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Plant calorimetry: how to quantitatively compare apples and oranges

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Abstract

Methods for the study of living plants or tissues by calorimetry have developed to the point that increasingly complex questions about plant physiology can be examined. Equipment now available or being developed for plant studies includes simple isothermal calorimeters with sample volumes from 1 ml to several liters, isothermal perfusion calorimeters, photocalorimeters, differential temperature scanning calorimeters, and calorimeters with multiple sample and CO_2 and O_2 sensing capabilities. Isothermal calorimetry, both with and without perfusion attachments, can be used to examine total metabolic rates of plant samples and to study effects of a wide variety of naturally occurring or artificially added factors on those rates. Moderately large numbers of plants can be examined for properties such as growth rates and tolerance limits to selected stimuli. Short term predictors of long term plant response can aid in selection of desired growth or tolerance characteristics and should prove of value in both classical plant breeding and future uses of biotechnology. Plant metabolism may be modeled with two inputs (photosynthate and oxygen) and three outputs (CO_2, biomass, and heat). Understanding the transform function between input and output requires measuring at least four of the five parameters. Calorimetric measurements in conjunction with measurement of the other input–output parameters provides a quantitative understanding of metabolism in apples and oranges.

INTRODUCTION

In his early experiments on plants, Henri Prat outlined many of the characteristics of plant metabolism and development that could be studied by calorimetric techniques [1]. He made two very important observations that, in contrast to heat generation in animals, (a) "In plants as in microorganisms, the thermic flux is mostly a function of cell multiplication." and (b) "specific features of the thermograms and modifications of thermogenesis due to aging and to physical, chemical and biotic external factors" could be examined to help define processes of growth and differentiation. Extension of these two observations leads to the suggestion that, under appropriate conditions, calorimetry can be used to monitor plant growth rates and the effects of factors that modify growth rate.

To illustrate the validity of these conclusions, Prat demonstrated that seed germination produced distinct patterns of thermogenesis. He then showed that the influence of physical, chemical, and biotic factors on the germination process could be investigated [2,3]. Alcohol and chlorpromazin reduced the rate of thermogenesis of germinating seeds; indoleacetic acid, gibberellin, calcium sulfate, and potassium nitrate increased it. He also examined differences in thermogenesis at different levels of root, stem, and flowering tissues and proposed that such studies could be utilized to examine effects of hormonal actions or additions of inhibitors. These early experiments demonstrated the general nature and feasibility of plant calorimetry in answering many current questions in plant physiology. Prat's success in these early studies prompted him to write in a 1969 review [1] that "we have been able to give only a short account of the innumerable applications of microcalorimetry in the physiological study of higher organisms. The field is large and promising ...".

In spite of this promising beginning, applications of calorimetry to plant studies have been limited. The recent increase in numbers of in vivo biological studies employing calorimetric methods has centered on microbial and animal systems; plants have been largely ignored. Indeed, Wadsö as recently as 1988 stated, "It is striking that very little calorimetric work has been performed on cellular materials from plants" [4]. This situation has now begun to change. With advances in equipment design and methods, there have been important developments in plant calorimetry that confirm and extend the predictions of Prat.

Measurements of plant metabolism are now possible under a wide range of conditions to help define plant metabolic activities. Still, a large portion of the plant calorimetry studies to date have retained a focus on development of calorimetric methods and demonstration of the types of information obtainable, rather than on answering specific questions in plant biology. This review will, accordingly, be directed more towards a review of the past, present, and to some extent, future uses of calorimetry for the study of plants rather than a summary of significant new findings about plant biology resulting from calorimetric studies. We will also focus attention on measurements that can be made simultaneously with heat rate measurements to help define metabolic pathways and bioenergetic efficiencies of plants.

There is an extensive body of literature on calorimetric studies of plant materials or plant products such as vegetable oil oxidations, or fermentive reactions on plant materials. Also, many studies have been conducted on plant membrane lipids and plant proteins to examine transitions that may be related to thermally induced changes in plant metabolism [5,6]. Varner and co-workers [7] have recently demonstrated calcium dependent, thermally induced transitions in preparations of soybean hypocotyl cell wall materials with studies using differential scanning calorimetry (DSC). There are also

reports of combustion calorimetric measurements on dried plant materials which are important in the quantifying of plant metabolic bioenergetics [8]. We shall not include any of these kinds of studies in this review but will focus only on studies of living tissues.

COMPARISON OF APPLES AND ORANGES: AN INTRODUCTION TO THE TYPES OF INFORMATION OBTAINABLE ON PLANTS USING CALORIMETRIC METHODS

Figure 1 illustrates some of the extensive, quantitative, physiological information that can be obtained from a single calorimeter experiment. With such experiments, it is possible to compare important features of the metabolic responses of different plants, such as apples and oranges. A heat conduction DSC with 1 cm^3 sample chambers was used to continuously examine metabolic rates [9] of about 100 mg fresh weight of orange and apple leaf segments over a wide temperature range. The activity profiles indicate major differences in the high temperature stabilities, i.e. apples are more heat sensitive than oranges. This hardly ranks as an unexpected finding, but the experiment is significant because it demonstrates the ability to use simple, rapid calorimetric techniques to quantitatively describe differences in thermal responses of plants. Other important features of the curves are the differences in temperature dependence of the rate limiting steps in metabolism, the temperature and time dependence of the inactivation portion of the curve, and the relative heat rates per gram of leaf tissues. Clearly, the reversibility of the thermal effects, the magnitudes and kinetics of the effects of chemical and biological activators, inhibitors, uncouplers, herbicides, etc. on the heat rates, and the effects of stress conditions on inactivation could also be examined.

WHAT IS MEASURED IN PLANT CALORIMETRY?

