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Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species

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Abstract

We investigated the extent to which leaf and root respiration (*R*) differ in their response to short- and long-term changes in temperature in several contrasting plant species (herbs, grasses, shrubs and trees) that differ in inherent relative growth rate (RGR, increase in mass per unit starting mass and time). Two experiments were conducted using hydroponically grown plants. In the long-term (LT) acclimation experiment, 16 species were grown at constant 18, 23 and 28 °C. In the short-term (ST) acclimation experiment, 9 of those species were grown at 25/20 °C (day/night) and then shifted to a 15/10 °C for 7 days. Short-term Q_{10} values (proportional change in *R* per 10 °C) and the degree of acclimation to longer-term changes in temperature were compared. The effect of growth temperature on root and leaf soluble sugar and nitrogen concentrations was examined. Light-saturated photosynthesis (A_{sat}) was also measured in the LT acclimation experiment. Our results show that Q_{10} values and the degree of acclimation are highly variable amongst species and that roots exhibit lower Q_{10} values than leaves over the 15–25 °C measurement temperature range. Differences in RGR or concentrations of soluble sugars/nitrogen could not account for the inter-specific differences in the Q_{10} or degree of acclimation. There were no systematic differences in the ability of roots and leaves to acclimate when plants *developed* under contrasting temperatures (LT acclimation). However, acclimation was greater in both leaves and roots that developed at the growth temperature (LT acclimation) than in pre-existing leaves and roots shifted from one temperature to another (ST acclimation). The balance between leaf *R* and A_{sat} was maintained in plants grown at different temperatures, regardless of their inherent relative growth rate. We conclude that there is tight coupling between the respiratory acclimation and the temperature under which leaves and roots developed and that acclimation plays an important role in determining the relationship between respiration and photosynthesis.

Keywords: acclimation, photosynthesis, Q_{10}, relative growth rate, respiration, soluble sugars, temperature

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Introduction

Plant respiration (*R*) plays a critical role in determining a wide range of ecological phenomena, from the performance of individual plants to global atmospheric CO_2 concentrations. *R* couples the production of energy and carbon skeletons (necessary for biosynthesis and cellular maintenance) to the release of large amounts of

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CO_2. For example, up to two thirds of daily photosynthetic carbon gain is released into the atmosphere by leaf, stem and root *R* in plants grown under controlled environment conditions at a single constant temperature (Van der Werf *et al*., 1994; Atkin *et al*., 1996). Globally, plant *R* releases approximately $60\,Gt\,C\,yr^{-1}$. This compares with the relatively small release of CO_2 from the use of fossil fuels and cement production ($5.5\,Gt\,yr^{-1}$) and changing land use ($1.6\,Gt\,C\,yr^{-1}$) (Schimel, 1995). Understanding the effect of environmental variations (e.g. temperature) on *R* in a wide range of plant species is therefore a prerequisite for predicting both plant growth in contrasting habitats, and future atmospheric CO_2 concentrations.

Respiration is sensitive to short-term changes in temperature (Forward, 1960). As a result, diurnal and seasonal temperature fluctuations can influence rates of *R* (Körner & Larcher, 1988). The temperature sensitivity of *R* (i.e. the Q_{10}; the proportional change in respiration rates per 10 °C) varies among plant species (Larigauderie & Körner, 1995). Previously reported Q_{10} values vary from 1.1 (Higgins & Spomer, 1976) to 2.9 (Tjoelker *et al*., 1999a) for roots, and from 1.4 to 4.0 for leaves (Azcón-Bieto, 1992; Larigauderie & Körner, 1995). Variability in Q_{10} values may reflect differences in growth conditions, measurement temperatures and/or physiological state of the tissues (Atkin *et al*., 2000a, b; Tjoelker *et al*., 2001; Bruhn *et al*., 2002; Covey-Crump *et al*., 2002; Griffin *et al*., 2002a, b).

Long-term exposure to a change in temperature can result in respiratory acclimation. Acclimation is the adjustment of the rates of respiration to compensate for a change in temperature (Atkin *et al*., 2000b). This encompasses recovery of *R* in warm-grown plants exposed to cooler temperatures and a decline in rates after exposure of cold-grown plants to a warmer temperature. Acclimation can be rapid; for example, acclimation occurs within two days of a temperature change in some species (Rook, 1969; Billings *et al*., 1971; Atkin *et al*., 2000a). Invariably, thermal acclimation results in cold-grown plants exhibiting higher rates of *R* at a set measuring temperature than plants grown at warmer temperatures (Klikoff, 1968; Billings & Mooney, 1968; Körner & Larcher, 1988; Arnone & Körner, 1997; Atkin *et al*., 2000a, b; Covey-Crump *et al*., 2002). Acclimation may also result in respiratory homeostasis (i.e. identical rates of *R* in plants grown at contrasting temperatures; Atkin *et al*., 2000a). Moreover, it could substantially reduce annual respiratory CO_2 release (Atkin *et al*., 2000b). As a result, failure to take into account acclimation results in an over-estimate of the effects of global warming on respiratory CO_2 release over long periods (Luo *et al*., 2001), particularly in models that assume a positive feedback of global warming on *R*.

There is growing evidence that the degree of respiratory acclimation varies substantially among species (Larigauderie & Körner, 1995; Tjoelker *et al*., 1999b). However, it is not known whether variations in acclimation can be *predicted* from functional traits such as the maximum relative growth rate (RGR, increase in mass per unit starting mass and time) and/or chemical composition. Compared with slow-growing plants, fast-growing species might be expected to exhibit a higher degree of acclimation due to greater inherent physiological plasticity (e.g. Aerts & Decaluwe, 1994; Huante *et al*., 1995; Valladares *et al*., 2000). The degree of acclimation may also be greatest in leaves and roots that exhibit large changes in the availability of respiratory substrate (i.e. soluble sugars) and/or nitrogen (i.e. protein) concentrations. Several studies have reported a strong correlation between rates of *R* and the concentration of N (Ryan, 1995; Reich *et al*., 1998; Mitchell *et al*., 1999; Tjoelker *et al*., 1999b; Griffin *et al*., 2002a). Another unknown is the extent to which leaves and roots (which typically experience contrasting daily and seasonal thermal regimes) differ in their degree of acclimation. Similarly, it is not known if the degree of acclimation differs between pre-existing leaves and/or roots *shifted* from one temperature to another and leaves/roots *developed* under contrasting temperatures. While only limited changes in leaf structure occur when leaves are shifted from one temperature to another, large changes in leaf thickness, density, specific leaf area and nitrogen concentration occur when leaves develop under contrasting temperature regimes (Woodward, 1979; Tjoelker *et al*., 1999a). Clearly, further work is needed if we are to predict the extent to which *R* acclimates in contrasting tissues.

The short-term temperature sensitivity of light-saturated photosynthesis (A_{sat}) typically differs from that of leaf *R*. For example, a decline in temperature from 25 to 15 °C reduces leaf *R* and A_{sat} by 55 and 21%, respectively, in *Eucalyptus pauciflora* (Atkin *et al*., 2000a). As a result the balance between leaf *R* and A_{sat} varies with short-term changes in temperature (Woodwell, 1983; 1990). However, prolonged exposure to a new growth temperature can result in photosynthetic (and respiratory) acclimation, with the result that the balance between leaf *R* and A_{sat} is re-established (Dewar *et al*., 1999). Numerous studies have shown that photosynthesis can acclimate to changes in growth temperature, with the result that rates of photosynthesis are similar in plants grown under contrasting thermal regimes (e.g. Berry & Björkman, 1980; Hurry *et al*., 1998; Bunce, 2000). The degree of photosynthetic acclimation differs amongst species, with high sink activity and developmental plasticity being necessary for maximal photosynthetic acclimation (Huner *et al*., 1993). When coupled to interspecific differences in respiratory acclimation, such

variations in photosynthetic acclimation may result in the balance between leaf *R* and A_{sat} being re-established to a greater extent in some species than others. To our knowledge, no study has investigated whether contrasting species differ in their ability to re-establish the balance between leaf *R* and A_{sat} following prolonged exposure to a new growth temperature.

