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Seasonal variation of photosynthesis and photosynthetic efficiency in *Phalaenopsis*

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Abstract

Nowadays, a quest for efficient greenhouse heating strategies, and their related effects on the plant's performance, exists. In this study, the effects of a combination of warm days and cool nights in autumn and spring on the photosynthetic activity and efficiency of *Phalaenopsis* were evaluated; the latter, being poorly characterised in plants with crassulacean acid metabolism (CAM) and, to our knowledge, not reported before in *Phalaenopsis*. 24-h CO_2 flux measurements and chlorophyll (Chl) fluorescence analyses were performed in both seasons on *Phalaenopsis* 'Hercules' exposed to relatively constant temperature regimes, 25.5/24.0°C (autumn) and 30/27°C (spring) respectively, and distinctive warm day/cool night temperature regimes, 27/20°C (autumn) and 36/24°C (spring), respectively. Cumulated leaf net CO_2 uptake of the distinctive warm day/cool night temperature regimes declined with 10–16% as compared to the more constant temperature regimes, while the efficiency of carbon fixation revealed no substantial differences in both seasons. Nevertheless, a distinctive warm day/cool night temperature regime seemed to induce photorespiration. Although photorespiration is expected not to occur in CAM, the suppression of the leaf net CO_2 exchange during Phase II and Phase IV as well as the slightly lower efficiency of carbon fixation for the distinctive warm day/cool night temperature regimes confirms the involvement of photorespiration in CAM. A seasonal effect was reflected in the leaf net CO_2 exchange rate with considerably higher rates in spring. In addition, sufficiently high levels of photosynthetically active radiation (PAR) in spring led to an efficiency of carbon fixation of 1.06–1.27% which is about twice as high than in autumn. As a result, only in the case where a net energy reduction between the temperature regimes compensates for the reduction in net CO_2 uptake, warm day/cool night temperature regimes may be recommended as a practical sustainable alternative.

Additional keywords: chlorophyll fluorescence; CO_2 assimilation; crassulacean acid metabolism; irradiance; photorespiration; temperature; quantum yield.

Introduction

Phalaenopsis is an epiphytic orchid exhibiting CAM photosynthesis (McWilliams 1970, Lootens and Heursel 1998, Guo and Lee 2006) and originating from tropical and subtropical areas of the South Pacific Islands and Asia (Pridgeon 2000). During greenhouse production, average daily temperatures of 25–30°C are maintained to promote leaf production and inhibit flower initiation (Chen *et al.* 1994, Blanchard and Runkle 2006). Growing

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Abbreviations: ADT – average daily temperature; CAM – crassulacean acid metabolism; Chl – chlorophyll; DIF – the difference between average day- and average night temperature; EC – electrical conductivity; F′ – steady-state fluorescence from the light-adapted state; F_0 – minimal fluorescence from the dark-adapted state; F_0' – minimal fluorescence from the light-adapted state; F_m – maximal fluorescence from the dark-adapted state; F_m' – maximal fluorescence from the light-adapted state; F_q' – difference in fluorescence between F_m' and F′; F_v/F_m – maximum quantum efficiency of PSII photochemistry; F_q'/F_m' – PSII operating efficiency; PAR – photosynthetically active radiation; PEP – phosphoenolpyruvate; PEPC – phosphoenolpyruvate carboxylase; PSII – photosystem II; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; VPD – vapour pressure deficit; ε – efficiency of carbon fixation.

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Phalaenopsis therefore implies high heating expenses, especially in northern latitudes during winter. To reduce energy consumption, warm day/cool night temperature regimes have been proposed (Buwalda *et al.* 2000, Lund *et al.* 2006). However, taking into account that temperature is a main environmental factor influencing the metabolism and development of CAM plants (Lüttge 2004), further improvement of *Phalaenopsis* production in terms of greenhouse heating strategies in northern latitudes demands a better understanding of the plant-temperature responses.

The prominent characteristics of CAM plants are the nighttime fixation of atmospheric CO_2 *via* open stomata and nocturnal malic acid synthesis (Phase I). During the following daytime, malic acid is decarboxylated and released CO_2 is used by photosynthetic assimilation *via* the Calvin cycle (Phase III) (Dodd *et al.* 2002). The transition from night to day and *vice versa* encompasses Phase II and Phase IV, respectively. These transient phases exhibit a shift in CO_2 uptake as a result of competition between phosphoenolpyruvate carboxylase (PEPC) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) for CO_2. Phase II is dominated by PEPC CO_2 uptake with a gradual increase in Rubisco activity, while Phase IV is dominated by the Rubisco-mediated uptake of atmospheric CO_2 (Osmond 1978, Griffiths *et al.* 2002).

In vitro PEPC studies showed that cool night temperatures stabilize the active form of phosphorylated PEPC causing less inhibition by its product, malate, and as such favouring nocturnal carboxylation (Buchanan-Bollig and Kluge 1981, Buchanan-Bollig *et al.* 1984, Carter *et al.* 1995). Warm day temperatures, however, promote dephosphorylation of PEPC, which increases the sensitivity to malate inhibition, and also benefit the decarboxylating enzymes (*e.g.* malic enzyme or phospho*enol*pyruvate carboxykinase). Subsequently, it was claimed that cool night temperatures (15–20°C) and temperature differences between day and night were both required for maximum nocturnal malic acid accumulation in CAM plants (Buchanan-Bollig and Kluge 1981, Buchanan-Bollig *et al.* 1984, Carter *et al.* 1995, Nimmo 2000). Indeed, several authors recommended an optimal night temperature of 18–22°C and day temperatures between 21°C and 28°C for net CO_2 uptake of *Phalaenopsis* (Ota *et al.* 1991, Chen *et al.* 2008, Ichihashi *et al.* 2008).

