Plant, Cell and Environment (2007) **30**, 820–833 doi: 10.1111/j.1365-3040.2007.01672.x

Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves

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ABSTRACT

Understanding the response of leaf respiration (*R*) to changes in irradiance and temperature is a prerequisite for predicting the impacts of climate change on plant function and future atmospheric CO_2 concentrations. Little is known, however, about the interactive effects of irradiance and temperature on leaf *R*. We investigated whether growth irradiance affects the temperature response of leaf *R* in darkness (R_{dark}) and in light (R_{light}) in seedlings of a broad-leaved evergreen species, *Quercus ilex*. Two hypotheses concerning R_{dark} were tested: (1) the Q_{10} (i.e. the proportional increase in *R* per 10 °C rise in temperature) of leaf R_{dark} is lower in shaded plants than in high-light-grown plants, and (2) shade-grown plants exhibit a lower degree of thermal acclimation of R_{dark} than plants exposed to higher growth irradiance. We also assessed whether light inhibition of R_{light} differs between leaves exposed to contrasting temperatures and growth irradiances, and whether the degree of thermal acclimation of R_{light} is dependent on growth irradiance. We showed that while growth irradiance did impact on photosynthesis, it had no effect on the Q_{10} of leaf R_{dark}. Growth irradiance had little impact on thermal acclimation when fully expanded, pre-existing leaves were exposed to contrasting temperatures for several weeks. When R_{light} was measured at a common irradiance, R_{light}/R_{dark} ratios were higher in shaded plants due to homeostasis of R_{light} between growth irradiance treatments and to the lower R_{dark} in shaded leaves. We also showed that R_{light} does not acclimate to the same degree as R_{dark}, and that R_{light}/R_{dark} decreases with increasing measuring and growth temperatures, irrespective of the growth irradiance. Collectively, we raised the possibility that predictive carbon cycle models can assume that growth irradiance and photosynthesis do not affect the temperature sensitivity of leaf R_{dark} of long-lived evergreen leaves, thus simplifying incorporation of leaf *R* into such models.

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Key-word: Plant respiration, shade, Q_{10}, acclimation, *Quercus ilex*.

INTRODUCTION

Plant respiration (*R*) oxidizes photosynthetically fixed products to provide the energy (ATP and NADH) and carbon skeletons necessary for growth, maintenance and ion uptake. *R* also plays a critical role in determining global atmospheric CO_2 concentrations, with plant *R* releasing approximately 60 Gt C yr^{-1} into the atmosphere (Schimel 1995); leaf *R* accounts for approximately half of whole plant *R* (Poorter *et al.* 1991). Understanding the effect of environmental variations (e.g. temperature and irradiance) on leaf *R* is therefore a prerequisite for predicting the impacts of global climate change on plant function and atmospheric CO_2 concentrations (Ryan 1991; Larigauderie & Körner 1995; Atkin & Tjoelker 2003).

Several studies have modelled the impacts of temperature-dependent changes in leaf *R* in darkness (R_{dark}) on plant function and/or atmospheric CO_2 concentrations (e.g. Wythers *et al.* 2005; King *et al.* 2006). Often, models assume that R_{dark} increases exponentially with temperature with the Q_{10} value (i.e. the proportional change in R_{dark} per 10 °C rise in temperature) being assumed to be 2.0 (Ryan 1991; Aber & Federer 1992; Schimel *et al.* 1997; Amthor 2000; Cox *et al.* 2000; White, Cannell & Friend 2000; Cramer *et al.* 2001). However, the response of R_{dark} to temperature is highly dynamic. For example, Q_{10} values are highly variable in response to diurnal changes in temperature (Tjoelker, Reich & Oleksyn 1999; Tjoelker, Oleksyn & Reich 2001; Atkin & Tjoelker 2003). The Q_{10} values typically range from 1.2 to 4.0, with variability occurring even within individual plants (Breeze & Elston 1978; Ryan 1991; Azcón-Bieto 1992; Dewar, Medlyn & McMurtrie 1999; Tjoelker *et al.* 2001). Potential factors that underpin the variability in Q_{10} values include changes in the control exerted by maximum enzyme activity, as well as changes in substrate availability and turnover of ATP to ADP (Atkin & Tjoelker 2003).

A number of studies have shown that variations in substrate availability can lead to predictable changes in the Q_{10} of plant R_{dark}, with the Q_{10} values being the highest at high concentrations of substrates. For example, adding exogenous glucose solution to detached roots of *Plantago lanceolata* increased the temperature dependence of R_{dark} (Covey-Crump, Attwood & Atkin 2002). Moreover, a similar response was observed in mitochondria isolated from soybean cotyledons (Atkin, Zhang & Wiskich 2002). To date, no study has established whether the Q_{10} of leaf R_{dark} varies in response to direct manipulations in substrate availability. Hartley *et al.* (2006) attempted to address this question by investigating the effect of changes in *Populus deltoides* leaf photosynthesis driven by increases and decreases in atmospheric CO_2 concentration at Biosphere 2 in Arizona; although they found no correlation between the Q_{10} of leaf R_{dark} and atmospheric CO_2 concentration, soluble sugar concentrations remained high in all treatments irrespective of the prevailing rate of photosynthesis. It thus remains possible that the Q_{10} values of leaf R_{dark} may vary with substrate availability, and that light-mediated change in photosynthesis, which impact on the concentration of total soluble sugars, may result in associated changes in the Q_{10} of leaf R_{dark}.

Studies investigating the phenotypic response of leaf R_{dark} to sustained changes in growth irradiance have generally shown that respiratory rates are lower in shaded leaves than in their high-light-grown counterparts (Boardman 1977; Sims & Pearcy 1989, 1991; Turnbull, Doley & Yates 1993; Sims & Pearcy 1994; Noguchi & Terashima 1997; Noguchi, Nakajima & Terashima 2001). Moreover, an analysis of global patterns of leaf R_{dark} found that rates of respiration are generally higher in plants growing in bright climates than in their low-light climate counterparts (Wright *et al.* 2006). Underpinning the higher rates of R_{dark} in high-light leaves are increases in the concentration of mitochondrial protein per unit leaf area and mass (Noguchi *et al.* 2001), substrate availability (Azcón-Bieto & Osmond 1983) and/or increase in ATP turnover (Lambers 1985; Noguchi, Sonoike & Terashima 1996; Noguchi & Terashima 1997). In addition to affecting overall rates of leaf R_{dark}, such changes in respiratory machinery and demand for respiratory products could also impact on the response of leaf R_{dark} to other abiotic factors such as temperature. However, little is known about the interactive effects of growth irradiance and temperature on leaf R_{dark}, which is essential to understand the carbon economy of plants growing in contrasting light environments under variable temperature conditions.