Two major metabolic processes can be defined in plants for study by calorimetry, the light driven reactions of photosynthesis and the multiple

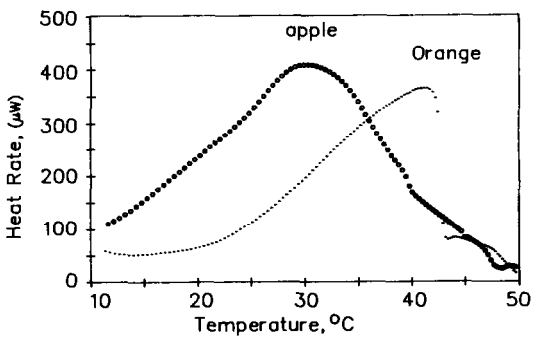

Fig. 1. Metabolic heat rates of orange (· · ·) and apple (○) leaf segments as a function of temperature.

steps of so called dark metabolism. Photosynthesis produces carbohydrates that are subsequently used in a complex series of reactions for plant biomass production. While photosynthesis remains the major subject of study by most plant scientists examining bioenergetics and plant productivity, it has not been a major focus of calorimetric studies because of the obvious difficulties in dealing with the heat associated with illuminating a plant in the calorimeter. However, this is not an insurmountable obstacle. Innovative, state of the art instrumentation will almost certainly bring about a change in this situation. Some steps in this direction have already been taken. In 1939, Magee et al. [10] constructed a photocalorimeter for measurements of the quantum efficiency of photosynthesis in algae and demonstrated that the potential exists to conduct a wide range of energy measurements on green plants in the light. Further indications of this possibility come from studies by Cooper and Converse [11] who modified an LKB batch calorimeter with fiber optics for thermochemical studies of rhodopsin and by Teixeira and Wadsö [12] who described modification of an LKB 2277 4-channel heat-conduction microcalorimeter to introduce monochromatic light via fiber optic guides for study of photochemical processes in solution.

Studies of metabolism in the dark can be carried out with much simpler equipment using any plant tissues with sufficient stored photosynthate to last the duration of the experiments, or using those that can be exogenously supplied with the necessary substrates during study. While it will be important to be able to study plant bioenergetics by calorimetric methods in the light as well as in the dark, in many instances nonphotosynthetic reactions may well be preferable indicators of plant growth rates and responses to many stresses. Calorimetry may prove to be uniquely suited for studying these subjects. Unless stated otherwise, the remainder of the studies reviewed in this paper are studies of metabolism occurring in the dark.

The output data from calorimetric measurements on living plant tissues is the rate of heat production as a function of time, temperature, or other experimental variable. The values of heat rate/mass of tissue are proportional to metabolic rates and can be compared for different plants, tissues, growth states, environmental conditions, etc. Most rapidly growing plant tissues produce heat rates of about 10 to 30 $\mu W\ mg^{-1}$ dry weight or 1 to 2 $\mu W\ mg^{-1}$ wet weight when measured at 25 °C.

THE NATURE OF PLANT MATERIALS THAT HAVE BEEN STUDIED BY CALORIMETRY; SAMPLE PREPARATIONS, SPECIAL TECHNIQUES, AND PROBLEMS

Calorimetry of plant tissues has been conducted using cells in liquid culture, callus cultures, root tips and other tissue cuttings, leaves, and whole plants. Three major questions must be considered prior to studies with any

plant material. First, can the tissues be maintained in a (near) viable state long enough to measure metabolism? Second, does the tissue sample produce a measurable metabolic heat under desired test conditions? Third, can conditions be maintained during measurement to ensure that any heat contributions caused by microbial growth are negligible? Satisfying these conditions for plant tissues is generally much simpler than for animal tissues.

Maintaining tissues in a viable, rapidly metabolizing state requires an adequate supply of nutrients and oxygen. Energy reserves must be supplied via a nutrient medium, stored photosynthate, seed reserves, etc. Generally, plant cuttings from light grown plants have sufficient reserves for several hours of study in the dark without supplying additional substrates. For cultured tissue, an adequate supply of water, minerals, and nutrients can generally be maintained by addition of small amounts of nutrient solution to cells in the calorimeter ampules.

Oxygen supply to the tissues is a more critical limitation to metabolism, since closed sample chambers are necessary to avoid the large heat effects associated with evaporation of water. Fortunately, metabolism of most plant tissues appears to be relatively independent of atmospheric oxygen levels until oxygen is nearly depleted, making it unnecessary to provide constant oxygen levels [13]. With an appropriate balance of ampule volume, tissue mass, and heat rate, the oxygen present in headspace gas is often adequate for several hours of calorimetric measurements. When measurements are to be prolonged, starting with a pure oxygen atmosphere generally has no measurable effect on plant metabolic rates, yet extends measurement times 5 fold. Interrupting measurements to resupply oxygen to the tissues is possible, as is continuous gas perfusion [14,15]. Assuming a constant heat rate per mole of O_2 allows a simple relationship to be defined between heat rates of metabolizing tissue and oxygen consumption rates that allows approximations of time to depletion of oxygen in given test conditions [9].

A problem more limiting than total oxygen supply in the sample containers is ensuring that tissues have adequate access to the oxygen that is available. With tightly packed tissues, or tissues such as roots or cell cultures that may be submerged in unstirred liquids, diffusion of oxygen to the plant materials can become limiting. This is often avoidable by using less tissue, by providing soluble nutrients to tissue sections via wetted fiber filters upon which the samples rest so that samples are not submerged, or by floating cells at the air–media interface by using media supplemented with an inert, high density preparation such as Percoll [16].

Calorimeters have been constructed to minimize nutrient supply problems by perfusion of liquid media through the sample vessel to ensure nutrient and oxygen supply (see refs. 4, 15, 17–19). Such perfusion equipment also allows addition of plant growth effectors to define tissue responses. Anderson et al. [20,21] have examined the effect of indole-3-acetic acid (IAA) on

the metabolism of corn with such apparatus. Perfusion capabilities have been essential for the calorimetric study of many animal tissues and offer some distinct advantages in long term plant studies or in studies requiring constant addition of test substances to the plant tissues. The disadvantages are a limited throughput in sample numbers for comparative or genetic studies, the inability to obtain thermal scans or rapidly change isothermal temperatures and, usually, increased calorimeter baseline noise. Studies of plant cells using flow calorimetry are hampered by the tendency for plant cells to clump, become inhomogeneous in the cultures, and to clog flow lines.

