

Respiratory Q_{10} of marigold (*Tagetes patula*) in response to long-term temperature differences and its relationship to growth and maintenance respiration

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Acclimation of respiration to temperature is not well understood. To determine whether whole plant respiration responses to long-term temperature treatments can be described using the Q_{10} concept, the CO_2 exchange rate of marigolds (*Tagetes patula* L. 'Queen Sophia'), grown at 20°C or 30°C, was measured for 62 days. When plants of the same age were compared, plants grown at 20°C consistently had a higher specific respiration (R_{spc}) than plants grown at 30°C (long-term $Q_{10} = 0.71\text{--}0.97$). This was due to a combination of greater dry mass at 30°C and a decrease in R_{spc} with increasing mass. When plants of the same dry mass were compared, the long-term Q_{10} was 1.35–1.55; i.e. R_{spc} was higher at 30°C than at 20°C. Whole plant respiration could be accurately described by dividing respiration into growth and maintenance components. The maintenance respiration coefficient was higher at 30°C than at 20°C, while the growth respiration coefficient was lower at 30°C, partly because of temperature-dependent changes in plant composition. These results suggest difficulties with interpreting temperature effects on whole plant respiration, because conclusions depend greatly on whether plants of the same age or mass are compared. These difficulties can be minimized by describing whole plant respiration on the basis of growth and maintenance components.

Introduction

Temperature greatly affects the carbon exchange rate and growth of plants. However, the exact method by which it effects carbon accumulation is not always clear, because temperature affects both photosynthesis and respiration. Although temperature effects on photosynthesis and its biochemical components are fairly well understood (see reviews by Medlyn et al. 2002 and Hikosaka et al. 2006), our understanding of how long-term warming affects respiration is, unfortunately, limited (Amthor 2000). Temperature effects on respiration are commonly ex-

pressed using the Q_{10} concept, where the Q_{10} is the relative increase in respiration with a 10°C increase in temperature. The Q_{10} is often within the range 2–2.5 (Larigauderie and Körner 1995, Amthor 2000), although a literature survey found Q_{10} values, based on short-term responses to temperature, ranging from 1.36 to 4.2 (Larigauderie and Körner 1995). Although some of the reported differences in Q_{10} may be due to differences among species or measuring techniques, there also are reports that the Q_{10} can vary throughout the year (Carey et al. 1997), with the nitrogen concentration of

Abbreviations – C_f , carbon content; C_{glu} , carbon content of glucose; CUE, carbon use efficiency; g_{20} , estimated growth respiration coefficient at 20°C; g_r , growth respiration coefficient; IRGA, infrared gas analyzer; M, plant dry mass; m_{20} , estimated maintenance respiration coefficient at 20°C; m_r , maintenance respiration coefficient; P_g , daily average gross photosynthesis; P_n , daily average net photosynthesis; R_d , average night-time respiration; R_g , growth respiration; RGR, relative growth rate; R_m , maintenance respiration; R_{spc} , specific respiration; T, temperature.

leaves (Turnbull et al. 2005), or as a function of the relative growth rate (RGR) and dry mass of plants (van Iersel 2003a). It has been suggested that an increase in RGR, and the accompanying increase in the ratio of growth respiration (R_g) to maintenance respiration (R_m), decreases the respiratory Q_{10} (van Iersel 2003a), because growth respiration is considered to be less sensitive to temperature than maintenance respiration.

There is controversy concerning the concept of dividing respiration into growth and maintenance respiration, partly because there is no clear biochemical distinction between the cellular processes required for maintenance and growth (Hansen 1998). In addition, certain energy-requiring processes (e.g. phloem loading) may be necessary for both growth and maintenance, while other processes (e.g. nutrient uptake) cannot easily be classified as growth or maintenance (Gifford 2003).

Despite its shortcomings, the separation of respiration into growth and maintenance components provides a more mechanistic approach to modeling plant or ecosystem respiration than other, more common, approaches. Many productivity models assume a simple, positive correlation between photosynthesis and respiration (Gifford 2003). Since photosynthesis and respiration respond differently to environmental conditions, such simple approaches are unlikely to accurately describe respiratory responses to changing environmental conditions. However, if the effect of these environmental conditions on growth and maintenance respiration are known, then models that use the growth and maintenance concept may be able to accurately describe respiration under different conditions. Despite their shortcomings, Amthor (2000) and Cannell and Thornley (2000) conclude that models that separate respiration into growth and maintenance components can be valuable in exploring the importance of respiration in plant growth.

Separation of respiration into growth and maintenance components may be especially useful when looking at respiratory responses to temperature. Growth respiration can be described as the growth rate \times growth respiration coefficient (g_r), and g_r is generally considered to be temperature insensitive, unless temperature affects either the chemical composition of the plant material or the biochemical pathways by which new plant material is synthesized (Penning de Vries et al. 1974). Szaniawski and Kielkiewicz (1982) indeed found that root temperatures ranging from 10°C to 30°C had no effect on g_r of roots or shoots. Thus, the effects of temperature on growth respiration probably depend mainly on effects on the growth rate of plants and not on changes in g_r . Maintenance respiration is generally expressed as the maintenance respiration coefficient (m_r) \times plant size.

Unlike g_r , m_r is considered to be highly sensitive to temperature, mainly due to increased protein turnover and membrane leakage at higher temperatures (de Wit et al. 1970, Thornley and Johnson 1990). For example, van Iersel (2003a) estimated the Q_{10} for m_r to be 2.5–2.6 for four bedding plant species exposed to short-term temperature changes. Thus, maintenance respiration responses to temperature are likely to depend both on temperature effects on plant size (and thus indirectly on plant growth rate) and on m_r . Therefore, to develop a mechanistic description of how temperature affects respiration, plant growth rate, size, g_r and m_r need to be determined simultaneously.

Much research has focused on the short-term effects of temperature on respiration (e.g. Larigauderie and Körner 1995). However, long-term effects often differ from short-term effects (Gifford 2003), since plant respiration can acclimate to temperature (Larigauderie and Körner 1995, Atkin and Tjoelker 2003, Bolstad et al. 2003). Because of this acclimation, short-term responses of respiration to temperature may not be indicative of long-term responses (Amthor 2000). The phrase 'long-term Q_{10} ' is used here to describe long-term respiratory responses to temperature, while Q_{10} is used for short-term responses. Since acclimation of photosynthesis and respiration can occur within as little as 2 days (Rook 1969), temperature effects that occur after exposing plants to a different temperature for more than 1–2 days may be considered to be long-term effects, while effects that occur within hours may be considered to be short-term effects.

