ELSEVIER

Thermochimica Acta 309 (1998) 5–16

thermochimica acta

Direct and indirect calorimetry of thermogenic flowers of the sacred lotus, *Nelumbo nucifera*[1]

Ingolf Lamprecht[a,*], Roger S. Seymour[b], Paul Schultze-Motel[b]

[a] *Institute for Biophysics, Free University of Berlin, D-14195 Berlin, Germany*
[b] *Department of Zoology, University of Adelaide, Adelaide SA 5005, Australia*

Received 19 June 1997; accepted 17 July 1997

Abstract

Direct and indirect calorimetric experiments were performed on flowers of the sacred lotus, *Nelumbo nucifera*, and compared with temperature measurements. To this end, a simple, light and cheap heat-flow calorimeter of the twin type was developed to monitor the heat output of lotus flowers in an outdoor pond. Each side of the calorimeter consisted of a water jacket as a heat sink surrounding a 730 ml concentric can as a calorimetric vessel. The vessel and heat sink were thermally connected *via* a Peltier element but otherwise thermally isolated. Both water jackets were housed in a styrofoam box and connected in parallel to a thermostated water bath. The calorimeter exhibited a mean sensitivity of 25.8 mV W^{-1}, a time constant of 8 min and a 24 h baseline stability better than 1% of the chosen range. This differential calorimeter was placed around lotus flowers ≈1 m above the water level. Direct calorimetry was accompanied with indirect calorimetry by measuring oxygen consumption rates of the flowers with open-flow respirometry, and the patterns of temperature change were recorded with thermocouples. Flowers maintained mean temperatures of ca. 30.7° and 34.2°C at mean calorimeter temperatures of 18.4° and 30.4°C, respectively, demonstrating good thermoregulatory ability. Metabolic heat production averaged ca. 0.51 W at the low temperature and 0.25 W at the high temperature. Dry heat loss to the calorimeter averaged −0.62 W and −0.17 W, respectively, which indicated that there was a small condensation of atmospheric water vapor inside the calorimeter at the low temperature, but net evaporation from the flower at a level of ca. 33% of heat production occurred at the high temperature.

In a set of laboratory experiments on cut lotus flowers, a heat-flux budget was constructed from measurements of heat production (open-flow respirometry), heat loss (gradient-layer calorimeter of the Benzinger/Kitzinger type), and evaporative heat loss (gravimetric). Heat production rate was ca. 0.3 W and was balanced almost completely by evaporative heat loss into the calorimeter air (25°C; 37% relative humidity). Therefore, total heat flux by convection, conduction and radiation was essentially zero, despite the flower's heat-producing receptacle prevailing ca. 5°C higher than the calorimeter air. Heat from the receptacle was apparently transferred to the petals which, in turn, lost it mainly through evaporation. Equivalence of direct and indirect calorimetry substantiated the assumed caloric equivalent of oxygen consumption of 21.1 J ml^{-1} and indicated that there was no conservation of energy in metabolic processes during thermogenesis. © 1998 Elsevier Science B.V.

Keywords: Lotus flower; Metabolism; Plant calorimetry; Thermogenesis; Thermoregulation

*Corresponding author: Fax: 0049308384323; e-mail: biophys@zedat.fu-berlin.de

[1] Presented as the Fourth Lavoisier Medal Lecture.

PII S0040-6031(97)00423-1

1. Introduction

Plants are generally near ambient temperature because of their low metabolic rates and high surface-to-volume ratio. However, a few "thermogenic" plants are able to increase the temperatures of their flowers, inflorescences or cones by a highly increased energy metabolism [1–3]. Over short periods of time, the mass-specific metabolic rate of the flower may equal that of small mammals or even that of hovering hummingbirds [4]. The main purpose of this high rate of heat production is thought to be volatilization of floral scents to attract insect pollinators, protection of the flower at low temperatures, and perhaps provision of a warm shelter for pollinators during cool nights [3]. During the thermogenic period, flower temperatures may exceed ambient temperature by as much as 35°C [4,5].