While microbial contamination is a serious problem that can never be overlooked, careful experimental procedures can effectively eliminate significant microbial contributions to the experimental heat rate measurements. With the exception of cells in culture, most plant samples are not sterile when introduced into the calorimeter. The potential for bacterial contribution to heat rate measurements is significant. In general, with intact tissues and sections, contamination can only be minimized, not eliminated. Surface sterilization of tissues and germinating seeds with dilute hypochlorite, minimizing immersion of tissue sections in liquids, general care in laboratory techniques, and avoiding solution conditions that favor bacterial growth are generally sufficient to ensure small contributions by microorganisms that may be neglected. Microbial inhibitors can be used, but such inhibitors are not highly specific and frequently influence plant metabolism.

Obviously, care must be taken in all studies, and particularly during longer experiments, since an initially low level of microbial contamination coupled with the short generation times of microorganisms may result in a significant metabolic heat output before an experiment is finished. Such growth does, however, produce a characteristic exponentially increasing heat rate superimposed on the plant metabolic rate and can generally be recognized. All samples should be examined microscopically at the conclusion of an experiment to demonstrate the absence or presence of microorganisms.

VARIATIONS IN METABOLIC HEAT RATES AMONG TISSUES AND SPECIES OF PLANTS

Simple isothermal measurements of heat rates per milligram for various tissues provide interesting insights into the wide range of differences in physiological states and biochemical efficiencies of plant tissues. For example, the heat rates per milligram for leaf tissues vary with leaf development. Young, expanding leaf tissues have higher heat rates per milligram and respiration rates than older, fully formed tissues [22,23]. Differences in heat rates per milligram are particularly notable with evergreen leaves or needles, which allow comparisons of metabolic heat rates of different aged leaves of a single plant. Comparison of needles from rapidly expanding current year's

growth of coast redwood (*Sequoia sempervirens*) with needles from the previous 2 years' growth shows that heat rates per milligram decrease to about 1/3 and then to 1/6 respectively [23]. Buds from completely dormant (continuously exposed to temperatures below −29 °C for 3 weeks) tamarack (*Larix laricina*, a deciduous conifer) have been studied and shown to give heat rates of 8 $\mu W\ mg^{-1}$ within 30 min of being raised to 25 °C [24a]. Answers to such questions as: what metabolic paths are involved?; what is the physiological function of this metabolism?, etc. are all unknown.

Heat rates per milligram of certain specialized plant tissues differ widely from typical plant values to raise even more perplexing questions about energy metabolism. Tissue from the spadix of voodoo lily (*Sauromatum guttatun*, Schott) has an extraordinarily high heat rate near 1 $mW\ mg^{-1}$ (wet weight) when measured under optimum conditions during the high rate of thermogenesis present in the odiferous stage of flowering [24b]. This value is 500 to 1000 times the average for most plant tissues examined.

Observations of such differences among plants and tissues will remain largely phenomenological until more fully understood. The coupling of calorimetry to additional measurements of respiration, discussed below, promises to make this possible.

BIOENERGETICS AND PLANT GROWTH

The basis for relating calorimetric measurements of dark metabolic heat rates to plant growth rates and efficiencies arises from the following considerations [22,25]. The growth rates of many plants under optimum nutrient conditions with ample light supply do not appear to be limited by photosynthetic rates. "Photosynthesis has been found to be a relatively unimportant character in determining variation in yield and growth rate." [25]. Instead, growth rates depend on the rates at which the products of photosynthesis can be utilized by metabolic reactions in forming cellular biomass [26–29].

One major determinant of the rate of biomass production is the absolute metabolic rate. A second determinant is how effectively plants utilize metabolic energy. Both of these are measurable by calorimetry combined with determination of the fluxes of substrates and products through plant metabolism.

Plants may differ greatly in efficiency of ATP production and in the efficiency of ATP utilization in biomass production. With inefficiency in either case, the fraction of the total photosynthate required to produce the high energy phosphate compounds necessary for growth will increase. The fraction remaining for biomass production will be reduced accordingly.

In the dark, metabolic heat measurements on meristem tissue examine the total metabolic utilization of available photosynthate for plant growth. To the extent that the metabolic efficiency of conversion of photosynthate to biomass is constant for a given species at specified conditions [24a,30–33],

variability of metabolic heat rates is an indicator of genetic potential for growth. It has been shown that when rapidly growing tissues are studied, significant positive correlations often exist between growth rates and various measures of dark metabolic rates [22,24a,33]. When a significant inefficient component of metabolism prevails within a plant, this correlation may disappear or even be negative.

In a study of rapidly growing meristem tissue from branch tips of larch trees [24a], it was shown that, (i) there is a wide variation of metabolic rates among larch clones, even among populations that have been selected for growth rate by traditional methods, (ii) for some populations a correlation exists between measured metabolic rates and growth rate of trees in field test plots, and (iii) in these populations the metabolic rates could probably be used to select trees with a potentially fast growth rate. Thus, differences in efficiencies among trees of this population were not sufficiently large to mask a general relationship between metabolic rates and growth rates. Another study has shown that the relative growth rate potential of coastal redwood (*Sequoia sempervirens*) clones can also be predicted from measurements of relative metabolic heat rates [33]. Calorimetrically measured metabolic heat rates of meristem tissue were found to be linearly related to the growth rate of both 60-day-old unrooted clones and 25-year-old trees. Although further development of the method is necessary, it appears that calorimetric measurement of the metabolic heat rate may be developed in some instances into a general, rapid, and simple method for culling trees of low growth potential. Based on the probable error in predicted diameter of redwoods, the method should be useful for removing about 80% of a normal population at a 95% confidence level. Because only very small amounts of material are required, the calorimetric measurement can be made while the trees are still in the seedling stage and before major cloning or propagation work has begun.