The objective of this research project was to quantify the effect of temperature on growth and maintenance respiration of whole marigold plants, throughout plant development. The hypothesis was that a long-term increase in temperature would increase m_r , because it is generally sensitive to temperature changes. However, since the plants were grown at constant temperatures, allowing the plants to acclimate, temperature effects on m_r would be expected to be less than in studies with short-term temperature changes, where plants do not acclimate to the rapidly changing temperature. Unlike m_r , g_r was not expected to be affected by temperature.

Materials and methods

Plant material and growing conditions

Plants were grown in $53 \times 27 \times 6$ cm³ (length \times width \times height) trays, with 20-cm slits cut at both ends of the tray. These trays were placed on top of a 32×58 cm² acrylic sheet, which had narrow slits to match those in the trays, and served as the base of the gas exchange chambers.

Seventy-centimeter pieces of capillary mat (Vattex-F, OS Plastics, Norcross, GA) were inserted through these slits, so that the mats covered the bottom of the trays, and approximately 15 cm of the capillary mat extended from the bottom of each side of the acrylic plate. The acrylic plate was put on top of a 9-cm-high wooden frame, and a second tray (filled with nutrient solution) was placed inside this frame. The two ends of the capillary mat were placed inside the bottom tray, and thus allowed for movement of the nutrient solution to the tray inside the gas exchange chamber (Fig. 1). The nutrient solution was made with a complete, water-soluble fertilizer (Miracle-Gro Excel 15-5-15 CalMag, The Scotts Co., Marysville, OH) dissolved in deionized water, resulting in a concentration of $100 \text{ mg L}^{-1} \text{ N}$. The trays containing the fertilizer solution were refilled daily, and the solution level in these trays was approximately 5 cm below the bottom of the trays with the plants. This irrigation system prevented sudden changes in the water content of the growing medium, which can have large effects on respiration (van Iersel and Bugbee 2000).

The tray inside the gas exchange chamber was lined with a second capillary mat, which was treated with CuSO_4 to prevent roots from growing into the capillary mat. This facilitated complete recovery of the root system at the end of the experiment. The trays were filled with a 50 : 50 (v/v) mixture of fine sand and perlite, and watered thoroughly with nutrient solution, before seeding each flat with 36 seeds of marigold 'Queen Sophia'. The eight trays were placed inside two growth chambers (four trays per growth chamber) and enclosed in CO_2 exchange

chambers ($0.58 \times 0.32 \times 0.60 \text{ m}^3$, length \times width \times height). The growth chambers were programmed for a 14-h light period and had a photosynthetic photon flux of $410 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at the surface of the growing medium, which resulted in a total daily photon flux of 20.7 mol m^{-2} .

Treatments

Temperature set points in the gas exchange chambers were 20°C or 30°C , and were kept constant throughout the entire experiment. The temperature of the air inside the gas exchange chambers was measured every 2 s with type T thermocouples connected to a thermocouple multiplexer (AM25T, Campbell Scientific, Logan, UT) and datalogger (CR10T, Campbell Scientific). Based on these temperature measurements, the datalogger controlled 200-W heaters in each chamber, using a relay driver (SDM-CD16AC, Campbell Scientific). The average temperature in each chamber was within 0.2°C of the set point, with short-term fluctuations of approximately 0.5°C .

Relative humidity averaged 83% and 64% at 20°C and 30°C , resulting in vapor pressure deficits of 0.4 and 1.5 kPa at 20°C and 30°C , respectively. No attempt was made to maintain similar vapor pressure deficits at the two temperatures, because under natural conditions the vapor pressure deficit generally increases with increasing temperature as well. The CO_2 concentrations inside the gas exchange chambers were not controlled, and thus depended on the CO_2 concentration of the ambient air

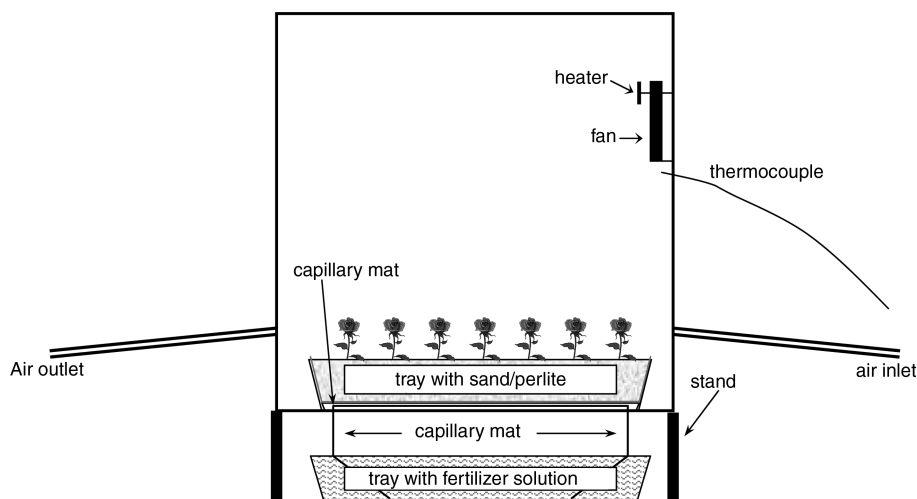


Fig. 1. On overview of the set-up for a single CO_2 exchange chamber. This set-up automatically maintained a constant moisture level in the trays, without the need to open the CO_2 exchange chambers for irrigation. Temperature was monitored with a type T thermocouple, and when the temperature dropped below the 20°C or 30°C set point, a datalogger automatically turned on the heater in the chamber. Eight of these CO_2 exchange chambers were placed in two reach-in growth chambers (four CO_2 exchange chambers per growth chamber).

and the CO₂ exchange rate of the plants. The CO₂ concentration inside the gas exchange chambers during photosynthesis measurements averaged 380 and 350 μmol mol⁻¹ at 20°C and 30°C, respectively.

