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# Compensatory effects of elevated $CO_2$ concentration on the inhibitory effects of high temperature and irradiance on photosynthetic gas exchange in carrots

A. THIAGARAJAN, R. LADA*, and P. JOY

*Department of Plant and Animal Sciences, Nova Scotia Agricultural College, P.O. Box 550, Truro, NS Canada B2N 5E3*

## Abstract

We determined the interactive effects of irradiance, elevated $CO_2$ concentration (EC), and temperature in carrot (*Daucus carota* var. *sativus*). Plants of the cv. Red Core Chantenay (RCC) were grown in a controlled environmental plant growth room and exposed to 3 levels of photosynthetically active radiation (PAR) (400, 800, 1 200 μmol $m^{-2}$ $s^{-1}$), 3 leaf chamber temperatures (15, 20, 30 °C), and 2 external $CO_2$ concentrations ($C_a$), AC and EC (350 and 750 μmol $mol^{-1}$, respectively). Rates of net photosynthesis ($P_N$) and transpiration ($E$) and stomatal conductance ($g_s$) were measured, along with water use efficiency (WUE) and ratio of internal and external $CO_2$ concentrations ($C_i/C_a$). $P_N$ revealed an interactive effect between PAR and $C_a$. As PAR increased so did $P_N$ under both $C_a$ regimes. The $g_s$ showed no interactive effects between the three parameters but had singular effects of temperature and PAR. $E$ was strongly influenced by the combination of PAR and temperature. WUE was interactively affected by all three parameters. Maximum WUE occurred at 15 °C and 1 200 μmol $m^{-2}$ $s^{-1}$ PAR under EC. The $C_i/C_a$ was influenced independently by temperature and $C_a$. Hence photosynthetic responses are interactively affected by changes in irradiance, external $CO_2$ concentration, and temperature. EC significantly compensates the inhibitory effects of high temperature and irradiance on $P_N$ and WUE.

*Additional key words*: *Daucus*; stomatal conductance; transpiration rate; water use efficiency.

## Introduction

Eco-physiological adaptations in plant systems provoked by global climate change are a subject of intensive investigation with plant physiologists. Increases in atmospheric $CO_2$ associated with a raise in temperature and irradiance are critical environmental parameters warranting attention due to their direct influence on the photosynthetic processes. The individual and/or interactive effects of some of these parameters on photosynthesis have been investigated in many crops. Generally, net photosynthetic rate ($P_N$) increases with irradiance in cucumber, tomato, pepper, and carrots (Warren Wilson *et al.* 1992, Nederhoff and Vegter 1994, Kyei-Boahen *et al.* 2003b). Also, an increase in PAR results in increases in stomatal conductance ($g_s$) and transpiration rate ($E$) to certain extent. This is followed by a steep decline phase resulting in a sharp increase in water use efficiency (WUE). In carrots, the optimum PAR exists between 600 and 800 μmol $m^{-2}$ $s^{-1}$ (Kyei-Boahen *et al.* 2003a). The effects of increasing temperature on $P_N$ and gas exchange processes are also well documented (Pastenes and Horton 1996, Law and Crafts-Bradner 1999). Influence of temperature on $P_N$ varies depending on crop species and their optimal temperature range. Above-optimal temperatures change the solubility of gases and a decline in the $CO_2/O_2$ specificity of ribulose bisphosphate (RuBP) carboxylase/oxygenase (RuBPCO) and ultimately reduce the carboxylation efficiency ($C_i/C_a$). With elevated $CO_2$ concentration (EC), a positive influence on $P_N$ is widely reported. EC increases $P_N$ in several crops until down-regulation process take-over (Sage 1994). Decrease in supply of inorganic phosphates ($P_i$) and/or limitations in regenerative capacity of RuBP may cause such adaptive reaction (Kyei-Boahen *et al.* 2003b, Thiagarajan and Lada 2007).

The previous findings on the interactive effect of irradiance with temperature on photosynthetic efficiency are either complementary or inhibitory. The damage to photosystems 1 (PS1) and 2 (PS2) caused by high temperature

