MICROCALORIMETRY: A NOVEL APPROACH TO DECISION MAKING IN CUT ROSE PRODUCTION

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Abstract

Since its innovative use by Lavoisier at the end of the eighteen-century, calorimetry has been one of the most important foundations of modern science. However, only recently, with the advent of modern and accurate sensors, has it to play a central role in the life sciences. The combination of calorimetric and respirometric studies resulted in a thermodynamic model that represents the rate of storage of chemical energy in structural biomass or Specific Growth Rate (RSG) that serves as an indicator for potential growth rate (PGR) of the tested tissue. Simultaneous measurements of metabolic heat rate (q) and respiratory heat production ($R_{CO2}$) at the relevant range of environmental conditions such as temperature provides an insight into plant’s response to these factors and allow us to define their optimal range. The actual measurement is destructive in nature but with microcalorimetry only a small amount of plant tissue (2-3 mg DM) is required for each observation.

This report demonstrates the use of calorespirometric analyses for two processes, using Rosa x hybrida ‘Kardinal’:

- RSG as a function of temperature of young rose leaflets and sprouting axillary buds.
- RSG as a function of moisture tension of young rose leaflets.

Optimal temperature for young leaflets was found to be 25°C while that of young buds was below 15°C. Optimal temperature control in rose greenhouses is discussed in view of these results.

The effect of physical characteristics of the root zone, such as moisture tension and unsaturated hydraulic conductivity ($K(h)$) on (Specific Growth Rate) RSG of young leaflets was evaluated at the optimal temperature. It was found that the higher the tension, the lower was the RSG. However, based on comparing the physical properties of 2 different media: UC mix and coir, it can be assumed that at high moisture tension, $K(h)$ is more effective than moisture tension per se in moderating RSG.

1. Introduction

Roses represent one of the most important cut-flower crops in many countries. This crop is typically grown in climate-controlled greenhouses where optimal conditions are sought to maximize production and optimize timing of harvest to satisfy market needs. Flower quality is also a parameter of major importance when making decisions involving several cultural practices (e.g., pruning, disbudding, etc.).

For most horticultural crops, the growth rate must be maximized to achieve maximum profitability. A plant's actual growth rate can generally be characterized as the mediation of its potential growth rate (PGR) by the multitude of environmental factors to which it is exposed. While it is a relatively simple matter to quantify growth, modeling potential growth has been impossible in the past.
Since its innovative use by Lavoisier at the end of the eighteenth-century, calorimetry as a discipline has been one of the most important foundations of modern science. However, only recently, with the advent of modern and very accurate measuring devices, has it reacquired a central role in the life sciences. Microcalorimetry has been suggested as a method to characterize PGR along with plant adaptation to various environmental conditions (Criddle et al., 1991). While calorimetric measurement has an inherent disadvantage of being destructive, only a small amount of plant tissue (typically, 2-4 mg DM) is required for each replicate in microcalorimetry.

The combination of extensive calorimetric and respirometric studies enabled the development of a thermodynamic model that allows the prediction of PGR from such data. Simultaneous measurements of metabolic heat rate (q) and respiratory heat production rate ($R_{CO2}$) at the relevant range of environmental conditions such as temperature may provide an insight into the plant response to these factors and define their optimal range. The calorimetric growth potential theory is based on the assumption that growth is maximal when the difference between $R_{CO2}$ (energy used in respiration) and q (energy “lost” as heat) is the largest (Hansen et al., 1994). The balance of these two represents the rate of storage of chemical energy in structural biomass or Specific Growth Rate (RSG) and serves as an indicator for growth potential of the tested tissue (Hansen et al., 1997). The theoretical basis offered by Hansen, Criddle and co-workers for predicting optimum conditions for growth has been supported by several experimental systems. Anekonda et al. (1994) found that in Sequoia sempervirens q and $R_{CO2}$ were highly correlated (r=0.85) to various measures of growth (height, basal diameter, stem volume). Similar results have been obtained when using microcalorimetry to predict the optimum growth conditions for tomato and cabbage (Criddle et al., 1991) as well as with larch (Hansen et al., 1989).