Measurements

The CO₂ exchange rate of whole trays of plants was measured every 10 min for 62 days, using a multi-chamber, semi-continuous CO₂ exchange system (van Iersel and Bugbee 2000). Approximately 0.5 L s⁻¹ of ambient air was blown into the acrylic gas exchange chambers, and airflow into the chambers was measured with mass flow meters (GFM37-32, Aalborg Instruments and Controls, Monsey, NY). The CO₂ concentration of the incoming air was measured with an infrared gas analyzer (IRGA) (SBA-1, PP-systems, Haverhill, MA). The difference in the CO₂ concentration of the air entering and exiting the chamber was measured with a pressure-corrected IRGA in differential mode (LI-6251, LI-COR, Lincoln, NE). Airflow to the differential IRGA was controlled by opening and closing solenoid valves so that air from each gas exchange chamber could be sampled separately. The solenoid valves were controlled by an SDM-CD16AC relay module and CR10T datalogger (Campbell Scientific). Whole chamber CO₂ exchange (μmol s⁻¹) was calculated as the product of mass flow (mol s⁻¹) and the difference in CO₂ concentration of the air entering and exiting the chamber (μmol mol⁻¹). Every chamber was measured for 30 s, once every 10 min. There was a 30-s delay in data collection after solenoids were switched to measure the next chamber, to ensure that all air from the previous gas exchange chamber was purged from the tubing and differential IRGA. The data from the 30-s measuring period were automatically collected, averaged and stored by the datalogger. Errors in the measurements due to zero drift of the differential IRGA were corrected by subtracting the CO₂ exchange rates of two empty gas exchange chambers (placed outside of the growth chambers) from the measured CO₂ exchange rate of the plants. The effects of water vapor in the air on the IRGA measurements were minimized by drying the air with a 4°C condenser and draining the condensed water.

The accuracy of the gas exchange system was tested before the start of the experiment by reacting approximately 1.3 g of NaHCO₃ with 2 M HCl inside the gas exchange chambers and measuring the amount of CO₂ that resulted from this reaction. The measured CO₂ exchange accounted for 97.2 ± 2.9% (mean ± SD, n = 8) of the CO₂ expected to evolve from the NaHCO₃.

At the end of the experiment, all plants were harvested, and leaf area, leaf, stem, flower and root dry mass, and leaf tissue nutrient concentrations, were determined. Leaf

area ratio (leaf area/total dry mass), specific leaf area (leaf area/leaf dry mass) and leaf mass ratio (leaf dry mass/total dry mass) were calculated from these data. Total C, N and S were determined with a CNS 2000 analyzer (LECO Corp., St Joseph, MI), while other nutrients were determined by dry ashing and inductively coupled plasma spectrophotometry (Jarrell-Ash ICAP 9000, Thermo Jarrell Ash Corp., Franklin, MA).

Calculations and data analysis

Daily average net photosynthesis (P_n, mol s⁻¹) and nighttime respiration (R_d, mol s⁻¹) were calculated from the gas exchange measurements and are expressed on a per-plant basis. Although P_n and R_d represent CO₂ fluxes in opposite directions, both are represented as positive values. Gross photosynthesis was estimated as the sum of P_n and R_d. Plant growth rate (g day⁻¹) was calculated from these data as:

$$\text{Growth} = 3600 (14P_n - 10R_d)12/C_f \quad (1)$$

where 3600 is the number of seconds in an hour, 14 h is the duration of the light period, 10 h is the duration of the dark period, 12 is the atomic mass of C (g mol⁻¹), and C_f is the carbon content of the plants, as determined by tissue analysis at the end of the experiment (g g⁻¹). It is assumed that C_f was constant throughout plant development. In these and all subsequent calculations, R_d is the average of the respiration during the dark periods before and after a particular light period. Plant dry mass (M) throughout the experiment was calculated as the integral of the growth rate. This calculation ignored the dry mass of the seed (approximately 4 mg per seed). To ensure that this had a negligible effect on the analyses, only data of plants with an estimated dry mass of more than 200 mg were used in further calculations. It took 26 and 17 days for plants to reach a dry mass of 200 mg at 20°C and 30°C, respectively. RGR (g g⁻¹ day⁻¹) was calculated as the ratio between the growth rate and dry mass of the plants.

Growth (g_r, g g⁻¹) and maintenance respiration coefficients (m_r, g g⁻¹ day⁻¹) were estimated from the correlation between the specific respiration rate (R_{spc}) and RGR (Hesketh et al. 1971):

$$R_{\text{spc}} = R_d/M = m_r + g_r \text{RGR} \quad (2a)$$

For consistency with other research on growth and maintenance respiration, R_d is expressed in units of grams of glucose per gram dry matter per day, so m_r is also in

$g\ g^{-1}\ d^{-1}$ and g_r is in $g\ g^{-1}$. To test for temperature effects on m_r and g_r , Equation 2a was modified as follows:

$$R_{spc} = m_{20} + m_T T + (g_{20} + g_T T) RGR \quad (2b)$$

where T is the temperature at which the plants were grown minus 20°C (i.e. 0°C for plants grown at 20°C , and 10°C for plants grown at 30°C), m_{20} and g_{20} are estimated maintenance and growth respiration coefficients for plants grown at 20°C , and m_T and g_T indicate the change in m_r and g_r per celcius degree, respectively (i.e. $m_{30} = m_{20} + m_T \times 10^\circ\text{C}$ and $g_{30} = g_{20} + g_T \times 10^\circ\text{C}$). Thus, if m_T and/or g_T are significantly different from zero, this indicates that m_r and/or g_r are significantly affected by temperature. To obtain estimates of m_r and g_r at 20°C and 30°C , Equation 2b was applied to the combined dataset of all experimental units (i.e. regressions were done on all data combined, not data from individual experimental units). In this approach, g_r and m_r are assumed to be constant throughout plant development. If there are important changes in g_r or m_r during ontogeny, this would be evident from a non-linear relationship between RGR and R_{spc} , and it is possible to modify Equation 2b to include an age-dependent component in addition to the temperature component. However, for the current dataset, Equation 2b provided a more than adequate fit ($R^2 = 0.981$). A Durbin-Watson test ($d = 1.881$) indicated that there was no positive ($P = 0.13$) or negative autocorrelation ($P = 0.87$) among the residuals of the regression with a first-order autocorrelation of 0.041.

Using the estimates of m_r and g_r , maintenance (R_m) and growth respiration (R_g) were calculated as:

$$R_m = m_r M \quad (3)$$

and

$$R_g = g_r \times \text{growth rate} \quad (4)$$

Finally, carbon use efficiency (CUE, the ratio between carbon incorporated into dry matter and the amount of carbon fixed in gross photosynthesis; $\text{mol}\ \text{mol}^{-1}$) was calculated as:

$$\text{CUE} = (14P_n - 10R_d) / (14P_n + 14R_d) \quad (5)$$

These calculations assume that respiration, excluding photorespiration, occurred at the same rate in the light and dark. Leaf respiration is generally considered to be inhibited in the light, a response that may be mediated by photorespiratory products (Bykova et al 2005). However, Wohlfahrt et al. (2005) point out that there is much uncertainty about the extent of the reduction of leaf

respiration by light, citing literature estimates ranging from about 15% to 100% inhibition of leaf respiration. On the other hand, Loreto et al (2001) used an infrared CO_2 analyzer that is insensitive to $^{13}\text{CO}_2$ to measure the respiratory $^{12}\text{CO}_2$ efflux from leaves in air with $^{13}\text{CO}_2$ as the only form of CO_2 . Based on this innovative technique, they concluded that leaf mitochondrial respiration was unaffected by light. Reviewing these findings, Gifford (2003) concluded that 'it now appears that the assumption usually adopted in whole-plant and ecological studies of autotrophic respiration continuing by day as at night, may be correct'.

