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Influence of age on dark respiration in eucalypts

T.S. Anekonda[a,*], R.S. Criddle[a], M.J. Bacca[b], L.D. Hansen[c]

[a]*Section of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA*
[b]*Simpson Tehama Fiber Farm, Corning, CA 96021, USA*
[c]*Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA*

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Abstract

Understanding age-related changes in respiratory parameters is required if measurements of juvenile metabolic properties are to be used for predicting long-term growth rates of trees. This study examines the influence of tree age on respiration in 13 *Eucalyptus* species, in different genotypes of the same species, and in rooted cuttings (ramets) of a single genotype. All of the respiratory parameters measured — metabolic heat rates, CO_2 production rates, and temperature coefficient of heat rate — showed systematic changes with tree age. Substrate carbon conversion efficiency and specific growth rates calculated from the respiratory data generally decreased with tree age.

Keywords: Calorimetry; *Eucalyptus*; Metabolic heat rate; Respiration; Temperature coefficient

Abbreviations: R_{CO_2}, CO_2 production rate; q, metabolic heat rate; q/R_{CO_2}, energy use efficiency; μ_q, temperature coefficient of metabolic heat rate.

1. Introduction

Prediction of long-term rates of biomass formation from rapid measurements on juvenile trees has had limited success [1–4]. Comparisons of growth rates of plants at juvenile stages usually do not extrapolate well to predictions of integrated growth over later stages of development. A method is needed to predict biomass accumulation at any stage from rapid measurements made on trees at the earliest possible stage. Predictive methods based on correlations of biomass production rate with physiological or morphological properties such as photosynthetic rates, the rates of individual biochemical reactions, or the growth of particular plant structures are often poor or are valid only under a specific set of conditions [5–7]. Measurements of respiration rates offer more promise. Simultaneous measurement of metabolic heat rate and CO_2 production rate may be used to predict relative growth rates and conversion of photosynthate carbon to biomass carbon (i.e., substrate carbon conversion efficiency) of plants in a given environment [8,9].

The relationship between q and temperature (T) can be represented in Arrhenius plots, where $\log_{10} q$ is plotted against absolute T in kiloKelvin [10]. For the eucalypt tissue used in this study, at lower temperatures (i.e., up to 30°C), the slope is linear and equal to μ_q. μ_q values have been shown to be related to the response of plants to climatic temperature and moist-

* Corresponding author. Tel.: +1-541-737-2244; fax: +1-541-737-1393.
E-mail address: anekondat@fsl.orst.edu (T.S. Anekonda).

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ure-stress [10,11]. Thus, it is possible to directly measure differences in respiratory parameters between tissues of juvenile and mature plants and use the respiration-based plant growth rate model of Hansen et al. [9] to define differences in specific growth rates and temperature coefficients at different developmental stages.

The main objective of this study was to quantify respiratory properties of eucalypts as a function of age and maturation state. Naturally occurring taxonomic hierarchy in eucalypts along with the availability of diverse plant materials allowed testing the influence of age on respiratory parameters among species, among different genotypes of a single species, and among different age ramets (rooted cuttings) of the same genotype.

2. Materials and methods

Plant materials were obtained by the Simpson Tehama Fiber Farm, primarily from CSIRO Division of Forestry, Australian Tree Seed Center. Some additional collections were from other seed collectors in Australia and from the USDA *Eucalyptus* program in FL. The following three types of plant material were used in this study.

2.1. Eucalyptus species

Thirteen *Eucalyptus* species were grown both in field and shade house environments. Field trees were planted on 22 May 1992 in a plantation at Corning, CA. The planting site was prepared thoroughly by ripping and disking prior to planting. Fourty nine, 3 month-old seedlings of each species were planted in square plots with a spacing of 3 m × 3 m. The plantation was drip irrigated in 1992 and 1993 and fertilized at irrigation. Trees were protected from insect and rodent pests with standard agricultural management practices. Among the available 1–3 provenances for each species, the most rapid growing was selected based on its first-year height. In the selected provenance, the five tallest trees were identified and used for study. Different seedlings of the same 13 *Eucalyptus* species were grown for 6 months during 1992 in pots containing peat/sand/perlite soil mix in a 50% shade house at the University of California at Davis, CA. All plants were irrigated with 50% Hoagland nutrient solution. 1–3 provenances were examined from each species. An average of two healthy and vigorous seedlings from each provenance were selected, and two measurements of q and R_{CO_2} were made on each seedling.