Some thermogenic plants were already known to Lamarck and described in his famous *Flore Françoise* published in 1794 [6], while most of them were detected much later. Many of these interesting plants belong to the Araceae family [1,7], but some palm trees [8–10], namely cycads [11,12], custard apples [9], water lilies [13,14], and lotus [14,15] developed similar mechanisms of increased heat production associated with reproduction. A few thermogenic plants are known to regulate flower temperature during anthesis. In particular, the epiphytic arum lily, *Philodendron selloum*, keeps maximum inflorescence temperatures between ca. 37° and 44°C for most of the day, despite ambient temperatures close to freezing [4,16], the American skunk cabbage, *Symplocarpus foetidus*, maintains temperatures between ca. 15° and 24°C for 2 weeks in an air of −15° to 15°C [5], and the sacred lotus, *Nelumbo nucifera*, regulates flower temperatures between 30° and 36°C for up to 4 days in environments between 10° and 45°C [17,18]. Such thermoregulation requires that plants increase their metabolic heat production when decreasing ambient temperature causes increasing heat loss, and vice versa.

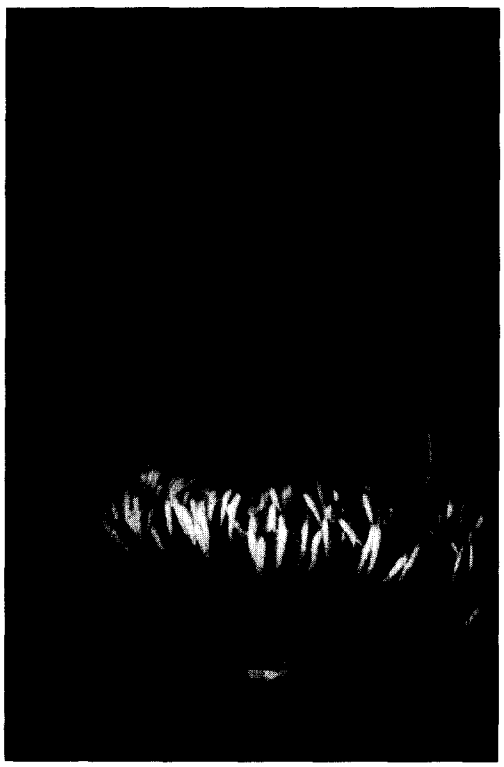
Most investigations of thermogenesis in flowers have been performed thermometrically, through biochemical analyses, or by determination of oxygen consumption and carbon-dioxide production (indirect calorimetry). Only two studies involved direct calorimetry. One concerned the inflorescence of *Philodendron selloum*, and produced a quantitative heat budget which balanced heat production with evaporative and non-evaporative (radiation, convection and conduction) heat loss and heat storage [16,19]. The other measured heat loss in the inflorescence of the voodoo lily, *Sauromatum guttatum* [20,21].

The present paper deals with the flower of the sacred lotus, *Nelumbo nucifera* (Fig. 1). Its flower consists of a central conical receptacle surrounded by stamens and petals which, in their closed state, form an empty floral chamber above the heat-producing carpellary receptacle. The flower is strongly thermogenic with unexpectedly precise thermoregulatory ability [17,18]. The early buds track ambient temperature until slight temperature elevations become detectable one or two days before the thermoregulatory phase commences [17]. Temperatures of thermoregulatory flowers remain between ca. 30° and 36°C even when ambient temperatures drop below 10°C at night and reach 45°C during the day [18]. As the temperature gradient between the hot flower and the environment increases, the flower compensates the higher heat loss by an elevated metabolic turnover. On the other hand, metabolism is reduced during daytime, and evaporative heat loss becomes important in reducing flower temperature below ambient. As the thermogenic period continues for up to 4 days, this regulation is completely reversible, resembling the well-known thermoregulation of homeothermic animals.

During the thermoregulatory period, the lotus flower opens its floral chamber by day, and closes at night. This promotes the idea that pollinating insects are rewarded with a warm, energy-saving shelter for the night and, with high temperatures, for departure in the morning [3,17]. Temperatures >30°C in the floral chamber correspond to the typical thoracic temperatures necessary for take-off in many insects [22]. Preflight shivering occurs near the maximum metabolic rate and is as energy-consuming as flight for many insects. In addition to some benefit from remaining warm throughout the night while the insects feed and copulate, they would save the energy otherwise needed for pre-flight warm-up.