It is worth mentioning that most studies looking at differences in respiration in the light and dark have focused on leaf respiration. Other organs may respond differently. For example, root respiration of wheat (*Triticum aestivum* L.) is higher in the light than in the dark, possibly because of a larger supply of carbohydrates (Monje and Bugbee 1998), so even if leaf respiration is inhibited by light, it is not clear how this would affect whole plant respiration. Thus, there are conflicting reports on how leaf respiration is affected by light, and effects on whole plants are even less understood (Frantz et al 2004). Thus, the assumption that respiration rates are equal in the light and dark is common in whole plant CO_2 exchange studies (e.g. McCree 1982, Monje and Bugbee 1998, van Iersel 2003b, Frantz et al, 2004).

The experiment was designed as a randomized complete block with repeated measures and four blocks. Each of the two growth chambers used in this study contained two blocks, i.e. two gas exchange chambers at 20°C and two chambers at 30°C . The experimental unit was a single tray of plants. Data were analyzed with analysis of variance and both linear and non-linear regression.

Results and Discussion

CO_2 exchange rates

Plants grown at 30°C consistently had higher R_d than those at 20°C ($P < 0.05$, for each day except day 3) (Fig. 2). The higher R_d at 30°C immediately after seeding may have been caused by germination-related metabolism, since seedlings emerged faster at 30°C than at 20°C . Because of faster germination at 30°C , these plants started photosynthesizing sooner than those at 20°C ; P_n was close to zero (i.e. $P_g = R_d$) after 5 and 7 days at 30°C and 20°C , respectively. Plants grown at 30°C consistently had higher P_n and P_g than those at 20°C from 4 to 41 days after seeding ($P < 0.05$). At 36 days after seeding, P_n of plants grown at 30°C had reached a maximum, and it decreased slowly thereafter. Presumably, at this stage the plants had developed a large enough leaf area to intercept

practically all the available light, so that a further increase in leaf area did not result in increased P_n . The subsequent decrease in P_n may have been caused by the continuing increase in R_d , since P_g was fairly constant from 32 to 60 days after seeding. Plants grown at 20°C reached their maximum P_n after approximately 44 days.

Plant growth and relative growth rate

Differences in CO_2 exchange rates were reflected in the calculated plant growth rates. Plants grown at 30°C had a significantly higher growth rate than those grown at 20°C from 7 until 41 days after seeding. Differences in dry mass between the treatments were significant throughout the entire experiment (Fig. 3). After 62 days, plants grown at 30°C had higher leaf area and leaf, stem, root and total dry mass, but fewer flower buds and lower flower dry mass, than plants grown at 20°C (Table 1). Total dry mass

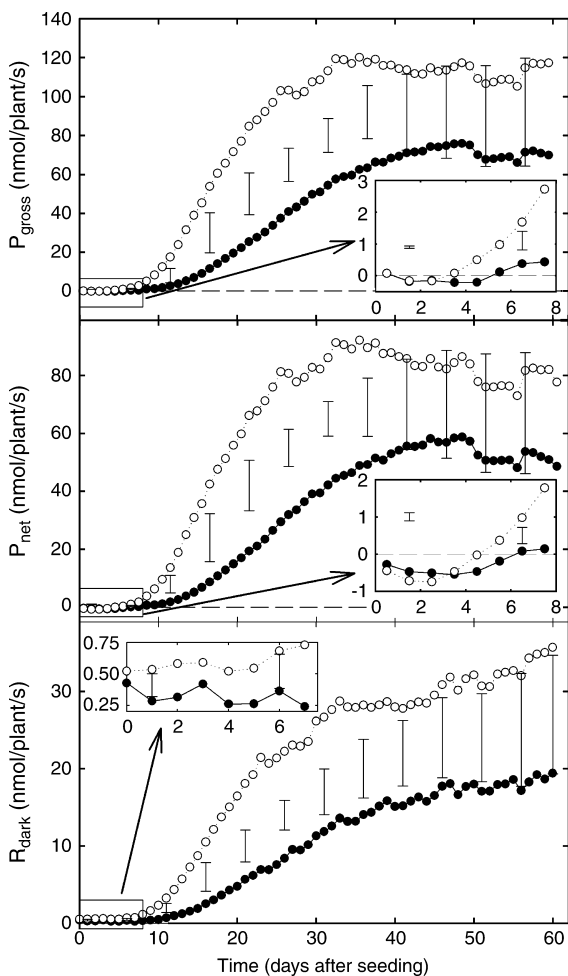


Fig. 2. Gross photosynthesis (P_g), net photosynthesis (P_n) and dark respiration (R_d) of marigold plants grown at 20°C (●) or 30°C (○) for 2 months. Error bars represent Fisher's least significant difference ($P = 0.05$).

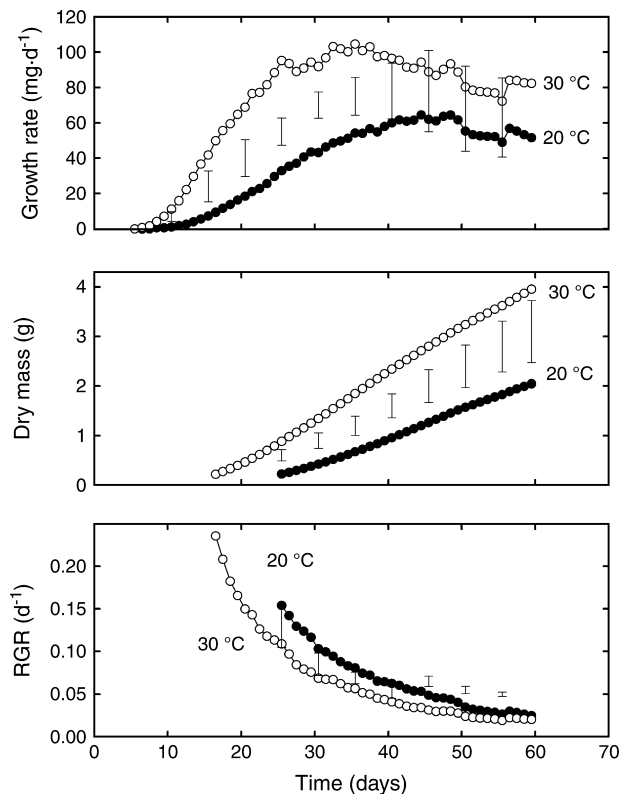


Fig. 3. Growth rate, dry mass, and relative growth rate (RGR). Growth rate was calculated from CO_2 exchange measurements and the carbon content of the plant material, while dry mass was calculated as the integral of the growth rate, and RGR as the ratio of growth rate to dry mass. Data are expressed on a per-plant basis. Error bars represent Fisher's least significant difference ($P = 0.05$).

