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Dark metabolic heat rates and integrated growth rates of coast redwood clones are correlated

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Abstract

The metabolic heat rate per gram of apical meristem tissue of coast redwood (*Sequoia sempervirens*) is measured to examine the variation of dark respiration rate among clones. Metabolic heat rates are found to be linearly related to integrated growth rates of both 60-day-old unrooted clones and 25-year-old trees. The correlation suggests that metabolic heat rate measurements may provide a means for rapid selection of coast redwood trees with high genetic potential for rapid growth.

INTRODUCTION

The rate of growth of an individual plant is a function of both the environment and the genotype of the plant [1]. Laboratory tests using near optimal, controlled environmental conditions can, in principle, be used to minimize environmental variations while measurements are made of the genetic potential for growth. However, in spite of many efforts, as yet no general, short-term laboratory test of plant growth potential has been developed. Predictive tests for genetic potential for high growth rates of plants require one to know which metabolic process limits the growth rate of an individual plant under optimum conditions where nutrients, light, water and environment are not limiting. We propose that the dark metabolic rate is directly proportional to growth rate under optimum conditions if the efficiency with which the photosynthate is converted into biomass is constant [2, 3]. If the efficiency is not constant, this simple relationship will not hold, and measurements of both efficiency and metabolic rate will be required to predict growth rate.

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Metabolic heat rate measuremens are easily performed, moderately rapid, and provide a general measure of the total utilization of available photosynthate for plant growth [4]. Consequently, the genetic variability of dark metabolic heat rates under constant, near optimum conditions may be an indicator of genetic potential for plant growth.

Coast redwood (*Sequoia sempervirens*) trees growing in common environments exhibit large differences in growth rates. Figure 1 illustrates such differences in growth with a photograph of a small grove of 25-year-old coast redwoods inhabiting an essentially common environment in the Davis University of California (UCD) arboretum. Size differences such as these are common in naturally established, even-aged stands. The UCD grove of trees and small samples of cloned redwoods provide an opportunity to develop and evaluate laboratory methods for correlating tree growth with measurements of metabolic rates. The establishment of such a correlation would provide a possible means for selection of fast growing coast redwood for reforestation programs. In a previous publication, we demonstrate a positive correlation between metabolic heat rates of apical meristem cuttings from larch (*Larix laricina*) clones and growth rate potential in some populations of this species [2].

This paper evaluates the relation between mtabolic heat rates and

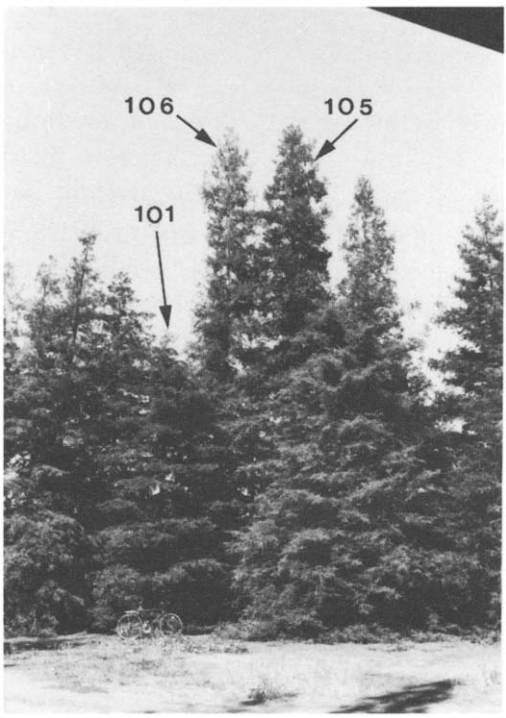

Fig. 1. Photograph of a portion of the UCD grove looking east from the faculty club. The largest and smallest "normal" trees included in the study are shown by arrows.

growth rates of coast redwood. The results show that (a) coast redwood clones have a wide variation in metabolic rate, (b) a correlation exists between measured metabolic heat rate and integrated rate of growth of unrooted clones in vitro, and (c) a correlation exists between measured metabolic heat rate and integrated growth rate for individual trees in a 25-year-old grove of coast redwoods with a known history. The methods presented in this paper may possibly be used to assist in the selection of coast redwood clones with potentially fast growth rates.