To investigate thermoregulation by the lotus, we used direct and indirect calorimetric methods simultaneously in two studies – one involving long-term measurements of intact flowers in an outdoor pond,

(A)



(B)

Fig. 1. (A) Flower of the sacred lotus, *Nelumbo nucifera*, at the second day of the thermogenic period in the pond. (B) Cross-section of a cut lotus flower showing the large floral chamber surrounded by the petals, and at the bottom the stamens around the conical receptacle.

and the other concerning short-term laboratory measurements on cut flowers. For the outdoor study, we developed a light-weight, portable calorimeter to work sufficiently well under the extreme conditions of the Australian summer. Because ambient temperatures could change considerably during day and night, we required a differential calorimeter consisting of two identical units (experimental and reference) connected in opposition. We also equipped the calorimeter for indirect calorimetry by flow-through respirometry. This paper describes the development and characteristics of the differential calorimeter, the experimental setup in the pond, and the results of direct and indirect calorimetry and thermometry. It also includes similar data obtained with a gradient layer calorimeter from cut flowers in the laboratory.

2. Materials and methods

2.1. Plant material

Sacred lotus, *Nelumbo nucifera nucifera* [23], is cultivated in an outdoor pond of ca. 40 m diameter in the Adelaide Botanic Gardens. The pond was surrounded by lawn and by reeds. Several thousand flowers appeared in the pond during the summer season, between early December 1996 and the end of February 1997.

Flowers were chosen at the beginning of their 3-to-4*d* thermogenic phase when the tips of the petals became dark pink. The bud was completely closed at this stage and opened slightly the following day, known as the "first day" [24]. The slightly opened petals closed on the evening of the first day and reopened widely on the "second day" which marks the end of intense thermogenesis [17,18]. The diameter of closed buds was ≈65 mm and the height of the stem was ca. 90 mm. The conical receptacle, which contains the carpels and is the main source of heat production, was 40 mm in diameter, 30 mm in height, and weighed ca. 8.5 g.

2.2. Differential calorimeter

When the plan to construct this calorimeter was born, a well-established thermometry and indirect calorimetry of lotus flowers in the pond already

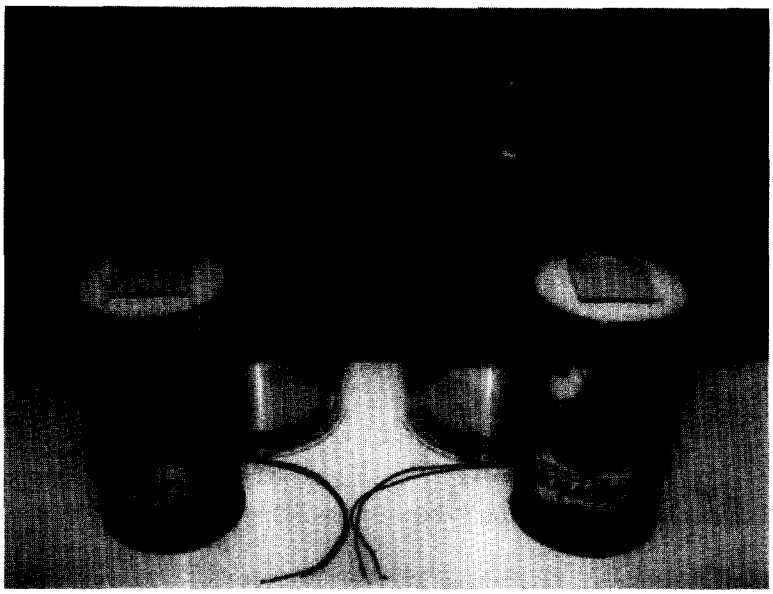


Fig. 2. Main elements of the differential calorimeter. In the back, two dry wine coolers equipped with water inlets and outlets; aluminum plates at the bottom. In front, the cans with aluminum plates and Peltier elements.

existed [17]. Hoods made from used plastic bottles were put over unopened buds and closed around the stem by plastic wrap so that air could be pumped through the hood and sent to an oxygen analyzer. Thermocouples were placed inside the hood and in the receptacle of the flower, and the hoods were protected from direct sunlight by umbrellas. To control ambient temperature during indirect calorimetry, some flowers were equipped with a clear plastic water-jacket connected to a water bath [25].