at the end of the experiment was closely correlated with the calculated dry mass, using the integral of Equation 1 (total dry mass = $1.12 \times$ calculated dry mass; $R^2 = 0.96$, slope is not significantly different from 1, $P = 0.2$), indicating that gas exchange measurements were a good indicator of plant growth.

Plants grown at 20°C had a higher specific leaf area, but lower leaf mass ratio, than those grown at 30°C. These temperature effects on specific leaf area and leaf mass ratio largely offset each other, and the leaf area ratio was similar in both treatments (Table 1). Shoot/root ratio was not affected by temperature (2.29 in both treatments). Although plants grown at 20°C had a lower growth rate than those grown at 30°C throughout much of the experiment, their RGR was higher from 27 until 50 days after seeding.

Respiration and long-term Q_{10}

Because there were large differences in dry mass between the two temperature treatments, long-term Q_{10}

Table 1. Temperature effects on the dry mass (M) and morphologic parameters of marigold. Data represent the total of all plants in a gas exchange chamber. NS, not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$.

Temperature (°C)	Flower buds	Leaf area (cm ²)	Leaf M (g)	Stem M (g)	Flower M (g)	Root M (g)	Total M (g)	Leaf area ratio (cm ² g ⁻¹)	Specific leaf area	Leaf mass ratio (g g ⁻¹)
20	51	3010	17.7	5.8	6.3	13.8	43.6	70	170	0.410
30	10	6356	41.5	16.4	0.7	31.2	92.2	75	154	0.485
Significance	**	***	***	**	**	*	**	NS	***	*

calculations based on whole plant R_d are difficult to interpret, since it is impossible to determine whether differences in R_d are due to differences in plant size or true temperature effects on respiration. Thus, to account for differences in plant size, long-term Q_{10} calculations are based on R_{spc} , instead of R_d . At both temperatures, R_{spc} decreased throughout the experiment, and with increasing dry mass (Fig. 4A,C). Such a decrease in R_{spc} during ontogeny is typical (Winzeler et al. 1976, van Iersel 2003b), and could be described accurately with exponential decay functions ($R^2 > 0.98$).

When plants of the same age were compared, the 20°C treatment consistently resulted in a higher R_{spc} than the 30°C treatment (Fig. 4A). The long-term Q_{10} for R_{spc} was calculated on the basis of the exponential decay curves, and gradually increased from 0.71 at 25–30 days after seeding to 0.97 at the end of the experiment (Fig. 4B). In contrast, Frantz et al. (2004) reported that changing only the night temperature resulted in a long-term respiratory Q_{10} of 1.2–1.5. An important difference between the

current study and those of Frantz et al. (2004) is that in the current study, temperature had an important effect on plant growth, while Frantz et al. (2004) did not find an effect of different night-time temperatures on growth.

Determination of temperature effects on R_{spc} of plants of the same age was complicated by the fact that this resulted in comparisons of plants of different sizes. Since R_{spc} decreased with increasing plant size (Fig. 4C), it is difficult to make meaningful comparisons based on plants of the same age, but different size. Thus, the long-term Q_{10} for R_{spc} was also calculated for plants of the same dry mass (based on the regression curves in Fig. 4C). The long-term Q_{10} for R_{spc} increased from 1.35 for plants with a dry mass of 0.2 to 1.55 for plants with a dry mass of 1 g, and decreased again with a further increase in dry mass (Fig. 4D). These long-term Q_{10} values are similar to those reported by Frantz et al. (2004).

Clearly, the calculated long-term Q_{10} depends greatly on whether plants of the same age or the same dry mass are compared, and determining the long-term Q_{10} based

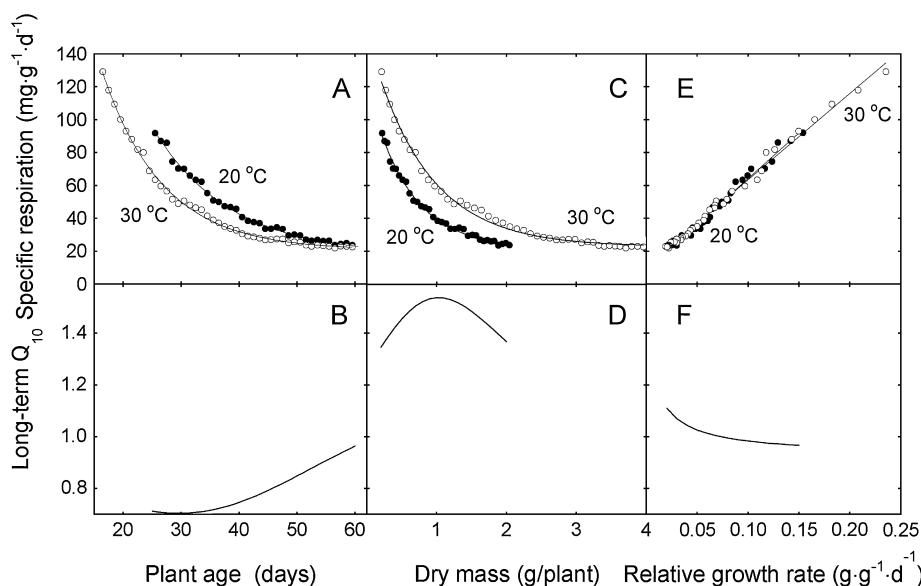


Fig. 4. Specific respiration rate (top) of marigolds grown at 20°C or 30°C for 60 days. Data are expressed as a function of plant age (A), dry mass (C) or relative growth rate (E). Regression lines are either exponential decay curves (A, C; $R^2 > 0.99$) or straight lines (E; Equation 2b, $R^2 = 0.97$). Long-term respiratory Q_{10} values (B, D, F) were calculated as the ratio between the results from the regression lines at 30°C and 20°C.

on measurement of plants of the same age, but different size, may not be meaningful, unless the potential effects of plant size are taken into consideration. The decrease in R_{spc} with increasing plant size is especially important in this regard. For example, when the long-term Q_{10} is compared among treatments that result in different plant sizes, the larger plants may have a lower Q_{10} , even if the treatments do not affect acclimation. Differences in the long-term Q_{10} could be due to direct treatment effects on respiration, to differences in plant size among treatments, or to a combination of both factors. Thus, when larger plants have a lower long-term Q_{10} , it may be difficult or impossible to determine whether this is caused by a direct treatment effect on respiration, or on an indirect effect on R_{spc} due to an increase in dry mass.

