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Extreme growth phenotypes of trees are caused by differences in energy metabolism☆

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Abstract

Rapid and slow growth phenotypes of same-age-trees can result from differences in genetic growth potential due to differences in the metabolic properties of the phenotypes or by differences in the match between their metabolic characteristics and environmental factors. In this study, paired rapid and slow growing trees from two species were examined to define physiological properties that determine the growth rate differences. Because plant structural biomass production depends on energy production via aerobic respiratory metabolism, respiration rate and energy use efficiencies of the rapid and slow growing trees were compared. Growth rates were calculated for each tree from the measurements of metabolic heat rates and CO_2 production rates of meristems as functions of temperature. The rates of metabolic heat and CO_2 production by respiration were higher, the energy use efficiency was higher, and the rate of storing chemical energy in structural biomass (calculated growth rate) was higher in the large trees than in the small trees, showing that the respiratory metabolic properties define growth rate differences. Ratios of calculated growth rates of large and small trees varied with temperature. Therefore, classification of trees into rapid growth or slow growth phenotypes is dependent upon growth temperature and the match between metabolic characteristics and environment. These findings suggest that respiratory parameters may be used in identifying trees most suitable for rapid growth in a given environment.

Keywords: Calorimetry; *Eucalyptus camaldulensis*; Metabolic heat; Respiration; *Sequoia sempervirens*; Specific growth; Temperature coefficient

1. Introduction

Plant growth requires metabolism of the carbon products of photosynthesis into structural biomass. A fraction of the photosynthetic carbon is respired in catabolic reactions that produce CO_2, ATP, and NADH. The remaining carbon is used as the substrate for anabolic reactions (biosynthesis) producing plant structures. The rate of biosynthesis is equal to the rate of respiration multiplied by a function of the substrate carbon conversion efficiency (ε), i.e. the fraction of substrate C incorporated into structural biomass (Eq. (1)) [1,2].

$$R_{SG} = R_{CO_2}\frac{\varepsilon}{1-\varepsilon} \quad (1)$$

where R_{SG} is the specific growth rate and R_{CO_2} is the rate of respiratory CO_2 production.

Calorespirometric measurements of metabolic heat loss (q) and CO_2 production rates from growing

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tissues can be used to determine ε (Eq. (2)).

$$\frac{q}{R_{CO_2}} = -\frac{1-\gamma_P}{4}\Delta H_{O_2} - \frac{\varepsilon}{1-\varepsilon}\Delta H_B \approx 455 - \frac{\varepsilon}{1-\varepsilon}\Delta H_B \quad (2)$$

where ΔH_{O_2} is the enthalpy change for combustion of substrate per mols of oxygen and γ_P is the oxidation state of the substrate C (equal to zero for carbohydrates); thus, the term $(1-\gamma_P/4)$ has units of moles O_2 mol^{-1} CO_2. ΔH_{O_2} is the calorespirometric constant with a value near -455 kJ mol^{-1}. ΔH_B is the enthalpy change per mol of substrate carbon incorporated into biomass during biosynthesis, with units of kJ mol^{-1} C. ΔH_B has a small, positive value dependent only on biomass composition and thus can be considered constant for each of the species studied. The ratio q/R_{CO_2} is inversely related to the substrate carbon conversion efficiency (ε) [1,2]. High values of q/R_{CO_2} (or low ε) indicate inefficient growth.

The R_{SG} is the rate of incorporation of carbon into new growth per mass of tissue (mol C s^{-1} mg^{-1} dry wt.). $R_{SG}\Delta H_B$ is therefore the growth rate as the rate of accumulation of chemical energy into structural biomass (kJ s^{-1} mg^{-1} DW) with carbohydrate as the reference state [1,2]. By combining Eqs. (1) and (2), $R_{SG}\Delta H_B$ may also be written as Eq. (3).

$$R_{SG}\Delta H_B = 455R_{CO_2} - q \quad (3)$$

In Eq. (3), $455R_{CO_2}$ equals the rate of energy produced by respiration, q is the rate of energy lost as heat, and the difference $R_{SG}\Delta H_B$ is therefore the rate of accumulation of energy into structural biomass (growth rate). Thus, with this model, phenotypic differences in growth rates of trees can be quantified in terms of measurable differences in metabolic rates and efficiencies.

The temperature range for growth and survival of different plant species, cultivars, and accessions has been successfully predicted with the above plant growth model, measurements of q and R_{CO_2}, and preparation of plots of $R_{SG}\Delta H_B$ and q/R_{CO_2} versus temperature [3–7]. The respiration parameters have been shown to be heritable and correlated with growth and biomass production rates as a function of temperature [5,8,9].