2.2. Phenotypically superior 'plus' trees

A second group of plants studied consisted of 27 phenotypically superior 'plus' trees of *E. camaldulensis*. These trees were selected from open-pollinated progeny trials planted with a spacing of 2.4 m × 3 m during 1984–1990 at Tehama Fiber Farm, CA. Of the 27, 17 were 3 years-old and the remaining 10 trees were 7–9 years-old. Irrigation, fertilizer application and plant protection measures undertaken in these progeny trials were similar to those for the species trail above. Metabolic properties of the plus trees were measured during late Summer 1993. For further details on plus trees refer to Anekonda et al. [8]. Respiration parameters were similar for the genets included in 7–9 age class, therefore we refer to this group of genets as the 8 year age class. The ''genet age'' is the age of the plus tree since the seed was originally planted. Trees in the 8 year class were cloned from other plantations in CA and the ramets planted at the Tehama Fiber Farm. Although this group was 8 years-old at the time of this study, the clones at Tehama Fiber Farm had only been growing in the field for 3 years (i.e., ramet age = 3).

2.3. One genotype (clone) at different developmental stages

A single *E. camaldulensis* plus tree (4016) was selected to test the effect of ramet age (not genet age) on values of the respiratory parameters. This plus tree was originally planted as a seed in 1984 (genet age = 9 years) and has since been cloned by using tissue culture to produce copies that have been planted at different locations in the Corning plantation over the years. Tissue culture usually does not rejuvenate plants, therefore, these tests measured respiratory and growth characteristics of the same genet in different ramet-age classes. Age groups of the ramets in the field were 2, 12, and 24 months at the time of experiments. 2–3 ramets were measured in each class and

calorespirometric measurements were made on two samples in each ramet.

2.4. Sample collection

Samples were harvested near 08:00 h in the shade house and near 07:00 h in the field. Shoot apices including terminal buds and subapical portions were collected from two actively growing primary branches for studies of different species and from the top branches of the plus trees. Samples were immediately placed in small open-top vials with cold, half-strength Hoagland's solution containing 1% sucrose. The vials were placed on ice during transport and stored in an aerated refrigerator at 5°C until measured in the calorimeter. Respiratory rates declined during the first 0.5 h and then remained nearly constant for the next 2–3 days. Thus, samples collected from the shade house were stored at least 0.5–1 h in the refrigerator before start of the first experiment. The field to lab transport time was about 2 h; hence field samples were used as soon as they arrived. For shade house trees, fresh samples were collected every day the experiment was run, whereas samples from the field trees were collected on alternate days or twice weekly.

2.5. Calorespirometric measurements

Calorespirometric measurements on the shade house samples were conducted during August and September, 1992. The field samples were measured during May and June of 1993. Measurements of heat rate (q) and CO_2 production rates (R_{CO_2}) in the dark at fixed temperatures were made using a Hart Scientific model 7707, heat-conduction, differential scanning calorimeter operated in the isothermal mode [12]. Metabolic heat rate is dq/dt, i.e., the time derivative of heat production by plant metabolic reactions. R_{CO_2} is d[CO_2]/dt, i.e., the time derivative of CO_2 production by plant metabolism. Approximately 1 cm long sections, including the apical meristem with subtending developing stem and leaves, were placed in the 1 cm^3 calorimeter ampules along with a 50 µl vial. Metabolic heat rates were measured with 40 µl of H_2O in the vial. Then, H_2O was removed and replaced with 40 µl of 0.4 M NaOH. CO_2 produced by the respiring tissues was absorbed by the NaOH to produce carbonate ion and liberate additional heat at a rate proportional to the CO_2 production rate. Isothermal metabolic heat rate measurements were made at 15°C and 25°C. R_{CO_2} was measured only at 25°C. The temperature coefficient of metabolic heat rate (μ_q in kiloKelvin (kK)) between 15°C and 25°C, and the ratio of heat rate to CO_2 rate (q/R_{CO_2}) were derived from the original variables [9,10].