MATERIALS AND METHODS

Instrumentation

Calorimetric measurements were made using a Hart Scientific Model 7707 heat conduction differential scanning calorimeter operated in the isothermal or scanning mode [4, 5]. Heat rate measurements were made on approximately 0.8 cm samples of growing tips. Measurements were made immediately following sampling except as explained below.

Growth and preparation of redwood samples

Two-year-old potted trees

These trees were prepared as part of an entirely different previous study. The top 25 cm was removed from 14 coast redwood trees (approximate ages 50–90 years) growing in natural stands in Mendocino and Humboldt counties in northern California. The trees selected showed outstanding height and diameter growth relative to other second-growth trees in the vicinity, but the ages are unknown and the trees cannot be relocated. The 25 cm cuttings were sterilized, sliced into 1 mm thick disks and grown in vitro, following the micropropagation techniques of Boulay [6] and Poissonnier et al. [7]. The slices were initially placed on a multiplication medium containing cytokinin to produce a proliferation of shoots. The proliferated shoots were divided and transferred to flasks containing elongation media (multiplication medium minus the cytokinins), eventually producing hundreds of single stemmed fast growing shoots. A random sample of shoots from each of the fourteen original trees was removed from sterile culture and rooted following the procedures of Poissonnier et al. [7]. These rooted trees were grown in gallon pots in a greenhouse under natural light with a 24 h maximum temperature of 30°C and watered daily with one-half strength Hoagland's nutrient solution from October until April, when calorimetric measurements on actively growing tips were made.

Unrooted clones

Six of the original fourteen trees were selected for further study. Ten randomly chosen shoots of each of these six trees (60 shoots total) were cut to a standard length of 1.5 cm and transferred under sterile conditions to elongation media [6]. These explants were grown in flasks with artificial lighting (16 h photoperiod) at 22°C. In addition to the six selected trees, in vitro explants of two other trees were included in this test: (a) shoots of an albino coast redwood (white with no pigmentation) introduced into culture from stump sprouts discovered in the forest in nothern California, and (b) shoots taken from a rooted cutting (TT) that was made several years ago from the tallest tree in the world, a tree 112 m tall growing in Redwood National Park. After 60 days in the elongation media, the entire plant was removed, and sections approximately 1 cm in length were cut from the growing tip for calorimetric measurements. Subsequently, the remainder of the plant was combined with the section used for the calorimetric measurement, dried, and weighed to determine the total increase in dry mass over the two-month period.

Mature trees

The twenty-five-year-old trees used in this study are growing in a small grove near the UCD faculty club. These trees were planted as two-year-old containerized seedlings in the winter of 1965. The trees are in a level area and are irrigated regularly. Trees to be used in this study were selected from the small L-shaped section of the grove directly east of the faculty club. The trees are planted in staggered rows, four rows wide in each leg of the L. The stem of the L runs north–south and is 40 m long by 15 m wide (on the outside of the L). The base of the L is also 40 m long by 15 m wide. The corner of the L is in the northeast. The requirements for selection were (a) the trees had to be healthy, well formed with clear apical dominance, and with a normal amount of foliage, (b) the location of the trees had to exclude heavy shade, and (c) the selected population had to include as wide a range of tree size as possible. After the initial selection of 12 trees, five more trees were selected to investigate the influence of each of the selection criteria on the results. Each of these latter five trees violated one of the selection rules. The trees were then given a code number on a map of the grove. Trees 1, 2, 9, 104 and 105 were the exceptions. Tree 1 is straggly and 105 is heavily foliated. Tree 2 has a split top. Trees 9 and 104 are shaded by larger trees. Figure 1 is a photograph of part of the north–south portion of the grove taken looking east from inside the L. The trees marked 101 and 106 in the photograph are respectively the smallest and the largest of the normal trees selected.

Trunk circumferences were measured 1.4 m above ground level to the nearest 1 cm with a steel tape. Heights were measured to the nearest dm with an inclinometer and tape. All tree size measurements were made in May 1988.

