

GROWTH AND MAINTENANCE RESPIRATION OF *CATHARANTHUS ROSEUS* L. ESTIMATED FROM CO₂ EXCHANGE

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Keywords: *Catharanthus roseus*; growth respiration; maintenance respiration; photosynthesis; vinca

Abstract

Growth and maintenance respiration in plants are difficult to separate, because they occur simultaneously in the same plant parts. If growth rate, plant size, and respiration rate are measured concurrently, respiration can be divided into growth and maintenance respiration using regression analysis. This is based on the assumptions that growth respiration is a function of growth rate and maintenance respiration is a function of biomass. Whole plant carbon exchange of eight vinca crops (*Catharanthus roseus* L.) was measured in a continuous photosynthesis system for approximately three weeks. Daily carbon gain (mol C/day) was used as measure of growth and cumulative carbon gain (mol C) was used as an estimate of plant size. Multi-variable regression analysis indicated that there was a linear correlation between growth and growth respiration. The slope of the regression line was 0.193 mol/mol, indicating that 0.193 mol of C was lost in growth respiration for every mol of C that was incorporated into dry matter. Maintenance respiration increased throughout the experiment, but maintenance respiration per unit dry mass decreased. The decrease in maintenance respiration per unit dry mass is probably due to a change in the chemical composition of the plants. Vinca has significant lignification in its cell walls. Lignified tissue normally requires little maintenance and lignification would thus be expected to result in a decrease in maintenance respiration per unit biomass.

1. Introduction

Maintenance (R_m) and growth respiration (R_g) are two different processes that occur simultaneously in plants. Maintenance respiration is defined as generation of energy from carbohydrates used for the resynthesis of enzymes, nucleic acids, lipids and other plant components and for the maintenance of ion and metabolite gradients across membranes (Penning de Vries, F.W.T., (1975)). Growth respiration is the respiration that results from the synthesis of new biomass. Thus, maintenance respiration is dependent on the biomass of the plants, while growth respiration depends on the growth rate of plants (Amthor, J.S., (1989)). Respiration normally is measured as CO₂ efflux or O₂ uptake by plants or plant parts, but these measurements cannot differentiate among R_m , R_g , and photorespiration. Separation of R_g and R_m is particularly difficult, because they occur simultaneously in the same plant parts. There is no strict physiological separation between R_g and R_m in plants. For example, ATP and NADP that are produced by plants can be used either for growth or maintenance purposes. Similarly, maintenance of ion and/or metabolite gradients across membranes may be needed for growth, maintenance, or both.

Reliable estimates of the different components of respiration are important for the development of simulation models and for improving our basic understanding of the physiological processes involved in the growth of plants. Several different approaches have been used in the past to estimate growth and/or maintenance respiration. Growth respiration has been estimated based on the chemical composition of plant material (Penning de Vries, F.W.T. (1983)), the increase in respiration with a short-term increase in growth (McCree, K.J. (1970); Gifford, R.M. (1995)), the carbon content (Vertregt, N. (1987)), the heat of combustion (Williams, K. (1987)), or the elemental composition of the plants (McDermitt, D.K. (1981)). All these methods are indirect estimates and most do not rely on actual respiration measurements.

Similarly, R_m estimates have been made in different ways. It has been estimated as the respiration rate under conditions of no net growth (McCree, K.J. (1970); Gifford, R.M. (1995)), and from dry weight, growth rate, and respiration measurements (Marcelis, L.F.M. (1995)). Since R_g depends on the growth rate of the plant, and R_m depends on plant dry mass (Amthor, J.S., (1989)), R_g and R_m can be expressed as a function of growth rate and plant size, respectively. However, the exact relationship between these parameters is unknown and depends on environmental conditions. If total respiration, growth rate, and plant size are measured simultaneously over an extended period, R_g and R_m can be estimated from regression analysis. This was the idea behind the approach of Marcelis, L.F.M. (1995). They estimated fruit dry mass non-destructively from fruit volume measurements and measured fruit respiration once during every dark period.

Since most of the dry mass increase of plants is the result of carbon accumulation, plant size and growth rate can be estimated from CO_2 exchange measurements, if that data is collected semi-continuously. The cumulative carbon accumulation of a plant during an experiment is a measure of the increase in the carbon content of the plants and can be used as measure of plant size, while the carbon gain in a 24-hour period is a measure of growth rate. The objective of this study was to use long-term (28 days) semi-continuous measurements of CO_2 exchange (net photosynthesis or dark respiration once every 20 minutes) to determine plant growth rate and size, and to use this data to estimate R_g and R_m of vinca. Vinca was used, because it has been found to grow well under the experimental conditions.