The success of the water-jacket prompted us to build a differential calorimeter that used water-jackets as heat sinks (Figs. 2 and 3). The water-jacket was made from a double-walled, clear styrene wine cooler (Decor, Australia) of 100 mm inner and 120 mm outer diameters, 185 mm internal and 220 mm external heights, and ca. 5 mm water layer between the two walls. The walls were sealed with silicon rubber, equipped with a water inlet and outlet, and connected to a refrigerated, thermostatic water bath (Julabo F20, Germany). An aluminum plate, 3 mm thick and 80 mm in diameter, was glued to the inside bottom of the cooler to render a good thermal contact to the heat sink. The calorimetric vessel proper was a thin-walled tinned steel can of 80 mm diameter and 145 mm height. A Peltier element (40×40×4 mm, type TEC1-12705) was fixed with a double-sided carpet tape to the outside bottom of this can. After application of a sufficient layer of silicone heat-transfer compound (Unick), the can was firmly pressed against the aluminum bottom plate. It was positioned inside the cooler with four plastic foam strips. The space between the strips was filled with styrofoam beads for additional insulation. In this way, the largest part of heat produced within the can flowed through the Peltier element to the heat sink. Both can and cooler were closed with stoppers cut from 20 mm foam plates. The stoppers on the experimental side were slit to the center to seal around the flower stem.

To allow simultaneous respirometry without interfering with the direct calorimetry, a silicon rubber tube of ca. 1.5 m length and 7 mm inner diameter was wrapped around the water-jacket and then inserted into the can. Thus, the outside air was preconditioned by this heat exchanger before entering the can. The temperature of the inflowing air was determined by a thermocouple. As the temperature of the can was monitored simultaneously, the heat flux due to air flow could be calculated.

The complete device with water-jacket, can and Peltier element was placed in a styrofoam box of 160×160×320 mm inner dimension and 45 mm wall

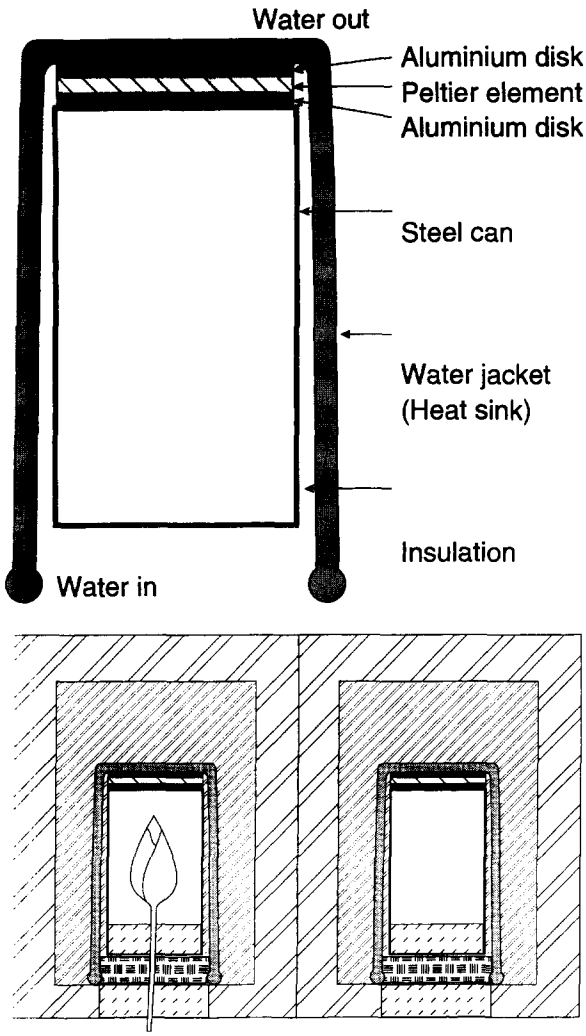


Fig. 3. Diagram of the construction of the lotus calorimeter (top) and differential setup (bottom), showing the three foam stoppers for the calorimeter vessel, the water-jacket and the external insulation.