The temperature response of CAM assimilation has received relatively high attention (*see* reviews of Nimmo 2000, Dodd *et al.* 2002, Lüttge 2004, 2006). On the contrary, only 5 studies deal with CAM photosynthetic efficiency (Spalding *et al.* 1980, Nobel and Hartsock 1983, Adams *et al.* 1986, Rascher and Lüttge 2002, Chen *et al.* 2008). The effect of a combination of warm days and cool nights on CAM photosynthetic efficiency remains unclear and to our knowledge no study covers a semiseasonal or seasonal response for *Phalaenopsis*.

A widely accepted method for the determination of photosynthetic efficiency relies on Chl fluorescence measurements (Maxwell and Johnson 2000). However, these instantaneous measures of photosynthetic efficiency, such as the photosystem II (PSII) operating efficiency (F_q'/F_m') and the maximum quantum efficiency of PSII photochemistry (F_v/F_m), may not be confused with the photosynthetic efficiency derived from CO_2 flux measurements, of which the latter can be denoted as the efficiency of carbon fixation (ε). F_q'/F_m' is a measure of the proportion of light absorbed by Chl associated with PSII that is used in photochemistry and can give an indication of ε. However, a discrepancy between F_q'/F_m' and ε might occur, especially under stress conditions, due to changes in photorespiration or pseudocyclic electron transport (Fryer *et al.* 1998). The fluorescence parameter F_v/F_m is a sensitive indicator of the photosynthetic performance with values ranging from 0.74 to 0.85 for nonstressed plants (Lichtenthaler *et al.* 2005). This parameter provides information about the processes at PSII level, which in turn can alter the photosynthetic efficiency.

According to Skillman (2008), the photosynthetic efficiency of CAM plants can only be determined in a meaningful way by carrying out 24-h gas exchange studies because that allows the temporal separation between nocturnal CO_2 uptake (phase I) and diurnal photosynthetic CO_2 fixation (phase III). In addition, the overall energetics of the transitional phases II and IV are not obvious, since they might involve varying levels of PEPC and Rubisco activity (Dodd *et al.* 2002, Wild *et al.* 2010). Finally, photorespiration is believed to be suppressed as the decarboxylation of malate during phase III generates high partial pressures of CO_2 around Rubisco.

In this study, 24-h CO_2 flux measurements and Chl fluorescence analyses were performed on warm day/cool night temperature-exposed *Phalaenopsis* 'Hercules' plants during a 28- and 32-d period in late autumn and early spring, respectively. The hypothesis was tested whether warm day/cool night temperature regimes applied during different seasons affect the photosynthetic efficiency. This allowed us to gain new insights in the seasonality of both the photosynthetic activity and the photosynthetic efficiency of *Phalaenopsis*.

Materials and methods

Plant material and experimental setup: 26-month-old nonflowering plants with at least 5 fully developed leaves of the *Phalaenopsis* hybrid 'Hercules' were purchased from a commercial grower (*Verdonck Van de Weyer Florist's business*, Wetteren, Belgium). Plants were grown in 12-cm pots (0.6 L) containing pine bark (*Pinus maritima* Lam.) supplemented with 4 kg of slaked lime (10%), 2.5 kg of *Sphagnum* and 0.5 kg of PGmix (15N-10P-20K) per m^3 (*Slingerland*, Zoeterwoude, the Netherlands), with an initial pH of 5.8 and electrical conductivity (EC) of 0.15 µS cm^{-1}. Plants were fertigated (pH of 5.6 and EC of 1 µS cm^{-1}) once a week according to good horticultural practices and planting density was 9 plants m^{-2}.

The experiment was carried out in two identical greenhouse compartments of 81 m^2 (9 × 9 m; height of top 5.5 m; height of side walls 3.8 m) of the Research Centre for Ornamental Plants (51° 03’ N, 3° 48’ E) in late autumn (30 November till 27 December 2005) and early spring (15 April till 16 May 2006). For both experimental periods, new plants were used. The purchased plants were divided over the two greenhouse compartments and subjected to either a rather constant temperature regime (according to commercial practice) or a distinctive warm day/cool night temperature regime. The daytime and nighttime heating set points were adjusted according to the season to limit the resulting energy consumption (Körner and Van Straten 2008). In late autumn, the day- and nighttime heating set points of the constant temperature regime and distinctive warm day/cool night temperature regime were set to 25.5/24.0°C (DIF 1.5) and 27/20°C (DIF 7), respectively. In early spring, the day- and nighttime heating set points were changed to 30/27°C (DIF 3) and 36/24°C (DIF 12) for both regimes. The ventilation set point was 1°C above the heating set points. Air temperature and relative air humidity were measured every 20 s with a RH/T-sensor (*225-500-A, NovaLynx Corporation*, Auburn, California, USA) and 20-min means were registered by the climate computer (*AEM/Mereg*, Maasbree, the Netherlands). Relative air humidity was however uncontrolled. In late autumn, a 12-h photoperiod was supplied using artificial lighting (*SON-T 400W, Philips*, Amsterdam, the Netherlands) with a radiation intensity of 50 µmol PAR m^{-2} s^{-1} at canopy level. In early spring, the photoperiod depended on the natural day lengths and shifted from 13 h 50 min to 15 h 53 min. A shade screen (*PH 66 O, Bonar Technical Fabrics*, Zele, Belgium) closed at 200 µmol PAR m^{-2} s^{-1} to avoid photodamage. Quantum sensors (*QS, Delta-T Devices*, Cambridge, UK) measured irradiance at canopy level every 10 s and 1-min means were recorded by a datalogger (*DL2, Delta-T Devices*, Cambridge, UK).