In this study we assess the extent to which Q_{10} values and the degree of respiratory acclimation vary among leaves and roots of several contrasting plant species that differ in maximum inherent RGR. We also assess whether acclimation is greatest in plants *developed* under contrasting temperatures (18, 23, 28 °C) than in warm-grown (25/20 °C) plants *shifted* to a lower temperature (15/10 °C) for several days. The effect of differing growth temperatures on soluble sugar concentrations, nitrogen concentrations and the relationship between these factors and variations in Q_{10}, the degree of acclimation and *R* rates *per se* are examined. The impact of growth temperature on the relationship between *R* and sugar and nitrogen concentrations in leaves and roots is assessed, as is the effect of growth temperature on the balance between leaf *R* and A_{sat}.

Materials and methods

To assess the extent to which contrasting plant species differ in their respiratory response to short- and long-term changes in temperature, we conducted two experiments. In the long-term (LT) acclimation experiment, we compared Q_{10} values and degrees of acclimation in plants developed at three constant temperatures (18, 23 and 28 °C); 16 species that differed in relative growth rate were used. Nine of those 16 species were used in the short-term (ST) acclimation experiment in which warm-grown plants (25/20 °C) were exposed to a lower temperature (15/10 °C) for 7 days; this enabled us to assess the extent to which pre-existing leaves and roots acclimated to a change in temperature. The impact of exposure to a lower growth temperature for 7 days (i.e. cold-treated) on the respiratory Q_{10} was also assessed in the ST acclimation experiment. In both the LT and ST acclimation experiments, the response of leaf and root *R* to temperature was investigated.

Plant material

Long-term acclimation experiment Seven pairs of fast- and slow-growing plant species in a single genus, representing a range of growth forms were chosen: grasses – *Poa trivialis* L., *P. costiniana* J Vickery; forbs – *Achillea millefolium* L., *A. ptarmica* L., *Geum rivale* Linn., *G. urbanum* L., *Plantago lanceolata* L., *P. euryphylla* BG Briggs., *Silene dioica* L. *and S. uniflora* Roth.; shrubs – *Acacia melanoxylon* R.Br. and *A. aneura* F. Muell Ex Benth.; trees – *Eucalyptus delegatensis* R. Baker. and *E. dumosa* Cunn. Ex. Schauer. In addition, the fast-growing *P. major* L and the slow-growing *Luzula acutifolia* Nordenskiöld were included. Details of the origin and natural distribution of these species can be found in Loveys *et al*. (2002). The seeds of *A. melanoxylon* were sterilized in 0.3% NaOH and then nicked with a razor blade. The *A. aneura* seeds were boiled in deionized H_2O for one minute then left to soak for 24 h. After pre-treatment, the seeds of both *Acacia* species were sown in Petri dishes lined with filter paper dampened with H_2O and placed in growth cabinets. Once the radicle had emerged, the germinated seeds were transferred to trays of 1 : 1 sand : vermiculite and placed in growth cabinets at 22 ± 2 °C (day) and 14 ± 2 °C (night) with a 16-h day. The seed of the *Eucalyptus* species required vernalization at 4 °C for six weeks prior to germination. Seed from all other species were sown on trays of John Innes F2 compost and placed in the growth cabinets described above. Once the roots of all species had reached at least 3 cm in length, the seedlings were carefully removed from the compost or sand : vermiculite and the roots thoroughly washed with H_2O. They were then transferred to 16 L hydroponics tanks filled with a fully aerated modified Hoagland's nutrient solution (Poorter & Remkes, 1990). An additional nitrogen supply (2 mM NH_4NO_3) was added to tanks in which *Luzula acutifolia* were to be grown as it exhibited nitrogen deficiency symptoms when provided with NO_3^- nitrogen alone. The pH of the solution was maintained at 5.8 and the solution was changed weekly. The tanks were placed in growth cabinets (Conviron E15, Winnipeg, Canada) with a 14-h day, 300 μmol m^{-2} s^{-1} PPFD provided by a combination of 400 W metal halide and 400 W high pressure sodium blubs. The temperature treatments were constant 18, 23 and 28 °C.

Short-term acclimation experiment To assess the extent to which preexisting leaves and roots, developed at a warmer temperature, are able to acclimate to a cooler temperature a subset of nine species (*A. millefolium*, *A. ptarmica*, *G. urbanum*, *G. rivale*, *L. acutifolia*, *P. lanceolata*, *P. euryphylla*, *S. dioica* and *S. uniflora*) were germinated as described above and transferred to hydroponics in the growth cabinets described above set at 25/20 °C day/night regime. After 14 days half of the plants were transferred to a 15/10 °C day/night regime for seven days.

Gas exchange

Light-saturated photosynthesis (A_{sat}) and dark leaf respiration (*R*) in leaves were measured using a Li-Cor 6400 infra-red gas analyser (Lincoln, NE, USA). In the LT acclimation experiment, four plants from each species

were selected. Rates of A_{sat} and leaf dark *R* were measured on an attached youngest fully expanded leaf after the plants had been growing in hydroponics for approximately 20–30 days, at the growth temperature. All measurements took place on plants that had experienced at least four hours of illumination in the growth cabinets (after 10 am and before 4 pm). Once the leaf was secured in the cuvette, leaves were exposed to saturating irradiance (1200–2000 μmol photons $m^{-2} s^{-1}$) and A_{sat} measured after 15 min. The LED light source was then switched off and the entire plant then covered with black cloth for 10–20 min prior to *R* being measured. *R* was monitored over this time period and recorded once respiration had stabilized. For plants grown at 18 and 28 °C, a further set of measurements of *R* were then made at a set temperature of 23 °C. Consequently, leaves developed at 28 and 18 °C were measured at two temperatures (28 and 23 °C and 18 and 23 °C, respectively) whereas the 23 °C grown plants were measured at 23 °C only. The short-term temperature coefficient (i.e. Q_{10}) could therefore be calculated only for the 28 and 18 °C grown plants. In all leaf CO_2 exchange measurements, the concentration of CO_2 in the cuvette was set to 370 μmol mol^{-1}. In ST acclimation experiment, six plants from each species were selected and dark *R* measured as described above on the youngest fully expanded leaf. Measurements were made at 25 and 15 °C for both the warm-grown and the 7-day cold-treated plants. Importantly, all leaves measured after 7-days of cold treatment were leaves that had previously developed at 25/20 °C. Thus, the ST acclimation experiment assessed the extent to which acclimation occurs in warm-grown plants *shifted* to low-growth temperature. In contrast, the LT acclimation experiment assessed the degree of acclimation in leaves *developed* under contrasting temperatures. In all leaf *R* measurements (LT and ST acclimation experiments), the leaf and whole plant temperatures were equal; this ensured that the leaf used for measurements was not uncoupled from the physiology of the rest of the plant (Amthor, 2000; Atkin *et al*., 2000a; Griffin *et al*., 2002b). Moreover, in all cases, *R* was first measured at the growth temperature followed by measurements at the second temperature (following 30–60 min exposure to the second temperature).

In both the LT and ST acclimation experiments, root *R* was measured on detached whole root systems using Clarke type O_2 electrodes (Rank Brothers, Cambridge, UK) coupled to a computer-based data acquisition system (NI-DAQ for Windows 2000; National Instruments, Newbury, Berkshire, UK). The roots were placed in cuvettes with a known volume of fully aerated modified Hoagland's nutrient solution (see above, pH 5.8). In the LT acclimation experiment, limitations in the number of plants meant that root *R* could only be measured at the growth temperature. [Measurement of *R* at two temperatures on a single root system resulted in substantial underestimates of *R* at the second temperature, due to decreases in *R* with time. Consequently, it was not possible to obtain accurate estimates of root *R* at two temperatures using the same root system]. Plant availability was not limiting in the ST acclimation experiment; we therefore measured root *R* at 25 and 15 °C using separate plants.

Chemical analyses

The fresh mass of leaf and root samples was recorded and then samples were freeze-dried in an Edwards EF4 Modulyo freeze-drier (Northern Scientific, York, UK). After the dry mass had been recorded, the replicate plant parts were pooled at each harvest and ground to a fine powder using either a mortar and pestle or a hammer mill (31–700 Hammer Mill, Glen Creston, UK) and analysed by mass spectrometry (CE Instruments NA2100 Brewanalyser, ThermoQuest Italia S.p.A. Milan, Italy) for total C and N concentration.