Finally, R_{spc} was plotted vs. RGR (Fig. 4E), which is a classic way to estimate g_r and m_r (Hesketh et al. 1971, Amthor 2000). Statistical analysis using Equation 2b indicated that both m_r and g_r were affected by temperature ($P < 0.001$); m_r was estimated to be 8.3 ± 0.6 and $11.2 \pm 0.5 \text{ mg g}^{-1} \text{ day}^{-1}$, while g_r was 0.562 ± 0.082 and $0.522 \pm 0.049 \text{ g g}^{-1}$ at 20°C and 30°C , respectively (estimate \pm SE, $R^2 = 0.981$). Long-term Q_{10} values for m_r and g_r were 1.35 and 0.93, respectively. Even though the analysis indicated that g_r and m_r were temperature sensitive, the correlation between R_{spc} and RGR could be described almost as accurately without taking the temperature sensitivity of g_r and m_r into account ($r^2 = 0.980$; $m_r = 10.4 \pm 0.3 \text{ mg g}^{-1} \text{ day}^{-1}$, $g_r = 0.533 \pm 0.43 \text{ g g}^{-1}$). The good fit of the regression suggests that both changes in respiration during the development of the plants and long-term temperature effects on respiration can be explained using the concept of R_g and R_m . In addition, it may not be necessary to take the possible temperature sensitivity of m_r and g_r into account. Although temperature had a highly significant effect on these two parameters, this effect was so small that it had a trivial effect on the fit of the regression model (Equation 2B, Fig. 4E).

When long-term Q_{10} values for R_d were based on data from plants with the same RGR, the long-term Q_{10} decreased with increasing RGR, from 1.11 for plants with an RGR of $0.02 \text{ g g}^{-1} \text{ day}^{-1}$ to 0.97 for plants with an RGR of $0.15 \text{ g g}^{-1} \text{ day}^{-1}$ (Fig. 4E).

In previous studies on respiratory responses to temperature, RGR has rarely been taken into account, although van Iersel (2003a) found that the Q_{10} for short-term responses of four species of bedding plants decreased with increasing RGR. Loveys et al. (2003) did not find a correlation between long-term Q_{10} and RGR across a wide variety of species. Important differences between the research by Loveys et al. (2003) and the research reported here include that Loveys et al. (2003) tried to correlate long-term Q_{10} with the RGR of different species and not to differences in RGR within a single species. In addition, Loveys et al. (2003) did not determine the RGR of the plants that were used for the long-term Q_{10} determinations; instead, they used the maximum RGR of the different species as determined in a separate study (Loveys et al. 2002). The RGR may have an important effect on the long-term Q_{10} , because it determines the ratio of R_g to R_m , and R_g and R_m may respond differently to temperature.

Growth and maintenance respiration

The decrease in g_r with increasing temperature was unexpected, because g_r is generally considered to be temperature insensitive (Penning de Vries et al., 1974). This temperature effect on g_r may have been related to differences in the chemical composition of the plants between temperature treatments. Plants grown at 20°C had a lower mineral nutrient content (96 mg g^{-1}) than those grown at 30°C (111 mg g^{-1}) (Table 2). It has previously been reported that a high mineral content, and the subsequent dilution of organic matter, results in a decrease in the construction costs of plant tissue (Gary et al. 1998). Construction cost is defined as the amount of carbohydrate needed to produce 1 g of tissue (excluding any maintenance costs), and therefore is closely related to g_r . The main difference between construction cost and g_r is that construction cost includes the carbohydrates needed for to provide carbon skeletons for organic compounds, while g_r does not. A dilution of the organic compounds by increased accumulation of mineral nutrients did indeed appear to have happened, since plants grown at 20°C had a higher carbon content than

Table 2. Temperature effects on the mineral composition of marigolds. NS, not significant; * $P < 0.05$; ** $P < 0.01$.

Temperature $^\circ\text{C}$	mg g ⁻¹							$\mu\text{g g}^{-1}$							
	C	N	P	K	Ca	Mg	S	Al	B	Cu	Fe	Mn	Mo	Na	Zn
20	437	37.6	5.2	27.0	18.7	5.6	0.8	171	60	82	91	72	4.6	566	117
30	417	45.8	5.4	31.3	20.2	6.8	0.6	169	75	139	97	104	4.4	342	101
Significance	**	*	NS	NS	*	*	*	NS	NS	NS	NS	NS	NS	**	NS

those grown at 30°C (Table 2). Other differences in the chemical composition of the plants may have occurred as well.

The g_r estimates were used to estimate construction costs [$g_r + (C_i/C_{glu})$, where C_{glu} is the carbon content of glucose] as 1.65 and 1.56 $g\ g^{-1}$, at 20°C and 30°C respectively. These values are slightly higher than the commonly used values of 1.39 and 1.45 $g\ g^{-1}$ for leaves and stems of non-leguminous species respectively (Penning de Vries et al. 1989), or those of leaves of yellow poplar (*Liriodendron tulipifera* L.) (1.46 $g\ g^{-1}$) (Wullschleger et al. 1997), but similar to estimates for sorghum [*Sorghum bicolor* (L.) Moench] plants (1.54 $g\ g^{-1}$) (Amthor et al. 1994). Small differences in estimates of construction cost among studies may be due to species differences or differences in the techniques used to obtain these estimates and their underlying assumptions. A glucose requirement of 1.65 $g\ g^{-1}$ yields a conversion efficiency (dry matter produced per gram of glucose) of 0.61 $g\ g^{-1}$, at the low end of the normal range of 0.6–0.8 $g\ g^{-1}$ (McCree 1982).