Samples from trees in the 25-year-old UCD redwood grove were used to determine the best physiological stage to obtain samples from mature trees and how best to treat the samples after collection. Samples of the first new growth that appeared in late January of the 1988 growing season (basal sprouts from the root crown) were collected and metabolic heat rates were measured. The heat rates measured were not stable and no correlation was seen with tree size. In late February, vegetative buds were collected and metabolic heat rates were again measured. The metabolic heat rates of these samples were neither reproducible for a given tree nor correlated with tree size. In mid-April, new vegetative growth was sampled and meristematic metabolic heat rates were determined. There was some correlation of metabolic rates with tree size, but metabolic rates were unstable during the measurements, i.e., drifting downward at such a rate that we were unable to obtain acceptable reproducibility. The study was therefore expanded to find a way of stabilizing the tissue.

Experiments done with and without water added to the calorimeter ampule demonstrated that desiccation of the tissue was not a problem over the time of the experiments.

Depletion of a necessary metabolite from the tissue samples following removal from the tree could also cause decreased metabolic activity with time. Accordingly, it was found that stable metabolic heat rates could be obtained by placing the cut end of the cuttings in one-quarter strength Hoagland's solution containing 1% of glucose immediately following removal from the tree and then storing in a glass-front refrigerator at 5°C for at least 2 h before measurement of the metabolic heat rate. Figure 2

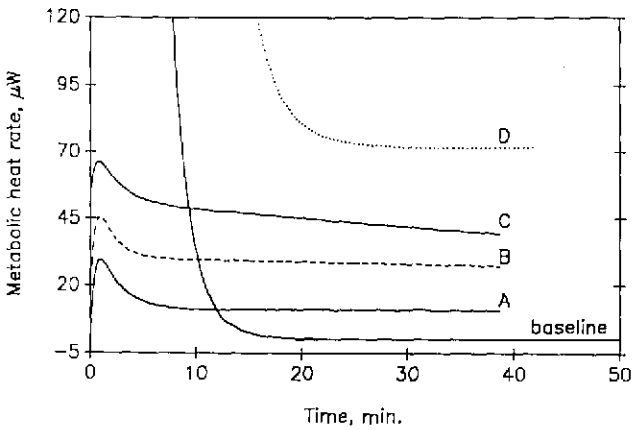

Fig. 2. Calorimetric data taken at 24°C on a sample of new growth stored in nutrient media (one-quarter strength Hoagland's + 1% of glucose) at 5°C for three days (D), and on samples of various ages, freshly cut from the tree; two-year-old tissue (A), one-year-old tissue (B), and new growth (C). The samples had approximately the same fresh weight. The initial rapid changes in the signals at time <20 min are caused by thermal equilibration of the ampules and samples to the calorimeter temperature. A baseline experiment with an empty ampule is shown for reference.

shows a comparison of data collected on a cutting of new growth stored for three days in the above fashion and cuttings of tissue of various ages freshly removed from the tree. Cold-stored cuttings gave the same metabolic heat rates (measured daily) for at least one week. Samples stored on nutrient media at room temperature exhibited gradually decreasing metabolic heat rates from day to day.

The age of the tissue also had an effect. Metabolic heat rate declined with age although the stability of the heat rate improved (see Fig. 2). New growth is preferable for measurement of metabolic heat rate, however, because it is easier to select samples of tissue which are undamaged. (More recent studies have shown that the nutrient/cold treatment is unnecessary for cuttings collected later in the season, i.e. late May to late July [8].)

The response of the metabolic rate to temperature was measured to evaluate possible thermal damage. Two samples were examined by scanning calorimetry to determine metabolic rate as a function of temperature [5]. Three additional samples were examined isothermally at temperatures over the normal range of growth [4]. There was no evidence of unexpected or abrupt changes in the metabolic rate between 5 and 30°C. A heat shock response was observed at temperatures above ≈30°C.

The absolute value of the metabolic heat rate of any given tree in the UCD grove was found to vary from week to week, probably depending on rainfall and irrigation, temperature, light conditions, and stage of development of new growth on the tree. The relative order of the metabolic heat rates remained the same, however, as long as the samples were collected at the same time and treated identically. We therefore selected one tree in the population being studied as a reference tree, and sampled it every time any of the trees in the population were sampled. The measured metabolic heat rates were then expressed as a ratio to the metabolic heat rate measured for the reference tree. In this way, data taken on samples collected at different times could be compared.