Soluble sugars were extracted from root and leaf samples by hot ethanol extraction. 500 μL of 80% (v/v) ethanol was mixed by vortex action with 5 mg of ground plant material in an eppendorf tube. The sample was then incubated at 80 °C for 20 min with vortex mixing every 10 min. The sample was centrifuged at 12000 r.p.m. for 5 min and the supernatant removed. This process was repeated a further two times each time with 500 μL of 80% (v/v) and the supernatant combined. Soluble sugars (glucose, sucrose and fructose) were estimated by three microtitre plate based assays. For glucose, extracts were incubated in the presence of hexokinase and glucose-6-phosphate dehydrogenase. The reduction of NADP to NADPH was then followed spectrophotometrically at 340 nm (Dynatech Laboratories MRX, Guernsey, UK). For fructose, extracts were incubated in the presence of hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase and the reduction of NADP to NADPH was followed (Scholes *et al*., 1994). The concentration of sucrose plus glucose was estimated via incubation of the extract with invertase (Sigma, St Louis, MO, USA), followed by measurement of the total glucose concentration as above.

Data analysis

The ST temperature response (i.e. the Q_{10}) of leaf respiration for the 18 and 28 °C grown plants in the LT acclimation experiment was determined via comparisons of the rates exhibited by those plants at 18 and 28 °C, respectively, with the rates exhibited at 23 °C:

$$Q_{10} = 10^{\left[10^{*}((\log R1 - \log R2)/(T1 - T2))\right]} \quad (1)$$

where R1 is the respiration rate measured at the warmer temperature (either 23 or 28 °C) and R2 is respiration measured at the cooler temperature (either 23 or 18 °C) and T1 and T2 are the respective measurement temperatures.

Calculation of the short-term Q_{10} for leaves and roots of the temperature-shifted plants and their controls in the ST acclimation experiment, with a temperature difference of 10 °C, took the simpler form:

$$Q_{10} = R \text{ at } 25\,^{\circ}C / R \text{ at } 15\,^{\circ}C \quad (2)$$

Estimates of how well *R* acclimates can vary depending on the method used to quantify the degree of acclimation and the region of the temperature response curve used for calculating acclimation ratios (Atkin *et al*., 2000b). We therefore decided to assess acclimation using several defined approaches, with acclimation ratios being calculated over temperatures (between 15 and 28 °C) that are well below the temperature optima of *R* for the selected species (Tjoelker *et al*., 2001). The first was the *Homeostasis Method*, which requires a comparison of *R* rates at the respective growth temperature. The ratio of *R* exhibited by cold-grown plants divided by *R* of the warm-grown plants (each measured at their respective growth temperature) can then be used to assess the degree of acclimation (e.g. Figure 1, $Acclim_{Homeo} = D/A$). If $Acclim_{Homeo}$ is greater than or equal to 1, then acclimation must have occurred. This $Acclim_{Homeo}$ ratio is the inverse of 'LT Q_{10} ratio' as measured by Larigauderie & Körner (1995); for our method the degree of acclimation increases as the ratio increases. The second approach was the *Set Temperature Method*. This requires a comparison of *R* at a set measuring temperature; 23 °C for plants grown at 18 and 28 °C (LT acclimation) or 25 °C for plants cold-acclimated to 15 °C or grown at constant 25 °C (ST acclimation) (e.g. Figure 1, $Acclim_{SetTemp} = C/A$). High ratios indicate high degrees of acclimation. Although it is not possible to determine if a plant has fully acclimated using this method, the method does provide a way of comparing the extent of acclimation in contrasting plant species. The final approach was the *Degree of Recovery Method*. This method enables the extent to which leaves and roots partly acclimate to changes in temperature to be accurately quantified. The *Degree of Recovery Method* assumes that exposure to a lower growth temperature initially results in a decline in respiration rates (e.g. Figure 1, A minus B). The extent of recovery (i.e. the proportion of the initial decline that is recovered following acclimation) is used to quantify the degree of acclimation [e.g. Figure 1, $Acclim_{Recovery} = (D–B)/(A–B)$]. Full recovery (and thus full acclimation) has occurred when $Acclim_{Recovery}$ is 1.0. Conversely, the degree of acclimation declines as *Acclim*-Recovery approaches zero. This method was used only in the ST acclimation experiment.

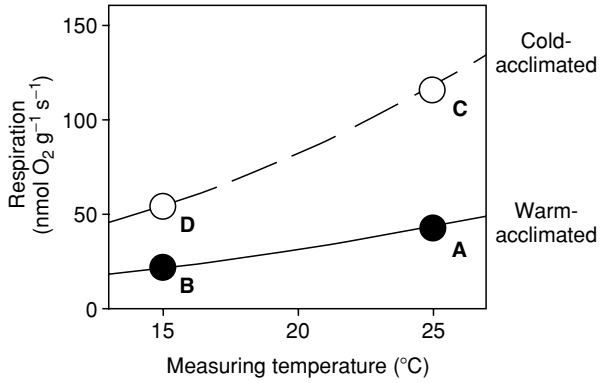

Fig. 1 Theoretical temperature response curves of respiration in warm-acclimated and cold-acclimated plants. In this example, the degree of acclimation to the low temperature can be quantified using three approaches. The first is the *Homeostasis Method*, which requires a comparison of *R* rates at the respective growth temperatures (e.g. $Acclim_{homeo} = D/A$). The second approach is the *Set Temperature Method* which requires a comparison of *R* at a set measuring temperature (e.g. $Acclim_{SetTemp} = C/A$). The final approach is the *Degree of Recovery Method* [e.g. $Acclim_{Recovery} = (D–B)/(A–B)$]. See 'Materials and methods' section for further details.

Statistical analysis

All data were tested for normality and homogeneity of variance using a Kolmogorov-Sminov test in SPSS v10 (SPSS Science, Birmingham, UK). For rates of leaf and root *R*, these tests were conducted using individual plant values. When comparing Q_{10} and acclimation ratios between temperature treatments, mean values for each species/temperature treatment combination were used; consequently, normality was tested on the mean values (i.e. $n = 16$). All data were found to be suitable for parametric tests. Linear regressions were performed in Sigma Plot v5 (SPSS Science, Birmingham, UK). One-way ANOVA with Tukey post-hoc tests, Student's *T*-tests and Pearson's or Spearman's correlations were performed using SPSS v10.

Results

Variations in the ST and LT temperature sensitivity of respiration

The response of leaf and root *R* to different growth temperatures varies among the species (Fig. 2). For example, there was no significant difference in leaf or root *R* rates when *G. rivale* was grown at 18, 23 or 28 °C (Fig. 2E)