Since water availability greatly affects growth rate, it was suggested by Hansen et al. (1997) that the soil water's status should also be reflected in calorimetric measurements. One of our goals in this study was to verify this hypothesis using young leaflets as these grow exponentially and are extremely sensitive to water stress.

The objectives of the present research were:

1. To study temperature effect on RSG of 2 rapidly growing tissues of rose plants: sprouting axillary buds and young leaflets.
2. To study the effect of water availability in the root zone on PGR of very young leaflets, at optimal temperature.

2. Materials and methods

2.1. Plant growth

Rose plants (Rosa x hybrida L. ‘Kardinal’, grafted onto Rosa canina L. ‘Natal Brier’ rootstock) were planted in 5-liter containers, filled with one of two artificial media: coconut coir made of shredded, partly composted husk fibers and UC mix (42% composted fir bark, 33% peat and 25% sand). The plants were grown in a glass-covered greenhouse at the Department of Environmental Horticulture, University of California at Davis, CA. The experimental set up consisted of 10 pots per medium, one plant per pot. Each pot had a space of 2700 cm² (including path space). Since the plants were located within a rose stand, no border plants were assigned. The plants were treated as a commercial rose crop. Weak stems were pinched and bent down to maximize photosynthesizing area. Flowers of commercial value were harvested at the normal opening stage. Stem length, fresh mass and leaf area were measured and flower quality was evaluated on a scale of 1-4, 4 being an excellent flower in commercial terms.

Within each treatment group the moisture tension ($Ψ_m$) of 4 plants per medium was logged at 15-minute intervals, using tensiometers equipped with high-flow ceramic tips and electronic pressure transducers (model “LT”, 15 cm. long, Irrometer Corp. Riverside, CA). In addition to logging on-going moisture tensions, the tensiometers
(inserted to a depth of 10-11 cm, 7-8 cm above the bottom of the container), were used to actuate an automated irrigation system based on tension set points. Usually, the set point for irrigation was 3 kPa. On some days, higher tension was applied to enable determination of RSG over a wider range of moisture conditions. The surface of the medium was covered with aluminum foil to minimize evaporation. The irrigation pulses were of adequate volume to allow some water to drain out of the containers after each pulse, so as to prevent salinity build-up. This was done in order to ascertain that only matric potential and not osmotic potential was serving as the water stress agent. The irrigation solution consisted of half-strength Hoagland solution. The electrical conductivity of the solution was 1.0 dS/m.

2.2. Calorimetric measurements

Calorimetric measurements were made with a CSC (Calorimetry Sciences Corporation, Provo, UT) Model 4100 differential scanning calorimeter operated in isothermal mode in the range 10-35°C for rose buds and 15-35°C for leaflets. Water condensation on the ampoules prevented us from measuring tissue response below 10°C. Young developing buds (upper buds taken from stems, 4-5 days after cutting them back) and leaflets of uniform size and developmental age (ca. 10 mm, 8-13 mg FM, 2.0-3.5 mg DM) were sampled from plants growing in the greenhouse at known Ψm. Once removed from the plant, the samples were kept in a shaded and humid box until the initiation of the calorimetric analysis that started no later than 5 minutes from their detachment and lasted less than 70 minutes. The calorimeter had four removable ampoules, three of which were used for simultaneous measurements of rate of heat production with the remaining ampoule used as a reference. Ampoules were thin-walled cylinders of 1 cm³ volume, constructed of Hastelloy C, with a screw cap sealed with a Viton gasket. Fifty μl of water was dispensed into the ampoules and the leaflets' base was inserted in the water. It is assumed that the RH within the ampoule reached close to saturation shortly after sealing the cap. The RCO₂ was determined via the methods of Criddle et al. (1991) and Fontana et al. (1990). RCO₂ measurements were facilitated using the bottom 3 mm of a thin-walled micro Eppendorf tube. Called a "base trap", these small containers were filled with 40 μl of 0.4 N NaOH and inserted into the ampoules to absorb CO₂ produced by respiring tissue samples. The heat of the exothermic reaction between CO₂ and NaOH forming carbonate was used to calorespirometrically estimate respiration rates. The CO₂ reaction heat rate divided by the change in enthalpy between the reaction of CO₂ with NaOH in the base trap (108.5 J mol⁻¹) was used to determine RCO₂ (Hansen et al., 1994). Once an optimal temperature was determined for young leaflets, they were sampled at this temperature, at different Ψm. At this stage of the work, only a small amount of plant material was removed from the plant for the calorimetric analyses so that the assay was essentially non-destructive for the whole plant. This enabled flower harvests to be conducted according to commercial practices.

