAM-Planze. Gärtnerbörse 98:30–33.

McWilliams, E.L. 1970. Comparative rates of dark CO_2 uptake and acidification in *Bromeliaceae*, *Orchidaceae*, and *Euphorbiaceae*. Bot. Gaz. 131:285–290.

Nobel, P.S. and T.L. Hartsock. 1986. Short-term and long-term responses of crassulacean acid metabolism plants to elevated CO_2. Plant Physiol. 82:604–606.

Ota, K., K. Morioka, and Y. Yamamoto. 1991. Effects of leaf age, inflorescence, temperature, light intensity and moisture conditions on CAM photosynthesis in *Phalaenopsis*. J. Jpn. Soc. Hort. Sci. 60:125–132. (Hort. Abstr. 64:630.)

Röber, R. 1994. Topfplanzen Kulturen. Verlag Eugen Ulmer, Stuttgart.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03. SAS Inst., Cary, N.C.

Van Der Ende, W. 1985. Teelttechnische gegevens over *Phalaenopsis* voor de snij. Vakb. Bloem. 20:44–45.

Winter, K. and G. Awender. 1989. Crassulacean acid metabolism and photochemical efficiency of photosystem II in the adaxial and abaxial parts of the succulent leaves of *Kalanchoë draigremontiana* grown at four photon flux densities. Plant Physiol. 90:948–954.

Winter, K. and B. Demmig. 1987. Reduction state of Q and nonradiative energy dissipation during photosynthesis in leaves of a crassulacean acid metabolism plant, *Kalanchoë daigremontiana* Hamet et Perr. Plant Physiol. 85:1000–1007.

Winter, K. and B. Engelbrecht. 1994. Short-term CO_2 responses of light and dark CO_2 fixation in the crassulacean acid metabolism plant *Kalanchoë pinnata*. J. Plant Physiol. 144:462–467.