2. Materials and methods

2.1. Plant material

Vinca 'Cooler Peppermint' seeds were planted in a peat-based growing mix (Redi-Earth, The Scotts Co.) and germinated in the dark at 20 C for 5 days. The seedlings were then transferred to a double-layer polyethylene greenhouse and grown until the second pair of true leaves appeared. The seedlings were then transplanted into cell packs (166 mL/cell) filled with diatomaceous earth (Isolite CG-2, Sundine Enterprises). Diatomaceous earth is a chemically inert growing substrate with a low cation exchange capacity ($<0.02 \text{ meq g}^{-1}$), consisting mainly of SiO_2 (78%), Al_2O_3 (12%), and Fe_2O_3 (5%). Eight groups of seedlings were placed in watertight trays (28 plants/group) and transferred to transparent acrylic chambers, which were placed inside two larger growth chambers. Plants were watered as needed with a complete 20N-4.4P-16.6K water-soluble fertiliser (20-10-20 Peat-Lite Special, The Scotts Co.), containing $100 \text{ mg L}^{-1} \text{ N}$. Environmental conditions inside the chambers were 22/18 C day/night, with a 14 hour light period and a photosynthetic photon flux density of $425 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the canopy level, which resulted in a total daily photon flux of 21.4 mol m^{-2} . Relative humidity was approximately 75% during the light period and 100% at night.

2.2. Measurements

CO₂ exchange rate of the eight groups of plants was measured with a ten-chamber, open, CO₂ exchange system. Ambient air was blown into the acrylic chambers and airflow was measured with mass flow meters (GFM37-32, Aalborg Instruments and Controls). The difference in the CO₂ concentration of the air entering and exiting the chamber was measured with an infrared gas analyser (Li-6251, Li-Cor). Whole chamber CO₂ exchange ($\mu\text{mol}\cdot\text{s}^{-1}$) was calculated as the product of mass flow ($\text{mol}\cdot\text{s}^{-1}$) and the difference in CO₂ concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$). Gas exchange data of every group of plants was collected at 20-minute intervals until four weeks after transplant. Daily carbon gain (DCG in mol C/day) was calculated as the total net carbon exchange of the plants in a 24-hour period and was used as a measure of plant growth. Cumulative carbon gain (CCG in mol) was calculated as the integral of the CO₂ exchange data and it is an indicator of plant growth since the start of the experiment. Assuming uniform plant material at the start of the experiment, CCG can be used as a measure of plant size. Carbon use efficiency (CUE, dimensionless) was calculated as the DCG divided by the total amount of C fixed in gross photosynthesis during the light period (estimated as net photosynthesis + dark respiration). This is the fraction of C fixed in photosynthesis that is incorporated into plant dry matter.

2.3. Data analysis

Estimation of R_m and R_g was based on the assumption that R_m can be expressed as a function of plant size and R_g as function of the growth rate of the plants. To minimise the effect of small differences in the size of the plants at the start of the experiment, respiration measurements were used only after CCG was higher than 0.05 mol. This resulted in 98 data points for regression analysis. Since the form of these functions was unknown, the first step was to determine their approximate shape. Initially, a seventh order polynomial was used to estimate R_m and R_g :

$$R = R_m + R_g \\ = x_0 + x_1 \cdot \text{CCG} + x_2 \cdot \text{CCG}^2 + \dots + x_7 \cdot \text{CCG}^7 + y_1 \cdot \text{DCG} + y_2 \cdot \text{DCG}^2 + \dots + y_7 \cdot \text{DCG}^7, \quad (1)$$

$$\text{where: } R_m = x_0 + x_1 \cdot \text{CCG} + x_2 \cdot \text{CCG}^2 + \dots + x_7 \cdot \text{CCG}^7, \quad (2)$$

$$\text{and } R_g = y_1 \cdot \text{DCG} + y_2 \cdot \text{DCG}^2 + \dots + y_7 \cdot \text{DCG}^7, \quad (3)$$

since R_g by definition is zero when there is no growth (DCG=0).