The impact of long-term temperature changes on R_{dark} depends on the degree of thermal acclimation (i.e. its ability to readjust and re-establish R_{dark}). Acclimation can occur rapidly, for example, within 2 d of a temperature change in some species (Rook 1969; Billings *et al.* 1971; Atkin, Edwards & Loveys 2000a; Bolstad, Reich & Lee 2003). Acclimation is generally more complete when plants develop new tissues under new growth temperature compared to when pre-existing leaves are exposed to a new growth temperature (Loveys *et al.* 2003). However, it has not been established whether pre-existing leaves exhibit higher degrees of acclimation when exposed to a new growth temperature for extended periods. Although it is known that thermal acclimation of R_{dark} is linked to changes in substrate supply and/or enzyme capacity (Atkin & Tjoelker 2003; Armstrong *et al.* 2006b), and that growth irradiance can influence both factors, it is unclear whether the degree of acclimation is directly affected by growth irradiance. There is growing evidence that thermal acclimation of photosynthesis is dependent on the irradiance experienced by leaves, with acclimation serving to maintain the balance between energy supply versus energy consumption (Huner *et al.* 1993; Anderson, Prasad & Stewart 1995; Gray *et al.* 1996; Huner *et al.* 1996; Gray *et al.* 1997). Given the tight coupling that exists between photosynthetic and respiratory metabolism (Raghavendra, Padmasree & Saradadevi 1994; Krömer 1995) and the fact that ratios of R_{dark} to photosynthesis are often homeostatic (Gifford 1995; Loveys *et al.* 2003), one hypothesis is that the thermal acclimation of leaf R_{dark} is also irradiance dependent. Alternatively, the metabolic conditions that trigger the thermal acclimation of R_{dark} might differ from those underpinning the acclimation of photosynthesis. So far, no study has investigated whether the degree of thermal acclimation of leaf R_{dark} differs between plants experiencing high and low irradiance.

To date, most studies investigating the effects of temperature and growth irradiance on leaf R have focussed on responses of R_{dark}, with little attention given to the impacts of temperature and growth irradiance on leaf R in light (R_{light}; non-photorespiratory mitochondrial CO_2 release in light). In most studies, the rate of leaf R in light is lower than that in darkness (i.e. light inhibits leaf R; Brooks & Farquhar 1985; Avelange & Rebéillé 1991; Atkin *et al.* 2000b), with the degree of light inhibition often being the greatest at high measuring temperatures (Atkin *et al.* 2000b); there is also some evidence that R_{light} can acclimate to contrasting growth temperatures in two herbaceous species (Atkin, Scheurwater & Pons 2006). Whether R_{light} acclimates to sustained changes in growth temperature and/or irradiance in pre-existing leaves, which is particularly important in long-lived leaves of evergreen plants, is not known.

We investigated whether growth irradiance affects the temperature response of leaf R_{dark} and R_{light} of a widespread evergreen Mediterranean dry-land plant species, *Quercus ilex* ssp. *ballota*. In nature, *Q. ilex* experiences large diurnal and seasonal variations in temperature, often under contrasting irradiances (Corcuera *et al.*, 2005). Two hypotheses concerning R_{dark} were tested: (1) the Q_{10} of leaf R_{dark} is lower in shaded plants than in plants exposed to higher irradiance, and (2) shade-grown plants exhibit a lower degree of thermal acclimation of R_{dark} than plants exposed to higher growth irradiance. The impacts of growth irradiance and changes in temperature (both short and long term) on the balance between R_{dark} and net photosynthesis were quantified. We also assessed if light inhibition of R_{dark} differs between leaves exposed to high and low

temperatures under two different growth irradiances, and whether the degree of acclimation of R_{light} is dependent on growth irradiance.

MATERIAL AND METHODS

Plant material

Q. ilex L. ssp. *ballota* (Holm oak) acorns were obtained from the National Centre of Forestry Improvement 'El Serranillo' from the Ministerio de Medio Ambiente (Guadalajara-Spain). Plants were grown from seed in soil under controlled environment conditions. The oaks were sown into individual pots [7 in. (20 cm) diameter and four acorns per pot] containing 50/50 sand/vermiculite and then left in a temperature-controlled glasshouse (25/20 °C). The *Q. ilex* seedlings were approximately 21 cm in height, with a total of 20–60 leaves on each plant.

Glasshouse experiment

A glasshouse experiment was conducted to assess the impact of growth irradiance on short-term temperature dependence of leaf R_{dark}. To account for localized environmental variations within the glasshouse, a randomized split block-pot design was adopted. Six blocks were used, consisting of three replicate control blocks (at ambient irradiance) and three replicate shade blocks (using green mesh cloth that reduced irradiance by >80%). Each block contained three pots of *Q. ilex*, which were averaged to yield a single replicate per block. Within each pot were four plants (to allow for four destructive harvests over the experimental period); randomized numbers were used to select individual plants from each pot. The plants were watered daily and fed once a week with a nutrient solution containing 2 mL of Phostrogen per litre of water [It contains the following standard nutrient solution: 4.4% P_2O_5, 22.4% K_2O, 1.5% MgO, 6.0% SO_3, 0.012% B, 0.0055% Cu, 0.04% Fe, 0.02% Mn, 0.0016% Mo, 0.0055% Zn, 1.43% CaO, 2.5% Ureic nitrogen, 3.5% NH_4 and 8% NO_3^- (Solaris, Buckinghamshire, UK)]. The temperature in the glasshouse was set to a constant 25 ± 2 °C and a relative humidity (RH) of 70%. Automatic supplementary lighting (using 400 W high-pressure sodium bulbs) was provided to maintain a day length at 16 h and to increase irradiance to a minimum of 300 μmol photons m^{-2} s^{-1} at plant height on cloudy, low-irradiance days.

The impact of altered growth irradiance on leaf R_{dark} (and photosynthesis) was assessed via first measuring gas exchange in plants before imposition of the shade treatment, for both control and treatment blocks (hereon termed period 1). In period 2, measurements were carried out after the treatment plants had been under shade for 3 weeks. These measurements were compared with the responses of plants kept under ambient conditions. The shaded plants were returned to ambient conditions for 1 week of recovery under ambient irradiance (period 3). In period 4, the shaded treatment was re-applied for another 3 weeks.

Measurements of the leaf gas exchange were carried out on attached, fully expanded, mature leaves (approximately 6 weeks old) using the Li-Cor 6400 gas exchange system (Li-Cor, Inc., NE, USA); the leaves were exposed to an atmospheric CO_2 concentration of 400 μL L^{-1} [using the built-in Li-Cor 6400 CO_2 controller (Li-Cor, Inc.)], and the measurements were made at 25 °C (i.e. the growth temperature) and an RH of 55–60%. Photosynthesis measurements were made at ambient irradiance in periods 1 and 3 for all blocks. In periods 2 and 4, measurements of photosynthesis were either performed at ambient irradiance (i.e. in non-shaded, control blocks) or at 25% of ambient irradiance in shaded blocks.

Following each set of photosynthetic measurements, leaf R_{dark} was measured at two contrasting temperatures (28 and 7 °C) to assess the short-term temperature sensitivity of R_{dark} (i.e. Q_{10}) using the same leaves as used for photosynthesis. These measurements were made in two growth cabinets (Microclima 1750; Snijders Scientific BV, Tilburg, the Netherlands), which were set to constant 28 and 7 °C (irradiance was 300 μmol photons m^{-2} s^{-1}, with an RH of 70%); these temperatures did not match those of the glasshouse, but they did enable the Q_{10} of leaf R_{dark} to be measured over a broad range of temperatures. The plants were initially moved from the glasshouse to the 28 °C growth cabinet for an hour (in the light), with the whole plants subsequently being covered with a dark cloth for 30 min before the measurement of leaf dark R (necessary to avoid transient changes in CO_2 release associated with post-illumination changes in metabolism; Azcón-Bieto & Osmond (1983). Afterwards, the plants were moved to the 7 °C cabinet for an hour in the light, and then covered for 30 min before performing the measurement of leaf R_{dark}. The leaves were harvested after the plants had experienced 8–10 h of illumination, and the fresh mass and leaf area of sections used in the CO_2 exchange measurements were quantified with a LI-3000A leaf area meter (Li-Cor, Inc.). Samples were then frozen in liquid N_2 and stored at –20 °C. Dry mass of each segment was recorded after freeze-drying under vacuum (Edwards Modulyo Freeze Drier; York, UK); the leaves were then pooled for each harvest and ground to a fine powder using a hammer mill (31-700 Hammer Mill; Glen Creston Ltd, Stanmore, UK). Soluble sugars and starch were extracted and measured as reported previously (Loveys *et al.* 2003).