Though a correlation exists between growth rates and metabolic rates of growing tissues in many samples tested, the data show significant scatter and some major outlier points, confirming the hypothesis that efficiency or other factors must be determined to better define the relationship. We have postulated that the metabolic efficiency of a plant can be calculated from knowledge of the metabolic heat rate, the rate of production of carbon dioxide, and the rate of consumption of oxygen, but the mathematical relationship between metabolic efficiency and these three parameters is not yet fully defined. Nevertheless, we have shown that measurement of these three parameters allows an empirical development of models that fit growth data sufficiently well to predict the known growth rates of several corn cultivars and clones of larch trees [24a].

When alternate pathways of metabolism become important or when maintenance requirements contribute a significant portion of the total energy production of the cells, increasing respiration may not translate into

increased biomass formation. An example of a weak negative correlation between respiration and plant growth has been presented by Wilson and co-workers [34–36] who point out that, "during early stages of crop development, plant energy utilization is dominated by the requirements for cell division and expansion and those for maintenance are relatively small. However, as the crop increases in size, so does the proportion of fully developed, degradable plant material requiring maintenance". Differences in respiration measured on mature leaves then largely reflect maintenance requirements. Those plants with minimal maintenance requirements can expend a larger portion of their total energy production on growth and it may be expected that a negative correlation between maintenance energy requirements and growth rate would be observed. Wilson and co-workers have demonstrated that selection of ryegrass plants with low rates of mature leaf respiration can lead to significantly increased growth yields in field studies, showing the importance of further studies in this area [35].

Failure to carefully specify the age of the tissue and an inability to separate maintenance and growth components of respiration have led to much confusion in the literature concerning correlations between growth rates and metabolic rates. Calorimetry will have a major role to play in properly defining this correlation. Calorimetric measurement in conjunction with substrate and product flux analysis is currently the only feasible means for measurement of efficiencies.

While it is possible that further selection of plants with low maintenance energy requirements or with limited alternative pathway capacity could increase yields of plants, such selections could also produce negative effects. For example, plants with limited capacity for development of alternate pathways of metabolism could have enhanced sensitivities to certain stress conditions such as cold exposures.

Simultaneous measurements of the rates of heat production, CO_2 production, and O_2 consumption by living tissues provide a useful means of detecting and quantifying metabolic rates and general features of the metabolic pathways active in the tissues. The ratios of heat rate to the rate of CO_2 production, or to the rate of O_2 utilization, have been called the calorespirometric ratio, calorespirometric constant, the oxycaloric equivalent, and the indirect calorimetric constant (without always distinguishing whether the ratio referred to was obtained with O_2 or CO_2 measurements). This ratio has a value of approximately 460 kJ mol^{-1} for most animal tissues with tightly coupled, aerobic, steady-state metabolism [37], but is not a constant in other organisms and varies widely in plants. Even individual plants of the same species can have widely varying values for this ratio [24b].

The experimentally determined ratio for any tissue depends on the stoichiometry and metabolic heat associated with each of the different metabolic pathways in the integrated metabolism. Consequently measurements of the rates of CO_2 production and of O_2 consumption, along with

metabolic heat measurements contain information on tissue metabolic pathways [17,38–41] and efficiency of energy utilization. The rate of heat production by respiring plant cells has not generally been measured directly. Most studies that have included heat rate considerations have simply calculated a value from O_2 consumption rates and an assumed calorespirometric constant. Assuming a constant value for heat produced per oxygen metabolized is not acceptable for plants.

Measurements of the rates of change of CO_2 and O_2 can be made directly in calorimetric reaction vessels, most commonly using microelectrodes [17,42]. Such measurements are readily performed in circumstances where the electrode can be submerged in a uniform, stirred solution in the reaction vessel. These electrodes do not function well, however, if the solution cannot be stirred, if tissue samples are solid, or if insufficient liquid is present to cover the electrodes. Accordingly, electrodes do not work well in small microcalorimeter vessels and with plant tissue samples. Other types of sensors for CO_2 and O_2 generate too much heat or are too large to be used within the reaction vessel or require gases to be flushed through the vessel and into the detector, causing attendant problems in heat measurements.

Calorimetric measurements have recently been used to determine CO_2 production rates by measurement of the heat resulting from reaction of metabolic CO_2 with a base in a separate container in the calorimeter reaction vessel [43]. Determination of the rate of CO_2 production by this method requires two measurements, one of the metabolic heat rate and one of the sum of the metabolic heat rate plus the heat rate from reaction of the CO_2. The difference between the two measured values gives the rate of CO_2 production. Figure 2 schematically illustrates how measurements are made and Fig. 3 illustrates the results for an isothermal experiment. Temperature scanning experiments can also be done to provide a measure of the CO_2 production rate as a continuous function of temperature. The detection limit for measurement of CO_2 production rates by this method is similar to

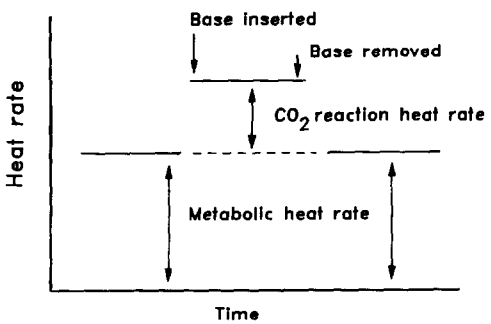

Fig. 2. Method for determination of CO_2 and heat production in calorimetry. (Adapted from ref. 43 with permission.)

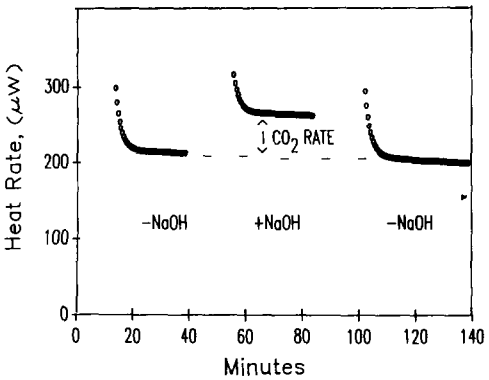

Fig. 3. Determination of CO_2 production rate for corn meristem tissue. (Adapted from ref. 43.)

detection limits reported for commercially available respirometers of various types [44–46].