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*Corresponding author; fax: +1 902 897 9762, e-mail: rlada@nsac.ca
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was alleviated by high irradiance in spinach and pea leaves (Weis 1982, Havaux *et al.* 1991) whereas it was aggravated in wheat (Al-Khatib and Paulsen 1999). Being extremely heat-sensitive, carrots are expected to experience severe reduction in $P_N$ under high temperature and irradiance. Little is known on the nature of this effect in carrots. Interaction between $C_a$ and irradiance can be paradoxical. EC can either aid in transportation of more electrons (Long 1991) or induce photoinhibition by affecting PS1 and PS2 (Roden and Ball 1996) under high irradiance. Temperature and EC exhibit complimentary effects on gas exchange. EC enhances carbon exchange rate and may reduce the likelihood of changes from high temperatures in the specificity of RuBPCO to $CO_2$ (Pastenes and Horton 1996). Also, $C_a$ compensates the negative photorespiratory effects of high temperatures through increased carbon flux in several $C_3$ plants such as beans and peanuts (Lambreva *et al.* 2005, Vu 2005). Under natural conditions plants are constantly exposed to collective influence of these parameters. Thus, examining interactive effects will help better comprehend the physiological adaptive mechanisms. Few studies have demonstrated these effects in rose (Urban *et al.* 2001), beans (Lambreva *et al.* 2005), and peanuts (Vu 2005), however, no studies have been conducted on carrots. Accordingly, this study was conducted with the following objectives: (*a*) identify the optimal temperature, PAR, and $C_a$ combination for maximizing photosynthesis and WUE, and (*b*) determine the nature of interactions between PAR, temperature, and EC under greenhouse conditions.

## Materials and methods

**Plants**: The carrot (*Daucus carota* L.) cv. RCC was sown in 10×15 cm pots filled with commercial pre-mixed growing media (*Pro-Mix BX*, Riviere-du-Loup, Canada). Plants were raised in a controlled environmental growth room maintained at 22/10 °C day/night temperature regime with a 16 h photoperiod under an irradiance of 450 μmol $m^{-2}$ $s^{-1}$ as measured by a *LI-188B* quantum radiometer (*Li-Cor*, Lincoln, USA). Irradiation was provided by a combination of incandescent, fluorescent, and high pressure sodium lamps. Relative humidity was maintained at 60 % throughout the study. After emergence, seedlings were thinned to 5 plants per pot at the first true leaf stage and allowed to grow for 3 weeks before being placed under specific experimental conditions. During the growing period, all the seedlings received 200–250 $cm^3$ of water daily, and 125 $cm^3$ per pot of 2.4 kg $m^{-3}$ 20N–20P–20K fertilizer, with micronutrients once a week. Experimental parameters included 3 levels of PAR (400, 800, 1 200 μmol $m^{-2}$ $s^{-1}$), 3 leaf chamber temperatures (15, 20, 30 °C), and 2 external $CO_2$ concentrations ($C_a$) (350 and 750 μmol $mol^{-1}$ = AC and EC).

**Gas exchange**: $P_N$, $E$, and $g_s$ were measured on the newest fully expanded intact leaf as described in Thiagarajan and Lada (2007) using a portable open-flow gas analyzer in conjunction with a portable Leaf Microclimate Control system temperature chamber (*LCA-4*, *Analytical Development Company*, Hoddesdon, UK). WUE was calculated as the ratio of $P_N$ to $E$. Leaf areas were calculated using a Computer Image Analysis System (CIAS) software (*Jandel Scientific*, Vancouver, WA, USA), and all measured parameters were adjusted to their respective leaf areas before statistical analysis.

**Manipulation of experimental parameters**: $CO_2$ concentration inside the leaf chamber LCA unit was controlled using a Leaf Microclimate Control System (LCMS, *Analytical Development Company*, Hoddesdon, UK). A $CO_2$ canister connected to the LCMS unit was used to modify the $C_a$ levels inside the leaf chamber to near constant levels. For altering PAR, a portable unit (*ADC* model *PLU-LMC-002*) with dichroic "white light" *60VWFL* lamp in combination with neutral density filters was used. Temperature inside the chamber was adjusted to desired levels with the use of the heating and cooling controls of the LCMS unit. For each treatment, four plants (4 replicates) were selected for observations. All readings were taken between 09:00 and 12:00 h daily. After imposition of each treatment effect, a 5-min interval period was practiced to allow for an equilibrium state before making measurements. An auto-mode function with the *LCA4* unit was invoked for recording gas exchange measurements at 1-min intervals in replicates of five. The $C_a$ was initially set to 350 μmol $mol^{-1}$ and the irradiance and temperature were maintained at 400 μmol $m^{-2}$ $s^{-1}$ and 15 °C, respectively. After measurements were taken at this combination, the temperature was increased from 15 to 20 °C and subsequently to 30 °C. Under each experimental condition, observations were recorded. The same procedure was repeated for treatment combinations with 800 and 1 200 μmol $m^{-2}$ $s^{-1}$ irradiances. Following this, the $C_a$ was increased to 750 μmol $mol^{-1}$ and the same protocol was practiced until all treatment combinations were examined.