Growth cabinet experiment

Following completion of the glasshouse experiments at the end of period 4, the plants left in the ambient control and shade-treatment blocks were shifted to one of four temperature-controlled cabinets (constant 7, 14, 21 or 28 °C) in order to assess the importance of growth irradiance on the long-term responses of leaf R (in the dark and light). The plants from the ambient controls were exposed to the highest irradiance possible at all temperatures in the growth cabinets (300 μmol photons m^{-2} s^{-1}), and the shade-treated plants were exposed to low irradiance (16 μmol photons m^{-2} s^{-1}) with day lengths again set to 16 h

day/8 h night and the RH at 70%. The growth irradiance was provided by a combination of fluorescent tubes (Sylvania, Yorkshire, UK); the tubes were shielded by thermal glass. The shade environment was achieved by using a green-shade cloth. The plants were watered daily and fed once a week with a nutrient solution as described earlier in the glasshouse experiment.

The measurements of leaf gas exchange were carried out using the attached, fully expanded mature leaves as described earlier. Photosynthesis at ambient irradiance (either 300 or 16 μmol photons m^{-2} s^{-1}, depending on the treatment) and R_{dark} (following 30 min darkness) were measured. All measurements took place on the plants that had experienced at least 3 h of illumination. The first set of measurements of photosynthesis and leaf *R* were recorded after the plants had been shifted to the growth cabinets (7, 14, 21 and 28 °C) and the two different light regimes for 1 h (day 0). The plants were then kept at new temperatures and two light treatments for several weeks. After 60 d in the growth cabinets, leaf *R* and photosynthesis were measured using the plants from each irradiance/growth temperature combination; preliminary experiments using hydroponically grown *Q. ilex* showed that pre-existing leaves can achieve near-full acclimation (i.e. homeostasis) when exposed to new growth temperatures (7–28 °C) for 60 d (Zaragoza-Castells 2006). However, whether such responses would also be exhibited by plants subjected to deep shade was not known.

Following photosynthesis and R_{dark} measurements on day 0 and day 60, rates of non-photorespiratory mitochondrial CO_2 release in light (R_{light}) were determined under each temperature and growth irradiance using the Laisk method (Laisk 1977, as modified by Brooks & Farquhar 1985). The Laisk method analyses the rate of net CO_2 gas exchange at low internal concentrations of CO_2 (C_i) and varying irradiances. R_{light} is the rate of CO_2 release at the photocompensation point, Γ_*. To establish Γ_* for *Q. ilex*, rates of net assimilation rate (A_{net}) were measured in preliminary experiments at several low C_i values (typically between 15 and 120 μL L^{-1}) at three irradiances: 500, followed by 150 and then 100 μmol photons m^{-2} s^{-1}. A linear regression of A_{net} versus C_i was calculated for each irradiance. The point at which three regressions intersected was then used to determine Γ_* (mean = 39.9 μL L^{-1} at 25 °C). Subsequently, R_{light} was estimated from net CO_2 exchange at Γ_* [using $A_{net} - C_i$ curves made for leaves exposed to a single measuring irradiance (300 μmol photons m^{-2} s^{-1})]. We decided to measure R_{light} at a common irradiance to facilitate comparison of the long-term effects of each treatment on R_{light} without having to account for the short-term effects of light on *R*. As Γ_* is temperature dependent, we applied the following equation (Brooks & Farquhar 1985) to predict Γ_* at each measuring temperature (Tx; i.e. 7, 14, 21 and 28 °C):

$$\Gamma_{*Tx} = \Gamma_{*25} + [1.88*(Tx - 25)] + \left[0.036*(Tx - 25)^2\right] \quad (1)$$

As CO_2 is known to diffuse through the gasket material in a predictable manner when the concentration of CO_2 inside the cuvette differs from that in the surrounding atmosphere, we corrected for gasket CO_2 diffusion (see Bruhn, Mikkelsen & Atkin 2002). The leaves were then harvested, and the fresh mass, leaf area and dry mass were recorded. Soluble sugar and starch concentrations were also analysed as described earlier.

Data and statistical analyses

The short-term temperature response (Q_{10}) of leaf R_{dark} for the glasshouse-grown plants was determined using the following equation:

$$Q_{10} = (R_{28}/R_7)^{[10/(28-7)]} \quad (2)$$

where R_{28} and R_7 represent the rates of R_{dark} measured at 28 and 7 °C, respectively. For plants subsequently exposed to 7, 14, 21 and 28 °C in the controlled environment growth cabinets, the Q_{10} was estimated by linear regression of ln-transformed values of R_{dark} plotted against measurement temperature (Atkin, Bruhn & Tjoelker 2005), with the slope of the temperature response curve (i.e. K) being used to calculate the Q_{10} according to

$$Q_{10} = e^{10K} \quad (3)$$

Estimates of the degree to which R_{dark} acclimated to long-term changes in temperature ($Acclim_{LTR\ 10}$) were assessed using the following equation (Atkin *et al.* 2005):

$$Acclim_{LTR10} = 1 - [(LTR_{10} - 1)/(Q_{10} - 1)] \quad (4)$$

where LTR_{10} represents the long-term temperature response of leaf R_{dark} (calculated using Eqn 3, but with K representing the long-term temperature response coefficient of R_{dark}), and where the Q_{10} is the short-term temperature response (also calculated using Eqn 3). LTR_{10} is defined as the proportional change in rates of R_{dark} of plants grown and measured at one temperature compared with a growth temperature 10 °C lower (Larigauderie & Körner 1995). The LTR_{10} values of the plants exposed to 7, 14, 21 and 28 °C were calculated using Eqn 3, using the rates of R_{dark} measured at each treatment respective growth temperature. Equation 4 provides a quantitative estimate of the degree of acclimation, with values ranging from 0 where no acclimation has taken place (i.e. where the LTR_{10} and Q_{10} are equal) to 1 (full acclimation).

Standard non-linear regression techniques were used to fit curves by iteration to existing data to the temperature responses of leaf R_{dark} using the following equation:

$$R_T = R_{10} * Q_{10}^{(T-10/10)} \quad (5)$$

where R_T is the rate of respiration at any given measurement temperature (T) and R_{10} represents R_{dark} at 10 °C.

All statistical analyses were conducted using SPSS version 11 (SPSS Science, Birmingham, UK). In cases where data remained non-parametric after transformation, equivalent non-parametric tests were used. For the

glasshouse experiment, independent sample *t*-tests were used to compare shaded and ambient treatments within a time period, one-way analyses of variance (ANOVA) were used to investigate whether there were differences between periods, and for the shaded treatment, whether rates of gas exchange at 28 °C (expressed as a proportion of the ambient treatment rate) changed between periods. Linear regressions were used to determine whether there were significant relationships between the rate of photosynthesis and the Q_{10} of leaf R_{dark}.