The estimated long-term Q_{10} for m_r was 1.35, indicating that m_r was lower at 20°C than at 30°C. This increase in m_r with increasing temperature was expected, partly because of increased protein turnover at higher temperatures (e.g. Thornley and Johnson 1990). There are few estimates of long-term temperature effects on m_r of whole plants. Winzeler et al. (1976) estimated m_r of barley (*Hordeum vulgare* L.) plants from R_d and dry mass measurements, and estimated a long-term Q_{10} for m_r of approximately 3 for plants grown at temperatures ranging from 15°C to 28°C. Mariko and Koizumi (1993) reported that the m_r of *Reynoutria japonica* was 1.9 times higher for plants grown at 25°C than at 15°C. Gifford (1995)

reported that the m_r of wheat increased with an increase in temperature from 15°C to 20°C, but was insensitive to a further increase in temperature. Others have estimated the temperature sensitivity of specific organs. Marcelis and Baan Hofman-Eijer (1995) found that m_r for cucumber (*Cucumis sativus* L.) fruit had a long-term Q_{10} of approximately 2, while Szaniawski and Kielkiewicz (1982) reported a long-term Q_{10} of 2.1 for m_r of sunflower (*Helianthus annuus* L.) roots. The estimated values for m_r in this study (8.3 and 11.2 $mg\ g\ day^{-1}$ at 20°C and 30°C) are well within the normal range of 3–50 $mg\ g^{-1}\ day^{-1}$ (Hesketh et al. 1980).

Maintenance respiration increased steadily throughout the growing period, as a result of increasing dry mass (Fig. 5). Differences in R_m between the two temperature treatments were larger than suggested by the differences in m_r , because plants grown at 30°C not only had a higher m_r , but also were larger than those grown at 20°C. Growth respiration changed less than R_m during the course of the experiment (Fig. 5).

Initially, R_g accounted for most of the respiration (approximately 90% at 27 and 17 days after seeding at 20°C and 30°C, respectively; Fig. 6), but the importance of R_m increased throughout the growing period, and R_m eventually accounted for 37% and 56% of the total respiration at 20 and 30°C, respectively. For plants of the same age, R_g accounted for a larger fraction of total respiration at 20°C than at 30°C. This difference could largely be explained on the basis of differences in dry mass among the treatments. When R_g as a fraction of total respiration was plotted vs dry mass, treatment differences were small (Fig. 6), especially for small plants (dry mass < 1.5 g). When plant with equal RGR were compared, plants grown at 20°C consistently allocated a larger

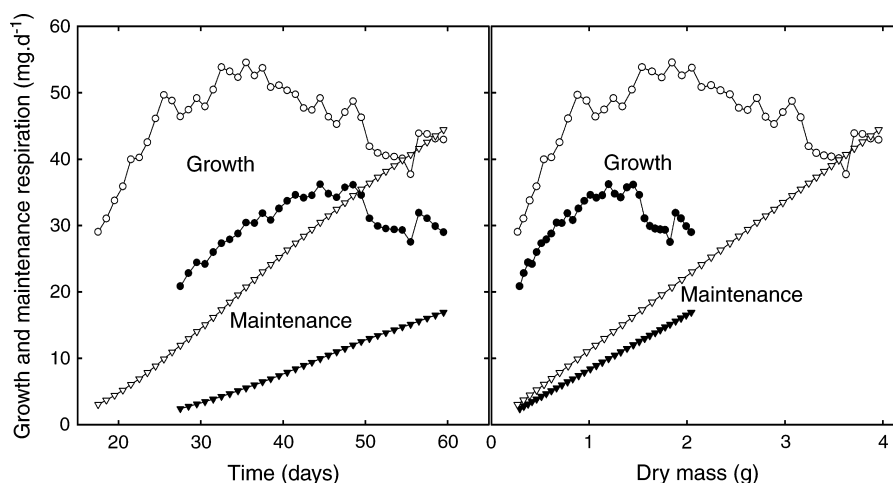


Fig. 5. Growth (●, ○) and maintenance respiration (▼, ▽) of marigolds grown at 20°C (closed symbols) or 30°C (open symbols) for 60 days. Data are shown as a function of plant age (left) or plant dry mass (right). Respiration rates were estimated using Equations 3 and 4.

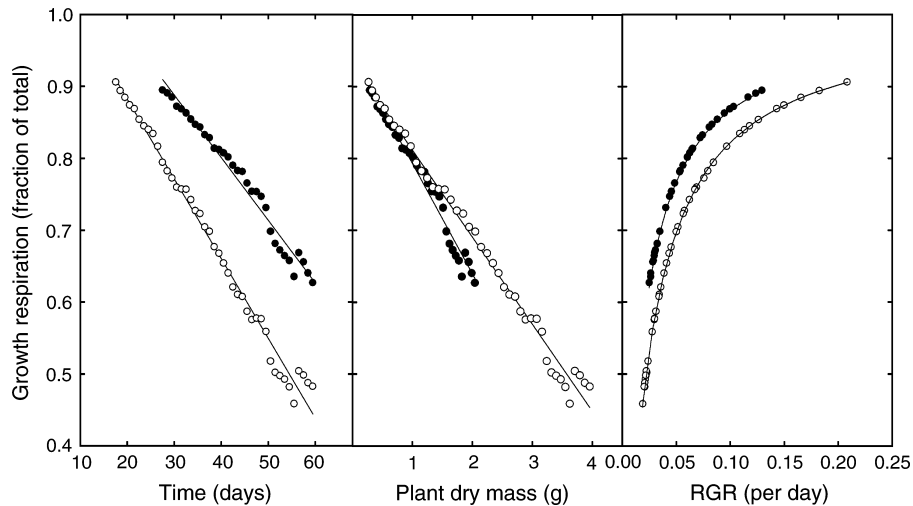


Fig. 6. Growth respiration of marigolds grown at 20°C (●) or 30°C (○), expressed as a fraction of total respiration and plotted as a function of plant age (left), dry mass (middle), or relative growth rate (right). Regression lines are either straight lines (left, middle; $r^2 > 0.977$), or a rectangular hyperbola (right, $R^2 = 1.00$).

fraction of their total respiration to growth than plants grown at 30°C (Fig. 6).

Carbon use efficiency

Carbon use efficiency increased sharply during the early part of the growing period, and decreased thereafter (Fig. 7). Van Iersel (2003b) showed that $1/\text{CUE} = (1 + g_r + m_r)/\text{RGR}$, and a decrease in RGR therefore results in a decrease in CUE, unless there are concomitant changes in g_r and/or m_r . Germinating seeds have a negative RGR as long as the catabolism of stored carbohydrates exceeds the production of new photosynthates. This

results in a negative RGR, and thus a low CUE. The CUE increases as seedlings start photosynthesizing and attain a positive growth rate and RGR. Likewise, the decrease in RGR during most of the plants' life cycle results in a concomitant decrease in CUE. The 30°C temperature resulted in a lower CUE from 40 to 51 days after seeding than 20°C, which can be explained by the combination of a higher m_r and lower RGR at 30°C. Frantz et al. (2004) studied the effect of different night-time temperatures on the CUE of several fast-growing species and found that increasing the night temperature by 10°C decreased CUE by 3.8–8.6%, with no significant effect on dry matter accumulation.