**Experimental design and statistical analyses**: A split-split plot design was followed for conducting the experiment inside the growth chamber. Main plot effect was $C_a$, sub-plot effect was irradiance, and sub-sub-plot effect was temperature. The WUE and $g_s$ values were log transformed before statistical analysis to conform to normality standards. However, the results reported in this paper are actual values. A 2×3×3 factorial design and repeated measures' model was assumed. The *proc Mixed* statement of *SAS* was used to analyze the data (*SAS*

*Institute*, Cary, USA). As the measurements were not taken from the same experimental unit each day, the independent assumption of error terms assumed with repeated measures was likely violated (Littell *et al.* 1998). Accordingly, the error term was assumed to be normally distributed with equal variance and the dependence was expressed as a covariance of Σ. An autoregressive covariance structure was sufficient for all obtained and calculated data for achieving convergence during the repeated measures procedure. Following significant effect of treatments, the means were separated using Tukeys LSD ($p$<0.05).

## Results

***P*$_N$**: An interaction between $C_a$ and PAR was observed in $P_N$ (Fig. 1*A*). This result suggests that $C_a$ and PAR had relatively more influence on the key components of the photosynthetic machinery than did the temperature. As PAR increased so did the $P_N$ under both $C_a$ regimes. At EC $P_N$ was at least twice as high as at AC under all PAR regimes (Table 1). $P_N$ was therefore maximized under the highest PAR and $C_a$ (1 200 μmol m$^{-2}$ s$^{-1}$ + 750 μmol m$^{-3}$) (Fig. 1*A*). Temperature independently influenced $P_N$. $P_N$ increased from 8.29 to 9.41 μmol m$^{-2}$ s$^{-1}$as temperature increased from 15 to 20 °C, reaching its highest. $P_N$ then declined to 7.18 μmol m$^{-2}$ s$^{-1}$ as the temperature increased to 30 °C (Table 2).

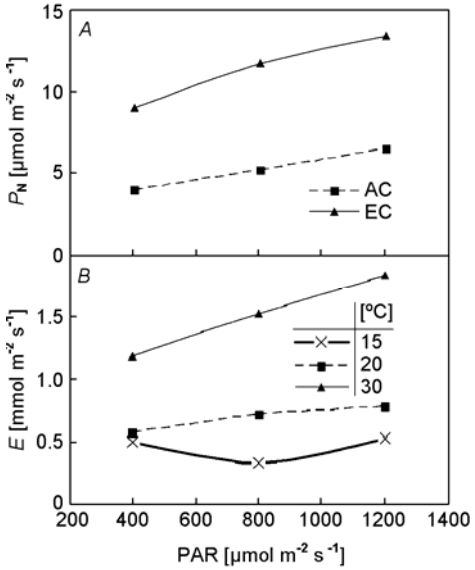


Fig. 1. (*A*) Net photosynthetic rate ($P_N$) influenced by different photosynthetically active radiation (PAR) and $CO_2$ regimes, and (*B*) transpiration rate ($E$) influenced by different PAR and temperature regimes.

***C*$_i$/*C*$_a$** was influenced by temperature and $C_a$ independently (Table 1). The lowest $C_i/C_a$ was found at 20 °C, indicating that the plant's carboxylation efficiency was the highest at this temperature. On the contrary, the highest value (0.57) was observed at 15 °C. At 30 °C, the efficiency increased by 12 % compared to the ones exposed to 15 °C. Increasing $C_a$ to 750 μmol mol$^{-1}$ altered the ratio from 0.53 to 0.47, thereby increasing the carboxylation efficiency by 11 %.

***g*$_s$ and *E***: The $g_s$ showed no difference for changes in $C_a$ ($p$=0.45). The $g_s$ responded negatively towards temperature and positively towards PAR (Table 1). As the temperature increased from 15 to 20 °C, $g_s$ declined by 50 % (0.10 to 0.05 μmol m$^{-2}$ s$^{-1}$). However, this diminishing effect did not last beyond 20 °C. This implies that the critical range for regulation of $g_s$ existed between 15 and 20 °C. A significant increase in $g_s$ was noticed only when PAR was increased from 400 to 1 200 mmol m$^{-2}$ s$^{-1}$, suggesting that drastic changes in PAR are required to evoke any change in $g_s$.