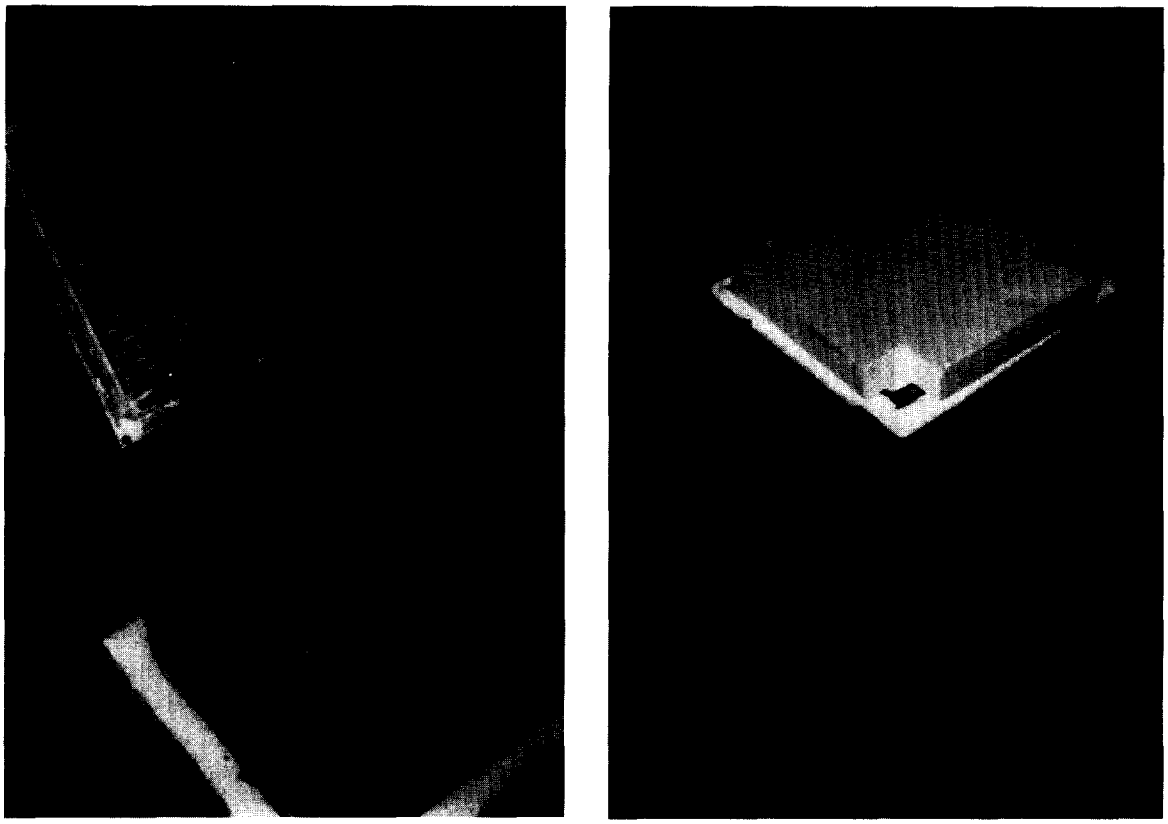
thickness. The space between the water-jacket and the walls was filled with styrofoam beads. Two halves of the twin setup were firmly connected to one another and covered with aluminum foil to reflect sunlight (Fig. 3). The whole assembly was fixed to 2 m wooden stakes rammed into the bottom of the pond. Another stake carried an umbrella to shade both the calorimeter halves.

Two precautions were taken to minimize thermal transients when the water-jacket temperature was changed. First, both water-jackets were connected in parallel to the ends of 6 m insulated hoses connected to the water bath. Thus, changes in water temperature affected both halves simultaneously and, therefore, had little influence on the calorimetric signal. Second, the total heat capacity of the average 42.2 g flower (ca. 80% of heat capacity of water [5]) in the measurement side of the calorimeter ($42.2\ g \times 3.34\ J\ g^{-1}\ °C^{-1} = 141\ J\ °C^{-1}$) was matched by a plastic container with styrofoam beads and 33.8 g of water ($33.8\ g \times 4.18\ J\ g^{-1}\ °C^{-1} = 141\ J\ °C^{-1}$) in the reference side.