Leaf net CO_2 exchange measurements: During each entire experimental period (*i.e.* 28 d in late autumn and 32 d in early spring), 24-h CO_2 flux measurements were performed on one leaf per plant and two plants per day/night temperature regime to quantify leaf net CO_2 exchange rates at 60-min intervals. The second leaf counted from the apex, which is the newly matured leaf (Guo and Lee 2006), was placed inside a Plexiglass gas exchange cuvette (length 15 cm, diameter 7 cm). The leaf-cuvette interface was sealed with a nonporous synthetic rubber sealent (*Terostat VII, Henkel-Teroson*, Heidelberg, Germany). The gas-exchange cuvette enclosed a fan (*KD0502PEB1.8, Sunon*, Brea, California, US) to obtain a homogeneous air mixture. Air with ambient CO_2 at concentrations of 700 and 565 ppm CO_2 in late autumn and early spring, respectively, was blown into a 50-L barrel (*Kartell*, Noviglio, Italy) using a pump (*N035AN.18, KNF Neuberger*, Freiburg, Germany) to level off possible CO_2 fluctuations and was sent through the leaf cuvette to a differential infra-red gas analyser (*ADC 225 MK3, ADC Bioscientific*, USA). Air transport occurred through PVC tubes (diameter 6 mm, *VWR*, Leuven, Belgium) surrounded by a heating cable (*RS-378-246*, 15 W m^{-1}, *RS Components*, Corby Northamptonshire, UK) to prevent condensation. The flow rate was determined upstream of the leaf cuvette with an electronic flow meter (*AWM5102, Honeywell*, Morristown, New Jersey, USA). Temperature of the sample air was measured with a thermocouple (*type T, Omega*, Stamford, Connecticut, USA). All variables were monitored every 10 s and 1-min means were recorded by a datalogger (*DL2, Delta-T Devices*, Cambridge, UK). The leaf surface enclosed in the leaf cuvette was determined with a portable leaf area meter (*LI-3000, LI-COR*, Lincoln, Nebraska, USA) coupled to a belt conveyer (*LI-3050 A, LI-COR*, Lincoln, Nebraska, USA).

Chl fluorescence measurements were conducted using a Chl fluorometer (*PAM-2000, Walz*, Effeltrich, Germany). Fluorescence was analyzed from the adaxial side of the second leaf counted from the apex, replicated for 6 plants and measured weekly (*i.e.* on day 1, 7, 14, and 28) during late autumn and on day 1, 7, 18, and 32 during early spring. Taking into account the diurnal cycle of fluorescence kinetics in *Phalaenopsis*, the fluorescence measurements were made early in the day (between 2–4 h after light on or sunrise in late autumn and early spring, respectively) when the plants were expected to be in phase III of CAM (Pollet *et al.* 2009). All plants were dark-adapted for at least 20 min. The minimal fluorescence from the dark-adapted state F_0 was measured under a weak modulated measuring beam (about 0.1 µmol PAR m^{-2} s^{-1}) and the maximal fluorescence from the dark-adapted state F_m was obtained during a 0.8-s saturation pulse (> 1,500 µmol PAR m^{-2} s^{-1}), allowing the determination of the maximum quantum efficiency of PSII photochemistry (F_v/F_m). After 5 min of illumination with continuous red, nonsaturating actinic light (≤ 150 µmol PAR m^{-2} s^{-1}) and saturating light pulses every 20 s, the PSII operating efficiency (F_q'/F_m') was calculated according to Baker (2008) with F_q' the difference in fluorescence between the maximal fluorescence from the light-adapted state (F_m') and the steady-state fluorescence from the light-adapted state (F').

Statistical analysis: Differences between temperature treatments and/or seasons were assessed statistically by *ANOVA* at the 5% probability level ($P \leq 0.05$) using *S-PLUS* (*v7.0, Insightful Corporation*, California, USA).

Results

Environmental conditions: The realised DIF of 1.5°C and 3.3°C was assigned as the more constant temperature regime in late autumn and early spring, respectively (Table 1). For the distinctive warm day/cool night temperature regime, a DIF of 6.7°C and 12.2°C was established in late autumn and early spring, respectively. The realised average daily temperatures between the constant and distinctive day/night temperature regime differed by 1.5°C and 2.2°C in late autumn and early spring, respectively. Within one season no differences were observed at the level of the daily PAR-sum. But spring daily PAR-sum almost doubled the PAR measured in late autumn. Notice that in early spring, in accordance with the increased daily PAR-sum, DIF of both temperature treatments raised to a similar extent. In addition, the daytime vapour pressure deficit (VPD) increased to a high level in early spring. At night, when most of the leaf CO_2 exchange is assumed to take place, VPD decreased in both seasons to an acceptable level ranging from 0.82 to 1.31 kPa.