RESULTS

Figure 3 shows metabolic heat rates of clones propagated from cuttings of 14 forest grown trees. Heat rates per mg of tissue within this population vary by a factor of ≈3. Reproducibility of measurements on a given tree was generally about $\pm 0.1\ \mu W\ mg^{-1}$. Long-term quantitative growth data are not available for these forest trees, but the data demonstrate a remarkable range of metabolic rates among this wild population.

Figure 4 shows a plot of the mean metabolic heat rate (at 24°C) against the mean growth rate (as total biomass produced in 60 days at 22°C) of the eight unrooted redwood clones. The correlation coefficient is 0.84 for the plot shown and 0.68 if the albino data are omitted. The variation

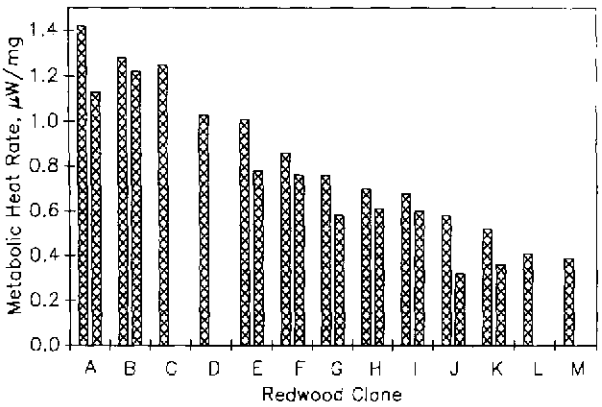

Fig. 3. Metabolic heat rate ($\mu W\,mg^{-1}$) at 24°C for two-year-old rooted clones derived from 14 different forest grown coast redwoods. Data are arranged by placing the trees in descending order of metabolic rates. Replicate values are presented, where tested, as an indication of the reproducibility of the measurement.

among individuals within a given clone is often larger than expected when compared with the variation in metabolic heat rate measurements for the redwood samples used to obtain the data in Fig. 3 or on larch [2]. The variation in biomass accumulation for these clones is also greater than expected in comparisons with clones of other species.

Table 1 gives metabolic heat rates and growth rates measured for the individuals within the clones. The data in Table 1 appear to be normally distributed about the mean for each clone. Note, however, that the data

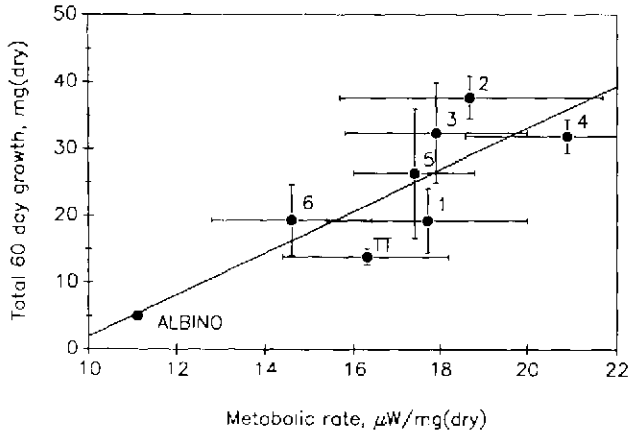

Fig. 4. Correlation between metabolic heat rate at 24°C and tissue mass at the end of two months growth for unrooted coast redwood clones growing in elongation media. The numbers refer to clones as indicated in Table 1; error bars indicate standard deviation of measurements on all explants. The equation for the least squares best fit line is "dry weight, mg" $= -(29.2 \pm 13.9) + (3.11 \pm 0.81) \times$ (metabolic rate, $\mu W\,mg^{-1}$) with a correlation coefficient of 0.84.