In the growth cabinet experiment, the thermal acclimation of leaf R_{dark} was investigated by applying the *F*-ratio method (Sokal & Rohlf 1981) to the curve-fitted data (see Results section). ANOVA (one-, two- and three-way) were used where necessary, as were independent sample *t*-tests. To determine whether light inhibited respiration, repeated measure ANOVA, with R_{light} and R_{dark} as the within-subject variables, and day and temperature as the between-subject factors, were carried out for each light treatment. These tests were followed up by paired *t*-tests on R_{light} and R_{dark} within light, day and temperature. Finally, to determine whether R_{light} was temperature dependent, one-way ANOVA within light treatment and day were carried out.

RESULTS

Glasshouse experiment: impacts of shading

Because of variations in total daily irradiance that resulted from intermittent cloud cover, substantial variations in photosynthetic rates (measured at ambient irradiance) were observed (Table 1). In period 1, prior to the onset of shading, there was no significant difference between the rates of photosynthesis in plants growing under ambient conditions and those that were to be subsequently shaded (Table 1; d.f. = 4, $t = -0.296$, $P = 0.782$). During subsequent periods (periods 2–4), exposure to and removal of shading (i.e. <20% of ambient irradiance) reduced or increased, respectively, the rate of photosynthesis relative to the ambient treatment (Table 1; $P < 0.001$). Similar to photosynthesis, the rates of leaf R_{dark} measured at 28 °C were reduced by shading (Table 1; $P < 0.05$). However, the shade treatment had no significant effect on the rates of R_{dark} measured at 7 °C (Table 1; $P > 0.174$). Collectively, the glasshouse experiment shows that changes in irradiance had a substantial impact on photosynthesis, with irradiance-mediated changes in leaf R_{dark} (at both measuring temperatures) being quantitatively less (Table 1).

Figure 1 demonstrates that there was no significant relationship between the Q_{10} of leaf R_{dark} and photosynthesis. There was also no significant relationship between the rates of leaf R_{dark} and sugar concentrations (irrespective of the temperature that leaf R_{dark} was measured), with the concentration of soluble sugars not differing between the plants exposed to ambient or shaded conditions (Table 1). However, starch concentrations decreased substantially when the leaves were shaded (Table 1). Shade treatment also affected the specific leaf area (SLA); during period 2, the SLA was significantly higher in shaded plants than in the ambient treatment (Table 1; $P = 0.008$), although there were no significant differences during the other three periods (including period 4 when the shade was re-applied).

Growth cabinet experiment: thermal acclimation under two growth irradiances

The shade-treated plants exhibited far lower rates of photosynthesis than their high-light-grown counterparts at all temperatures ($P < 0.001$; Fig. 2). In the high-light-treated plants, photosynthetic rates were maximal between 14 and 28 °C, depending on the sampling day (Fig. 2a). Although increasing temperature reduced photosynthesis under low light (Fig. 2b), the magnitude of the decrease was minor compared with the effect of growth irradiance on photosynthetic CO_2 uptake. When all temperatures were combined within a light treatment, a significant reduction in the rate of photosynthesis was observed between day 0 and 60 for high light ($P = 0.008$; Fig. 2a), and in contrast, under shade, a significant increase in the rate of photosynthesis was observed between day 0 and 60 ($P = 0.030$; Fig. 2b).

In both light treatments, leaf R_{dark} was temperature sensitive (i.e. respiration rates increased with measurement temperature), and with the rates of leaf R_{dark} being generally lower at any given temperature in low-light-treated plants than in the high-light controls (Fig. 3). Regardless of growth irradiance, exposure to 7 °C for 60 d resulted in the rates of leaf R_{dark} increasing compared with the rates exhibited at 7 °C on day 0 (i.e. leaf R_{dark} cold acclimated). Although the overall pattern of response was similar for high- and low-light-treated plants (with the rates of leaf R_{dark} being relatively homeostatic following 60 d temperature treatment), sustained exposure to 28 °C did result in greater decreases in the leaf R_{dark} of low-light-treated plants (Fig. 3b) than high-light-treated plants (Fig. 3a). The *F*-ratio method was used to investigate whether there were significant differences between the lines fitted to the R_{dark} and temperature data for day 0 and day 60 (Fig. 3a,b). In the shaded treatment, a highly significant difference between the two fitted lines was detected ($P < 0.01$), demonstrating that thermal acclimation occurred. Although the gradient of the high-light curve was reduced in the day 60 data set (compared with the day 0 data set), no significant difference was observed between the fitted lines ($P > 0.05$). $Acclim_{LTR\ 10}$ ratios (calculated using Eqn 4) were 0.65 and 0.83 for high- and low-light-treated plants, respectively. When the leaf R_{dark} was measured at 21 °C (after 60 d exposure to the four different growth temperatures), the rates of R_{dark} increased with decreasing growth temperature in both high- and low-light-treated plants (Fig. 4), further supporting the conclusion that R_{dark} can acclimate to low growth temperatures irrespective of the growth irradiance.

On day 0, short-term changes in temperature had an impact on the balance between leaf R_{dark} and net photosynthesis (P_{net}) measured at ambient irradiance (i.e. leaf R_{dark}/P_{net}), particularly in the plants exposed to the low-light treatment (Fig. 5); R_{dark}/P_{net} values of high-light-treated

Table 1. Variations in ambient irradiance and a range of leaf traits over the four experimental periods for glasshouse-grown *Quercus ilex*

	Period 1		Period 2		Period 3		Period 4	
	Ambient	Ambient	Ambient	Shade	Ambient	Recovery	Ambient	Shade
Irradiance (μmol photons m^{-2} s^{-1})	500	500	726	56	167	167	910	45
A_{net} (μmol CO_2 m^{-2} s^{-1})	9.11 ± 1.56^{ns}	9.80 ± 1.72^{ns}	15.49 ± 0.45***	2.07 ± 0.37***	4.66 ± 1.09^{ns}	5.34 ± 0.51^{ns}	15.74 ± 0.94***	1.80 ± 0.06***
$R_{dark\ 28}$ (μmol CO_2 m^{-2} s^{-1})	1.16 ± 0.28^{ns}	1.00 ± 0.09^{ns}	0.91 ± 0.15*	0.46 ± 0.10*	0.84 ± 0.08^{ns}	0.81 ± 0.09^{ns}	0.98 ± 0.06*	0.68 ± 0.07*
$R_{dark\ 7}$ (μmol CO_2 m^{-2} s^{-1})	0.28 ± 0.08^{ns}	0.17 ± 0.05^{ns}	0.32 ± 0.06^{ns}	0.21 ± 0.04^{ns}	0.14 ± 0.01^{ns}	0.21 ± 0.04^{ns}	0.10 ± 0.0^{ns}	0.11 ± 0.03^{ns}
Proportional A_{net} change (Shaded/Ambient plants)	1.07 ± 0.19^{a}		0.13 ± 0.02^{b}		1.14 ± 0.11^{a}		0.11 ± 0.00^{b}	
Proportional $R_{dark\ 28}$ change (Shaded/Ambient plants)	0.86 ± 0.08^{a}		0.50 ± 0.11^{b}		0.96 ± 0.11^{a}		0.69 ± 0.07^{ab}	
SLA (m^2 kg^{-1})	7.3 ± 0.2^{ns}	7.3 ± 0.3^{ns}	6.3 ± 0.1**	7.4 ± 0.2**	6.4 ± 0.8^{ns}	7.1 ± 0.3^{ns}	6.7 ± 0.3^{ns}	8.9 ± 1.9^{ns}
Sugar concentration (mg g^{-1})	77.6	49.6	38.0	33.3	37.9	37.9	34.9	26.2
Starch concentration (mg g^{-1})	18.5	20.5	17.6	2.0	9.2	23.2	7.3	5.7

Rates of net photosynthesis (A_{net}) measured at ambient irradiance and at 25 °C, leaf dark respiration measured at 28 and 7 °C ($R_{dark\ 28}$ and $R_{dark\ 7}$, respectively), specific leaf area (SLA, ratio of leaf area to leaf dry mass), the concentration of total soluble sugars (fructose + sucrose + glucose) and the concentration of starch are shown. Irradiances are the mean values observed on each measurement day. Total soluble sugar and starch concentrations were determined using pooled samples of three replicates harvested on each measurement day. Photosynthesis, respiration and leaf structural trait values are the mean of three replicates (±SE). Independent samples *t*-tests were carried out between the shaded and ambient treatments, within a period (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The ratio values represent rates exhibited by shade-treated plants divided by rates exhibited by control plants kept under ambient irradiance conditions ($n = 3$, ±SE). Different letters indicate significant differences between experimental periods [one-way analysis of variance (ANOVA) and *post hoc* tests; $P < 0.05$].