The calorimeter was electrically calibrated in the pond location with a 99.8 Ω resistor connected to a nominal 12 V truck battery; the actual heating voltage at the calorimeter was measured to 0.01 V with a voltmeter. The sensitivity of the calorimeter varied slightly with water temperature, being 27.2 mV W^{-1} at 15°C, and 24.3 mV W^{-1} at 30°C. The time constant of the calorimeter was 476 s for the time required to reach $1/e$ of an abrupt heat-flux change. When the water-bath temperature was changed from 10° to 30°C or vice versa, it took ca. 1 h for the calorimetric baseline to return to its former value. The baseline stability of the setup was tested under severe conditions on the roof of the institute. At a water-bath temperature set to 15°C, the can temperature remained between 15.7° and 16.1°C although the ambient temperature increased to 52°C in direct sun and to 33°C in the shade, and both calorimetric units were sometimes exposed to different conditions. At the same time, the baseline fluctuated less than 0.5 mV of the 50 mV range during 24 h, although the ambient temperature on the roof dropped to 15°C in the early morning. Such 1% deviations are negligible at the heat output of lotus flowers.

2.3. Gradient-layer calorimeter

We used an instrument constructed by H.T. Hammel, Department of Physiology and Biophysics, Indiana University (Figs. 4 and 5). It has inside dimensions of 150×150×150 mm and a total volume of 3375 cm^3. The cover is equipped with two ports to connect respirometry tubing and introduce thermocouples. The 9 mm aluminum walls bear an O-ring at their upper sides so that the cover can be firmly sealed with four screws to the main body of the calorimeter. The outside of the calorimeter is insulated by an 8 mm styrofoam layer, and the whole setup rests on four



(A) (B)

Fig. 4. (A) Internal view of the gradient layer calorimeter with the walls constructed as heat-flow sensors; and (B) the inlet and outlet openings, the external insulation and the four supporting plastic tubes.

plastic tubes of 100 mm length to ensure a good circulation of air at constant temperature around all sides (Fig. 4). All six walls are evenly covered with thermocouples connected in series, rendering a thermopile of 1575 elements and a resistance of 27.7 Ω. Both sides of each thermocouple are separated by the "gradient layer" of 1 mm, and they are electrically insulated with varnish. Detailed descriptions of such gradient-layer instruments can be found in the literature [26–29].

When calibrated electrically, the calorimeter showed a sensitivity of 0.715 mV W^{-1}, only 1/100 of the figure of modern heat-flux calorimeters. On the other hand, the time constant of the instrument was rather short: 125 s to 1/*e*. This attractive time constant for a 3.3 l instrument was obtained by using a gradient layer of relatively high thermal conductivity and a large number of thermojunctions in series [26,28].

The calorimeter was housed in a thermostated cabinet which remained at 25.0±0.1°C during the entire investigation. Plastic tubing for indirect calorimetry was passed through this cabinet so that a thermal equilibrium of air was obtained before entering the calorimeter. The calorimetric signal was passed to a double-channel flat-bed recorder (Rikadenki model R-22) set to 1 mV full scale and a paper speed of 5 cm h^{-1} (Fig. 5).

For laboratory studies, lotus flowers were cut underwater before noon. The stem was always kept under water to avoid entry of air into the vascular system. Each bud was transported to the laboratory and placed with its stem into a 30 ml vessel filled with a flower nutrition solution (Chrysal, Bendien-Naarden, Holland). The vessel opening was sealed around the stem with a film barrier (Parafilm M, American Can) to prevent evaporation from the solution. Copper–con-