TABLE 1

Metabolic heat rate and total biomass data on tip sections of unrooted clones of coast redwood growing on elongation media

Clone [a]	Dry Wt. (mg)	Metabolic heat rate ($\mu W\ mg^{-1}$)	Clone	Dry wt. (mg)	Metabolic heat rate ($\mu W\ mg^{-1}$)
RW1	29.0	18.8	RW5	43.7	19.4
	16.2	16.3		22.1	16.2
	21.0	22.0		20.1	16.7
	17.3	18.0		43.1	17.2
	14.8	15.5		33.1	19.3
	15.9	15.3		21.5	16.6
Mean	19.2	17.7		14.1	14.2
SE [b]	±4.8	±2.3		19.5	19.0
				24.7	17.6
RW2	36.0	13.9		20.9	17.7
	39.9	23.0	Mean	26.3	17.4
	34.8	19.5	SE	±9.7	±1.4
	36.4	16.3			
	43.5	18.6	RW6	27.5	12.2
	34.8	20.8		14.2	15.9
Mean	37.6	18.7		17.6	15.4
SE	±3.2	±3.0		23.4	16.7
				14.0	12.9
RW3	26.7	18.8	Mean	19.3	14.6
	33.8	18.8	SE	±5.3	±1.8
	20.7	18.8			
	45.2	13.2	World's tallest tree	16.1	14.7
	32.8	19.3		12.2	18.8
	34.4	18.3		12.9	17.1
Mean	32.3	17.9		13.7	16.3
SE	±7.5	±2.1		14.4	13.1
				13.7	17.5
RW4	33.5	24.4	Mean	13.8	16.3
	27.9	22.5	SE	±1.2	±1.9
	34.7	19.5			
	29.8	20.1	Albino	5.0	11.1
	33.0	17.8			
Mean	31.8	20.9			
SE	±2.5	±2.3			

[a] Clonal designations refer to numbers assigned by RAW as part of a collection of clonally propagated forest trees.

[b] SE = standard error.

TABLE 2

Metabolic heat rates for replicate samples of coast redwood clones RW5 and an albino growing in multiplication media

Clone	Metabolic heat rate (μW mg^{-1})	Clone	Metabolic heat rate (μW mg^{-1})
RW5	5.02	Albino	6.04
	4.51		8.11
	7.23		7.44
	4.86		2.70
	7.46		11.59
	7.80	Mean	7.18
	8.21	SE	±2.89
	5.93		
	4.47		
Mean	6.35		
SE	±1.47		

TABLE 3

Results of measurements on meristem tissue from 25-year-old coast redwood trees growing in a grove on the UCD campus

Tree	Metabolic heat rate at 18°C relative to tree no. 3 Mean ± standard error	No. of determinations	Height (m)	Diameter at breast height (cm)
1[a]	1.08 ± 0.04	2	8.2	12
2[b]	0.65 ± 0.04	4	14.9	34
3	1.00 ± 0.02	9	17.1	34
4	0.96 ± 0.03	3	12.2	32
5	0.73 ± 0.02	2	11.3	20
6	0.85 ± 0.01	2	17.1	34
7	0.97 ± 0.02	3	18.0	33
8	1.43 ± 0.04	4	17.4	45
9[c]	1.06 ± 0.01	2	10.4	23
10	0.93 ± 0.02	2	11.9	31
11	0.98 ± 0.04	2	14.6	34
101	0.80 ± 0.05	3	8.8	30
102	1.31 ± 0.03	2	16.4	45
103	1.11 ± 0.01	2	16.8	40
104[c]	1.04 ± 0.03	2	8.5	23
105[d]	0.97 ± 0.01	2	21.6	53
106	1.19 ± 0.04	3	16.8	46

[a] Tree is in very poor condition.
[b] Tree has a split top.
[c] Tree is heavily shaded by surrounding, larger trees on the south, east and west.
[d] Tree has very heavy foliage compared with others.