Table 1. Daytime and nighttime air temperature, difference between average day- and average night temperature (DIF), average daily temperature (ADT), daily PAR-sum, daytime and nighttime vapour-pressure deficit (VPD) for a more constant (*i.e.* DIF 1.5 and DIF 3) and a distinctive (*i.e.* DIF 7 and DIF 12) day/night temperature regime in late autumn and early spring. Data presented are the means over 28 d ($n = 28$; mean ± SE) and 32 d ($n = 32$; mean ± SE) for late autumn and early spring, respectively.

Temperature treatment	Temperature [°C] daytime	nighttime	DIF [°C]	ADT [°C]	PAR [MJ m^{-2} d^{-1}]	VPD [kPa] daytime	nighttime
Late autumn							
DIF 1.5	25.7 ± 0.1	24.3 ± 0.1	1.5 ± 0.1	25.0 ± 0.1	0.62 ± 0.02	1.32 ± 0.02	1.26 ± 0.02
DIF 7	27.0 ± 0.2	20.3 ± 0.3	6.7 ± 0.3	23.5 ± 0.2	0.62 ± 0.02	1.41 ± 0.06	0.82 ± 0.03
Early spring							
DIF 3	30.4 ± 0.2	27.1 ± 0.1	3.3 ± 0.2	29.1 ± 0.1	1.02 ± 0.07	2.01 ± 0.08	1.31 ± 0.05
DIF 12	36.1 ± 0.2	24.0 ± 0.4	12.2 ± 0.3	31.3 ± 0.2	1.02 ± 0.07	3.00 ± 0.14	1.14 ± 0.05

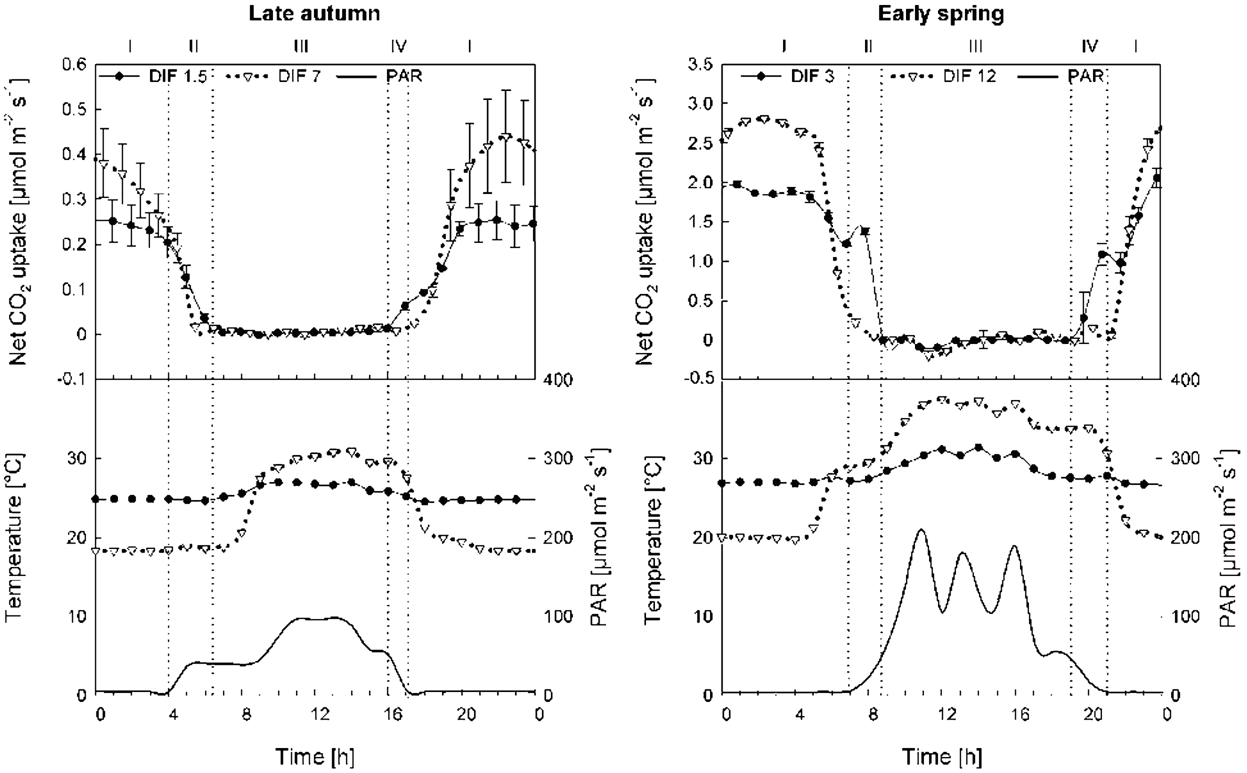

Fig. 1. Net CO_2 uptake of the second leaf counted from the apex of *Phalaenopsis* ($n = 2$, mean ± SE), the photosynthetically active radiation (PAR) and air temperature evolution during day 2 in late autumn and early spring with a more constant (*i.e.* DIF 1.5 and DIF 3) and a distinctive (*i.e.* DIF 7 and DIF 12) day/night temperature regime. *Dotted lines* are indicative for the different CAM-phases (I–IV) as identified by Osmond (1978).