3. Results

3.1. Species study

Fig. 1 shows a plot of R_{CO_2} for field grown *Eucalyptus* species (genet age = 18 months) vs. R_{CO_2} for the same 13 species grown in the shade house (genet age = 6 months). The relation between the field grown species and shade house grown species is moderate ($R^2 = 0.49$) with relatively consistent rank order. Exact correspondence between R_{CO_2} for field and shade house samples should yield a line with slope of 1 and passing through the origin. In Fig. 1, the

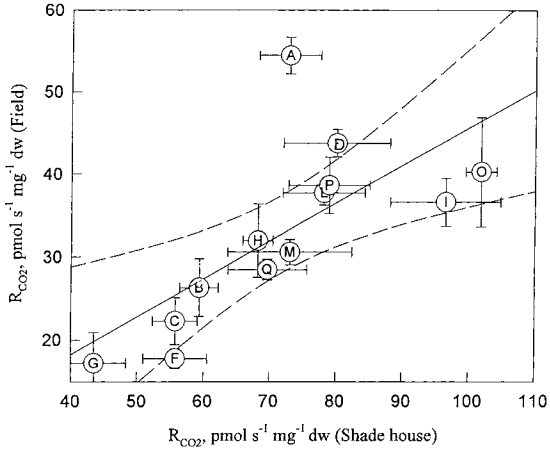

Fig. 1. Relation between rate of CO_2 production (R_{CO_2}) at 25°C by trees from 13 *Eucalyptus* species grown in shade house with that of the same species grown in field: (A) *E. brookerana*, (B) *E. camaldulensis*, (C) *E. cypellocarpa*, (D) *E. dalrympleana*, (F) *E. fastigata*, (G) *E. fraxinoides*, (H) *E. maidenii*, (I) *E. glaucescens*, (L) *E. ovata*, (M) *E. radiata*, (O) *E. rubida*, (P) *E. smithii*, and (Q) *E. tereticornis*. Error bars indicate the s.d. of the mean of five trees for the field grown species and an average of four trees for the shade house grown species. Equation for the regression line is $R_{CO_2,\text{Field}} = -0.05 + 0.46 \times R_{CO_2,\text{Shade house}}$ ($R^2 = 0.49$). The dashed lines indicate 95% confidence interval.

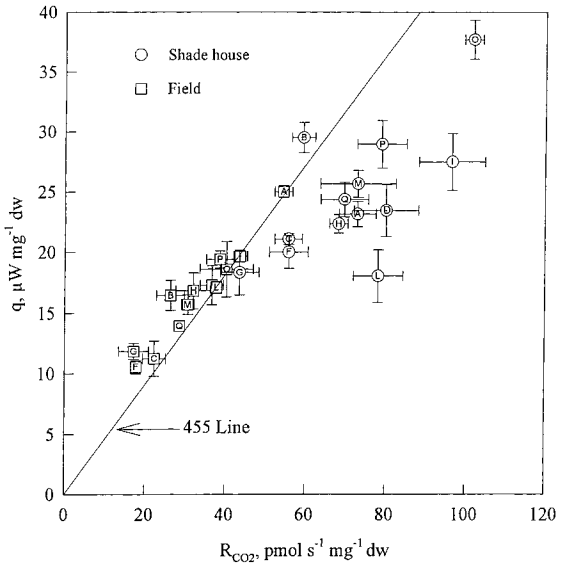

Fig. 2. Relation between metabolic heat rate (q) and the rate of CO_2 production (R_{CO_2}) by trees from 13 *Eucalyptus* species grown in shade house (open circles) and the same species grown in field (open square). Refer to Fig. 1 for species names. A line passing through the origin and with slope equal to 455 kJ mol^{-1} facilitates comparison of shade house and field values. Error bars indicate the s.d. of the mean of five trees for the field grown species and an average of four trees for the shade house grown species.

intercept of the regression line is close to 0 but the slope is less than 1. Shade house trees at genet age of 6 months have uniformly higher CO_2 rates than 18 months-old field grown trees by a factor of 2 This difference may result from age differences or different growth environments or both.

In Fig. 2, q is plotted against R_{CO_2} for both field grown and shade house grown trees. A line representing q/R_{CO_2} equal to 455 kJ mol^{-1} CO_2 separates q/R_{CO_2} values for trees grown in the shade house from those of the same species grown in the field. The number 455 kJ mol^{-1} is a constant equal to the heat produced per mole of oxygen consumed during combustion of carbon compounds [9]. Heat per mole of CO_2 is generally <455 kJ mol^{-1} for the younger, shade house grown trees and >455 kJ mol^{-1} for older trees of the same species grown in the field. This difference could again be due to an age or a growth condition difference, but results from a difference in biomass composition, a difference in substrate carbon conversion efficiency, and/or a difference in the oxidation state of the substrate carbon used for respiration/biosynthesis in the two sets of trees.