In this study, it is hypothesized that differences in growth rates arise either from different combinations of respiration rate and efficiency and/or from differences in genotype by environment interactions. Combinations of high rates and high efficiencies and a near optimal match between the temperature dependence of growth rate and the temperature pattern of the environment are predicted for the rapid growing trees. Low respiration rates and efficiencies and/or a growth–environment mismatch are predicted for the slow growing phenotypes. These predictions are tested in this study by direct measurement of q and R_{CO_2} in extreme phenotypes of rapid and slow growing trees.

In previous studies, we focused on metabolic rate and growth rate distribution within broad taxonomic groups: related genera, sub-genera, species, and cultivars within species. In this study, extreme growth phenotypes are compared with further establish the growth rate dependence on rates and efficiencies of respiratory metabolism and to define the near-maximum range of rates and efficiencies existing within these species. Large and small trees representing the extreme growth rate phenotypes of *Eucalyptus camaldulensis* in a plantation are compared. In addition, rapid growing 'wild-type' redwoods are compared with slow growing 'dwarf' redwoods to demonstrate that genetic changes altering respiration properties change growth rates.

The two tree species included in this study are taxonomically very different. River redgum is an angiosperm native to Australia, whereas coast redwood is a gymnosperm native to the California coast of North America. Thus, this study examines relationships among respiration rate, efficiency, and tree size for two widely divergent species and for plants that developed size differences by very different pathways. Success in predicting growth rate differences in these disparate examples supports use of the growth rate model employed in developing predictive, respiration-based physiological models for growth.

2. Materials and methods

2.1. Plant propagation and selection

Open-pollinated seedlots of river redgum (*E. camaldulensis* Denn.) were obtained primarily from the CSIRO Tree Seed Center in Canberra, Australia. During the spring of 1989, these seedlots were planted

in replicated, open-pollinated family plots with a spacing of 2.1 m × 3 m in a commercial planting site of Tehama Fiber Farm at Corning, CA. Standard irrigation and fertilizer schedules and plant protection measures were applied.

In July of 1992, nine phenotypically superior, large trees were chosen from among these for measurement. A phenotypically inferior, small tree adjacent to each large tree was also selected for respiration measurements. The pairing of large and small trees from the same plot minimized environmental differences and maximized the probability of genetic differences that determine growth properties. At the time of selection, total tree height and diameter (d) at 1.37 m above ground were measured. Stem volume index was calculated as height × d^2.

The coast redwood (*Sequoia sempervirens* (D. Don) Endl.) trees used in this study were grown from rooted cuttings collected in 1966 from the boles and crowns of six just-felled, 300–500-year-old trees. All six came from the same 200 m diameter plot near Korbel in Humboldt County, CA (personal communication, W.J. Libby). By 1968, six trees from the original cuttings had been shown to be consistently and substantially smaller than other trees in the experiment. This 'dwarf' genotype was referred to as 'The Ronald Regan Redwood' and later named KKT. The dwarf grows more slowly than the other redwoods, which will be referred to here as 'wild', and has a denser, more symmetric crown resembling giant sequoia (*Sequoiadendron giganteum*). Its foliage is bluish-green in winter and turns bright green in spring (personal communication, W.J. Libby). It survives well under normal growth conditions.

In 1975, six wild rooted cuttings (S1–S6) and six dwarfs were planted in two six-tree rows at the Russell Reservation of the University of California, Berkeley. Wild types S1–S5 all originated as cuttings from lower boles, near ground level, and were thus moderately juvenile (probably early adolescent). The S5 soon died, thus it was replaced later that year with a seedling of Humboldt County origin. Although S3 resembles the other wild plants, it originated from the tree that also produced the dwarf KKT. Thus, S3 should be genetically identical to KKT except for the changes. The S6 ramet originated as an upper-crown cutting and thus may be initially much more mature than other wild types. In spite of these substantial initial maturation differences among the six wild-type trees, phenotypic appearance and growth rates were generally similar after 25 years of growth in the field though they differed somewhat in crown structure, growth rate, and stem volume. Of the six original dwarfs, three had been cut when they were about 2–3 m tall, to demonstrate their value of using them as Christmas trees. However, only two of the cut trees re-sprouted well and grew to a height of about half the size of the uncut dwarfs. Total tree height and diameter at 1.37 m above the ground level were measured, in January 2001, for both wild and dwarf trees.