CO_2 assimilation and photosynthetic efficiency: Fig. 1 shows the leaf net CO_2 exchange, PAR and air temperature evolution as well as the four-phase framework described by Osmond (1978) for the second day of the late autumn and early spring experiment. The leaf net CO_2 exchange pattern was typical for CAM with a predominating nocturnal CO_2 uptake (phase I) and no net CO_2 exchange during daytime due to stomatal closure (phase III). The nighttime leaf net CO_2 exchange (phase I) of DIF 7 and DIF 12 plants averaged 92.2% and 98.6% of the total daily net CO_2 assimilation, respectively. For the DIF 1.5 and DIF 3 leaves, only 85.0% and 85.9% of the total daily net CO_2 assimilation took place during phase I, respectively. The contribution of both transient phases (*i.e.* phase II and phase IV) to the total daily net CO_2 assimilation was limited to 6.5% and 2.0% in DIF 7 and DIF 12, respectively. For the DIF 1.5 and DIF 3 plants, this contribution was considerably higher and reached 14.0% and 14.9%, respectively. Notice also that the absolute leaf net CO_2 exchange rate was a 6- to 8-fold higher at PAR values of about 200 µmol m^{-2} s^{-1} in early spring as compared to late autumn with PAR intensities of 100 µmol m^{-2} s^{-1}.

The enhanced daily net CO_2 assimilation in DIF 7 and DIF 12 leaves was however not preserved throughout the experiment. From day 20 and day 10 in the late autumn and early spring experiments, respectively, the DIF 1.5 and DIF 3 cumulated leaf net CO_2 uptake had the tendency to exceed the DIF 7 and DIF 12 cumulated leaf net CO_2 uptake (Fig. 2). As a consequence, the cumulated leaf net CO_2 uptake of DIF 7 and DIF 12 plants was with respect to DIF 1.5 and DIF 3 plants 1.7 g m^{-2} (*i.e.* 9.8%) and 12.0 g m^{-2} (*i.e.* 15.8%) lower after about one month since the start of the experiments in late autumn and early spring, respectively.

Up to day 15, the efficiency of carbon fixation (ε) was substantially lower in late autumn than in early spring (Fig. 3). Starting from day 18 of the late autumn

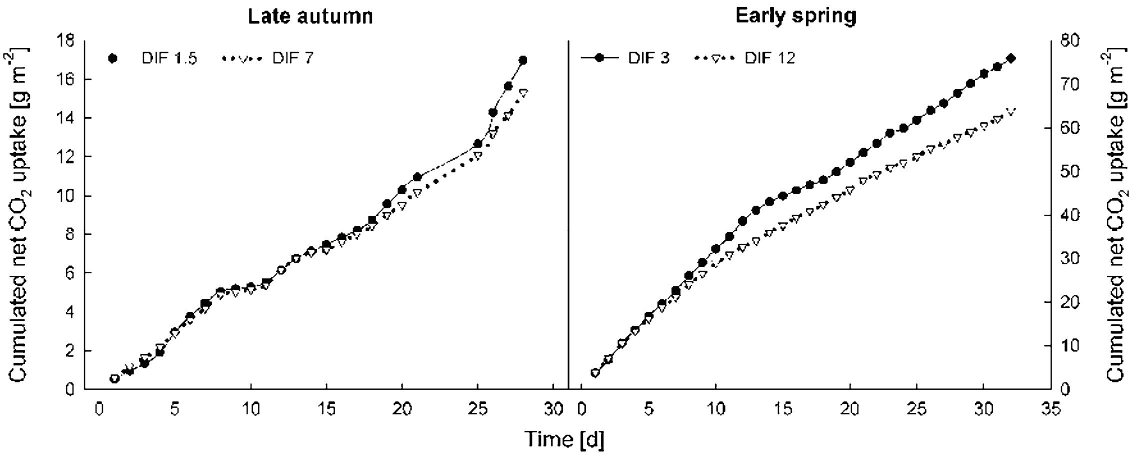

Fig. 2. Cumulated net CO_2 uptake of the second leaf counted from the apex of *Phalaenopsis* in late autumn and early spring with a more constant (*i.e.* DIF 1.5 and DIF 3) and a distinctive (*i.e.* DIF 7 and DIF 12) day/night temperature regime.