When using the calorimetric method for determination of CO_2 production rate, it is convenient to express experimental results from CO_2 and metabolic rate measurements as a unitless ratio, R,

$$R = (q_{met} + q_{CO_2})/q_{met} = 1 + q_{CO_2}/q_{met} \quad (1)$$

where q_{CO_2} is the heat rate produced by the CO_2 reaction and q_{met} is the metabolic heat rate. R is obtained directly from measured values (see Fig. 2). From eqn. (1) it is apparent that

$$(R - 1) = q_{CO_2}/q_{met} = (-\Delta H)/R_c \quad (2)$$

where R_c is the calorespirometric ratio and ΔH is the enthalpy change for reaction of CO_2 with the base. The commonly reported ratio of 460 kJ mol^{-1} for animal respiration combined with the $-\Delta H$ value for CO_2 reaction with 0.4 M NaOH gives a value of $(R - 1)$ of 0.24.

In the presence of a CO_2 absorber in a sealed vessel, uptake of O_2 results in a pressure decrease. Thus, a sealed calorimeter vessel can be used to simultaneously measure metabolic heat rate, CO_2 evolution rate, and O_2 consumption rate [47]. Measurements of oxygen uptake by pressure changes and gas trapping methods for both O_2 and CO_2 date back to instruments described by Barcroft and Haldane [48] and to Warburg [49].

Figure 4 illustrates the measurement of heat rate, O_2 consumption rate, and CO_2 production rate by corn shoots in a large volume (75 cm^3), isothermal calorimeter [47]. The curves of heat rate show an initial period (about 1.5 h) of changing power output as the calorimeter approaches thermal equilibrium, followed by a steady state rate indicative of the metabolic heat rate of the tissue. Reliable pressure measurements were obtained within about 10 min after closing the calorimeter.

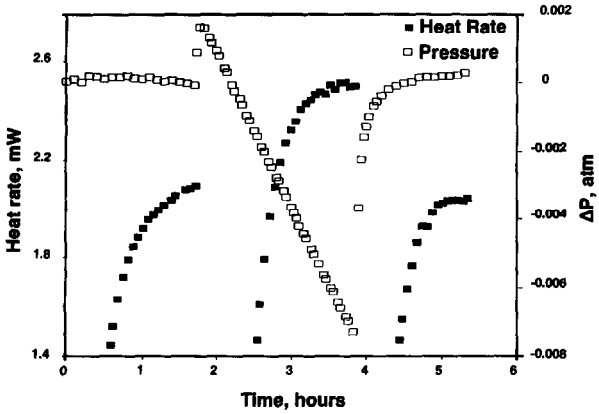

Fig. 4. Heat rate, O_2 consumption rate, and CO_2 evolution rate measurements in a large volume (75 cm^3) calorimeter at 25°C for 10 corn seedling axes from seed corn germinated 90 h at 25°C. (Reproduced from reference 47 with permission.)

As seen in Fig. 4, the pressure remains nearly constant during the initial measurements of heat rate of this corn tissue, when there is no CO_2 trapping agent present. The small pressure change with time indicates a near equality in the flux rates of CO_2 and O_2. Other plant species, and even some different inbred lines of corn tested under these same conditions, do not show an equality between O_2 consumption and CO_2 production during metabolism, i.e. the respiratory quotient (RQ) is not equal to unity. The slope of the pressure versus time plot in the absence of a CO_2 trap is a sensitive indication of the sum of O_2 and CO_2 rates.

After a NaOH trap was added at about 1.8 h, a period of thermal equilibration was again noted as indicated in Fig. 4. Then, a steady state heat rate was obtained that was higher than the value for tissue alone. The differences of heat produced by samples with and without NaOH present represent the heat rate due to trapping, and consequently the rate of CO_2 formation. A linear decrease in pressure reflecting O_2 uptake is evident within 5 min of the time of NaOH addition. Accordingly, the O_2 consumption rate can be determined and compared with CO_2 rates and with the measured heats of metabolism.

As a check on the CO_2 rate measurements and the stability of metabolism during measurements, ampules containing the sample are generally opened again, and the NaOH is removed. When the NaOH was removed, the heat rate returned to the initial steady state value for the metabolic rate of the corn tissue, and pressure again remained constant.

Repeated tests of heat rates per milligram and of the heat rate per rate of O_2 or CO_2 on different tissue samples from the same plant or from plants with the same genotype show high reproducibility. Repeated heat rate per milligram tests with corn seedlings from a single inbred line demonstrated a standard deviation of 5%; and the ratios of heat rate per CO_2 rate have a

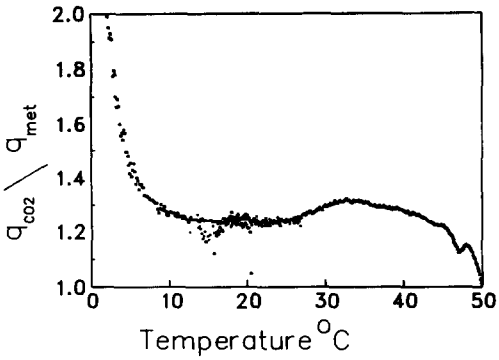

Fig. 5. Changes in $(R-1)$ with temperature for tomato leaflets. (From ref. 43.)

standard deviation of 4%. In contrast, measurements of heat rates per milligram of tissue from 14 different inbred lines of corn grown under identical conditions gave values differing by as much as 28% and heat rates per mole O_2 or CO_2 varied by as much as 2 to 3 fold.