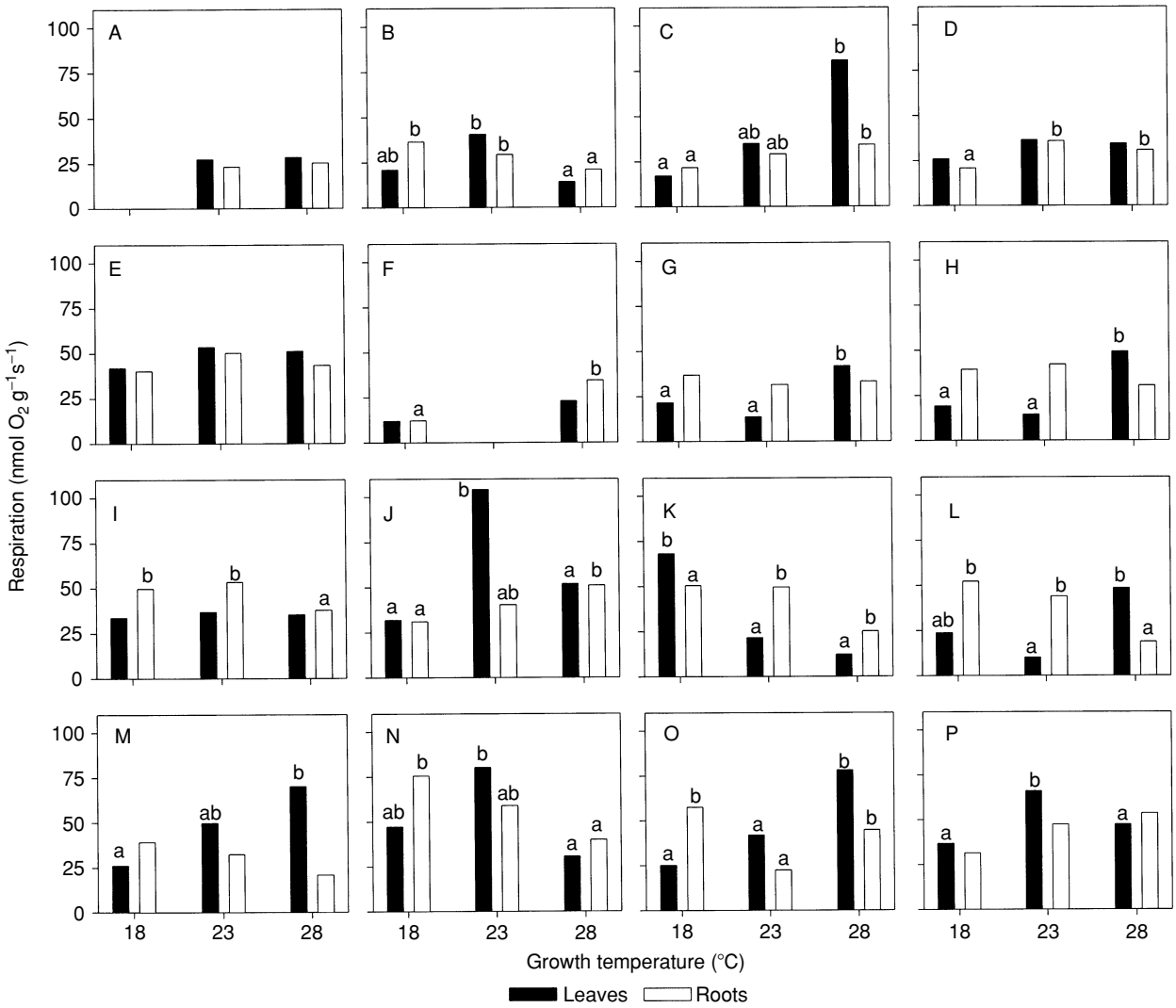

Fig. 2 Leaf (nmol CO_2 g^{-1} s^{-1}) and root respiration (nmol O_2 g^{-1} s^{-1}) of (A) *A. aneura*, (B) *P. euryphylla*, (C) *A. melanoxylon*, (D) *E. dumosa*, (E) *G. rivale*, (F) *P. costiniana*, (G) *L. acutifolia*, (H) *E. delegatensis*, (I) *G. urbanum*, (J) *S. uniflora*, (K) *A. ptarmica*, (L) *P. trivialis*, (M) *S. diocia*, (N) *A. millefolium*, (O) *P. major*, and (P) *P. lanceolata* measured at the growth temperatures of 18, 23 and 28 °C. Data are the averages of all replicate plants (4–6). Different letters indicate statistical difference (where b is greater than a) at $P = 0.05$ as determined by ANOVA with Tukey's post hoc tests between each temperature treatment within an organ. Where no letters are shown, there was no significant difference between temperature treatments within an organ.

suggesting that this species exhibits a high degree of acclimation to the different growth temperatures. In contrast, the leaf *R* rates of *A. melanoxylon* (Fig. 2C) increased with growth temperature ($P = 0.01$). Rates of root *R* of *A. melanoxylon* were also significantly higher when grown at 28 °C compared to 18 °C ($P = 0.04$). Several species showed a decline in *R* rates when grown at 28 °C (e.g. *A. ptarmica, A.millefolium*; Fig. 2K and N, respectively).

Although there was substantial inter-specific variation in the Q_{10} of leaves and roots (Tables 1 and 2), Q_{10} values were generally greater in leaves than roots (Tables 1 and 2) in ST acclimation experiment; the average Q_{10} values for leaves and roots were 2.0–2.2 and 1.55–1.61, respectively (Table 3). Growth temperature had no systematic or significant effect on the Q_{10} of leaf *R* in the LT acclimation experiment (Table 1) or root *R* in the ST acclimation experiment (Table 2), with the inter-specific averages being similar for the contrasting growth temperatures (Table 3). Leaf Q_{10} values were *higher* in warm-grown plants than their cold-acclimated counterparts in six of the nine species used in the ST acclimation experiment (Table 2); average leaf Q_{10} values were also significantly ($P = 0.03$) higher in the warm-grown plants (Tables 2 and 3).

No systematic or significant relationship was found between the Q_{10} of leaves or roots of each growth

Table 1 Maximum relative growth rates (RGR, $mg\,g^{-1}\,d^{-1}$), short-term Q10 values of the 18 and 28 °C grown plants and average acclimation ratios (using the *Set Temperature* and *Homeostasis Methods*) for each of the 16 species grown in the Long-Term (LT) acclimation experiment (see Materials and methods for details)

				Acclimation ratio		
		Leaf Q_{10}		Leaves	Roots	
Species	RGR_{max} mg $g^{-1}d^{-1}$	18 °C	28 °C	$Acclim_{SetTemp}$	$Acclim_{Homeo}$	$Acclim_{Homeo}$
Acacia aneura	63	ND	2.30	1.41	0.97	0.92
Plantago euryphylla	104	2.25	1.06*	1.56	1.68	1.32
Acacia melanoxylon	107	2.17	3.58 ns	0.71	0.46	0.80
Eucalyptus dumosa	111	1.86	1.14*	1.05	0.88	0.87
Geum rivale	116*	1.93	2.11 ns	1.29	0.91	0.98
Poa costiniana	134	ND	3.52	ND	ND	ND
Luzula acutifolia	138	2.02	1.49 ns	1.38	0.95	1.06
Eucalyptus delegatensis	150	2.65	2.96 ns	1.29	0.81	1.16
Geum urbanum	166	1.83	1.70 ns	1.27	0.98	1.17
Silene uniflora	168	2.68	0.84 ns	1.14	1.16	0.78
Achillea ptarmica	187	1.50	1.38 ns	2.83	2.47	1.50
Poa trivialis	210	0.76	1.26 ns	1.22	1.29	1.77
Silene dioica	228	2.38	1.73 ns	0.87	0.62	1.39
Achillea millefolium	251	2.67	1.75 ns	2.14	1.60	1.38
Plantago major	208	2.26	2.37 ns	0.82	0.57	1.53
Plantago lanceolata	267	1.65	2.43 ns	1.43	0.97	0.77

Respiration rates for each species/growth temperature (18, 23 or 28 °C) combination are shown in Fig. 1. For plants grown at 18 and 28 °C, values at a measuring temperature of 23 °C were used to calculate the leaf Q_{10} values shown. The acclimation ratios represent the average of the individual 18/23 and 23/28 °C ratios. ND denotes no data. Maximum RGR values were taken from Loveys *et al*. (2002). Statistical difference of leaf Q_{10} values determined by an independent *T*-test between temperature treatments. *$P < 0.05$. ns denotes not statistically different.

treatment/species combination, and their corresponding whole plant maximum RGR value (Tables 1 and 2). Moreover, there was no relationship between leaf and root Q_{10} values and the concentration of soluble sugars or total N (data not shown).

Comparison of methods to assess the degree of acclimation

A comparison of acclimation ratios for individual species calculated using the *Set Temperature Method* vs. those calculated using the *Homeostasis Method* is provided in Fig. 3. In the LT acclimation experiment, very similar leaf *R* acclimation ratios were obtained using the two methods ($P < 0.001$, $r^2 = 0.85$) (Fig. 3a). However, there was more variability in the ratios obtained with the two methods in the ST acclimation experiment. There was no correlation between the two methods for roots but there was a significant relationship ($P = 0.01$, $r^2 = 0.61$) between the *Homeostasis Method* and the *Set Temperature Method* for leaves (Fig. 3b).

Degree of acclimation in leaves and roots

Acclimation ratios were greater in roots than leaves in most species in ST acclimation experiment (*Homeostasis* and *Set Temperature Methods*, Fig. 4b). Moreover, average $Acclim_{Homeo}$ ratios were significantly greater in roots than leaves in the ST acclimation experiment (Table 4). In contrast, roots and leaves did not differ systematically in their ability to acclimate in the LT acclimation experiment ($Acclim_{Homeo}$; Fig. 4a; Table 4). There was no significant correlation between root and leaf acclimation in either experiment.