As expected, $E$ was strongly influenced by PAR and temperature interactively (Fig. 1*B*), however, it did not show any significant response due to increase in $C_a$. The $E$ at AC and EC remained the same (0.89 mmol m$^{-2}$ s$^{-1}$). A maximum (1.83 mmol m$^{-2}$ s$^{-1}$) and a minimum value (0.33 mmol m$^{-2}$ s$^{-1}$) of $E$ were found at 1 200 PAR + 30 °C and at 800 PAR + 15 °C combinations, respectively (Table 2). Increasing temperatures from 15 to 20 °C, generally showed no influence on $E$, irrespective of PAR. However, at 30 °C, and at PAR of 800 and 1 200, $E$ increased by 19 and 55 %, respectively, compared to PAR 400 (1.18 mmol m$^{-2}$ s$^{-1}$). At 400 PAR, $E$ and showed little or no increase (<1 %) for a temperature change from 15 to 20 °C, however, it increased by 107 % when the temperature was increased to 30 °C. The same trend continued for 800 and 1 200 PAR, however, with 800 PAR the increase between 15 and 20 °C was significant (Table 2).

**WUE**: Temperature, PAR, and $C_a$ interactively contributed to WUE. Increase in temperature reduced WUE, however, this negative consequence was compensated by the doubling effect of EC. The influence of PAR in improving the WUE along with temperature and $C_a$ was essential at 15 °C. Maximum WUE was observed when plants were subjected to a combination of 15 °C + 1 200 PAR + EC. Conversely, plants that were exposed to a combination of 30 °C + 400 PAR + AC had the lowest WUE. At 15 °C, PAR of 800 μmol m$^{-2}$ s$^{-1}$ proved to be the optimal irradiance to evoke the highest WUE at both AC and EC (Table 3 and Fig. 2). At 20 and 30 °C no increase was observed in WUE between PAR and within $C_a$. Seemingly, at higher temperatures, the influence of PAR was superseded by temperature and/or $C_a$.

At AC, WUE decreased at 30 °C when compared to those observed at 15 or 20 °C irrespective of PAR. Furthermore, at 800 PAR an increase in temperature

Table 1. Net photosynthetic rate, $P_N$ [μmol $m^{-2}$ $s^{-1}$], transpiration rate, $E$ [mmol $m^{-2}$ $s^{-1}$], stomatal conductance, $g_s$ [mol $m^{-2}$ $s^{-1}$], water use efficiency, WUE, and ratio of intercellular to ambient $CO_2$ concentration ($C_i/C_a$) at various irradiance, temperature, and $CO_2$ regimes. Values followed by the same letter are not significantly different at 5 % level.

| | | $P_N$ | $E$ | $g_s$ | WUE | $C_i/C_a$ |
|---|---|---|---|---|---|---|
| PAR [μmol $m^{-2}$ $s^{-1}$] | 400 | 6.49 c | 0.75 b | 0.06 b | 11.71 b | 0.52 a |
| | 800 | 8.48 b | 0.86 b | 0.07 ab | 16.57 a | 0.48 a |
| | 1200 | 9.92 a | 1.05 a | 0.08 a | 13.98 a | 0.49 a |
| Temperature [°C] | 15 | 8.29 b | 0.45 c | 0.10 a | 22.86 a | 0.57 a |
| | 20 | 9.41 a | 0.69 b | 0.05 b | 14.51 b | 0.42 c |
| | 30 | 7.18 c | 1.51 a | 0.05 b | 4.89 c | 0.50 b |
| $C_a$ [μmol $mol^{-1}$] | 350 | 5.20 b | 0.94 a | 0.07 a | 9.42 b | 0.53 a |
| | 750 | 11.39 a | 0.83 a | 0.07 a | 18.76 a | 0.47 b |
| PAR×Temperature | | NS | S | NS | NS | NS |
| PAR×$C_a$ | | S | NS | NS | NS | NS |
| Temperature×$C_a$ | | NS | NS | NS | S | NS |
| PAR×Temperature×$C_a$ | | NS | NS | NS | S | NS |

Table 2. Rates of net photosynthesis ($P_N$) and transpiration ($E$) at different photosynthetically active radiation (PAR) and $CO_2$ regimes (AC and EC). Values followed by the same letter are not significantly different at 5 % level.

| PAR | $C_a$ [μmol $mol^{-1}$] | | Temperature [°C] | | |
|---|---|---|---|---|---|
| [μmol $m^{-2}$ $s^{-1}$] | AC | EC | 15 | 20 | 30 |
| 400 | 3.94 f | 0.50 de | 0.57 de | 1.18 c | 9.03 c |
| 800 | 5.20 e | 0.33 e | 0.71 d | 1.52 b | 11.76 b |
| 1200 | 6.45 d | 0.53 de | 0.78 d | 1.83 a | 13.38 a |

Table 3. Water use efficiency under different PAR, temperature, and $CO_2$ regimes. Values followed by the same letter are not significantly different at 5 % level.