$(R-1)$ can also vary for an individual plant in response to stress when alternate metabolic pathways are engaged or induced. Measurement of this value can provide important information about plant metabolic differences and their responses to stress. Figure 5 combines the results of two temperature scanning experiments on tomato leaves. One experiment scanned temperature upward from 20 °C and the other downward from 25 °C. $R-1$ is approximately constant through the mid-range of normal growing temperatures, then varies as metabolic pathways vary at both high and low temperatures. The various temperatures, i.e. near 12, 32, and 45 °C, at which alterations in metabolic pathways occur are clearly evident in these data.

Heytler and Hardy [50] demonstrated an important use of adiabatic calorimetry in bioenergetic studies when they examined metabolic costs associated with the symbiotic N_2 fixation system. The heat production from isolated soybean nodules infected by *Rhizobium japonicum* was measured for various nitrogenase substrates. The metabolic cost of nitrogen fixation was determined to be 9.5 g glucose per gram of nitrogen fixed or 3.8 g of carbon per gram of nitrogen fixed.

TEMPERATURE DEPENDENCE OF PLANT METABOLIC RATES

Temperature dependences of plant respiration rates have long been of interest to plant physiologists seeking to describe plant responses, to examine ranges of thermal stability, and to infer mechanisms of plant responses to temperature [5]. The measurement of temperature dependence of plant metabolism yields an apparent activation energy (E_A) for the rate limiting steps of metabolism. Because of the complex nature of the interacting metabolic reactions, temperature dependences are commonly reported

simply as Q_{10} values, the factor by which respiration changes with each 10 °C change in temperature. Over physiologically relevant temperature ranges for most plants Q_{10} is about 2 [51]. Values of Q_{10} do, however, vary considerably among different plants and for individual plants grown at different temperatures [52], for different temperature ranges of measurement [53], for different substrate levels [54], and also may differ with season of the year [55]. Reports of Q_{10} values should always be accompanied by a report of the methods and conditions used to obtain these values.

Amthor [22] suggests that due to such universal differences in Q_{10}, even among genotypes of the same species, research on respiration should be conducted at several temperatures. It should be added that many of the reported changes in Q_{10} may also be a strong function of how respiration was measured. Changes in the respiratory quotient, RQ (rate of CO_2 production per rate of O_2 consumption) often accompany changes in temperature, indicating that respiration measured as CO_2 production or as O_2 consumption would also differ. While calorimetry is an ideal method for measuring temperature dependences because of the intrinsic accuracy of temperature control with this method, the values of Q_{10} obtained are just as method dependent as values obtained from O_2 and CO_2 measurements.

Isothermal calorimetric methods now allow relatively rapid measurements of the temperature dependences of the metabolic rates of many plants [13,24]. The values of apparent E_A for perennial (but not annual) plants fit a rather distinct pattern. Cold climate plants, from either northern latitudes or from high elevations, have apparent E_A values significantly lower than the values for plants from warmer climates (i.e. metabolic rates of cold climate plants are less dependent on temperature than their warm climate counterparts). Some examples are presented in Table 1 [56]. Tomato, a chilling sensitive plant, has a much higher apparent E_A below about 12 °C. The actual value for tomato at low temperature is not determinable because of a time dependent irreversible inactivation of tissue below 12 °C.

TABLE 1

Organism	E_A/R [a] (K)
Coast redwood	8.3
Sierra redwood	5.0
Southern black spruce	8.4
Northern black spruce	7.1
Southern magnolia	7.3
Northern magnolia	5.5
Tomato, 12–32°C	6.6
0–10°C	>11 [b]

[a] R is the gas constant.
[b] Time dependent [57].

It should be noted that the cold climate plants are generally subjected to a much wider range of temperatures and to much larger rates of temperature change than the warm climate plants. Regulation of metabolic activity over this wide temperature range may be simpler when temperature dependences are smaller. If temperature dependence of metabolism can be related to the activation energy of rate limiting enzymes, the decreased E_A of cold temperature plants indicates generally more efficient catalysis by enzymes in the cold weather plants. This is a question worth further serious study.

Calorimetry has become a major tool in contributing to the understanding of thermal injury of plants. The rate of loss of metabolic activity of plants at high temperatures (but less easily at low temperatures due to low metabolic heat rates) can be examined isothermally by rapidly raising temperatures to a selected value and observing the rate of activity decrease. The rate loss at different temperatures can give a general picture of rate of activity loss as a function of temperature. Using this technique, Criddle et al. [13] have studied tomato cells above 36 °C. Loss of metabolic activity with time follows first order kinetics. These workers have also looked at barley root tissues exposed to various high temperatures to follow the activity loss with time, the effects of salt on the high temperature loss, and the reversibility of high temperature losses [58,59].

Loseva et al. [60] used isothermal calorimetry to examine the effects of high temperature on wheat germination. They defined characteristic portions of the thermogram of germinating seeds that may serve as indicators of heat resistance of the plants. It was proposed that comparative evaluations of heat resistance by these methods could be used for selection of seeds for agriculture.

Wade et al. [61] have followed the germination of soybean seeds at two different temperatures in a fashion similar to some of the early studies of Prat [1–2]. They monitored differences in intraspecies responses of the germinating seeds at 30 °C and suggested that these arise from differences in metabolic efficiencies.

Studies by Rank et al. [57] reported development of a temperature cycling method that extends the isothermal methods for evaluating thermal stabilities of plants. The method was used to examine the ability of plants to recover from high and low temperature stresses. Cells and leaf tissues in calorimeter ampules were rapidly cycled between a reference temperature in the mid-range of thermal stability, a selected high or low stress temperature, and back to the reference temperature to examine the amount of recovered activity. This cycling of temperatures was continued so that plots of activity decrease versus time of exposure to the test temperature could be produced. Additional experiments were run using different test temperatures and the data combined to produce response surface plots of the type shown in Fig. 6. Both high and low temperature exposures of the tomato cells produced the same general form of response surface plot.