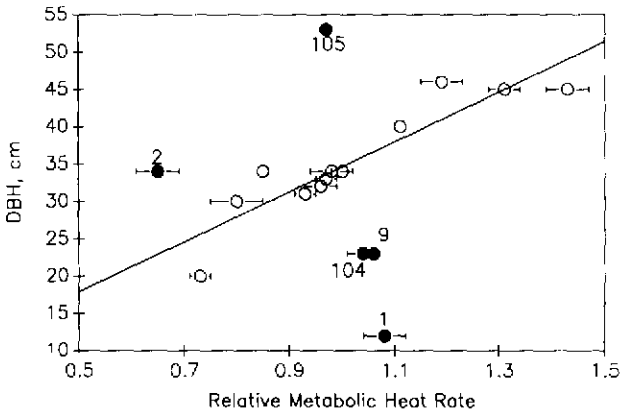

Fig. 5. Correlation between size and metabolic heat rate at 18°C for 25-year-old coast redwood trees growing in a grove on UCD campus. The regression equation for the line shown is "diameter at breast height, cm" = $(1.1 \pm 4.9) + (33.5 \pm 4.7) \times$ (metabolic heat rate, $\mu W\,mg^{-1}$) with a correlation coefficient of 0.91. The points shown as closed circles were not used in fitting the line, as explained in the text. Cuttings were taken from the tips of lower branches at various times during May and early June 1988. These samples were carbohydrate loaded before calorimetric measurement, as described in the text.

on some clones are tightly clustered whereas the data on other clones are very broad, sometimes in both variables and sometimes in only one.

To further examine variability within a single clone, the metabolic heat rates of additional samples of RW5 and albino clones were measured (Table 2). In contrast to the study of Table 1, these samples were growing in multiplication media rather than elongation media. The heat rate per mg of tissue is only about half that measured for the same clone growth in elongation media, but the variation is comparable. The albino samples exhibited very low growth rates in elongation media but grew at nearly the same rate as fully green samples in multiplication media.

Dimensions and relative metabolic heat rates of the trees in the UCD grove are given in Table 3. All samples used to collect these data were stored at 5°C in nutrient media for three days. A plot of diameter at breast height versus average relative metabolic heat rate is shown in Fig. 5. The metabolic heat rate is positively correlated with both diameter ($R = 0.91$) and height ($R = 0.61$).

DISCUSSION

The data in Figs. 4 and 5 demonstrate that a linear positive correlation exists between integrated growth rates and metabolic heat rates both for very early growth stages and for 25-year-old trees.

The points shown in Fig. 5 for the trees that violated the selection rules and which do not fit the linear correlation are also instructive. Tree 1, by far the smallest tree in the entire grove, has a near average metabolic

heat rate. The appearance of this tree, straggly with few branches only lightly foliated, also shows that this tree has failed to reach its growth potential. We postulate that this is caused by soil compaction around two sides of the tree because of the presence of a dirt roadway within 4–5 feet. Coast redwood has been shown to be weakened by soil compaction [9]. Tree 2 has a split top. Although we do not understand why this is related to lower metabolic rates, we have also observed this in two-month-old unrooted clones. Note that the metabolic rate measured for clone RW5 on media which promoted budding and multiple shoot formation (Table 2) was only about half that for RW5 on elongation media (Table 1). Trees 9 and 104 are heavily shaded on three sides and exhibit morphology characteristic of dense-growth redwoods. These trees have not grown at rates predicted from metabolic rate measurements; possibly because their photosynthesis is limited.

Tree 105 is much larger than predicted from the metabolic heat rate. This tree differs markedly from the others in growth habit. It is by far the most heavily foliated tree in the grove. We postulate that this tree has exceeded the growth rate limitations imposed by a moderate metabolic rate (in terms of metabolic rate per mass of foliage) to become a rapid grower by enhanced production of foliage, thereby increasing the total metabolism of the tree. This observation suggests that the metabolic rate of the entire tree would be a better predictor of growth potential than the metabolic rate per mass of tissue.

The excess variation unaccounted for in the data on repeated measurements of metabolic heat rates of clones (Tables 1 and 2) is clearly a property of the tissue and not of the measurement. We postulate that this variation may be caused by variations in the genotypes as well as phenotypes of replicate clones of the same tree. This variation may result from the hexaploid nature of the coast redwood [10]. Tissue cultures of normal diploid plants can give rise to surprisingly high rates of somaclonal variants [11].

CONCLUSION

This study shows that it may be possible to use calorimetry to select rapidly trees having high growth potential or, conversely, to eliminate trees that probably have poor growth potential. Combination of calorimetric measurements with traditional considerations such as growth habit could help in selection of stock for the commerical propagation of redwoods.

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