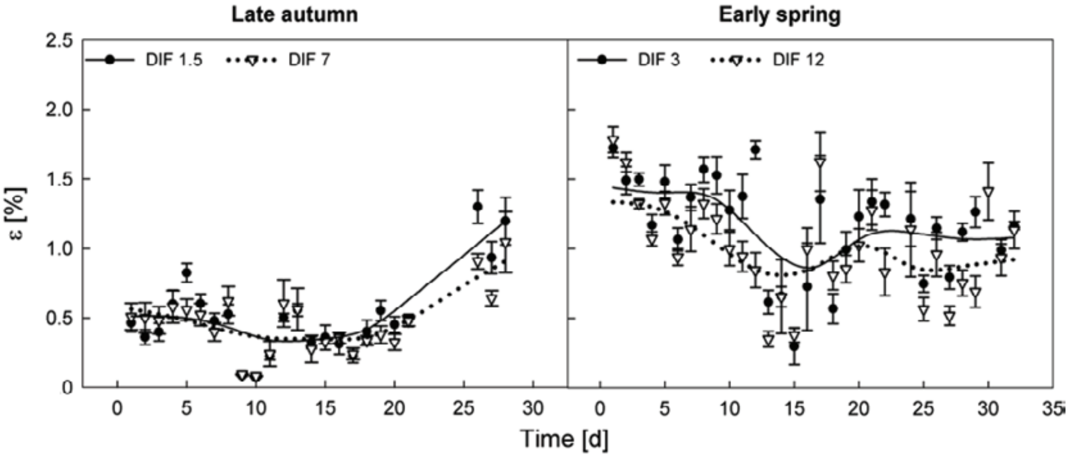

Fig. 3. Efficiency of carbon fixation (ε), calculated as the ratio of the 24-h integrated leaf net CO_2 exchange to the daily PAR-sum, of *Phalaenopsis* exposed to a more constant (*i.e.* DIF 1.5 and DIF 3) and a distinctive (*i.e.* DIF 7 and DIF 12) day/night temperature regime during a month in late autumn and early spring. *Bars* represent standard errors ($n = 2$).

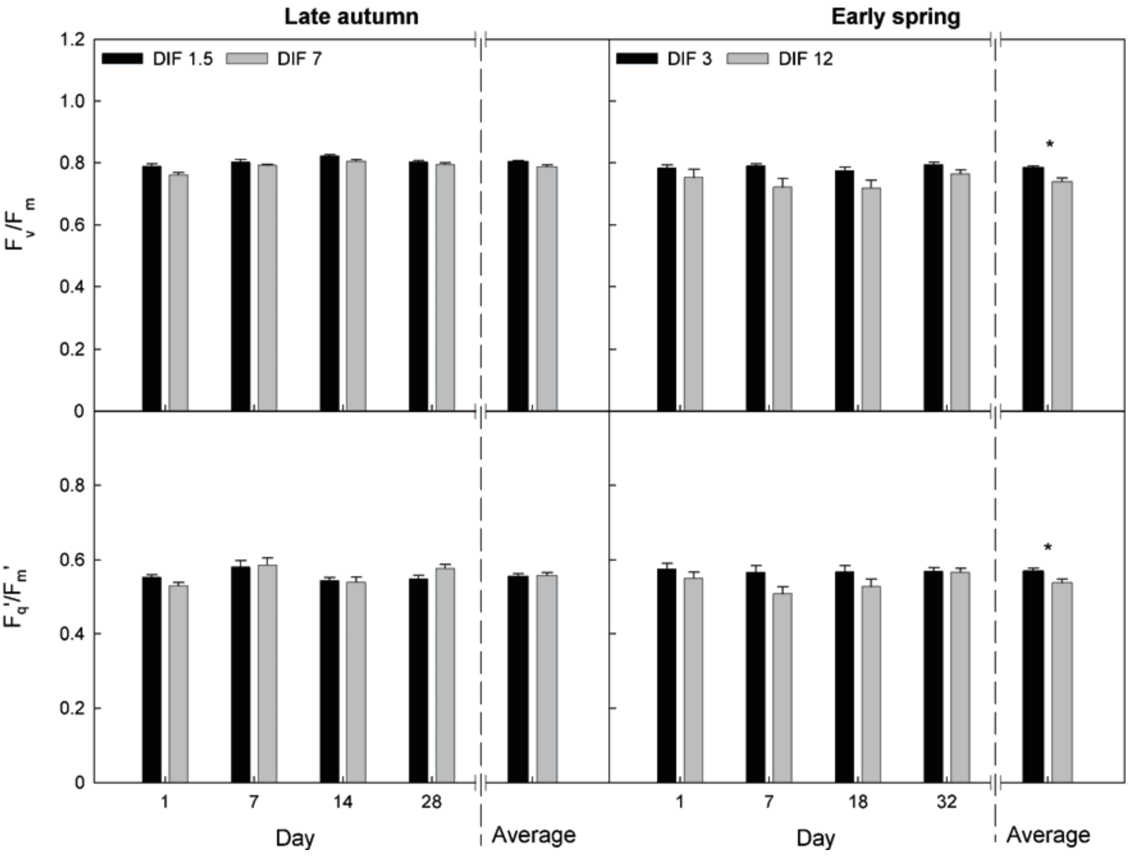

Fig. 4. Maximum quantum efficiency of PSII photochemistry (F_v/F_m), PSII operating efficiency (F_q'/F_m') and their seasonal averages for *Phalaenopsis* grown at a more constant (*i.e.* DIF 1.5 and DIF 3) and a distinctive (*i.e.* DIF 7 and DIF 12) day/night temperature regime in late autumn and early spring ($n = 6$, mean ± SE). Significant differences between temperature treatments are marked with * ($P \leq 0.05$).

experiment, however, ε increased in 10 d only to a level similar to ε in early spring. Nevertheless, average ε in late autumn was significantly lower than average ε in early spring. Within one season, no significant differences could be observed between DIF 1.5 and DIF 7, and DIF 3 and DIF 12, respectively. In late autumn, ε averaged 0.51 ± 0.06% and 0.46 ± 0.05% for DIF 1.5 and DIF 7 plants, respectively. The average ε in early spring was 1.27 ± 0.10% and 1.06 ± 0.07% for DIF 3 and DIF 12 plants, respectively.