3. Results and discussion

Temperature response curves for RSG of sprouting buds and young leaflets are distinctly different from each other (Fig. 1). RSG for buds declines in near linear fashion with increasing temperature, reaching zero at 22°C. RSG for leaves is always higher than that of buds, showing a pattern with a maximum value at 25°C. Since the highest RSG value for buds was measured at the lowest temperature used (10°C) in this study, it is possible that the actual maximum occurs at even lower temperature. Thus optimum growth of young leaflets is assumed to occur around 25°C. Above ~34°C the thermodynamic model for PGR predicts growth cessation. This optimum is very close to known values in the literature regarding optimal temperatures for rose development (Jiao et al., 1991). The response curve of young buds shows a clear preference toward lower temperatures. The model predicts gradually decreasing PGR
with increasing temperature and growth cessation around 22°C. This finding is supported by van den Berg (1987), who found that high temperatures resulted in lighter rose stems of the cultivar "Sonia". His tested range was 9-25°C with an optimum at 13°C. Shin et al. (2000) also observed the longest stems at lower temperature, but found that temperatures below 18°C resulted in lesser shoots, possibly due to the fact that at low temperatures the restricted photosynthetic rate results in reduced amount of biomass for shoot formation. It should be mentioned that our measurements were conducted only with a well-defined young buds and during a narrow time window, while van den Berg's and Shin’s test lasted from the time of releasing the bud from correlative inhibition until harvest. Since most of the cell division is completed within the first few days of bud emergence, one must assume that different optima might be found for more mature stem apices. It is of clear importance, however, that immediately after bud break, when most of the cell division takes place, requires low temperature in order to enhance maximal PGR. This is particularly important for rose growers since it is a common practice among some of them to elevate temperatures after pinching, in order to promote bud break. Our results suggest that if temperatures remain high after bud break, this practice may negatively affect flower quality.

After noting the predicted optimal temperature for PGR of young leaflets to be 25°C, we measured their RSG value at different moisture tensions at this temperature. We chose to analyze the response of leaflets and not that of buds since leaflets are damaged in response to water stress, while no such damage is visible in young buds. The result of this set of measurements is presented in Figure 2. In spite of the large variation, probably resulted from the effect of variable aerial conditions, two phenomena are clearly visible. For both media low moisture tensions are accompanied with maximal RSG values. This fact seems to support the hypothesis that the calorespirometric model might be of predictive value as far as water stress is concerned. In fact, this is the first documented support to this hypothesis. The other finding relates to the difference between the two media. Recently we demonstrated that differences in physical properties between these two media are closely associated with performance of roses growing in them (Raviv et al., 2000). Both specific transpiration rate and yield were affected. The two main influencing properties were unsaturated hydraulic conductivity in the range above ca. 3.3 kPa and free air space at container capacity. Of the observed factors only moisture tensions appeared to affect RSG. It is particularly interesting to note that in UC mix the RSG descends to zero with high tension, while coir-grown plants still have relatively high RSG values even at 20 kPa. It should be noted that a moisture tension of 20 kPa coincides with significant levels of stress in potted plants (Lieth and Burger, 1989). Optimal growing conditions for plants in UC mix has been shown to be between 1 and 7 kPa (Kiehl et al., 1993). Our current work suggests that plants response to tension above 5 kPa is significantly better in coir than UC in mix. This corroborates the findings of Raviv et al. (2000) that coir is a significantly better growing medium at high tensions due to its higher unsaturated hydraulic conductivity.

References


Figures

1. Temperature response curve of young rose leaflets (squares) and young rose buds (diamonds).
2. Specific Growth Rate (RSG) of rose leaflets, grown in coir or UC mix, as a function of moisture tension in the medium.

\[ y = -8.9 \ln(x) + 27.7 \]
\[ R^2 = 0.63; p < 0.01 \]

\[ y = -6.83 \ln(x) + 37.1 \]
\[ R^2 = 0.594; p < 0.01 \]