2.2. Sample collection

Four to six samples of meristematic tissue were collected from each large and small river redgum tree on 21 June 1993 and from three wild and three adjacent uncut dwarf (KKT) redwood plants on 6 June 1995. All samples were taken between 7:00 and 9:00 a.m. The collected tissue came from shoot-apices and subapical portions on two actively growing primary upper branches of the river redgum trees, and from easily reachable lateral branches located around the mid-crown of the redwoods. Samples were immediately placed in small vials with cold, half-strength Hoagland's solution containing 1% sucrose covering the cut sections of the branch. The vials were placed on ice during transport and stored in an aerated refrigerator at 5°C until calorespirometric measurements were completed (2–3 days). Respiratory values of individual samples remained nearly constant during the 2–3-day measurement period. This sampling and storage technique minimized injury-related respiration.

2.3. Calorespirometric measurements

Calorespirometric measurements were made with a Hart Scientific model 7707, heat-conduction, multicell, differential-scanning calorimeter operated in the isothermal mode [10]. A 1 cm long tissue section, including the apical meristem with subtending stem and needles or leaves, was placed in a 1 cm^3 calorimeter ampule and heat rate and CO_2 production rates measured by the methods used in Criddle et al. [11]. Measurements of q and R_{CO_2} were made at 25°C for

Table 1
Mean respiration parameters measured at 25°C for large and small phenotypes and their stem volume measurements of river redgum trees

Type	Respiration parameters				Stem volume index (m^3)
	q ($\mu W\ mg^{-1}$)	$455R_{CO_2}$ ($\mu W\ mg^{-1}$)	q/R_{CO_2} ($kJ\ mol^{-1}$)	$R_{SG}\Delta H_B$ ($\mu W\ mg^{-1}$)	
Large	17.1	19.6	400	14.8	0.231
Small	16.6	14.7	512	8.9	0.004
Significance[a]	NS[b]	*	**	**	**

[a] Significance of difference between means of large and small trees.
[b] NS: not significant at $P < 0.05$.
* $P < 0.05$.
** $P < 0.01$.

eucalypts and at both 15 and 25°C for redwoods. The ratio of heat rate to CO_2 production rate (q/R_{CO_2}) and $R_{SG}\Delta H_B$ were calculated using the methods of Hansen et al. [1]. For redwoods, the Arrhenius temperature coefficients of q, $455R_{CO_2}$, q/R_{CO_2}, and $R_{SG}\Delta H_B$ were calculated for the temperature range from 5 to 30°C [4,5,12]. For further details on measurement methods and calculations, see Criddle and Hansen [10].

2.4. Analysis

Differences between respiration parameters of large and small river redgum trees and between wild and dwarf redwoods were tested using Cochran's approximate t-statistic for unequal variances (TTEST procedure [13]). To visualize crossover points between wild and dwarf redwoods, Arrhenius plots of ln q and ln $455R_{CO_2}$ versus reciprocal temperature and plots of q/R_{CO_2} and $R_{SG}\Delta H_B$ versus temperature were prepared for the temperature range from 15 to 30°C.

3. Results

Table 1 shows that large river redgum trees with an average stem volume index of 0.231 m^3 produced respiratory energy ($455R_{CO_2}$) at a rate 33% higher ($P < 0.05$) than the small trees with stem volume index 0.004 m^3, and lost 28% less ($P < 0.0001$) metabolic heat per mol of CO_2 respired (q/R_{CO_2}). The specific growth rate in terms of the rate of accumulation of energy into structural biomass ($R_{SG}\Delta H_B$) was 66% higher ($P < 0.001$) at 25°C in large trees than in small trees, i.e. trees with high respiratory rate and high efficiency were larger than trees with lower rates and low efficiencies.

Table 2 shows that at 25°C, q, $455R_{CO_2}$, and $R_{SG}\Delta H_B$ were significantly ($P < 0.05$) higher and q/R_{CO_2} was lower for wild trees than for dwarfs. At 15°C, differences in respiration parameters were smaller with only R_{CO_2} being significantly different. On average, stem volume of the wild trees was 2.24 times larger than the dwarf trees.