Chl fluorescence: At individual days during late autumn and early spring, no significant differences could be observed between DIF 1.5 and DIF 7, and DIF 3 and DIF 12, respectively for F_v/F_m and F_q'/F_m' (Fig. 4). In early spring, however, average F_v/F_m and average F_q'/F_m' of DIF 3 plants were significantly higher than for DIF 12 plants. In late autumn, both average F_v/F_m and average F_q'/F_m' were not significantly different between DIF 1.5 and DIF 7, and DIF 3 and DIF 12, respectively.

Discussion

Warm day/cool night temperature effect on photosynthesis and photosynthetic efficiency: Many studies indicated that diurnal temperature alternations with a warm day and cool night, which is similar to a positive DIF, are favourable for nocturnal CO_2 uptake in CAM plants (Neales 1973, Osmond 1978, Nobel and Hartsock 1984, Carter *et al.* 1995, Lüttge 2004). Indeed, nocturnal CO_2 uptake (Phase I) of *Phalaenopsis* ‘Hercules’ was enhanced when growing at DIF 7 (actual day/night temperature of 27/20°C) and DIF 12 (actual day/night temperature of 36/24°C) (Fig. 1). These findings are also consistent with Ota *et al.* (1991), Lootens and Heursel (1998), Chen *et al.* (2008) and Ichihashi *et al.* (2008) who demonstrated for *Phalaenopsis* that net CO_2 uptake was maximal when grown at day temperatures of 21°C to 28°C and night temperatures of 18°C to 22°C. Nevertheless, nocturnal CO_2 uptake of DIF 7 and DIF 12 plants tended to be lower after 3 to 5 days compared to the nocturnal CO_2 uptake of DIF 1.5 and DIF 3 plants. Subsequently, the cumulated leaf net CO_2 uptake of the distinctive warm day/cool night temperature regimes (*i.e.* DIF 7 and DIF 12) declined with 10% and 16% as compared to the more constant temperature regimes (*i.e.* DIF 1.5 and DIF 3) after one month in late autumn and early spring, respectively (Fig. 2). In this context, it is however wrong to conclude that a distinctive warm day/cool night temperature regime is harmful for *Phalaenopsis* growth. Indeed, the cumulated net CO_2 uptake at DIF 12 in early spring is still a 4-fold higher than the cumulated net CO_2 uptake at DIF 1.5 in late autumn due to the difference in light intensity between both measurement periods. Therefore, providing a sufficiently high light intensity (*i.e.* 200 µmol m^{-2} s^{-1}) might contribute to a high daily net CO_2 uptake, even when a distinctive warm day/cool night temperature is maintained.

On the other hand, a considerable reduction in the contribution of the transient phases II and IV (Osmond 1978) to the daily net CO_2 assimilation was observed when exposing *Phalaenopsis* ' Hercules' to a distinctive warm day/cool night temperature regime, especially in early spring. Taking the involvement of Rubisco during these transient phases (Dodd *et al.* 2002) into account as well as the increased oxygenation activity of Rubisco at higher temperatures (Jordan and Ogren 1984) (*e.g.* ≥ 30°C in early spring), these finding suggest the occurrence of photorespiration in CAM. It is believed that photorespiration is suppressed in CAM due to carbon-concentrating mechanisms (Spalding *et al.* 1979). However, more recently Thomas *et al.* (1987), Maxwell *et al.* (1998), Lüttge (2002) and Duarte and Lüttge (2007) concluded that the suppression of photorespiration in CAM is incomplete. Consistent with earlier findings that photorespiration imposes a penalty on the efficiency of carbon fixation (ε), especially in C_3 plants (Singsaas *et al.* 2001), also our results revealed a slightly lower ε for the distinctive warm day/cool night temperature regimes (*i.e.* DIF 7 and DIF 12) as compared to the more constant day/night temperature regimes (*i.e.* DIF 1.5 and DIF 3) in CAM. Based on the suppression of the leaf net CO_2 exchange during Phase II and Phase IV, and the slight decline in ε, it can be concluded that photorespiration occurs in CAM.

Maximum quantum efficiency of PSII photochemistry (F_v/F_m) and PSII operating efficiency (F_q'/F_m') were only significantly reduced in early spring when the daytime temperature was high as observed in this study (*i.e.* 36.1°C). These findings are consistent with Ali *et al.* (2005), who reported a significant decrease of F_v/F_m after 24-h exposure of *Phalaenopsis* to 40°C due to temperature-associated oxidative damage**.** However, in our study, the involvement of oxidative stress could not be proven. So, the mechanism of temperature associated oxidative damage in *Phalaenopsis* still needs further investigation.