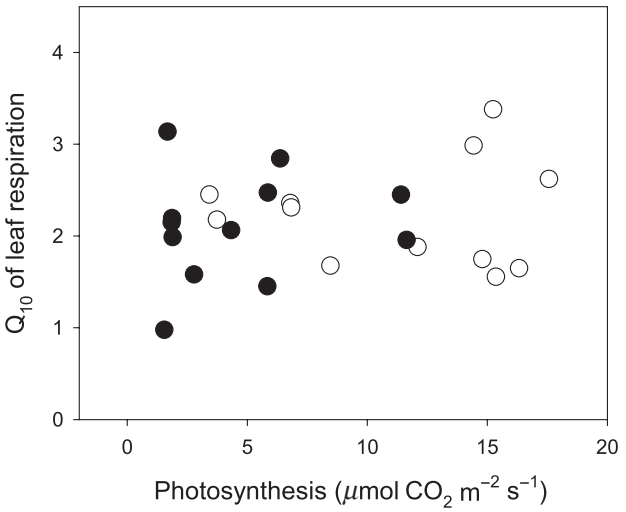

Figure 1. The Q_{10} of leaf respiration [calculated using leaf respiration in darkness (R_{dark}) rates measured at 28 and 7 °C] plotted against rates of photosynthesis exhibited by the same leaves when measured at the prevailing growth irradiance. The Q_{10} values are shown for plants experiencing ambient irradiance throughout periods 1–4 (open circles) and for plants exposed to shade-treatment during periods 2 and 4 (closed circles). Each data point represents an individual leaf measurement.

plants increased slightly, although not significantly ($P > 0.05$), from around 0.05 for 7 °C treated plants to near 0.085 for 28 °C treated plants (Fig. 5a). In contrast, for the low-light-treated plants, exposure to 28 °C resulted in R_{dark}/P_{net} rising to near 2.3 (Fig. 5b), and R_{dark}/P_{net} was significantly higher at 28 °C than at the other three temperatures ($P < 0.028$); thus, under low light on day 0, respiratory CO_2 release was greater than photosynthetic CO_2 uptake at 28 °C. R_{dark}/P_{net} values underwent substantial changes following 60 d exposure to the different growth temperatures; for high-light plants, R_{dark}/P_{net} at 7 °C increased significantly over two-fold ($P = 0.032$), and there was also a significant increase in R_{dark}/P_{net} at 14 °C ($P = 0.010$). These changes resulted in near-perfect homeostasis of R_{dark}/P_{net} across the four growth temperatures (Fig. 5a). For low-light-treated plants, R_{dark}/P_{net} remained relatively constant at 7 °C but decreased significantly at 28 °C ($P = 0.026$; Fig. 5b). As a result, low-light-treated plants were no longer in a negative carbon balance following 60 d at 28 °C.

In general, leaf R_{light} (measured at a common irradiance) was similar in high- and low-light-treated plants (data not shown), demonstrating that, in contrast to leaf R_{dark}, sustained exposure to low light did not alter the 'underlying' rates of R_{light}. Consequently, growth irradiance altered the ratio of R_{light}/R_{dark} at each temperature; whereas R_{light}/R_{dark} was typically less than unity in high-light-grown plants (Fig. 6a); R_{light}/R_{dark} ratios exceed unity in low-light-treated plants (Fig. 6b). Moreover, although R_{light}/R_{dark} ratios of low-light-treated plants decreased after 60 d exposure to each growth temperature, the ratios remained higher than exhibited by the high-light-treated plants (duration of exposure to each growth temperature had no significant effect on R_{light}/R_{dark} in high-light-treated plants). In both high- and low-light-treated plants, R_{light}/R_{dark} ratios were at their lowest at the highest temperature. We therefore conclude that when leaf R_{light} is measured at a common irradiance, R_{light}/R_{dark} ratios are higher in shaded plants than their high-light-grown counterparts, and that R_{light}/R_{dark} decreases with increasing temperature.

The SLA values of plants in the growth cabinets were significantly higher in shaded leaves than in high-light-treated leaves (Table 2; $P < 0.001$), with the relative difference between irradiance treatments varying as a function of growth temperature ($P = 0.008$) and duration of the treatments ($P = 0.021$). No significant differences in sugar concentration were observed between the plants grown under high and low light on day 0 or day 60 (Table 2); in contrast, starch concentrations were lower in shaded leaves than their high-light counterparts on day 0 (with no difference in starch concentration between high- and low-light plants on day 60).

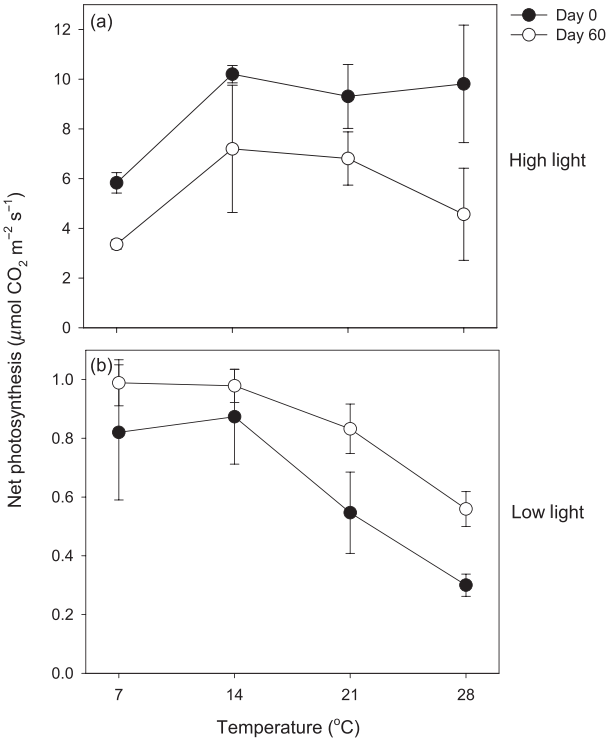

Figure 2. Effect of temperature (7, 14, 21 and 28 °C) in the controlled environment growth cabinets on rates of photosynthesis (P_{net}) of fully expanded, mature *Quercus ilex* leaves under (a) high and (b) low growth irradiance. Plants had previously experienced ambient irradiance in the glasshouse experiment (high-irradiance-treated plants) or shaded conditions in the glasshouse (shade-treated plants), and were then exposed to a growth irradiance of 300 and 16 μmol photons m^{-2} s^{-1} in the growth cabinets, respectively. Values are shown for leaves on day 0 and 60 d after shifting the plants from the glasshouse to the temperature-controlled growth cabinets. Photosynthesis was measured at 300 and 16 μmol photons m^{-2} s^{-1} for both high and low irradiance treatments, respectively. Values are the mean of three replicates (±SE).