Acclimation ratios ($Acclim_{Homeo}$) for leaves and roots were then plotted against the corresponding maximum RGR value for each species (Fig. 5). $Acclim_{Homeo}$ ratios varied substantially among the species. In the LT acclimation experiment this variation was not correlated with the maximum RGR of each species, either for leaves or roots (Fig. 5a). Similarly, there was no relationship between $Acclim_{Homeo}$ and the RGR for leaves or roots in the ST acclimation experiment. Inter-specific differences in the concentration of soluble sugars or nitrogen concentration could not account for the variations in acclimation ratios (data not shown).

R and variations in chemical composition

To assess whether the difference in *R* rates amongst species (Fig. 2) was associated with variations in soluble

Table 2 Respiration of leaves (nmol CO_2 g^{-1} s^{-1}) and roots (nmol O_2 g^{-1} s^{-1}) for the 9 species grown in the Short-Term (ST) acclimation experiment. Also shown are the short-term Q_{10} values of the 25 °C grown plants and plants treated with 15/10 °C for 7 days, as well as the average acclimation ratios (using the *Set Temperature*, *Homeostasis* and *Recovery Methods*) for each species (see Materials and methods for details)

	R at the growth T (nmol g^{-1} s^{-1})		Q_{10}			Acclimation ratio	
Organ/Species	15 °C	25 °C	15 °C	25 °C	Set T	$Acclim_{homeo}$	$Acclim_{recovery}$
Leaves							
Plantago euryphylla	24.5 ± 2.3	37.6 ± 6.4 ns	2.70	2.55 ns	0.72	1.92	0.55
Geum rivale	38.9 ± 3.8	50.6 ± 2.4*	1.65	1.68 ns	1.22	0.77	0.32
Luzula acutifolia	32.0 ± 6.4	39.9 ± 3.5 ns	1.75	3.03 ns	1.40	0.80	0.68
Geum urbanum	8.9 ± 1.2	47.2 ± 6.8**	2.77	2.64 ns	0.45	0.19	−0.41
Silene uniflora	35.7 ± 4.5	67.6 ± 5.0***	2.12	2.58**	1.12	0.53	0.25
Achillea ptarmica	50.4 ± 4.3	79.3 ± 4.9***	1.60	2.17 ns	1.00	0.63	0.31
Silene dioica	38.5 ± 4.4	62.5 ± 4.3*	2.01	2.50 ns	1.21	0.62	0.34
Achillea millefolium	44.4 ± 3.7	84.1 ± 4.3***	1.89	2.24 ns	0.96	0.53	0.12
Plantago lanceolata	45.8 ± 3.2	72.9 ± 3.9***	1.51	2.18**	0.94	0.63	0.31
Roots							
Plantago euryphylla	31.0 ± 2.8	39.8 ± 9.8 ns	1.94	1.47	1.51	0.78	0.50
Geum rivale	37.8 ± 4.0	45.0 ± 4.1 ns	1.47	1.15	1.23	0.84	−0.24
Luzula acutifolia	28.8 ± 14.47	49.1 ± 13.9*	2.42	1.58	0.58	0.66	0.18
Geum urbanum	19.7 ± 1.5	28.1 ± 3.2 ns	1.21	2.02	1.69	0.70	0.19
Silene uniflora	39.1 ± 6.2	51.2 ± 1.6***	1.41	2.18	1.22	0.87	0.72
Achillea ptarmica	26.2 ± 2.5	46.6 ± 2.9 ns	2.12	1.61	1.19	0.56	−0.15
Silene dioica	49.3 ± 7.8	34.4 ± 2.7 ns	0.85	1.85	1.22	1.43	1.94
Achillea millefolium	47.5 ± 4.0	51.1 ± 5.5 ns	1.41	1.40	1.31	0.93	0.75
Plantago lanceolata	33.6 ± 3.3	32.6 ± 1.5 ns	1.70	1.00	1.75	1.03	1.28

Respiration rates were measured at the growth temperature (25 °C for the 25/20 °C grown plants and 15 °C for the 7-day 15/10 °C treated plants) and are the mean of six replicate plants (± SE). Q_{10} values were calculated via comparison of rates exhibited by 25/20 °C-grown plants measured at 15 and 25 °C, and by 7-day 15/10 °C-treated plants measured at 15 and 25 °C. Statistical difference in respiration rates and Q_{10} values determined by an independent *T*-test between temperature treatments. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ns denotes not statistically different. Root Q_{10} values were determined from means of all replicate plants and therefore no statistics were performed.

Table 3 Comparison of the mean Q_{10} values exhibited by leaves and roots of all species (± SE) grown in the Long-Term (LT; $n = 16$) and Short-Term (ST; $n = 9$) acclimation experiments

	LT acclimation		ST acclimation	
Organ	18 °C grown	28 °C grown	15°C acclimated	25°C grown
Leaves	2.03 ± 0.13	2.04 ± 0.21 ns	2.00 ± 0.15	2.39 ± 0.13*
Roots	ND	ND	1.61 ± 0.16	1.58 ± 0.13 ns

See Fig. 2 and Table 1 for the individual species Q_{10} values used for calculating the mean Q_{10} values. In the LT acclimation experiment, values represent the average Q_{10} values exhibited by the 18 and 28 °C-grown plants. Statistical difference for leaves and roots determined by an independent *T*-test between temperature treatments. *$P < 0.05$. ns denotes not statistically different.

sugars and/or nitrogen concentration, we plotted leaf and root respiration rates (measured at growth temperature) against soluble sugar (Figs 6a, b) and total nitrogen (Figs 6c, d) concentrations for each species (LT acclimation). Contrary to expectations, leaf respiration was *negatively* correlated with soluble sugar concentration when all temperature treatments were considered together ($P = 0.006$, $r^2 = 0.2$) (Fig. 6a). In single species studies, leaf respiration rates are often *positively* correlated with sugar concentrations (e.g. Atkin *et al*., 2000d; Griffin *et al*., 2002a). The absence of any significant difference in the regressions amongst the growth temperatures suggests

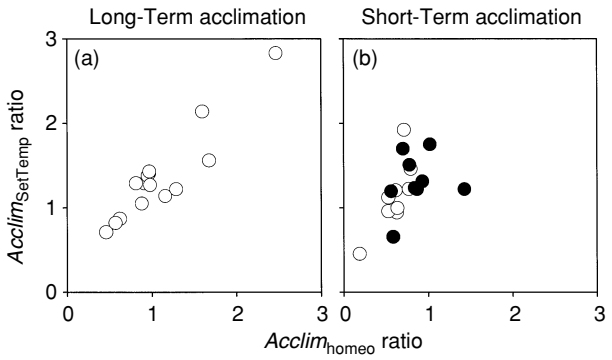

Fig. 3 Comparison of acclimation ratios using two methods (*Set Temperature* vs *Homeostasis Method*) in (a) Long-Term (LT) and (b) Short-Term (ST) acclimation experiments. $Acclim_{homeo}$ in the LT acclimation experiment represent the average of cold/warm-grown ratios for respiration rates measured at respective growth temperatures (i.e. 18/23 °C ratio plus 23/28 °C ratio, divided by two to obtain overall average acclimation ratio). $Acclim_{SetTemp}$ ratios in LT acclimation experiment represent the average cold/warm ratios of 18/23 °C and 23/28 °C grown plants (*R* measured at 23 °C in all cases). $Acclim_{homeo}$ and $Acclim_{SetTemp}$ values for the ST acclimation experiment were calculated as described in 'Materials and methods'. Symbols: (○), leaves; (●), roots. Increasing ratios indicate increasing degrees of thermal acclimation for both the *Homeostasis* and *Set Temperature Methods*.