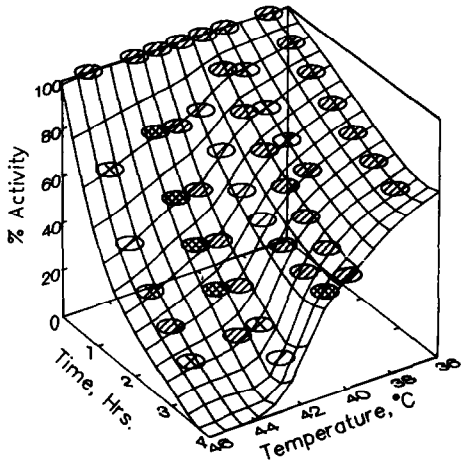

Fig. 6. Time–temperature surface function for high temperature inactivation of tomato cells. The time value plotted is the time at the elevated temperature. Metabolic heat rates were measured at 25°C and as such are a measure of the recovered activity after exposure to the stress temperature. (From ref. 58.)

Development of temperature scanning methods to follow plant metabolism as a continuous function of temperature [9] now allows ready definition of the operational temperature ranges for normal plant metabolism, identification of temperatures at which metabolism is altered, and preliminary indications of the nature of biochemical events responsible for high and low temperature inactivation. Figure 7 shows activity versus temperature profiles for 1 cm cuttings from growing branch tips from three clones of coast redwoods. These clones are all growing at a plantation near Berkeley, CA as a representative collection cloned from forest trees growing at locations throughout the range of native redwood growth [23]. All trees have similar

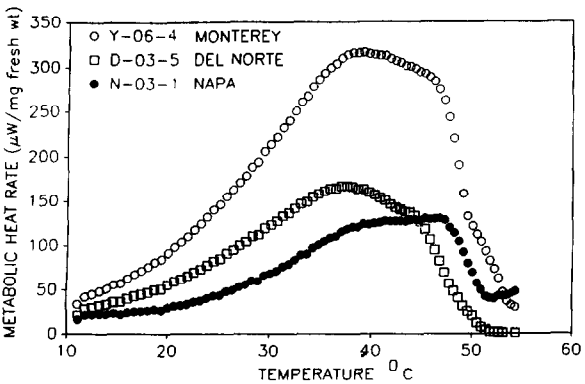

Fig. 7. Metabolic heat rate as a function of temperature for coast redwoods cloned from trees originating in Napa county (•), Monterey county (○), and Del Norte county (□).

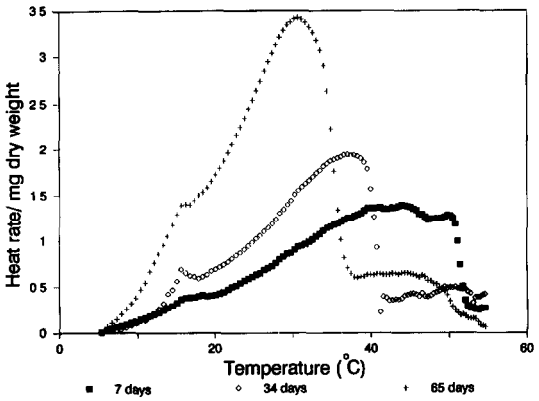

Fig. 8. Metabolic heat rates for germinating peach seedlings as a function of increasing temperatures. The temperatures of maximal activities were related to the length of vernalization treatments (7, 34, or 65 days).

temperature coefficients in the mid-range of their normal growth temperature range. There are obvious differences in high temperature activity profiles and maximum temperatures for stabilities of these trees. The differences reflect accurately the differences in average high temperatures experienced at their native locations in California. The range of difference showed the temperatures for maximum activity of northern samples to be about 4 °C lower than those for trees from the southern end of the range. These redwood trees from a relatively narrow geographical distribution have genetically adapted high temperature stress limits that are specific to local conditions and are maintained during growth at a common location. Such studies can guide successful selection of trees for transporting to other regions.

In another series of scanning calorimetry experiments, vigor and thermal tolerance of peach seedlings were examined as a function of the duration of vernalization treatment before germination. A quantitative relationship was established between vernalization time and vigor measured as metabolic heat rates by isothermal calorimetry, and between vernalization time and high temperature sensitivity as measured by scanning calorimetry (Fig. 8) [62]. The longer vernalization treatments gave greatly enhanced seedling vigor and lowered the maximum for high temperature stability by as much as 20 °C.

With such scanning calorimetry techniques, it may be possible to select from existing plants those strains suitably matched to various climatic requirements. However, it will require an understanding of the mechanisms of temperature induced stress responses in plants and of the operation of the primary temperature sensors in plants, if progress is to be made in molecular genetic modifications of plants for increased thermal tolerance. Currently

there is a limited understanding of what constitutes high and low temperature resistances and how temperature stresses result in injury.

RESPONSES OF PLANTS TO METABOLIC EFFECTORS

The metabolic heat rate of plant tissue appears to be an accurate and sensitive measure of stress response in the whole plant under field conditions. Increasing stress is generally indicated by decreasing metabolic heat rate in the studies done to date. Stresses studied by calorimetry are salt, high and low temperature (discussed earlier in this review), aluminum, and air pollutants.

The effect of salinity stress on metabolic heat output of barley root tips (*Hordeum vulgare* L.) has been measured by isothermal microcalorimetry [63]. Several barley varieties differing in tolerance to salinity were compared and differences were quantified. Figure 9 shows the data obtained as a function of salt concentration. Two levels of sensitivity to increasing salt were found. During the first activity transition, the metabolic heat rate decreased to about 50% of that of the zero salt treatment and leveled off. The concentration of salt required for the first transition was cultivar dependent. A second level of sensitivity occurred at high concentrations of salt (≈ 200 mM) and was not cultivar dependent. A first order decay in metabolic rate with time of salt exposure was found. The inhibition was irreversible in these experiments.

Cyanide, azide, and dinitrophenol all caused major reductions in heat rates. The decreased rate of metabolic heat output of the first metabolic heat rate transition (Fig. 9) paralleled decreases in uptake of NO_3^-, NH_4^+, and inorganic phosphate that occurred as the salt concentration was increased.