3.2. Plus trees

Table 1 summarizes average values of q, R_{CO_2}, q/R_{CO_2} and μ_q for two genet ages of plus trees. The respiratory variables change significantly with genet age. For example, ramets 3 years of age may have high or low values of each respiratory property depending on the genet age of the tree. Metabolic heat rates (q) and R_{CO_2} increased from genet age 3–8. Values of q increased more than R_{CO_2} so that q/R_{CO_2} also increased. Values of the temperature coefficient of q (μ_q) also increased with increase in genet age from 3–8 years.

3.3. One genotype at different developmental stages

Fig. 3 presents q, R'_{CO_2}, q/R_{CO_2} and μ_q plotted against ramet age of the 4016 clone. Metabolic rates for ramets measured as either q or R_{CO_2} for the ramets are very high during the first 2 months after planting, but decrease to a near constant value by 12 months. Values of q/R_{CO_2} are initially low, then increase by 12 months. The μ_q values steadily increase during the 24 months of ramet growth.

Table 1
Estimated means and the standard errors[a] of means of metabolic heat rate (q), CO_2 production rate (R_{CO_2}), the ratio (q/R_{CO_2}) and temperature coefficient of metabolic heat rate (μ_q) for two genet age classes of 3 year-old plus trees

Genet age[b]	Number of genets	q (μW s^{-1} mg^{-1} dw)	R_{CO_2} (pmol s^{-1} mg^{-1} dw)	q/R_{CO_2} (kJ mol^{-1})	μ_q (kK)
3	17	11.6 ± 0.3	29.3 ± 0.9	418 ± 11	7.25 ± 0.16
8	10	17.7 ± 1.3	37.1 ± 2.8	472 ± 26	8.84 ± 0.30

[a] Standard errors of means are estimated using measured respiration values on genets in each age class.
[b] Genet age as of June 1993; refers to age of the plus tree since the seed was originally planted.

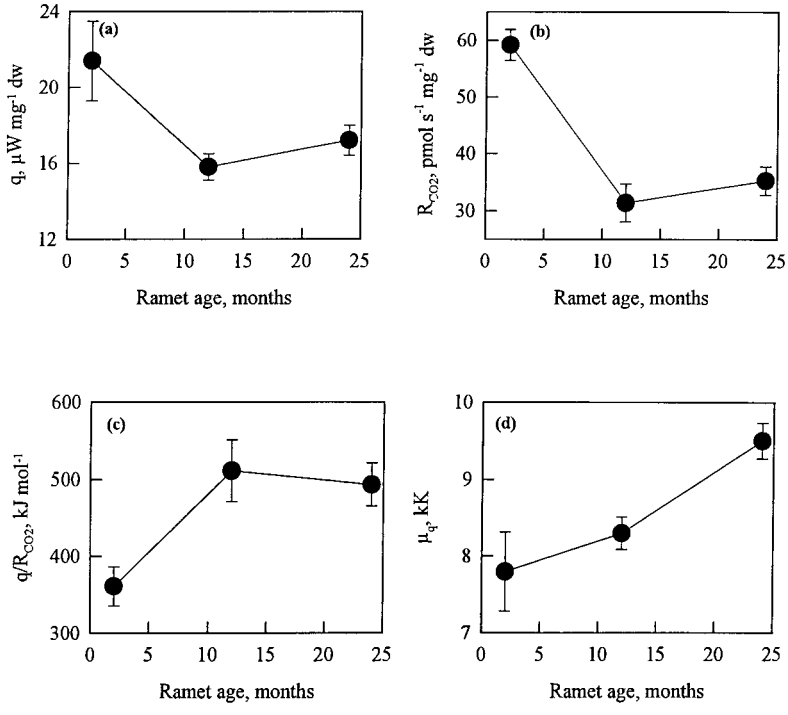

Fig. 3. Average values of (a) q, (b) R_{CO_2}, (c) q/R_{CO_2}, and (d) μ_q for ramets of the 4016 clone plotted against their ramet age. Error bars indicate the s.d. of the mean of 4–6 measurements from 2 to 3 ramets in each ramet age class.

4. Discussion

Distinct changes in respiratory rates and efficiency accompany aging in young *Eucalyptus* plants. Because the changes were similar in all 13 species, the relative order of species respiratory values was conserved during aging. Both q and R_{CO_2} were larger for young, shade house grown trees than for field grown trees. More scatter occurred in q values (not shown) than in R_{CO_2} values, suggesting that q is more influenced by experimental variables other than age and location. Values for both q and R_{CO_2} from shade house trees are more scattered than those from field grown trees, probably because trees in the shade house represent more than one provenance while a single provenance was selected for the field grown trees [8]. Additionally, respiratory parameters change rapidly in very young seedlings so that variability due to small differences in the time of measurement would cause larger variability in the data on younger trees.