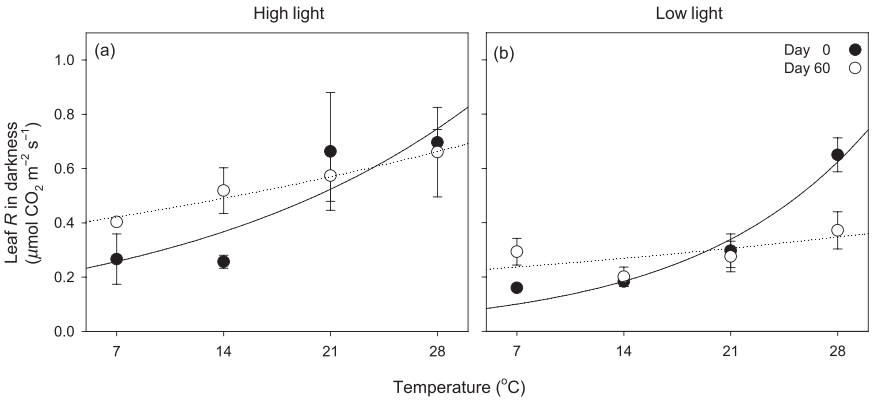

Figure 3. Rates of leaf dark respiration (R_{dark}) of *Quercus ilex* leaves plotted against temperature ($n = 3$, ±SE), both for plants exposed to (a) high and (b) low irradiance (300 and 16 μmol photons m^{-2} s^{-1}, respectively). In (a), plants had previously experienced unshaded, ambient irradiance in the glasshouse; in (b), plants had previously been shade treated (>80% reduction in ambient irradiance) for 3 weeks. In both (a) and (b), closed circles show the immediate effect of temperature on rates of leaf R_{dark} when glasshouse-grown plants were shifted to the four temperature-controlled growth cabinets (constant 7, 14, 21 or 28 °C). Open circles show subsequent rates of R_{dark} exhibited by pre-existing, fully expanded leaves following 60 d exposure to each growth temperature. Fitted lines were calculated using Eqn 4, day 0 (solid line) and day 60 (dotted line).

DISCUSSION

Respiration and growth irradiance

We investigated the effect of irradiance on the amount of substrates available for respiration, mediated through changes in photosynthesis (Azcón-Bieto & Osmond 1983; Lambers 1985; Azcón-Bieto 1992), and observed whether the temperature sensitivity of leaf R_{dark} was affected. Our results demonstrate that the Q_{10} of leaf R_{dark} of *Q. ilex* was not dependent on concurrent rates of photosynthesis (Fig. 1); the Q_{10} values of plants exposed to deep shade did not decrease despite the severe reduction in photosynthesis rates (Table 1). Although within-canopy variability in the

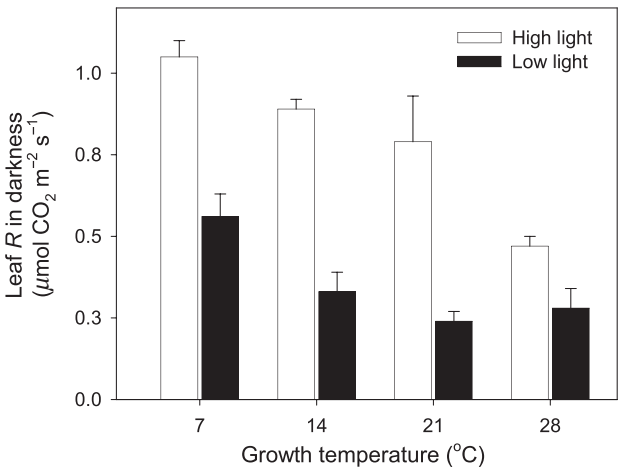

Figure 4. Effect of 60 d exposure to four growth temperatures (7–28 °C) on rates of leaf respiration in darkness (R_{dark}) of *Quercus ilex* measured at a set temperature (21 °C) ($n = 3$, ±SE), both for high- (open bars, 300 μmol photons m^{-2} s^{-1}) and low-light-treated plants (closed bars, 16 μmol photons m^{-2} s^{-1}).

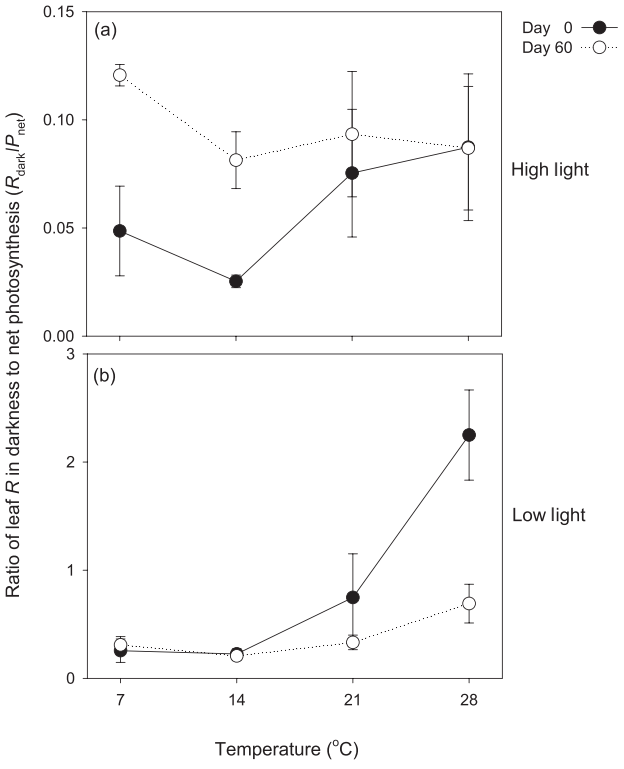

Figure 5. Ratios of leaf respiration in darkness (R_{dark}) to net photosynthesis (P_{net}) of fully expanded mature *Quercus ilex* leaves plotted against temperature ($n = 3$, ±SE), both for (a) high- (300 μmol photons m^{-2} s^{-1}) and (b) low-light-treated plants (16 μmol photons m^{-2} s^{-1}). Closed symbols represent the values of glasshouse-grown plants when first exposed to each new growth temperature on day 0. Open symbols represent ratios of R_{dark}/P_{net} following 60 d exposure to each respective growth temperature/irradiance treatment. High-light-treated plants had previously experienced ambient irradiance in the glasshouse experiment, whereas low-light-treated plants had previously experienced the shade treatment in the glasshouse experiment.