Seasonal effect on photosynthesis and photosynthetic efficiency: Leaf net CO_2-exchange rate of *Phalaenopsis* 'Hercules' was considerably higher when PAR was 200 µmol m^{-2} s^{-1} in early spring as compared to 100 µmol m^{-2} s^{-1} in late autumn. This seasonal PAR effect is consistent with studies on a series of CAM plants where PAR intensity during the day determined the magnitude of CO_2 uptake during the subsequent night (Nobel and Hartsock 1983, Nobel *et al.* 2002, Pimienta-Barrios *et al.* 2006). The key role for this seasonal PAR response can be ascribed to the amount of phospho*enol*-pyruvate (PEP). Nobel and Hartsock (1983) pointed out that photosynthesis and gluconeogenesis increase the carbohydrate storage pools required for PEP synthesis as a precursor for nocturnal CO_2 uptake by PEPC. Similar PAR responses have also been demonstrated for *Phalaenopsis*. At a PAR intensity of 180 to 200 µmol m^{-2} s^{-1}, leaf net CO_2 exchange rates were obtained between 4.5 and 6.0 µmol m^{-2} s^{-1} (Lootens and Heursel 1998, Guo and Lee 2006). Reducing irradiance levels to 90 µmol PAR m^{-2} s^{-1} led to a leaf net CO_2-exchange rate of 2.3 µmol m^{-2} s^{-1} (Chen *et al.* 2008). However, in our study absolute CO_2-exchange rates of *Phalaenopsis* 'Hercules' were substantially lower due to the relatively high nighttime vapour-pressure deficits (up to 1.7 kPa), which in agreement with Ichihashi *et al.* (2008) probably forced the stomata to close.

Also, ε indicated a seasonal PAR effect. In late autumn, daily PAR was not sufficient to meet the energy requirements for optimal CAM photosynthesis and ε was reduced by 50% compared to early spring. However, the relative quick increase in ε near the end of late autumn to a similar level as in early spring was remarkable. This increase could be attributed to a rise in daily PAR-sum. The average daily PAR-sum increased from 0.59 to 0.68 MJ m^{-2} d^{-1} for the period before and after day 16, respectively. Consistently, the growth rate calculated as the slope of the cumulated net CO_2 uptake evolution for equivalent periods, increased from 0.47 to 0.70 g(CO_2) m^{-2} d^{-1} and as such confirms the rise of ε towards the end of late autumn.

The ε in CAM plants has received relatively little attention. CAM ε appears to be highly variable and to our knowledge (semi-) seasonal responses of ε in CAM plants have not yet been reported before. For C_3 plants, 8 mol photons are required to fix 1 mol CO_2 assuming ideal conditions. Consequently, the theoretical maximum ε for C_3 plants is calculated to be 12.5% (Bolton and Hall 1991). Taking into account that only 45% of the incident PAR is absorbed by the photosynthetic apparatus and that only two thirds of the absorbed energy can be stored into chemical energy, the practical maximum ε under optimum conditions is estimated to be 8–9% (Bolton and Hall 1991, Melis 2009). The CAM pathway is however more energy-demanding for net CO_2 assimilation than the C_3 pathway (Winter and Smith 1996), and as such the ε for CAM plants could be expected to be lower than 8–9%. Yet, the average ε for CAM plants found in literature and pooled for all measurement methods and conditions, equals 7.3% (Skillman 2008). In our study, however, ε was calculated from CO_2-flux measurements only. When similar measurement methods were used, ε averaged 1.1% (Nobel 1977, Nobel and Hartsock 1983), which also is consistent with our findings for the ε of *Phalaenopsis* 'Hercules' during early spring.

Energy savings *via* warm day/cool night temperature regimes: Important energy savings can be achieved when allowing a positive difference between average day – and average night temperature (DIF) in spring and autumn. Above this, energy saving is additionally favoured by adjusting the DIF value to the season (Körner and Van Straten 2008). In this study, a positive DIF was therefore imposed with selection of daytime and nighttime heating set points in accordance with Belgian standard seasonal outdoor temperatures. During early spring, the greenhouse heated up mainly by irradiance leading to daytime temperatures up to 36°C, while at night ventilation with cool outdoor air allowed greenhouse air temperature to drop down to 24°C (Table 1). As a result, a positive DIF of 12°C could be obtained and saving energy is readily feasible according to Körner and Challa (2003), Lund *et al.* (2006) and Körner and Van Straten (2008). In late autumn, however, low outdoor temperatures required supplementary greenhouse heating. To limit energy consumption a maximum day- and night temperature of 27°C and 24°C, respectively, was selected and a DIF of 1.5°C and 7°C was established. This seasonal effect on DIF, with relatively high DIF values (*i.e.* 12) in early spring and relatively low ones (*i.e.* 7) in late autumn, was also obvious in the higher average daily temperature recorded in early spring as compared to late autumn.

Conclusions: This study evaluated the photosynthetic activity and efficiency of *Phalaenopsis* 'Hercules' grown under a constant temperature regime and a distinctive warm day/cool night temperature regime in late autumn and early spring. It was shown that a combination of warm days and cool nights did not impair the efficiency of carbon fixation. However, too high daytime temperatures (*e.g.* 36.1°C) are likely to decrease photosynthetic efficiency due to a temperature-related up-regulation of the Rubisco oxygenase activity and thus photorespiration and concomitant suppression of the leaf net CO_2 exchange during the CAM phases II and IV. The photosynthetic activity and photosynthetic efficiency revealed a pronounced seasonality. Leaf net CO_2 exchange rate as well as the efficiency of carbon fixation was substantially higher during early spring. The efficiency of carbon fixation amounted 1.06–1.27% in early spring suggesting that the light energy level (*i.e.* 200 μmol m^{-2} s^{-1}) was sufficiently high to meet the optimal energy requirements for optimal CAM photosynthesis in *Phalaenopsis*.

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