The ratio of q/R_{CO_2} is smaller in the younger, shade house grown plants than in older, field grown trees (Fig. 2). A tree with relatively high q/R_{CO_2} is either energetically less efficient, respiring more reduced substrate, or produces more oxidized biomass than one with a smaller value of this ratio [9]. Because the meristem tissue measured in this study is heterotrophic, i.e., not yet photosynthetically competent, respiration is based on substrate exported by older leaves [13]. As trees age, a higher proportion of the carbon from photosynthate is incorporated into more reduced compounds such as lignin and less into cellulose and sugars. Thus, use of more reduced substrate as trees age is the most likely explanation to account for the increase in q/R_{CO_2} changes with age noted here. Since all the species show approximately the same relative change in q/R_{CO_2} upon aging, the substrate and biomass oxidation states must undergo similar changes for each of the species.

A consistent relation exists between respiration and genet age. There appears to be a "memory" of initial

genet age. Such ontogenetic aging or maturation effects are common in woody plants [14,15]. Both q and R_{CO_2} increase rapidly first and then level off with genet age, irrespective of what age ramets are measured (Table 1). Values of q/R_{CO_2} and μ_q show a steady increase with genet age.

Although genet age of 4016 was 9 years, respiratory values for ramets of 4016 are unstable during the first few months and do not reflect the genet age values as expected from 8 year-old trees (compare values in Fig. 3 with values in Table 1). However, q, R_{CO_2}, and q/R_{CO_2} values of 12 month-old ramets are similar to the values expected for plants with the genet age of 8 years. Only μ_q values (9.4 kK) of ramets at age 24 months exceeded the expected values (8.84 kK) typical of 8 year genets. These data again support the earlier observations on plus trees that values of measured respiratory parameters are influenced by genet age of the propagules planted.

The observed changes in the metabolic parameters measured can be related to plant growth rates using the model of Hansen et al. [9]. Growth rates increase with metabolic rates and decrease with loss of substrate carbon conversion efficiency. Based strictly on a proportionality between growth rates and metabolic rates, growth rates for the plus trees would initially increase rapidly and then plateau by 8 years (Table 1). During aging of the young plants studied, metabolic rates increase and as judged by q/R_{CO_2} ratios, substrate carbon conversion efficiency may decrease. Growth rate at any time is a product of these two terms [16].

Genets with age 8 showed high μ_q values and would be expected to have relatively poor growth. As we have previously shown, when values of μ_q for *Eucalyptus* species exceed about 8.5 kK, growth rates at the field site in Corning are diminished [8].

Interpretation of causal factors in respiratory changes is complicated by experimental conditions, as the younger plants were grown in a shade house and the older plants in the field. However, our results suggest that age, not growth conditions, is the dominant factor in the difference measured. Very good growth conditions were maintained for both sets of plants. Even though the species studied differ markedly in growth responses to environmental differences, the order of responses remained nearly constant for both growth conditions.

4.1. Age-dependence of respiratory metabolism and its significance in forestry

There are important consequences of the relation between respiratory metabolism and genet age of eucalypts. First, the observed differences in metabolism among eucalypt species may reflect early adaptation of these species to their native environments [8]. For example, a species with low metabolic efficiency at early age may experience severe competitive disadvantage because it may be overtopped by more efficient species in the natural stands [17]. If age effects are species specific, severe interspecific competition may result as species grow and mature in mixed stands. Physiological changes associated with competitive stand growth could be effectively studied using metabolic parameters.

Second, maximum rates and efficiencies of biomass production are reached by genet age 4 and begin to decrease by 8 years. Third, this study shows that it may be possible to define maturation states based on measurements of respiratory parameters.

For cloning, it is important to obtain ramets from trees with low genet age to avoid the slower growth associated with mature propagules [18]. Seed germination initiates the maturation process. A stage of near optimum growth rate and efficiency is achieved by 4 years in eucalypts and then declines. Because ramets exhibit the maturation state of the source plant, a ramet from an 8 year-old tree begins growth with characteristics of an 8 year-old plant. This ramet may quickly mature into a state with lower metabolic efficiency and lowered production rate. Clearly it is advantageous to identify superior seedlings for cloning soon after germination so that ramets can experience a maximum growth time before entering the lower efficiency, slower growing, mature state. Maturation state consideration is particularly important when commercial planting is undertaken by using clonal forestry procedures [19]. Calorespirometric methods may be able to rapidly identify the superior genotypes for cloning early in their developmental stages.

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