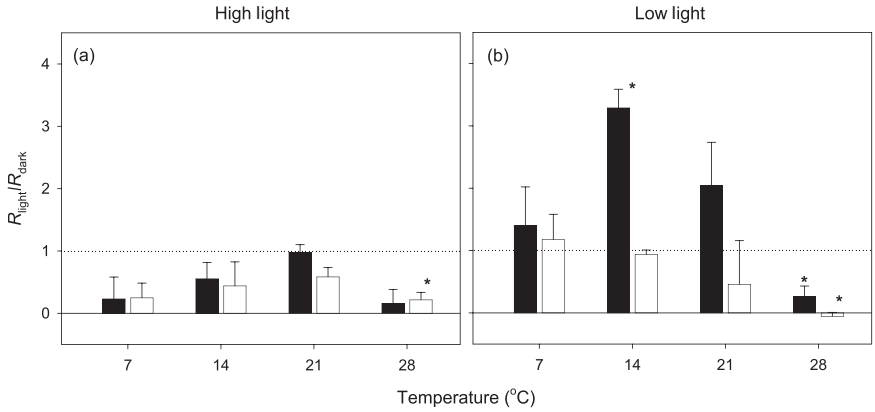

Figure 6. Effect of measurement and growth temperature (7–28 °C) on the ratio of leaf respiration in light to rates in darkness (i.e. R_{light}/R_{dark}) for *Quercus ilex* leaves that were grown under (a) high light and (b) low light, for fluxes measured when plants were first exposed to each new growth temperature (day 0, black bars) and after 60 d exposure to each growth temperature (day 60, white bars). Values are the mean of three replicates (±SE). The dashed line indicates a ratio of 1. Bars labelled '*' indicate where the proportion differs significantly from 1 (i.e. there is a significant different between R_{light} and R_{dark}; $P < 0.05$).

Q_{10} of leaf R_{dark} in trees has been reported in two studies (Griffin, Turnbull & Murthy 2002; Turnbull *et al.* 2003), in neither study were higher Q_{10} values found in leaves exhibiting higher rates of photosynthesis. Other studies have reported no variation in the Q_{10} of leaf R_{dark} in canopies, despite considerable within-canopy variation in rates of photosynthesis (Bolstad, Mitchell & Vose 1999). Similarly, in studies where rates of photosynthesis were manipulated, no relationship between variations in photosynthesis and the Q_{10} of R_{dark} was reported, either in leaves or roots (Hartley *et al.* 2006 & Atkinson *et al.* 2006, respectively). Thus, our results and those of previous studies suggest that environment-dependent changes in photosynthesis do not lead to concomitant changes in the Q_{10} of R_{dark}. The lack of a relationship between rates of photosynthesis and the Q_{10} of R_{dark} may reflect the fact that the shade-induced changes in photosynthesis can have relatively minor impacts on soluble sugar concentrations due to conversion of starch reserves to soluble sugars, as we (Tables 1 & 2) and others (e.g. Whitehead *et al.* 2004) found.

Given that soluble sugar concentrations and the Q_{10} values remained relatively constant in our study, and that there was no relationship between the rates of leaf R_{dark} and sugar concentrations, why was leaf R_{dark} lower in shade-treated plants? Two factors might have contributed to the shade-induced decline in leaf R_{dark}: decreases in capacity of the respiratory machinery and/or increases in adenylate restriction. However, the latter is unlikely as we found that using the approach of Atkin & Day (1990), addition of a respiratory uncoupler (CCCP) plus exogenous substrate (glucose) did not stimulate O_2 uptake in leaf slices of *Q. ilex* (data not shown), suggesting that

Table 2. Effect of growth temperature (7, 14, 21 and 28 °C) in the controlled environment growth cabinets on leaf mass/area relationships and sugar starch concentrations of *Quercus ilex* grown under high and low irradiance

		Growth temperature and sampling day							
		7 °C		14 °C		21 °C		28 °C	
Growth irradiance	Leaf trait	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60
High	SLA ($m^2\ kg^{-1}$)	6.9 ± 0.4	5.9 ± 0.2	6.8 ± 0.4	6.0 ± 0.5	6.1 ± 0.1	5.3 ± 0.2	6.8 ± 0.2	6.1 ± 0.2
	(Sugar) ($mg\ g^{-1}$)	34.7	36.6	36.4	35.1	39.7	58.1	29.9	41.4
	(Starch) ($mg\ g^{-1}$)	11.6	0.2	6.0	2.8	23.0	6.7	8.6	10.8
Shade	SLA ($m^2\ kg^{-1}$)	7.1 ± 0.1	6.4 ± 0.1	9.2 ± 1.7	10.6 ± 0.1	7.4 ± 0.2	9.5 ± 1.6	7.5 ± 0.2	7.7 ± 0.6
	(Sugar) ($mg\ g^{-1}$)	34	32.5	31	43.1	36.7	34.6	35.5	36.7
	(Starch) ($mg\ g^{-1}$)	0	2.2	0	1.6	0	2.0	0	1.9

Plants had previously experienced ambient irradiance in the glasshouse experiment (high-irradiance-treated plants) or shaded conditions in the glasshouse (shade-treated plants), and were then exposed to a growth irradiance of 300 and 16 μmol photons $m^{-2}\ s^{-1}$ in the growth cabinets, respectively. Specific leaf area (SLA, ratio of leaf area to leaf dry mass), concentration of total soluble sugars (i.e. fructose + sucrose + glucose) and concentration of starch are shown. Values are shown for leaves on day 0 and 60 d after shifting the plants from the glasshouse to the temperature-controlled growth cabinets. Total soluble sugar and starch concentrations were determined using pooled samples of three replicates harvested at each temperature and time. SLA values are the mean of three replicates (±SE).

respiration was not adenylate restricted in either growth irradiance treatment. Rather, the growth irradiance-mediated changes in *in vivo* rates of leaf R_{dark} likely reflected changes in overall respiratory capacity either via changes in the mitochondrial protein per unit leaf area and/or the rate of respiration per unit mitochondrial protein (Noguchi *et al.* 2005).

Although the rates of leaf R_{dark} were lower in shade-treated leaves than in their high-light-grown counterparts, growth irradiance had little impact on the degree of thermal acclimation when pre-existing leaves were exposed to contrasting growth temperatures for several weeks. The fact that leaf R_{dark} acclimates under shade, as well as under sun, was unexpected, as (1) rates of leaf R_{dark} and photosynthesis are often strongly coupled, and (2) thermal acclimation of photosynthesis is dependent on the irradiance experienced by leaves (Huner *et al.* 1993; Anderson *et al.* 1995; Huner *et al.* 1996). However, rather than exhibiting lower degrees of acclimation, the shaded leaves actually exhibited a slightly higher degree of acclimation than their high-light-grown counterparts, despite exhibiting lower rates of leaf R_{dark} at any given temperature.

Balance between respiration and photosynthesis

A number of studies have documented a balanced relationship between R_{dark} and photosynthesis (measured at saturated irradiance) after changes in the growth temperature (Dewar *et al.* 1999; Gifford 2003; Loveys *et al.* 2003; Atkin *et al.* 2006). The maintenance of this balance likely reflects interdependence between R_{dark} and photosynthesis (Raghavendra *et al.* 1994; Krömer 1995; Hoefnagel, Atkin & Wiskich 1998) for plants experiencing a single, common growth irradiance. Although the ratio of R_{dark} to photosynthesis varied with measuring temperature and differed between high- and low-light-grown plants, we observed a near-constant relationship between leaf R_{dark} and ambient light P_{net} across the growth temperatures (i.e. homeostasis of the R_{dark}/P_{net} relationship), both in high- and low-light-treated plants (Fig. 5). In high-light-grown plants, homeostasis was achieved via a substantial increase in the R_{dark}/P_{net} ratio after 60 d at 7 °C (Fig. 5), reflecting the increase in rates of leaf R_{dark} and decrease in rates of P_{net} at that low growth temperature. In contrast, near homeostasis of R_{dark}/P_{net} under low light was achieved via substantial decreases in leaf R_{dark} at 28 °C. Thus, while homeostasis of R_{dark}/P_{net} is approached across the contrasting growth temperatures in high- and low-light-grown plants, the underlying factors responsible for homeostasis are growth irradiance dependent.