Non-significant terms were dropped from the regression equation, starting with the highest order terms. Removal of all non-significant terms resulted in $R = R_m + R_g = x_0 + x_1 \cdot \text{CCG} + x_2 \cdot \text{CCG}^2 + x_3 \cdot \text{CCG}^3 + y_1 \cdot \text{DCG}$ ($r^2 = 0.976$). This indicated that R_g is linearly related to the growth rate of the plants (DCG), while R_m increased with increasing plant size (CCG), but decreased per unit plant mass. For subsequent analysis, R_g was considered to be a linear function of DCG:

$$R_g = y_1 \cdot \text{DCG}. \quad (4)$$

Several non-linear equations were used to describe the relationship between R_m and CCG. These equations had nearly identical results and the results of only one equation will be discussed. This equation is:

$$R_m = x_0 / (1 + x_1 \cdot e^{\text{CCG} \cdot x_2}) + x_3 \cdot \text{CCG}. \quad (5)$$

Hence, total respiration was modelled as:

$$R = R_m + R_g = x_0 / (1 + x_1 \cdot e^{\text{CCG} \cdot x_2}) + x_3 \cdot \text{CCG} + y_1 \cdot \text{DCG}. \quad (6)$$

3. Results

There was a close linear correlation between the CCG over the course of the experiment and the final dry mass of the plants (dry mass (g) = 2.77 g + 23.23 g·mol⁻¹ · CCG; $r^2 = 0.98$), where 2.77 g is the estimated dry mass of the plants at the start of the experiment and 23.23 g·mol⁻¹ is the increase of plant dry mass (g) with every mol of C incorporated into the plants. The strong correlation between dry mass and CCG indicates that the CO₂ exchange measurements did indeed give an accurate measurement of the growth rate of the plants.

The model used to estimate R_m and R_g resulted in a good fit of the calculated versus the measured respiration data (Fig. 1; $r^2 = 0.98$). Regression coefficients are shown in Table 1. Maintenance respiration was responsible for a larger fraction of the total respiration than R_g , but the ratio of R_g to R_m increased throughout the experiment and R_g would have been larger than R_m if the experiment had been continued longer (Fig. 1).

Growth respiration increased linearly with growth rate (DCG, Fig. 2). The slope of the regression line is 0.002236 (μmol·s⁻¹) / (mmol·d⁻¹) or 0.193 mol·mol⁻¹. This indicates that the plants respired 0.193 mol of C to incorporate one mol of C into dry matter.

The relationship between the modelled R_m and CCG was more complex. Maintenance respiration increased rapidly with an increase in CCG from 0.05 to 0.15 moland then approached an asymptote with a slope of 0.063 μmol·mol⁻¹·s⁻¹ or 5.44 mmol mol⁻¹·d⁻¹ (Fig. 3). After an initial increase, R_m per unit dry mass decreased throughout most of the experiment (data not shown). Carbon use efficiency of the plants increased from 0.53 when CCG was 0.1 mol to 0.69 when CCG was 0.5 mol (Fig. 4).

4. Discussion

The linear correlation between DCG and R_g is not surprising. It indicates that the conversion efficiency (total amount of carbohydrates needed for the production of 1 g of dry matter of new plant material) did not change significantly during the experiment. Since the plants remained vegetative during this experiment, the chemical composition of the newly produced plant material (leaves, stems, and roots) probably did not change much. Vinca normally experiences lignification of stem tissue, but this does not involve the incorporation of extra C, and the respiration associated with lignification would thus be part of R_m .

The estimated growth respiration can be used to calculate the conversion efficiency of vinca. Vinca plants contain approximately 45% C, so 0.45 g C or 1.125 g CH₂O is needed to provide the C in one gram of structural dry matter. In addition, 0.193 mol of CH₂O is respired for every mol of C incorporated into the plants. Per gram dry matter, 0.0375 mol C is incorporated and therefore 0.0072 mol C (0.216 g CH₂O) is respired to make this growth possible. Therefore, the conversion efficiency (total amount of carbohydrates needed for the production of 1 g of dry matter) is estimated to be 1.34 g CH₂O · g⁻¹ dry matter (1.125 + 0.216). This is slightly lower than the 1.39 and 1.45 g CH₂O · g⁻¹ dry matter that are often used for leaves and stems of non-leguminous species, respectively (Penning de Vries, F.W.T. (1989)). Gary C. (1998) reported a considerably lower conversion efficiency for tomato (0.99 and 0.94 g CH₂O · g⁻¹ dry matter for leaves and stems respectively). However, the conversion efficiency of tomato is low because of the high mineral content of tomato plants (Gary, C., (1998)) and the estimated conversion efficiency of 1.34 g·g⁻¹ seems reasonable.