Table 2
Metabolic heat rates and CO_2 production rates measured at 15 and 25°C for wild and dwarf redwoods[a]

T (°C)	Type	q ($\mu W\ mg^{-1}$)	$455R_{CO_2}$ ($\mu W\ mg^{-1}$)	q/R_{CO_2} ($kJ\ mol^{-1}$)	$R_{SG}\Delta H_B$ ($\mu W\ mg^{-1}$)
15	Wild	5.8	7.5	356	0.77
	Dwarf	5.0	6.4	360	0.64
	Significance[a]	NS	*	NS	NS
25	Wild	17.3	17.9	442	0.27
	Dwarf	15.3	14.3	495	−0.46
	Significance[b]	*	**	*	**

[a] On average, stem volume indexes of three wild and three dwarf redwoods were 6.79 and 3.03 m^3, respectively.
[b] Significance of difference between means of wild and dwarf trees; NS: not significant at $P < 0.05$.
* $P < 0.05$.
** $P < 0.01$.

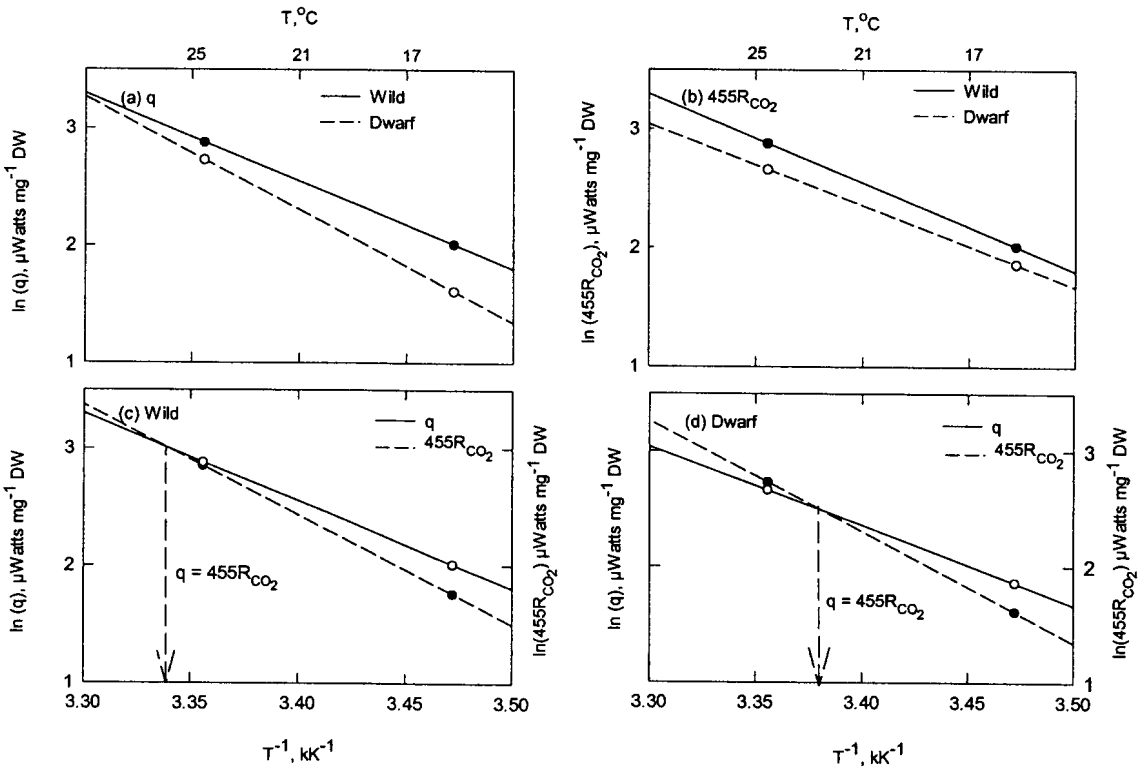

Fig. 1. Arrhenius plots for mean values of q and $455R_{CO_2}$ for wild and dwarf redwoods measured at 15 and 25°C. Symbols show the measured values and lines show the extrapolated portions of the curves. Extrapolated portions of the curves are valid to the extent that q and R_{CO_2} are described by Arrhenius functions.

Values of q and R_{CO_2} are higher for wild than for dwarf at both 15 and 25°C (Fig. 1a and b). Temperature dependencies of q and R_{CO_2} differ between wild and dwarf. In Fig. 1c and d, values of q and $455R_{CO_2}$ are plotted together for wild and dwarf redwoods to illustrate the crossover temperature, where $q = 455R_{CO_2}$. At the temperature where these curves cross $(455R_{CO_2} - q)$ becomes 0. This defines the upper temperature limit for growth. The crossover for the wild type occurred at 26°C and that for dwarfs at 23°C.