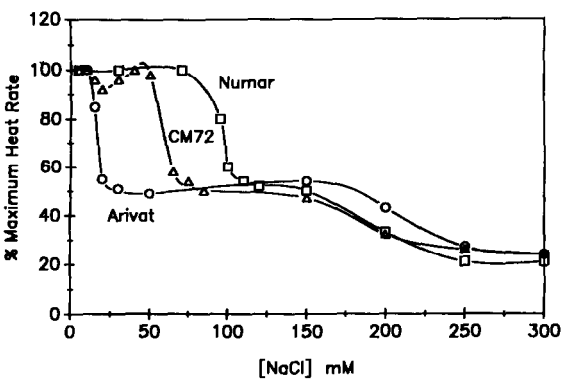

Fig. 9. Effects of NaCl concentration on metabolic heat rates of Arivat (○), CM 72 (△), and Numar (□) root tissues. Data for these experiments were all normalized to set rates at low salt to 100% and thus eliminate heat rate differences between samples due only to differing mass of root tissue. (Reproduced from ref. 63 with permission.)

Ca^{2+} was found to be protective against salt damage. Salt inhibition was shown not to be due simply to osmotic effects. Mannitol at 300 mM did not have any measurable effect on metabolic heat rate.

An unpublished study [56] on cottonwood root tissue showed a response in this species similar to that in barley. Forage Kochia, a desert shrub, responds differently, however. The metabolic rate was shown to decrease approximately linearly with both salt and mannitol concentration, although the response was quantitatively less with mannitol at the same osmotic pressure [56].

The effects of Al^{3+} on barley root metabolism are detectable by isothermal calorimetry as a decrease in metabolic heat rates relative to controls, at concentrations as low as 8 μM [24b]. Inhibition patterns obtained resemble those noted by others studying aluminum effects on root elongation [64].

An important research area in plant physiology to which calorimetry may make major contributions is the study of plant response to natural growth regulators. To date, little work has been done in this area. Bogie et al. [65] were the first to report effects of IAA promotion of metabolic rates by calorimetric studies of oat (*Avena soliva*) coleoptiles. More recently Anderson et al. [20] studied effects of IAA on elongation of corn (*Zea mays*) coleoptile segments as a function of time using flow calorimetry techniques [21]. They were able to describe the time dependence of IAA induced changes in heat production as well as the IAA concentration threshold of the response. Significant stimulatory effects were measured with IAA concentrations from 10^{-7} to 10^{-5} M. IAA became inhibitory with further increase in concentration to 10^{-3} M. The flow system allowed continuous measurements over a period of several hours on approximately 250 mg (wet weight) tissue, with time resolution in the 1 min range. A substantial portion of the effects on tissue metabolism was shown to occur within 20 min after IAA addition. The flow apparatus used also allowed alteration of solution pH or nutrients in the perfusion liquid, providing a flexibility that will allow a large number of further interesting plant studies to be performed.

Bower [66] has used isothermal calorimetry in a study of atmospheric pollutants on plant activities. He examined ozone injury to Ponderosa and Jeffrey pine needles. Using 1 cm needle segments he demonstrated a correlation between the extent of ozone damage, measured as lesions on the needles, and metabolic heat rates. He also measured increases in metabolic heat rates resulting from acid deposition on the needles. The response was more dependent upon the composition of the deposition than on the pH. Nitrate containing deposits had the largest effects, suggesting that nitrogen uptake through the needles may have been the primary contributor to the increased metabolic rate.

The metabolism of cauliflower florets has been followed isothermally [67]. The results illustrate another important consideration for plant studies in closed ampules. The cauliflower tissues appear to produce gas-phase meta-

bolic effectors that activate or inhibit metabolism depending upon concentrations. The effects of CO_2 build-up within the closed ampules on tissue activities must also be considered.

Studies of the effects of salt and other effectors are greatly facilitated by the ability to exchange the solution bathing the tissues without opening the calorimeter vessel. The flow calorimeter of Anderson and Lovrien [21] provides a convenient method for this. With some calorimetric equipment, addition of effectors can be done simply by addition of small bore tubing and syringes to the calorimeter [67].

SUMMARY

Methods for the study of living plants or tissues by calorimetry have developed to the point where increasingly complex questions about plant physiology can be examined. Equipment now available or being developed for plant studies includes simple isothermal calorimeters with sample volumes from 1 ml to several liters, isothermal perfusion calorimeters, photocalorimeters, differential temperature scanning calorimeters, and calorimeters with multiple sample and CO_2 and O_2 sensing capabilities.

Isothermal calorimetry, both with and without perfusion attachments, can be used to examine total metabolic rates of plant samples and to study effects of a wide variety of naturally occurring or artificially added factors on those rates. Moderately large numbers of plants can be examined for properties such as growth rates and tolerance limits to selected stimuli. The results can aid in selection of desired growth or tolerance characteristics and should prove of value in both classical plant breeding and future uses of biotechnology. Additional plant studies are required to further determine conditions in which calorimetric studies can best serve as reliable short term predictors of long term plant responses.

Scanning calorimetry is particularly useful for defining temperature sensitivities and discovering temperature related plant responses, but it also contributes to an understanding of the underlying mechanisms of the responses. For example, a physical change in the state of some plant component is evident in a scanning experiment but does not appear in isothermal data collected at various temperatures.

A model of metabolism in plants has two inputs (photosynthate and oxygen) and three outputs (CO_2, biomass, and heat). Understanding the transform function between input and output requires measuring at least four of the five parameters. Past thermodynamic models of plant metabolism have been written in terms of free energy changes, but have not proven as useful as desired because free energy changes are path dependent for irreversible processes and cannot be determined without knowledge of cell component concentrations and equilibrium constants at intracellular conditions. What is needed in the future, and can be developed using calorimetric

measurements in conjunction with measurement of the other input–output parameters, is an enthalpic model. Entropy changes are readily measurable state functions even for irreversible processes [68]. Thus, a quantitative understanding of metabolism in apples and oranges will require calorimetric measurements combined with a variety of other measures of plant metabolism.

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