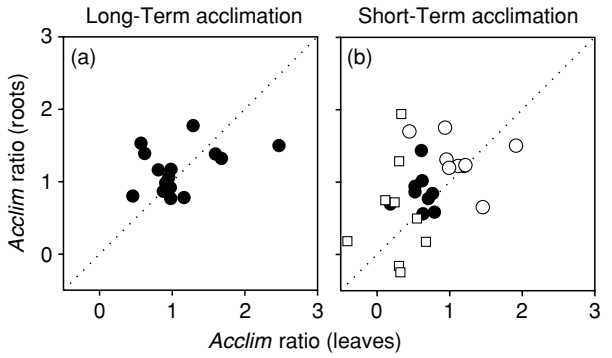

Fig. 4 Root vs leaf acclimation ratios of individual species grown in the Long-Term (a) and Short-Term (b) acclimation experiments. Symbols: (●), $Acclim_{homeo}$ ratio; (○) $Acclim_{SetTemp}$ ratio; (□) $Acclim_{Recovery}$ ratio. The $Acclim_{homeo}$ ratios in the LT acclimation experiment represent the average of 18/23 and 23/28 °C ratios. The ST acclimation values were calculated as described in 'Materials and methods'.

that despite differences in growth temperature (i.e. 28, 23 and 18 °C), similar leaf respiration rates were maintained for a given sugar concentration (Fig. 6a). No correlation was found between root respiration rates and soluble sugar concentrations ($P = 0.25$, $r^2 = 0.02$) (Fig. 6b). Leaf

Table 4 Comparison of the mean acclimation ratios exhibited by leaves and roots of all species (± SE) grown in the Long-Term (LT; $n = 16$) and Short-Term (ST; $n = 9$) acclimation experiments

Method	Organ	LT acclimation	ST acclimation
Homeostasis ($Acclim_{Homeo}$)	Leaves	1.09 ± 0.13 ns	0.59 ± 0.06**
	Roots	1.16 ± 0.08	0.90 ± 0.10
Set Temperature ($Acclim_{SetTemp}$)	Leaves	1.36 ± 0.14	1.11 ± 0.11 ns
	Roots	ND	1.35 ± 0.14
Recovery ($Acclim_{Recovery}$)	Leaves	ND	0.27 ± 0.10 ns
	Roots	ND	0.51 ± 0.23

See Tables 1 and 2 for the individual species acclimation ratios used for calculating the mean ratios. In the LT acclimation experiment, values represent the average acclimation ratios exhibited by the 18 and 28 °C-grown plants. Statistical difference between leaves and roots within each method of calculating acclimation determined by an independent *T*-test. *$P < 0.05$. **$P < 0.01$. ns denotes not statistically different.

and root respiration rates were positively correlated with nitrogen concentration at 18 °C (leaves $P = 0.003$, $r^2 = 0.68$; roots $P = 0.01$, $r^2 = 0.34$). The relationship between respiration and nitrogen was not significant when plants were grown at 23 or 28 °C (Fig. 6d). However, when all temperature treatments were considered together there were significant positive relationships between leaf respiration and leaf nitrogen ($P = 0.01$, $r^2 = 0.1$) and root respiration and root nitrogen ($P = 0.001$, $r^2 = 0.2$).

In the LT acclimation experiment, growth temperature had little effect on the concentration of soluble sugars, either in leaves or roots (Fig. 6). Similarly, acclimation to 15/10 °C had little effect on the average concentration of soluble sugars in the ST acclimation experiment (leaves: 99.2 ± 16.3 and 83.9 ± 11.6 mg g^{-1} for 15/10 °C-acclimated and 25/20 °C-grown plants, respectively; roots: 27.3 ± 4.6 and 24.2 ± 4.7 mg g^{-1} for 15/10 °C-acclimated and 25/20 °C-grown plants, respectively). Growth temperature also had no systematic or significant effect on the concentration of nitrogen in the contrasting species, either in leaves or roots in the LT (Fig. 6) or ST acclimation experiments (leaves: 2.78 ± 0.18 and 2.53 ± 0.25 mmol N g^{-1} for 15/10 °C-acclimated and 25/20 °C-grown plants, respectively; roots: 2.29 ± 0.19 and 2.51 ± 0.18 mmol N g^{-1} for 15/10 °C-acclimated and 25/20 °C-grown plants, respectively). Thus, our growth temperatures had little impact on the chemical composition of roots and leaves. No correlation was found between the maximum RGR (Table 1) and concentration soluble sugars (Fig. 6).

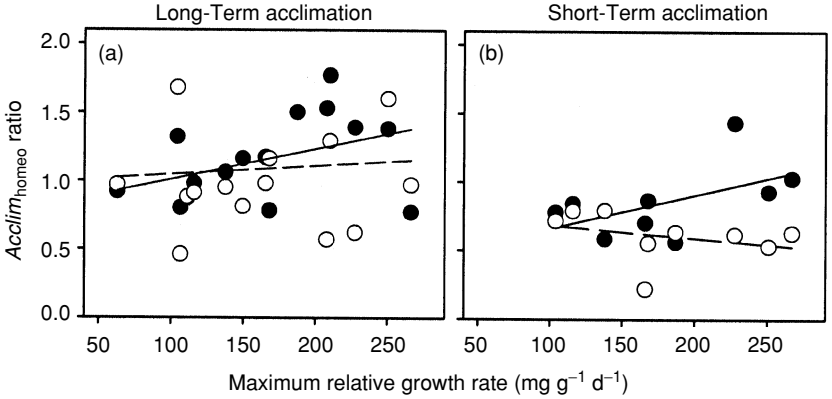

Fig. 5 Variations in acclimation ratios ($Acclim_{homeo}$) of leaf (○) and root (●) respiration for several plant species differing in maximum relative growth rate (mg g^{-1} d^{-1}). The $Acclim_{homeo}$ values in the LT acclimation experiment (a) represent the average of 18/23 and 23/28 °C ratios. The ST acclimation experiment values (b) were calculated as described in 'Materials and methods'. Values of RGR are from Loveys *et al.* (2002). Each symbol represents a different species. Lines are linear regressions; (a) Leaves (– –) $P = 0.61$, $r^2 = 0.02$, roots (–) $P = 0.13$, $r^2 = 0.16$; (b) Leaves (– –) $P = 0.42$, $r^2 = 0.09$, roots (–) $P = 0.13$, $r^2 = 0.29$.

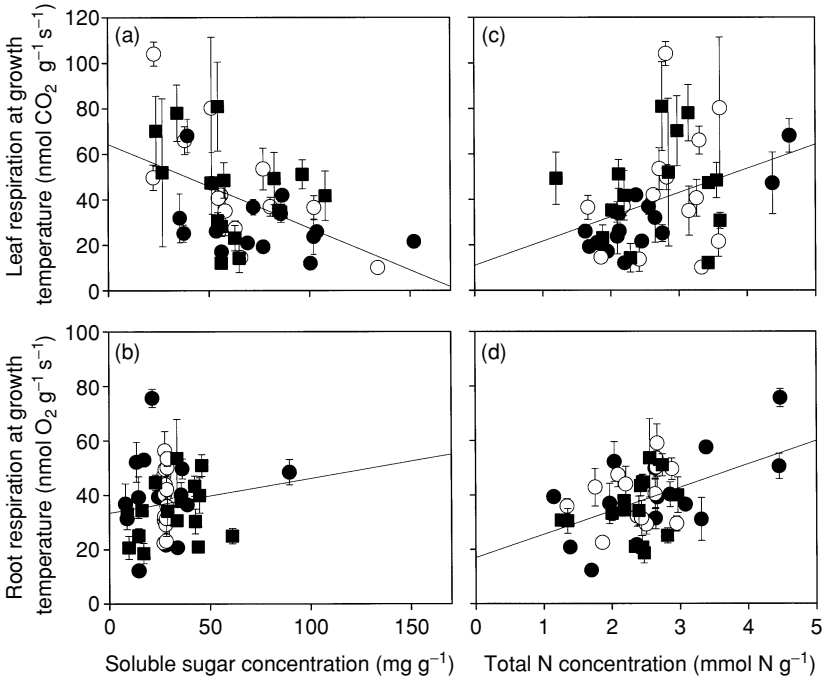

Fig. 6 Rates of leaf (a and c; nmol CO_2 g^{-1} s^{-1}) and root (b and d; nmol O_2 g^{-1} s^{-1}) respiration plotted against soluble sugar (a and b; mg g^{-1}) and total nitrogen (c and d, mmol N g^{-1}) concentration for individual species grown at 18 (●), 23 (○) and 28 (■) °C in the LT acclimation experiment. Respiration rates were measured at the growth temperature and represent the average of 4 individual replicates (± SE). Linear regressions [(a) $P = 0.006$, $r^2 = 0.2$; (b) $P = 0.25$, $r^2 = 0.02$; (c) $P = 0.01$, $r^2 = 0.1$; (d) $P = 0.001$, $r^2 = 0.23$] were fitted through all data (i.e. 18, 23 and 28 °C) as slopes and intercepts of regressions for each temperature were not significantly different.