| PAR | Temperature [°C] | | | | | |
|---|---|---|---|---|---|---|
| [μmol $m^{-2}$ $s^{-1}$] | 15 | | 20 | | 30 | |
| | $C_a$ | | | | | |
| | AC | EC | AC | EC | AC | EC |
| 400 | 11.79 cd | 22.57 bc | 8.30 de | 18.65 ab | 2.30 g | 6.64 e |
| 800 | 30.26 ab | 29.48 ab | 7.62 e | 22.10 ab | 2.79 f | 7.19 e |
| 1200 | 9.92 de | 33.14 a | 8.89 de | 21.50 ab | 2.89 f | 7.53 de |

from 15 to 20 °C showed a decline in WUE. At EC, WUE was higher than at AC at all temperatures and PAR, except at the 15 °C and 800 PAR combinations (Table 3). At 15 °C, the increase in PAR from 400 to 1 200 μmol $m^{-2}$ $s^{-1}$ increased WUE by 47 % under EC. However, this beneficial effect was not observed under other temperature regimes. The interaction between temperature and $CO_2$ ($p<0.0001$) was stronger than the interaction between temperature, $CO_2$, and PAR ($p<0.0346$), suggesting that temperature and $CO_2$ are the more contributing parameters to WUE.

## Discussion

***P*N**: This interaction between $C_a$ and PAR has been observed previously in several other crops, *e.g.* mung bean (Karim *et al.* 2003). We found that $P_N$ increased with increasing PAR and $C_a$. For change in PAR from 400 and 800 μmol $m^{-2}$ $s^{-1}$, $P_N$ experienced a 56 % enhancement. However, this improvement diminished by 5 % when PAR was changed from 800 to 1 200 μmol $m^{-2}$ $s^{-1}$ (Table 2). This phenomenon implies the up-regulatory mechanisms involved with augmenting $P_N$ begin to break down as PAR becomes too high. One of the possible

reasons for this is inducement of photoinhibitory processes due to high PAR. The consequences of photoinhibition are reduced quantum efficiency for $CO_2$ absorption and release of $O_2$ and reduced photochemical activity of PS2 (Alves *et al.* 2002). Since $g_s$ increased with elevated PAR up to 800 μmol $m^{-2}$ $s^{-1}$, the principal conduits for $CO_2$ fixation were opened, hence the carbon exchange was not affected, and photoinhibition did not take place. At 1 200 μmol $m^{-2}$ $s^{-1}$ these mechanisms may begin to break down as observed in the reduced enhancement of $P_N$ and beyond this level a further reduction may be expected.

The positive influence of doubling $C_a$ on $P_N$ is in accordance with findings of Kyei-Boahen *et al.* (2003b). However, with PAR only a hyperbolic response is widely reported (Kyei-Boahen *et al.* 2003a). Saturated supply of $CO_2$ to RuBPCO complex to compete against $O_2$ molecules is suggested to be reason for increase in $P_N$ in response to $C_a$ enrichment. Limited supply of $P_i$ that cannot meet the high demands of the photosynthetic apparatus was ascribed towards the down-regulation of $P_N$ under high PAR (>700 μmol $m^{-2}$ $s^{-1}$). However, our results did not show a complete down-regulation of $P_N$ in response to PAR. With AC, increase of $P_N$ in response to increase in PAR from 400 to 800 was 32 % whereas the increase from 800 to 1 200 caused only a 24 % increase. A similar pattern was observed under EC, however, the increase was lower (14 %) in response to PAR increase from 800 and 1 200 μmol $m^{-2}$ $s^{-1}$. Although there is a decline in the magnitude of $P_N$ enhancement, PAR values were not sufficiently high enough to manifest a complete down regulation. The optimal PAR for saturating $P_N$ varies among cultivars. Kyei-Boahen *et al.* (2003a) found that carrot cultivars were not saturated in their $P_N$ response even at 1 000 μmol $m^{-2}$ $s^{-1}$. Similar results were reported in potatoes and tomato (Midmore and Prange 1992, Warren Wilson *et al.* 1992).

With temperature, our results correspond well with findings described by other researchers confirming that $P_N$ will reach its optimum values at 20 °C and then decrease with temperatures above 25 °C (Table 1). Temperature primarily affects the functionality of RuBPCO protein. Low temperature provokes inadequate viscosity state inside thylakoid membrane (Gomes-Laranjo *et al.* 2005), therefore, electron transport functions could have been negatively affected. On the other hand, optimal temperature activates electron transport in PS2, increases RuBPCO functionality (Han *et al.* 2004), and hence promotes $P_N$. Nevertheless, high temperature (above 35 °C) denatures RuBPCO protein, promotes ion imbalance, and degrades the plasma membrane integrity in most crops (Larkindale and Huang 2004).