The estimated R_m also seems to be a reasonable estimate. At the end of the experiment, when CCG was 0.55 mol C, R_m was approximately 0.17 μmol·s⁻¹ or 0.18 g C·day⁻¹. Since the plants contained close to 0.1 mol C at the start of the experiment, the total amount of C in the plants was 0.65 mol or 7.9 g. Thus, the R_m coefficient [g C respired for maintenance ; g⁻¹ of C in plant material day⁻¹] was 0.023 g·g⁻¹·day⁻¹. This is identical to the 0.023 g · g⁻¹·day⁻¹ reported for wheat by Gifford, R.M. (1995).

Although R_m increased throughout the experiment, R_m per unit of plant dry matter decreased. This is probably related to the lignification that occurs in vinca stems. Lignified plant material requires little maintenance and lignification thus results in decreased R_m per unit of plant dry matter.

Carbon use efficiency of the plants increased throughout the experiment (Fig. 4), because a smaller fraction of the total available carbohydrates was used for R_m as the plants became larger (Fig. 3). Thus, more of the carbohydrates were available for plant growth as the plants became larger, increasing the fraction of the available C that was incorporated into the plants.

The good agreement between the data from this experiment and published values for conversion efficiency and R_m coefficient suggests that this new approach for estimating R_g and R_m from semi-continuous CO_2 exchange measurements yields reasonable results. Since these CO_2 exchange measurements are made in controlled environment chambers, they are well-suited to study the effect of environmental conditions on R_g and R_m . Conditions that can easily be controlled include temperature, light and humidity.

The effects of temperature on R_g and R_m would be particularly interesting. Protein turnover and many other physiological processes are temperature dependent and temperature would thus be expected to have a significant effect on R_m . Growth respiration would probably be less affected by temperature, unless the chemical composition of the plants is altered.

Acknowledgements

I thank Larry Freeman and Kevin Calhoun for their technical help and the Fred C. Gloeckner Foundation for their funding of this research..

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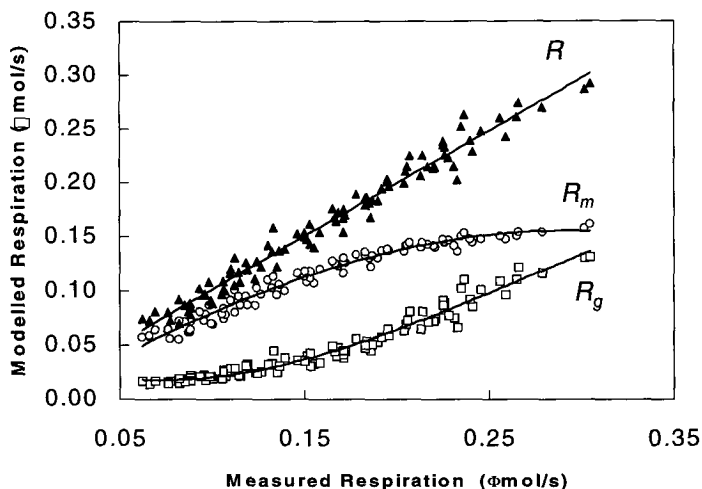
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Tables

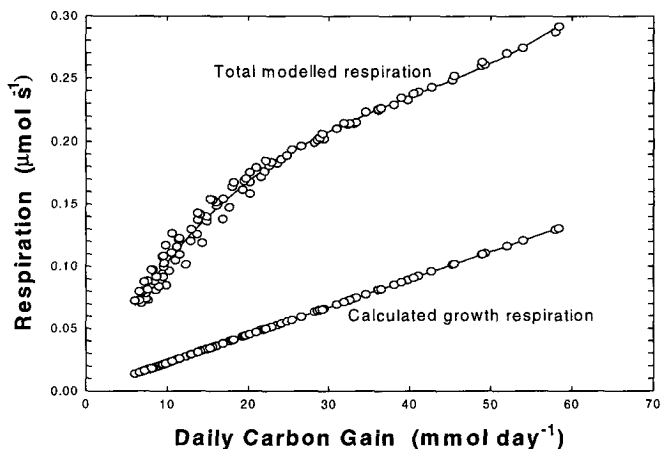
1. Regression coefficients used to estimate growth, maintenance, and total respiration, using equations 4, 5, and 6.