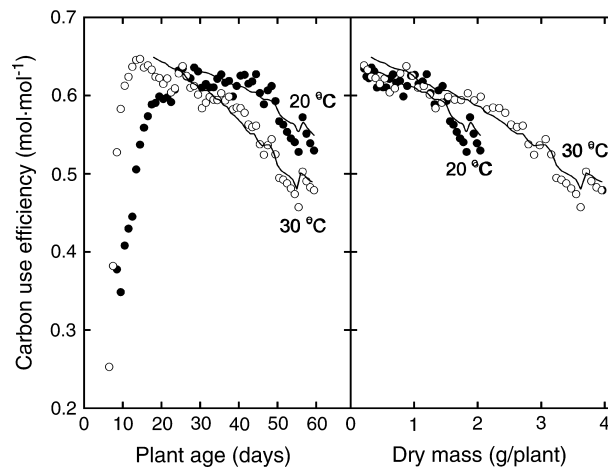


Fig. 7. The CUE of marigolds (moles of C incorporated into plant dry matter per mole of C fixed in gross photosynthesis) grown at 20°C (●) or 30°C (○) for 60 days. Data are shown as a function of plant age (left) or plant dry mass (right). The lines in the figure indicate that the CUE estimated from $1/\text{CUE} = (1 + g_r + m_r)/\text{RGR}$ with the g_r and m_r estimates from Fig. 4E and Equation 2b.

Calculations of CUE are based on the assumption that respiration in the light is the same as in the dark. If respiration in the light is lower, than the true P_g is lower than what is calculated by summing P_n and R_d . Thus, if respiration is lower in the light than in the dark, the true CUE would be higher than values calculated here. The sensitivity of CUE to the assumption that respiration rates are equal in the light and dark was tested by calculating CUE values assuming 10%, 30% and 50% reductions in whole plant respiration in the light. This analysis revealed that for every 10% reduction in respiration, CUE increased by 0.015–0.016 mol mol⁻¹. Thus, in a worst-case scenario of a 50% reduction of respiration in the light, the assumption of equal respiration in the light and dark would result in an underestimation of CUE by 0.080 ± 0.004 mol mol⁻¹ (mean ± SD). This error would be fairly stable throughout ontogeny. This analysis indicates that the assumption of equal respiration in the light and dark may cause an underestimation of CUE, but has little or no effect on treatment comparisons or changes in CUE throughout ontogeny. Earlier reports also indicated only a small effect of this assumption on CUE (Monje and Bugbee 1998, Frantz and Bugbee 2005).

Short-term vs long-term responses to temperature

The long-term Q_{10} for respiration (1.35–1.55 when plants with the same dry mass are compared) in response to long-term changes in temperature is lower than typical respiratory responses to short-term temperature changes (e.g. Larigauderie and Körner 1995). This is probably at least partly due to temperature acclimation of respiration. Such acclimation can occur rapidly (within 2 days) and is reversible (Rook 1969, Bolstad et al. 2003). Acclimation of respiration results in smaller effects of long-term changes in temperature than what would be predicted from short-term studies. Will (2000) has argued that such acclimation should be predicted on the basis of the concept of an 'effective acclimation temperature', which he defined as the temperature at which the mean daily respiration rate occurs. This effective acclimation temperature may differ from the daily mean temperature, because of the exponential increase in respiration with increasing temperature, and is linearly related to acclimation of leaf respiration of loblolly pine (Will 2000).

Ignoring acclimation effects in estimating respiration rates will probably result in an overestimation of respiration with increasing temperature, regardless of whether respiration is expressed on a whole plant, dry weight or ground area basis. Thus, models that use a simple Q_{10} equation (with R_d at a certain reference temperature and Q_{10} as the two variables) will not be able to accurately describe both short-term and long-term

effects of temperature on respiration. Acclimation of respiration to temperature may involve changes in both Q_{10} and R_d at the reference temperature (Atkin and Tjoelker 2003). Accurate estimates of whole plant or ecosystem respiration are further complicated by differences in respiration and its temperature sensitivity among different layers with a canopy (Griffin et al. 2002). Such effects need to be studied in more detail to allow for more accurate estimates of respiration rates. This would improve plant productivity models, since our current capacity to model respiration rates falls short of the capacity to model photosynthesis (Gifford 2003).

Conclusions

Determining the temperature sensitivity of whole plant respiration in response to long-term temperature differences is complicated by the fact that these temperature differences are likely to affect plant growth, and therefore result in plants of different sizes. Correcting for differences in plant size by comparing R_{spc} instead of whole plant R_d does not solve this problem, because R_{spc} decreases with increasing plant size. In this study, plants grew much faster at 30°C than at 20°C. The larger size of the plants grown at 30°C resulted in a lower R_{spc} at 30°C than at 20°C, and thus in a long-term Q_{10} of < 1 (0.71–0.97) when plants of the same age were compared. This illustrates the difficulty in comparing plants of the same age; temperature effects on R_{spc} may be due to a direct effect on R_d , differences in plant size, or both. This problem can be addressed by comparing the R_{spc} of plants of the same dry mass instead of the same age, which resulted in long-term Q_{10} values between 1.35 and 1.55. A more mechanistic approach is to separate respiration into growth and maintenance components. In this case, R_d could be accurately described as a function of R_g and R_m , and temperature effects on g_r and m_r could be estimated. There was a small decrease in g_r with increasing temperature (long-term $Q_{10} = 0.93$), while m_r increased with temperature (long-term $Q_{10} = 1.35$). Whether this approach is universally applicable remains to be seen. The unexpected decrease in g_r with increasing temperature was at least partially due to changes in the chemical composition of the plants (decrease in C content). Early during the growing period, R_g accounted for 90% of R_d , but this fraction decreased steadily as the importance of R_m in the carbon balance of the plants increased over time. Early in the growing period, CUE of the plants was low, presumably because of respiration of the germinating seeds. After germination was complete, CUE peaked to approximately 0.65. The following decline in CUE was the result of a decrease in RGR and a larger fraction of available carbohydrates being

allocated to maintenance rather than growth processes. These results suggest that long-term temperature effects can be accurately described as a function of growth and maintenance respiration. Whether short-term temperature effects on respiration can be described in a similar way needs further investigation.

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