One explanation why P_{net} decreased (and R_{dark}/P_{net} increased) after 60 d at 7 °C is that the combination of chilling temperatures and 'bright' light may have resulted in the onset of photoinhibition (Hurry *et al.* 1992; Nie, Long & Baker 1992). To assess this, we measured 30 min dark-adapted ratio of variable/maximum fluorescence (F_v/F_m) values [MINI–PAM (Walz, Effeltrich, Germany)] after 60 d exposure to each growth temperature in the 'high-light'-treated plants. No significant differences in F_v/F_m were found among the growth temperatures, with the overall average across the temperatures being 0.76 ± 0.02. The reduced rates of photosynthesis at 7 °C were not, therefore, because of loss of maximal efficiency of photosystem II (PSII). Moreover, it suggests that photoinhibition did not contribute to the higher R_{dark}/P_{net} exhibited by 60 d 7 °C treated plants (Fig. 5a). One factor that likely contributed to the observed R_{dark}/P_{net} patterns was the fact that 60 d exposure to 300 μmol photons m^{-2} s^{-1} (following growth under 910 μmol photons m^{-2} s^{-1} in period 4 of the glasshouse experiment) was associated with the decline in P_{net} (Fig. 2a). These reductions in P_{net} likely reflect acclimation to the lower growth irradiance, as reported in previous studies (e.g. Sims & Pearcy 1991).

Developmental dependence of leaf respiration

The majority of studies investigating the acclimation of leaf *R* have investigated plants with short-lived leaves (e.g. Armstrong, Logan & Atkin 2006a; Armstrong *et al.* 2006b; Loveys *et al.* 2003). Such studies have concluded that acclimation is developmentally dependent (i.e. new leaves need to be formed at a new growth temperature for leaf R_{dark} to fully acclimate). For example, in the short-lived *Arabidopsis thaliana*, acclimation to 5 °C was only maximal once new leaves developed in the cold (Armstrong *et al.* 2006a,b). Underpinning the increased respiratory capacity of cold-developed leaves was an increase in the density of mitochondria in epidermal cells, increased ratios of cristae to matrix in mesophyll cell mitochondria and increased oxidative capacity of individual mitochondria (Armstrong *et al.* 2006b). Such changes were not found in warm-grown, pre-existing leaves shifted to the cold for up to 3 weeks, suggesting that formation of new leaves is an essential requirement for full cold acclimation, at least in short-lived species (Armstrong *et al.* 2006b).

In our study, we measured respiration in the long-lived leaves of an evergreen Mediterranean tree species (*Q. ilex*; Crescente, Gratani & Larcher 2002), enabling us to test the hypothesis that acclimation can occur in pre-existing leaves, given sufficient time under a new thermal regime. We observed a high degree of thermal acclimation in R_{dark} in *Q. ilex* fully expanded, pre-existing leaves. Other studies have also reported evidence of substantial acclimation in leaves of other long-lived species (Bolstad *et al.* 1999; Atkin, Holly & Ball 2000c; Bolstad, Vose & McNulty 2001). Thus, it seems that full acclimation can occur in pre-existing leaves but only if the tissues are sufficiently long-lived to allow for changes in underlying cellular ultra structure and biochemistry to occur within the pre-existing leaves. In short-lived species, such changes cannot occur due to senescence of pre-existing leaves.

The temperature dependence of R_{light}

Vital in determining the extent to which leaf *R* impacts on net ecosystem CO_2 exchange is the extent to which leaf *R*

continues in the light. Although it is well known that leaf R takes place in both light and darkness, the rates of leaf R_{light} are typically lower when photosynthesis is also occurring (Brooks & Farquhar 1985; Pärnik & Keerberg 1995; Hoefnagel *et al.* 1998; Atkin *et al.* 2000b; Wang *et al.* 2001; Tcherkez *et al.* 2005) even when re-fixation of respiratory CO_2 is taken into account (Pärnik & Keerberg 1995). Our results demonstrate that light does inhibit leaf R in *Q. ilex* under some conditions (e.g. high measuring temperatures and low growth irradiance). However, unlike Atkin *et al.* (2000b, 2006), we found that R_{light} (measured at common irradiance) was substantially greater than R_{dark} in low-light-grown plants at low-moderate temperatures, suggesting that growth irradiance is important in determining the extent of light inhibition of leaf R. Previous studies have also reported that in some cases, R_{light} is greater than R_{dark}, particularly when leaves are chilled (Hurry *et al.* 1996). A likely explanation why the degree of light inhibition increases with temperature is that photorespiration is greater under hot conditions, with the greater rates of photorespiration leading to greater inhibition of key respiratory enzymes controlling carbon flow into the TCA cycle [e.g. pyruvate decarboxylase complex (PDC)]. PDC activity is known to be reduced under conditions of high photorespiration (Budde & Randall 1987, 1990; Atkin *et al.* 1998). Photorespiration-dependent inhibition of leaf R is likely to be particularly important in tree species experiencing hot, dry summers in Mediterranean regions (Peñuelas *et al.* 2004), where high rates of photorespiration are common (Filella *et al.* 1998; Peñuelas & Filella 1998). Collectively, such results highlight the potential importance of measuring both temperature *and* growth irradiance to determine the extent of light inhibition of leaf R.

CONCLUSIONS

Understanding the effect of environmental variations (e.g. temperature and irradiance) on leaf R is a prerequisite for predicting the impacts of global climate change on plant function and global atmospheric CO_2 concentrations (Ryan 1991; Larigauderie & Körner 1995; Atkin & Tjoelker 2003). It was with this in mind that we investigated the temperature and irradiance dependence of leaf R of long-lived leaves of an evergreen broad-leaved species (*Q. ilex*). Our finding that the Q_{10} values of leaf R_{dark} remain constant irrespective of the light treatment, if applicable under field conditions, has important implications for climate-carbon cycling models, as it may be possible for such models to assume that growth irradiance and photosynthesis do not affect the temperature sensitivity of leaf R_{dark}. Moreover, the fact that the acclimation of leaf R_{dark} occurs in both high- and low-light-grown plants may further simplify the incorporation of dynamic changes in leaf R_{dark} for plants growing under full sun and deep shade into large-scale models, assuming the same is found under field conditions. However, a greater challenge will be accounting for the fact that R_{light} rarely equals R_{dark}, with both measuring temperature and growth irradiance having substantial impacts on the ratio of R_{light} to R_{dark}; accounting for dynamic changes in such ratios is necessary if the modelling community is to more accurately predict rates of ecosystem gross primary productivity (GPP) and ecosystem-level R (R_e) (Wohlfart *et al.* 2005; Wythers *et al.* 2005).

ACKNOWLEDGMENTS

We thank D. Sherlock for his technical assistance, I. Hartley for his statistical help and the two anonymous referees for their useful comments. This work was funded by the Natural Environment Research Council (NERC) in the UK (NER/A/S/2001/01186; OKA), the Nordic Academy of Advanced Studies (NorFA) Temperature Stress Network and a University of York PhD studentship to JZC. Financial support was also provided by the Spanish Ministry of Education and Science (grants RASINV, CGL2004-04884-C02-02/BOS and PLASTOFOR, AGL2004-00536/FOR) to FV, and a scholarship from the Spanish Ministry of Education, Science and Sport (FPU fellowship, AP 2001-0193) to DSG.

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Received 26 January 2007; received in revised form 21 March 2007; accepted for publication 23 March 2007