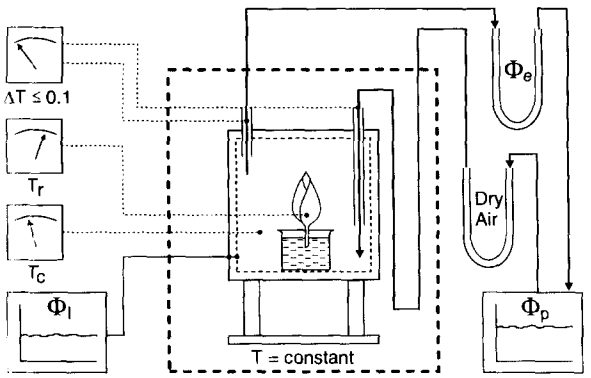


Fig. 5. Diagram of the gradient-layer setup with the instrument and flower in a constant temperature cabinet (T=constant), the heat-flow sensors (dashed lines inside calorimeter) connected to the heat-flow recorder (Φ_I), the oxygen sensor and recorder determining heat production (Φ_p), and the mass increase in Drierite due to evaporation (Φ_e). Simultaneously, the following temperatures were monitored: calorimeter (T_c), lotus flower receptacle (T_r) and the difference between inflowing and outflowing air ($\Delta T \leq 0.1$).

stantan thermocouples were placed in the receptacle, in the air inside the calorimeter laterally to the flower, and in the inflow and outflow respirometry tubes. Thermocouple outputs were measured by calibrated Fluke model 52 thermometers. Measurements began ca. 30 min after cutting and continued for 4 to 8 h during which thermogenesis gradually diminished.

2.4. Indirect calorimetry

During in situ measurements in the lotus pond, atmospheric air was sucked through the calorimeter vessel by means of a constant-flow pump at a rate of ca. 400 ml min^{-1}. The air passed through a mass flowmeter (Sierra Instruments) and part of it through a paramagnetic oxygen analyzer (Taylor–Servomex model 570A) that monitored oxygen concentration in both the atmosphere and the sample from the calorimeter. All temperatures, flow rates, analyzer outputs, and barometric pressures were recorded on a data logger (Grant Squirrel 1200 series). For laboratory measurements with the gradient-layer calorimeter, dry, CO_2-free air from outdoors was pumped at ca. 900 ml min^{-1} through the air cabinet, into the calorimeter, through a U-tube filled with $CaSO_4$ (Drierite) to absorb water vapor, and into an oxygen analyzer (Taylor–Servomex model OA184). Its output was monitored at 10 mV full scale on the same recorder as the calorimetric signal. The temperature difference of air at the entrance and exit of the calorimeter never exceeded 0.1°C so that a heat transport out of the calorimeter in the air can be neglected due to the low heat capacity of air. A full description of both respirometry systems and respirometry calculations is given elsewhere [18]. Oxygen-consumption rates were transformed to energy units by assuming a respiratory quotient RQ=1.00 [17] and a heat dissipation of 21.1 J ml^{-1} of consumed oxygen [30]. Evaporative water loss was measured by weighing the U-tubes at 1 mg approximately every hour. The latent heat of evaporation was assumed to be 2.44 kJ g^{-1}.

Statistics included calculation of 95% confidence intervals (CI) and model 1 least-squares regressions. Flowers were considered to be independent samples.

3. Results

3.1. Differential calorimeter

3.1.1. Thermometry

Fig. 6 shows the results of a five-day experiment on flower 13 with a reversed diel temperature regime

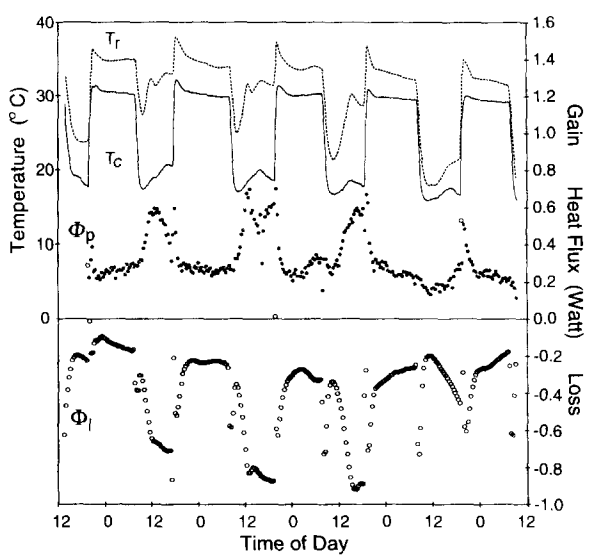


Fig. 6. Energetic parameters of a lotus flower during its 5d thermogenic period. T_c, temperature of the calorimeter; T_r, temperature of the floral receptacle; Φ_p, metabolic heat-production rate (from oxygen consumption rate); and Φ_I, dry heat-loss rate (calorimetric output).