Balance between leaf R and light-saturated photosynthesis

Did prolonged exposure to different growth temperatures alter the balance between leaf R and A_{sat}? To investigate this question, we plotted rates of leaf R against the corresponding rates of A_{sat} for each species/growth temperature combination used in the LT acclimation experiment (all measured at their respective growth temperatures). Leaf R was significantly positively correlated with rates of A_{sat} at all growth temperatures (18 °C, $P < 0.0001$, $r^2 = 0.9$; 23 °C, $P = 0.001$, $r^2 = 0.6$; 28 °C, $P < 0.0001$, $r^2 = 0.9$) (Fig. 7). Growth temperature had no effect on this relationship. This suggests that A_{sat} either acclimated to the same degree as leaf R, or that A_{sat} was relatively temperature insensitive across the 18–28 °C range.

Discussion

Acclimation in leaves and roots: importance of development

Previous studies on photosynthetic and respiratory acclimation have shown that acclimation to a new growth

temperature can occur in pre-existing leaves, formed at the previous growth temperature (Pisek *et al*., 1973; Atkin *et al*., 2000a). However, studies with winter rye and *Arabidopsis* have shown that for *maximal* acclimation of photosynthesis to a new growth temperature, new leaves need to be formed (Hurry *et al*., 1995; Strand *et al*., 1997). Newly developed leaves possess the chemical composition and enzymatic machinery to acclimate photosynthesis more fully than their pre-existing, temperature-shifted counterparts (Hurry *et al*., 1995; Strand *et al*., 1997). Our results suggest that the same may be true for leaf and root *R*, as the degree of acclimation was greater in the LT than ST acclimation experiment (Table 4).

Development also needs to be considered when dealing with the question of whether roots and leaves differ in their ability to acclimate to contrasting temperatures. Using the *Homeostasis Method*, we found no evidence that leaves and roots differ in their ability to acclimate when both organs develop at the prevailing growth temperature (LT acclimation; Fig. 4a, Table 4). Tjoelker *et al*. (1999b) also reported no systematic difference in the acclimation ratio (*Set Temperature Method*) of roots and leaves in tree seedlings. However, roots exhibited a higher degree of acclimation than leaves when plants were *shifted* from 25/20 to 15/10 °C for 7 days (ST acclimation; Figs 4b and 5b; Table 4). As we measured whole root systems (i.e. young and old roots), development of new roots at the new growth temperature would slowly increase the acclimation ratio of the whole root system. In contrast, our measurements of leaf respiration were on individual mature leaves only, with 25 °C-developed leaves being measured both in the 25 °C-maintained and 7-day 15 °C-shifted plants. Presumably, the degree of acclimation would have been greater in the leaves in the ST acclimation experiment if we had measured *R* in leaves that developed at the new low growth temperature.

Lower Q_{10} values in roots than leaves (Table 3) may be another factor that may have contributed to the higher degree of acclimation exhibited by roots in the ST acclimation experiment. Changes in temperature displace *R* from homeostasis to a lesser extent in tissues with a lower Q_{10} than in those with a higher Q_{10}. Thus, metabolic changes associated with acclimation are more likely to result in *R* exhibiting homeostasis.

Q_{10} values of leaves and roots

Why was leaf *R* more temperature sensitive than root *R*? Several studies have shown that the Q_{10} often decreases with increasing measuring temperature over the 15–25 °C range in leaves and roots (Palta & Nobel, 1989; Atkin *et al*., 2000a; Tjoelker *et al*., 2001; Bruhn *et al*., 2002; Covey-Crump *et al*., 2002). In contrast, the Q_{10} is relatively temperature insensitive across the 15–25 °C range in leaves of other species (e.g. Atkin *et al*., 2000a). Therefore, lower Q_{10} values in roots than leaves could reflect differences in the extent to which the Q_{10} decreased over the 15–25 °C range. For example, in roots, the Q_{10} might have been 2.0 at 15 °C and 1.2 at 25 °C, with the result that the average value was 1.6 over the entire range. For leaves, the Q_{10} might have been 2.5 at 15 °C and 1.5 at 25 °C (i.e. average of 2.0) or a constant value of 2.0 constant over the entire range. The higher Q_{10} values in leaves may possibly reflect the higher concentration of sugars in leaves (Fig. 6; Table 3). Previous studies have shown that Q_{10} values are higher in leaves and roots that exhibit higher concentrations of soluble substrates (Berry & Raison, 1981; Atkin *et al*., 2002; Covey-Crump *et al*., 2002) (but see Griffin *et al*., 2002a).

In our study, growth temperature had no effect on the Q_{10} of leaf *R* in the LT acclimation experiment (Table 1) or root *R* in the ST acclimation experiment (Table 2). However, leaf Q_{10} values were higher in warm-grown plants than in their 7-day, 15 °C-treated counterparts in 6 of the 9 selected species (Table 2). This result contrasts with that of other studies where exposure to a low growth temperature increases the Q_{10} (Atkin *et al*., 2000a; Covey-Crump *et al*., 2002) or where growth temperature either has no systematic effect on the Q_{10} (Tjoelker *et al*., 1999b; 2001; Gunderson *et al*., 2000). Therefore, the response of the Q_{10} to growth temperature is highly variable.

Traits to predict variations in Q_{10} and acclimation

One of our main aims was to determine whether we could predict the responses of leaf and root *R* to short- and long-term changes in temperature using traits such as the maximum RGR and concentrations of soluble sugars and nitrogen. Having the ability to predict the responses of leaf and root *R* to changes in temperature is fundamental to projecting the impact of global change on the biosphere (Long, 1991). We found that Q_{10} values and degree of acclimation are highly variable amongst the selected plant species (as did Turnbull *et al*., 2001); however, neither RGR, the concentration of soluble sugars or total nitrogen could account for this variability. Similarly, Larigauderie & Körner (1995) found no systematic difference in the ability of lowland (presumably fast-growing) and alpine (presumably slow-growing) species to acclimate leaf *R* to 10 vs 20 °C. In contrast, conifer leaves exhibit a greater degree of leaf *R* acclimation than broad-leaved deciduous tree species (Tjoelker *et al*., 1999b), with acclimation in the tree species being coupled to changes in foliar nitrogen and carbohydrate concentrations (Tjoelker *et al*., 1999a). This suggests that in some

cases (e.g. across contrasting functional groups) traits could be identified to predict thermal acclimation of plant respiration. Further work is needed to establish whether this is the case.

Several studies have shown that the Q_{10} often varies in a substrate-dependent manner within individual species (Berry & Raison, 1981; Atkin *et al*., 2002; Covey-Crump *et al*., 2002). However, variability in Q_{10} values can occur in the absence of changes in the concentration of soluble carbohydrate concentrations (Atkin *et al*., 2000d) or without expected changes in soluble carbohydrate concentrations (Griffin *et al*., 2002a). Thus, it seems that other factors must be responsible for the variability in temperature sensitivity of leaf and root *R* amongst contrasting species. For example, plant species may differ in the extent to which individual enzymatic steps (which differ in their temperature sensitivity) regulate respiratory flux.

Assessing acclimation

We used three methods to assess respiratory acclimation. For plants developed at the growth temperature, similar conclusions are reached when using the *Homeostasis* and *Set Temperature Methods* (Fig. 3a). However, results from the two methods are less consistent when pre-existing leaves and roots are shifted from one temperature to another (Fig. 3b). Moreover, although roots exhibit higher degrees of acclimation than leaves in most species when compared using the *Homeostasis* and *Set Temperature Methods*, there is no systematic difference between roots and leaves when using the recovery method. These results demonstrate that the conclusions reached in acclimation studies depend strongly on whether leaves and roots developed at the growth temperature and/or the method used to compare warm- and cold-acclimated plants. The method used will depend on the nature of the question being asked [e.g. is respiratory CO_2 release homeostatic across a range of growth temperatures, and to what extent does respiratory flux (and presumably ATP synthesis) recover following exposure to cold?]