The dwarf was less efficient (q/R_{CO_2} values were greater) than the wild type (Fig. 2) at temperatures above 15°C, but more efficient below this temperature. $R_{SG}\Delta H_B$ calculated as a function of temperature, by use of the data in Tables 2 and 3, shows that growth rate of wild and dwarf trees become more similar at low temperature (Fig. 3). The dwarf had a smaller specific growth rate ($R_{SG}\Delta H_B$) at both 15 and 25°C, but the differences are much smaller at 15°C. The growth rates of the dwarf may exceed that of the wild type below 6°C. Fig. 3 also shows that the dwarf has a smaller temperature range with positive values for $R_{SG}\Delta H_B$ than the wild type.

4. Discussion

Although the eucalyptus data in this study, with measurements made at only one temperature, cannot separate genotype and environmental contributions to growth rate differences, systematic respiratory differences between the phenotypic classes are clearly shown at 25°C. Because, this temperature is near the mean temperature for the growing season at Corning, the respiration rate measurements provide a reasonable estimate of growth rate differences between the rapid and slow growth phenotypes in the field. The rapid growth phenotypes are larger, because the large

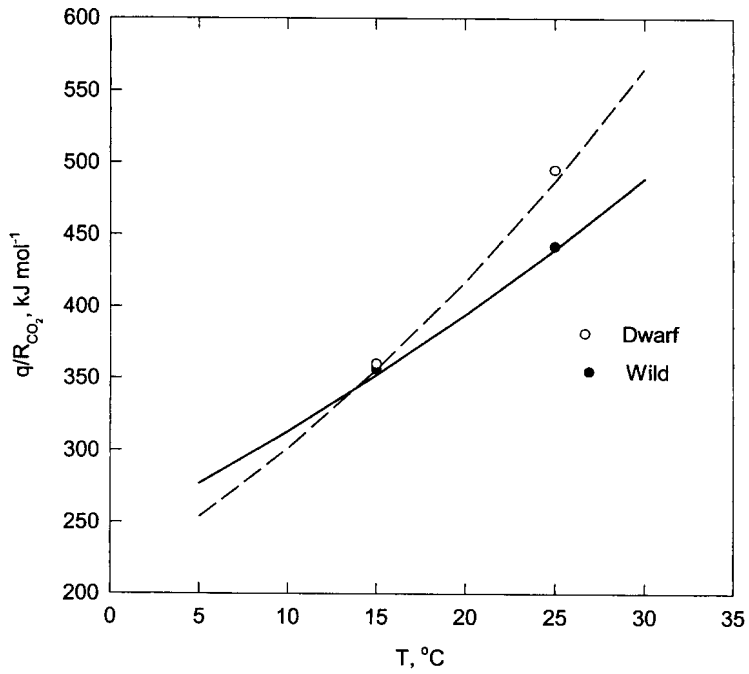

Fig. 2. Mean values of q/R_{CO_2} for wild and dwarf redwoods calculated from q and R_{CO_2} measured at 15 and 25°C. Symbols show the measured values and lines show the extrapolated portions of the curves calculated by Arrhenius functions.

trees had higher metabolic rate and greater carbon-conversion efficiencies. However, the influence of growth temperature on growth of three other genotypes can be seen unequivocally in data from a previous study, in which three selected eucalyptus genotypes were grown under three different temperatures (Table 4) [14]. The heat and CO_2 production rates either increased or remained constant as growth temperatures increased from 15 to 30°C for 4016 and C11, while these rates rapidly declined for GD1. The ratio q/R_{CO_2} steadily increased from 15 to 30°C for GD1, but this ratio slightly decreased for C11 and 4016. Respiration rates and efficiencies essentially determined the biomass production rates of these genotypes in response to changing growth temperature, so that the biomass production rate at 30°C was highest for 4016, intermediate for C11, and lowest for GD1.

The average genetic difference between the wild and dwarf redwoods was expected to be smaller than that between the large and small river redgums, because all the redwood trees originated from the same natural stand. The environmental differences between microsites of the wild and dwarf redwoods in this study must also be minimal, because all experimental trees were grown in near proximity in a nearly

Table 3
Temperature dependence of coast redwood metabolism

Type	ln A_q[a] (μW mg^{-1})	μ_q[b] (kK)	ln A_{CO_2}[a] (μW mg^{-1})	μ_{CO_2}[b] (kK)
Wild	34.3	9.38	27.9	7.47
Dwarf	34.9	9.60	25.8	6.90

[a] ln A_q and ln A_{CO_2} are the natural logarithms of the pre-exponential terms in the respective Arrhenius equations.
[b] Thse μ_q and μ_{CO_2} are the temperature coefficients of metabolic heat rate and CO_2 rate, respectively.