***C*i/*C*a** ratio is an indicator of the carboxylation efficiency of the $C_3$ plants. Temperature plays a pivotal role in the activation and deactivation processes of enzyme complex involved in photosynthesis. In most species, the activity of RuBPCO activase declines as temperature increases above the optimum range (Kim and Portis 2005). Based on our results the maximum efficiency was observed at 20 °C (Table 1). The sub-optimal and supra-optimal temperatures have recorded lower carboxylation efficiencies. Most probably, optimal temperatures provoked the active stages of RuBPCO and improved the carboxylation efficiency of the plants. This is established by the lower values of $C_i/C_a$ recorded at 20 °C. Another possible mechanism of achieving better carboxylation efficiency is the increased $C_a$ supply. Since the increase in temperature brought down the $g_s$ by more than 50 %, carbon exchange was limited and thus the possibility of this effect was negligible (Table 1). However, when $C_a$ increased the reduction in $C_i/C_a$ values was apparent. Increased supply of $C_a$ improved the probability of $CO_2$ molecules competing against $O_2$ molecules in binding with RuBPCO exchange sites.

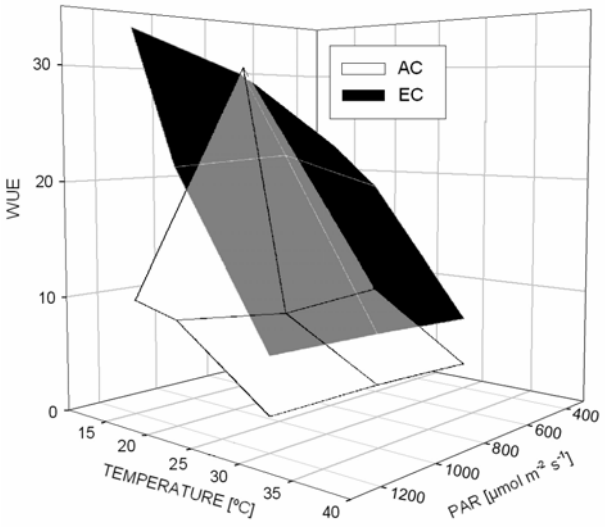


Fig. 2. Water use efficiency (WUE) as influenced by photosynthetically active radiation (PAR), temperature, and $CO_2$ regimes.

***g*s and *E***: Increase in $C_a$ decreases $g_s$ in many $C_3$ crops including carrots (Kyei-Boahen 2003b). However, our results have shown otherwise (Table 1). The increase or decrease in $g_s$ is suggested to vary depending on individual experiment, growth, and measurement conditions (Field 1995). Moreover, stomatal aperture is a primary regulatory mechanism to maintain normal tight coupling with photosynthesis (Morison 1998). The response in $g_s$ towards increase in temperature was typical as observed in many $C_3$ plants. Plants generally tend to shut down stomata under high temperatures attempting to maintain optimal leaf moisture potential. For instance, according to Morales *et al.* (2004) heat stress caused a decline in $g_s$ in tomatoes. With PAR, $g_s$ increased linearly. Increase in PAR produces increases in $g_s$ until certain levels beyond which it declines in a hyperbolic fashion (Kyei-Boahen

*et al.* 2003a). In the present study, however, $g_s$ did not reach the declining phase. This is further supported by the fact that the $P_N$ did not reach its acclimation phase.

The influence of temperature on *E* was more pronounced only above 20 °C at all PAR levels. The increases in *E* between 20 and 30 °C were 107, 114, and 134 % at PAR of 400, 800, and 1 200, respectively. Individual effects of temperature and PAR have been investigated in several $C_3$ crops. A linear relationship between temperature and *E* has been documented in cotton, wheat (Law and Crafts-Brandner 1999), and potatoes (Ghosh *et al.* 2000). Gao *et al.* (2002) indicated that *E* increases linearly with PAR, however, $g_s$ decreases with increasing vapor pressure deficit and *E* is a hyperbolic function of vapor pressure deficit. Our results in Tables 1 and 2 clearly provide evidence for this phenomenon. Few studies have reported interactive effects between PAR and temperature. Frak *et al.* (2002) found that *E* increases linearly with PAR and vapor pressure deficit. Our results corroborate with earlier findings that temperature effects became more influential as the PAR increased. Temperature directly governs stomatal regulation and consequently influences the rates of carbon exchange. Nevertheless, under sufficient influx pathways, efficiency of photosystems is controlled by optimal PAR. However, sufficient supply of $CO_2$ is essential to attain maximal WUE.