Integrating leaf level and whole shoot level studies

We recently reported on the effect of growth temperature (18, 23 and 28 °C) on the RGR and components of RGR [i.e. net assimilation rate (NAR), specific leaf area (SLA) and leaf area ratio (LMR)] (Loveys *et al*., 2002). When grown at 18 °C, NAR became more important than SLA for explaining inter-specific variations in RGR. Variations in whole shoot photosynthesis and carbon concentration could not explain the importance of NAR in determining RGR at the lower temperatures. Rather, variations in the degree to which whole plant *R* per unit leaf area acclimated to the different growth temperatures were responsible. More specifically, it appeared that the importance of NAR in determining variations in RGR at the lower growth temperature was due to a greater degree of thermal acclimation of *R* in shoots, but not the roots, in slow-growing species. In our current study, we have shown that the degree of acclimation does not differ systematically between whole root systems of fast- and slow-growing species in plants grown at 18, 23 and 28 °C (LT acclimation; Fig. 5). However, no systematic differences were found in the degree of acclimation exhibited by mature leaves of the fast- and slow-growing species (Fig. 5). How, then can we explain the results reported in Loveys *et al*. (2002)? One possibility is that Loveys *et al*. (2002) measured *R* rates in whole shoots (i.e. developing and mature leaves) whereas we have focused on the response of mature leaves only in the current study. If the degree of acclimation potential of developing leaves differs from that of mature leaves, then it may be that responses seen at the whole shoot level will differ from that of mature leaves alone. Further work is necessary to determine the extent to which growth temperature affects rates of *R* in developing and mature leaves.

Balance between respiration and photosynthesis

Our finding that the balance between leaf *R* and light-saturated photosynthesis is maintained across species and growth temperatures (Fig. 7) supports the findings of previous experimental and modelling studies that suggest that a balance is maintained between leaf *R* and A_{sat} (Gifford, 1995; Dewar *et al*., 1999). The maintenance of a balance is likely to reflect the fact that leaf *R* and A_{sat} are interdependent, with *R* relying on photosynthesis for substrate whereas photosynthesis depends on *R* for a range of compounds (e.g. ATP; see a recent review by Atkin *et al*., 2000c). What is surprising, however, is the extent to which contrasting species exhibit similar leaf *R* to A_{sat} ratios at all temperatures. Leaves of plant species from contrasting habitats differ substantially in chemical composition, metabolic fluxes and physical structures; we anticipated that such differences would result in inter-specific and inter-growth temperature differences in the amount of leaf *R* needed to support photosynthesis and vice versa. Thus, even though the relationship between leaf *R* and A_{sat} is affected by environmental factors such as water availability (Turnbull *et al*., 2001) and night-time temperature (Turnbull *et al*., 2001), our data shows that temperature-mediated differences in leaf *R* are closely linked to concomitant differences in leaf photosynthesis.

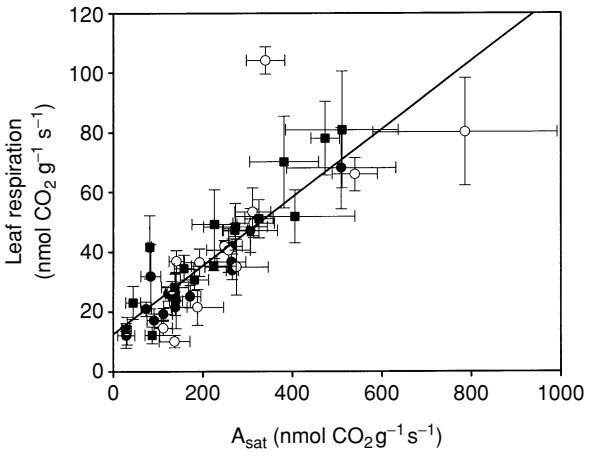

Fig. 7 Rates of leaf respiration (nmol CO_2 $g^{-1}s^{-1}$) plotted against light saturated photosynthesis (nmol CO_2 $g^{-1}s^{-1}$; measured at ambient CO_2) for individual species grown at 18 (●), 23 (○) and 28 (■) °C in the long-term acclimation experiment. Respiration and photosynthesis were measured at the growth temperature and represent the average of 4 individual replicates (± SE). A linear regression (Leaf $R = 12.53 + 0.115$ * A_{sat}; $P < 0.001$, $r^2 = 0.7$) was fitted through all data (i.e. 18, 23 and 28 °C) as slopes and intercepts of regressions for each temperature were not significantly different.

Inter-specific differences in soluble sugars and leaf respiration

Respiration is often stimulated by the addition of substrates such as glucose and sucrose (Atkin & Day, 1990; Noguchi & Terashima, 1997), with variations in *R* often correlating with variations in soluble sugar content. This suggests that substrate supply partly controls the rate of flux through the respiratory pathway (Breeze & Elston, 1978; Azcón-Bieto *et al*., 1983). Importantly, the concentration of soluble sugars is often affected by growth temperature, with cold-grown and cold-treated plants exhibiting higher concentrations of sugars than their warm-grown counterparts (Hurry *et al*., 1998; Atkin *et al*., 2000a). Moreover, Tjoelker *et al*. (1999a) and Griffin *et al*. (2002a) reported that inter-specific and inter-canopy variations in leaf *R*, respectively, are positively correlated with variations in leaf soluble sugar concentration. In contrast, we found a negative relationship between inter-specific variations in leaf *R* and soluble sugar concentration in our selected 16 herbaceous, grass and shrub/tree species (i.e. species with high leaf *R* exhibited low sugar concentrations; Fig. 6). This finding was all the more surprising given that high rates of leaf *R* are associated with high rates of A_{sat} (Fig. 7) and presumably high rates of soluble sugar synthesis. One explanation for these findings is that the pool of soluble sugars is kept low by the high rates of leaf *R*, and that leaf *R* does not become substrate limited due to the high rates of A_{sat}. This suggestion is supported by Turnbull *et al*., 2002), who found that respiratory-driven reductions in leaf carbohydrate concentrations are associated with an increase in photosynthetic capacity in *Populus deltoides.* Alternatively, species with high rates of leaf *R* may also exhibit high rates of sugar export to other parts of the plant. Regardless of the cause, our results suggest that inter-specific differences in respiration are not always positively correlated with variations in soluble sugar concentrations. Moreover, the lack of correlation between acclimation and sugar concentrations suggests that variations in the degree of acclimation are not always associated with the extent to which soluble sugars accumulate at different temperatures (e.g. cold-acclimation may be associated with an increase in substrate availability to the respiratory apparatus).

Implications for global CO_2 exchange models

Our findings that leaves exhibit higher Q_{10} values than roots over the temperature range 15–25 °C, and that the degree of acclimation depends to a large extent on whether a plant has developed at the new growth temperature have important implications for global CO_2 exchange models. Most climate-carbon cycle models assume that plant *R* will increase with increasing temperature in a predictable way that will remain constant over time (Rustad *et al*., 2001). In such models, *R* is assumed *not* to acclimate to future changes in temperature, while contrasting species/tissues/growth conditions are assumed to exhibit similar Q_{10} values. However, failure to take into account acclimation and/or variations in the Q_{10} results in an over-estimate of the effects of global warming on respiratory CO_2 release over long periods (Luo *et al*., 2001), particularly in models that assume a positive feedback of global warming on *R*. To illustrate the impact of acclimation on annual rates of respiratory CO_2 release, Atkin *et al*. (2000b) modelled the effect of acclimation (assuming homeostasis was achieved) and different Q_{10} values using field temperature data recorded over a full year. Annual CO_2 release was up to 47% higher in roots with a Q_{10} of 3.0 compared to a Q_{10} of 1.5, whereas rapid acclimation reduced annual CO_2 release by up to 40%. Therefore, the extent of annual respiratory CO_2 release will be overestimated whenever acclimation is not taken into account. This emphasizes the need for a more complete understanding of the extent to which the ST and LT temperature response of *R* differs within and among plant species.

Conclusions

Our study has highlighted the role development plays in determining the extent to which leaf and root respiration acclimate to different growth temperatures, with greater acclimation occurring when plants develop at a particular growth temperature than when pre-existing leaves and roots are shifted from one temperature to another. As a result, the speed with which a plant achieves *full* acclimation to a change in temperature is likely to be determined by the rate that it generates new leaves or roots. Moreover, our work suggests that, for a given species, roots exhibit lower Q_{10} values than leaves when compared over the same temperature range and that traits such as the RGR and concentrations of soluble sugars/nitrogen cannot account for inter-specific differences in the Q_{10} or the degree of acclimation. We have also shown that the balance between leaf R and A_{sat} is maintained in plants grown at different temperatures, regardless of their inherent RGR.

Acknowledgements

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