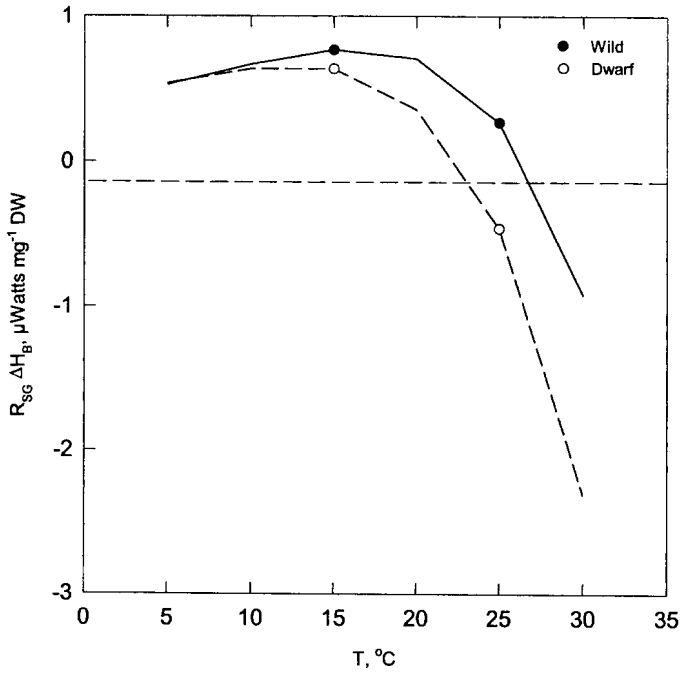

Fig. 3. Mean values of $R_{SG}\Delta H_B$ for wild and dwarf redwoods calculated from q and R_{CO_2} measured at 15 and 25°C. Symbols show the measured values and lines show the extrapolated portions of the curves. Negative values $R_{SG}\Delta H_B$ indicate temperatures at which tissue damage is predicted to occur and growth to stop. Extrapolated portions of the curves are valid to the extent that $R_{SG}\Delta H_B$ is described by Arrhenius functions.

homogeneous field. Therefore, growth and metabolic differences found between wild and dwarf redwoods are likely due to a large change in chromosome(s) or changes at many genes. Differences were found in the temperature coefficients of both q and R_{CO_2} and the high temperature limits for positive rates of accumulation of energy into structural biomass (Fig. 1). Because, temperature coefficients of energy production and energy loss differ, dwarf and wild types change their relative growth rates with temperature

Table 4
Respiration rates (q and R_{CO_2}), efficiencies (q/R_{CO_2}) measured at 25°C and biomass production rates for three eucalypt clones (C11, 4016, and GD1) when grown under three temperatures in controlled growth chamber environments[a]

Growth T (°C)	Clone	q (µW mg^{-1} DW)	R_{CO_2} (pmol s^{-1} mg^{-1} DW)	q/R_{CO_2} (kJ mol^{-1})	Biomass (mg per day)
15	C11	29	58.3	511	78
15	4016	24.1	50	497	77
15	GD1	19.3	44.4	487	42
20	C11	24.2	58.3	467	85
20	4016	24.3	55.6	450	137
20	GD1	19.7	38.9	552	71
30	C11	26	55.6	480	119
30	4016	30.9	63.9	492	134
30	GD1	11.6	19.4	653	8

[a] For details, see Criddle et al. [14].

[3–7]; thus, the difference must have evolutionary significance. The wild type is better adapted to temperatures above 15°C than the dwarf genotype with higher respiratory rate and efficiency (lower q/R_{CO_2}) (Fig. 2). The accumulation of chemical energy in structural biomass as predicted from the plant growth model (Eq. (1)) was consistently lower for dwarf than for wild trees (Fig. 3), but the difference narrowed at low temperatures. Perhaps the large chromosomal change in the dwarf tree is similar to the change noted in the Down's syndrome in humans, in which many traits, including growth rates and maturation state, are affected by having an extra copy of an entire (otherwise normal) chromosome.

This study shows a strong association between respiration and tree growth rates in widely varying taxonomic groups. Respiration parameters, therefore, can help in selecting trees for superior biomass production.

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