**WUE**: At 15 °C, maximum WUE (33.21) was observed at 800 PAR, irrespective of $C_a$ enrichment (Table 1 and Fig. 2). However, at 1 200 PAR, $C_a$ enrichment was required to maintain the maximum WUE, indicating that $C_a$ enrichment can compensate the negative effects of photoacclimation. Long (1991) showed that $C_a$ enrichment can positively influence the photon energy utilization by supporting a greater load of electron transport in $C_3$ plants. Hymus *et al.* (2001) reported dissipation of absorbed photons is more efficient under EC thus reducing photoinhibition. Our results corroborate well with previous findings. The $g_s$ is the primary site of $CO_2$ exchange directly influencing water loss and photosynthesis. Our data suggested that increase in PAR increased $g_s$ linearly (Table 1). This perhaps contributed to a better $CO_2$ exchange rate and improved WUE. Increase in $g_s$ in response to increase in PAR has been previously reported in beans by Lambreva *et al.* (2005).

At 20 °C, PAR had no influence on WUE. However, $C_a$ exhibited its maximum compensatory effects at this temperature regime. In fact, doubling of $C_a$ concentrations from 350 increased the WUE by 124, 190, and 141 % at 400, 800, and 1 200 PAR, respectively, indicating that $C_a$ can effectively overcome the stress imposed by sub-optimal temperatures (Table 3). Sub-optimal temperatures can reduce the exchange efficiency of RuBPCO, however, our results have shown increases in carboxylation efficiencies ($C_i/C_a$) at high temperatures (Table 1). Enrichment of $C_a$ likely compensated this impediment through saturation of $CO_2$ at exchange sites. Our data indicated a drop in $g_s$ under all PAR levels for increase in temperature (Table 1). Increasing temperature to sub-optimal range would cause partial closure of stomata and pose stomatal limitation for carbon exchange. Such impaired $CO_2$ influx rates would subsequently reduce $P_N$ under AC. The decrease in WUE at 20 °C as against 15 °C supports this hypothesis (Table 1). Conversely, a saturated supply of $C_a$ confers advantage to $CO_2$ molecules competing with $O_2$ molecules to bind with the RuBPCO and subsequently increase $P_N$ (Chaves and Pereira 1992). This positive effect of $C_a$ enrichment on $P_N$ was so strong that it overcame the negative impact of temperature on *E*.

At 30 °C, the WUEs were reduced by at least 70 % when compared to those observed at 20 °C, with still no effect of PAR (Table 3). Although $g_s$ remained constant between 20 and 30 °C, acute decrease in WUE can be attributed to increase in *E* and decrease in $P_N$ due to non-stomatal factors. The contribution of *E* was observed as the maximum values were registered at this temperature regime. Several non-stomatal factors could be attributed to decrease in $P_N$. Temperature around 30 °C causes deactivation of several enzymes, including RuBPCO consequently hampering $P_N$. Pastenes and Horton (1996) suggest that temperature >30 °C would limit the supply of NADPH available for the carbon assimilation process. In addition, it would also affect the electron transport functionality of PS2. The non-responsiveness for PAR at high temperature provides strong evidence for the proposed hypothesis. Nevertheless, $C_a$ enrichment exhibited similar compensatory effects for stomatal limitations as found at 20 °C. Indeed, the improvements ranged from 152 to 188 % across PAR levels.

In conclusion, $P_N$ was influenced by PAR and $C_a$ interactively and increased linearly with both parameters. The PAR and $C_a$ levels were not sufficiently high enough to cause a complete down-regulation. Maximum $P_N$ was registered at 1 200 μmol $m^{-2}$ $s^{-1}$ PAR and EC. Temperature individually influenced $P_N$. The optimal temperature was 20 °C for maximum $P_N$. A decline in $P_N$ was observed below and above the optimal temperatures likely due to enzyme deactivation and photo-inhibitory mechanisms, respectively. The $g_s$ responded only to temperature and PAR. The $g_s$ decreased when temperature was raised above 15 °C and when PAR was increased from 400 to 1 200 μmol $m^{-2}$ $s^{-1}$. Reaction towards temperature was regulatory mechanism to conserve leaf moisture potential. With PAR, normally a hyperbolic response is expected. However, since PAR did not saturate the photosynthetic apparatus, a decline in $g_s$ was not observed. *E* increased linearly with PAR and temperature. The influence of temperature was more effective at high PAR. Maximum WUE was noticed at 800 μmol $m^{-2}$ $s^{-1}$ PAR and at 15 °C combination. $C_a$ did not influence WUE at this temperature. However, at high PAR and high temperatures, enrichment of $C_a$ was necessary to attain high WUE. An increased load of electron transport provoked by enrich-

ment of $C_a$ was attributed to this compensatory effect on photoinhibition. This beneficiary effect was well established by the lower $C_i/C_a$ at high temperatures. This clearly suggests that carrots exhibit maximum WUE at low temperatures and moderate PAR, with no negative effects of EC. However, at high temperature and PAR, EC might improve the WUE.