Regression coefficients used to estimate growth (R_g), maintenance (R_m), and total respiration (R), as a function of daily (DCG) and cumulative carbon gain (CCG). $R = R_m + R_g = x_0 / (1 + x_1 \cdot e^{CCG \cdot x_2}) + x_3 \cdot CCG + y_1 \cdot DCG$.		
Coefficient	Value	Units
X_0	0.1275	$\mu\text{mol} \cdot \text{s}^{-1}$
X_1	5.582	dimensionless
X_2	26.81	mol^{-1}
X_3	0.063	$\mu\text{mol} \cdot \text{s}^{-1}$
Y_1	0.002236	$(\mu\text{mol} \cdot \text{s}^{-1}) / (\text{mmol} \cdot \text{d}^{-1})$

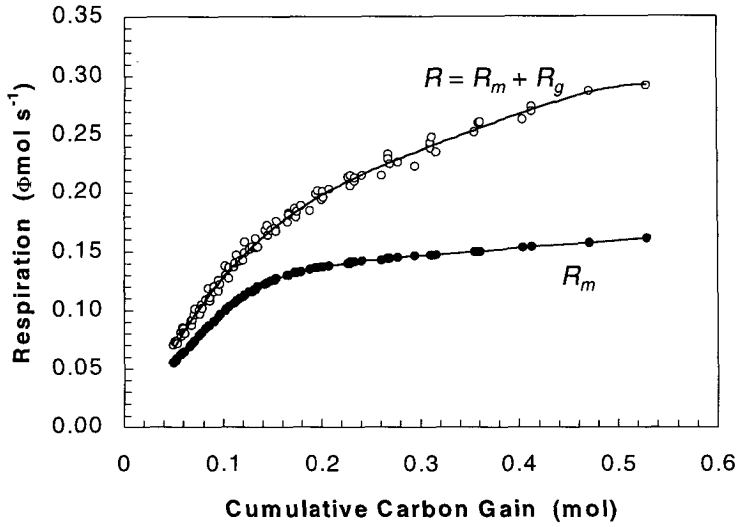
Figures



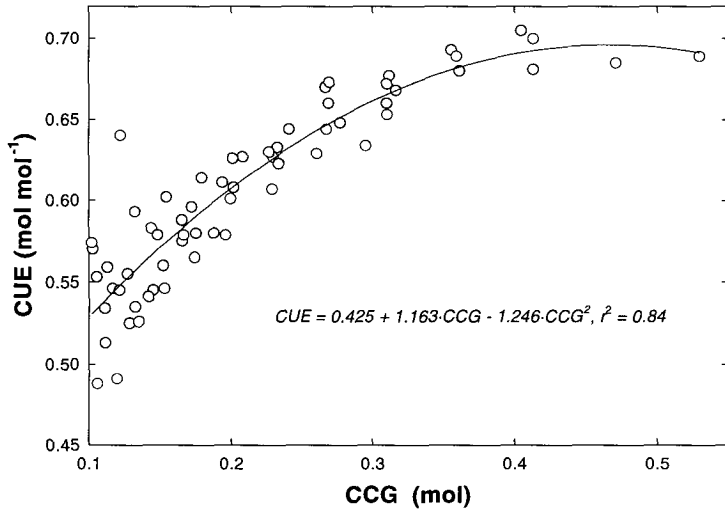
1. Comparison of modelled total (R), growth (R_g) and maintenance respiration (R_m) of vinca with measured dark respiration. Calculations are based on equations (4), (5) and (6).



2. Total modelled respiration ($R = R_m + R_g$) and calculated growth respiration (R_g) of vinca as a function of the daily carbon gain (DCG) of the plants. Daily carbon gain is an estimate of the growth rate of the plants.



- Total modelled respiration ($R = R_m + R_g$) and calculated maintenance respiration (R_m) as a function of the cumulative carbon gain of the plants since the start of the experiment (CCG). Cumulative carbon gain is an estimate of plant size.



- The relation between cumulative carbon gain (CCG) and carbon use efficiency (CUE) of vinca.