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Grimm, B., Porra, R.J., Rüdiger, W., Scheer, H. (ed.): **Chlorophylls and Bacteriochlorophylls. Biochemistry, Biophysics, Functions and Applications.** – Springer, Dordrecht 2006. ISBN-10 1-4020-4515-8 (hard bound). XXIX + 8 +603 pp., € 245.00.

The 25th volume of the well-known series "Advances in Photosynthesis and Respiration" is devoted to the major pigments of photosynthesis. It is a successor of the often cited voluminous book edited by Hugo Scheer that was published fifteen years ago by the CRC Press. It consisted of 42 chapters; this number is similar to the 37 chapters of the new book. It is again a multi-authored book and most of the seventy authors (some of them were authors in both issues) tried to present an up-to-date state of knowledge in the respective fields of chlorophyll and bacteriochlorophyll research. Nevertheless, I was surprised reading author's apology on p. 381: Harold Paulsen wrote the chapter 26 in mid-2002 (!) and states that Fig. 1 (sketch of LHCII structure) is outdated. I do not understand why it was not possible to replace the figure – did the editorial and publisher procedures take full four years?

The chapters belong to five topical parts. The first of them is on structures, chemistry, and analysis of chlorophyll pigments (9 chapters) and starts with an overview of the whole book that also gives practical information (*e.g.* comparison of ring numbering according to two nomenclatures on p. 3, possible substituents on pp. 5–6). The following chapters deal with synthesis, structure, and reactivity of chlorophylls, features of chlorophylls of the *c* group, unusual tetrapyrrole pigments in individual photosynthetic bacteria and algae, special chlorophylls in reaction centres, chlorophylls formed during heavy metal stress, and degradation products formed during digestion, extraction, and storage of plant materials. Four chapters are of an immense practical value: they deal with methodical questions. The first of them is on the use of spectroscopic methods in structure determination of pigments. The following chapter gives recommendations for spectrophotometric and spectrofluorometric assays of chlorophylls and bacteriochlorophylls and shows errors introduced by using old-fashioned methods and equations. Next chapters show the methods of analysis of chlorophylls by high performance liquid chromatography and by simple open-column chromatography (extraction and detection techniques are also given).

Ten chapters of the second part deal with metabolism of chlorophylls, beginning with biosynthesis of 5-aminolevulinic acid, the respective enzymes, the basic pathways and last steps leading to biosynthesis of chlorophylls *a* and *b* and bacteriochlorophylls *a* to *e*, involvement of tetrapyrrole compounds in cellular regulation, and with chlorophyll catabolism. The importance of chlorophyll evolution in phylogeny of oxygenic photosynthesis is stressed in last chapter of this part.

Nine chapters of part three are devoted mainly to bacteriochlorophylls and their reaction with the native environment in different organisms. Interactions with proteins in reaction centres, light-harvesting complexes, and chlorosomes, *etc*. are given here. Most chapters show models of the respective structures, global ring currents, macrocycles, protein maquettes, pigment-protein complexes, and so on.

The part dealing with functions of chlorophylls consists of four chapters only. Excitation energy transfers in different photosynthetic structures, carotenoid-to-bacteriochlorophyll energy transfers, and their dynamics are in the focus of these chapters.

The last part is on practical applications of chlorophylls and bacteriochlorophylls. Two chapters describe their use in diagnostics and photodynamic therapy as well as results of clinical trials, in complex electronic systems and biological and technical models, and in monitoring chlorophyll contents in oceans used to predict primary biomass production. Last chapter is on functionalized transformation products of chlorophylls and bacteriochlorophylls in natural sediments. Thus these pigments are very important in geochemical and paleoenvironmental studies.

The texts are accompanied by many figures and schemes (10 colour figures are present on pages CP1 to CP8), with tables and wide lists of references. As usual in this book series, there is a very good and detailed subject index (over 40 pages!). I welcome that three of the four editors work in Germany: this ensured a better balance of results produced in individual parts of world than that in some other volumes of this book series.

I fully recommend this book to all researchers, students, and teachers interested in natural pigments and in the processes of photosynthesis. As a university student I read cover-to-cover the "Untersuchungen über Chlorophyll" by Willstätter and Stoll published in 1913 – the difference in contents of that book and the reviewed one is certainly much larger than I did expect in my young days!

Z. ŠESTÁK (*Praha*)