Table 1
Mean direct and indirect calorimetric values during temperature stability in five lotus flowers measured outdoors in the differential calorimeter. The calorimeter temperature was maintained at low and high levels by a water-jacket

	Low temperature		High temperature	
	Mean	95% CI	Mean	95% CI
Chamber temperature (°C)	18.4	1.9	30.4	0.2
Receptacle temperature (°C)	30.7	1.0	34.2	0.5
Heat production rate (mW)	512	42	254	42
Dry heat flux (mW)	−623	104	−171	48
Evaporative heat flux (mW)	111	91	−83	43
Evaporation rate (mg min^{-1})	2.8	2.3	−2.1	1.1

required for another investigation [25]. The water bath was set to 30°C at ca. 18 : 00, and back to 15°C at ca. 8 : 00. The two upper traces represent the temperature of the receptacle (T_r) and the calorimeter (T_c). The shapes of the temperature curves, upon abrupt water-bath temperature changes, were influenced by lags in the thermoregulatory responses of the flower, which we have analyzed elsewhere [25]. Ignoring the overshoots and undershoots during these regulatory adjustments, we observed a long "plateau period" at 30°C, and a shorter one at 15°C, when readings did not change greatly. During the whole experiment T_r remained $>T_c$, but with decreasing differences as the flower gradually abandoned thermoregulation.

Mean temperatures of five flowers during the plateau periods demonstrated temperature regulation (Table 1). When the water bath was set to 15°C, heating by the flower and passage through the 6 m hoses raised the mean T_c to 18.4°C. Under these conditions, T_r was 30.7°C, a 12.3°C elevation. At a water-bath setting of 30°C, mean T_c was 30.4°C, and T_r was 34.2°C, only a 3.8°C elevation.

3.1.2. Direct and indirect calorimetry

Measurements of oxygen consumption were converted into rates of metabolic heat output (Φ_p). Fig. 6 illustrates this energetic response of a flower to temperature changes. In general, Φ_p decreased at high T_c and increased at low T_c, demonstrating the inverse pattern of the thermoregulatory responses. During the temperature plateau periods, the mean Φ_p at low T_c was about twice the value at high T_c (Table 1). Instabilities immediately following abrupt changes in water bath temperature are thought to result mainly from biochemical lags in the flower's regulatory mechanism [25]. For example, a quick rise in T_c resulted in a lower rate of heat loss from the flower and a rise in T_r. By the Q_{10} effect, this usually caused an upward spike in Φ_p before the regulatory adjustment brought it down. The opposite effect was sometimes seen after an abrupt drop in T_c.

The direct calorimetric signal, representing dry heat flux by convection, conduction and radiation (Φ_l), shows a pattern comparable to that of Φ_p (Fig. 6). The overall picture in the plateau period of five flowers is that the magnitude of Φ_l was inversely related to T_c (Table 1). Φ_l was roughly proportional to the temperature gradient, $T_r - T_c$; the thermal conductance at low and high temperatures was 51 and 45 mW $°C^{-1}$, respectively.

Differences between Φ_l and Φ_p may be used to estimate the contribution of evaporation to heat flux during the plateau period (Table 1). At the low temperature, this mean difference was positive, indicating that vapor from the outside air condensed inside the calorimeter. However, at the high temperature, there was always a significant evaporative heat loss, amounting to 33% of heat production. It is regrettable that we did not evaluate evaporation independently by measuring the humidity of the air flowing through the calorimeter.

Transient changes in Φ_l during changes in water-bath temperature were sometimes extreme (Fig. 6), probably artifacts resulting from thermal imbalances in the system. Such disturbances following rises in temperature are seen in Fig. 6, and in three cases the signal became positive. The fact that the calorimetric fluctuations find no correspondence in the respiration rate supports the assumption of an artifact. We evaluated heat transfer by the air passing through the

calorimeter and found that the temperatures of inflowing and outflowing air differed by <3.2°C. At the given flow rates and the small heat capacity of air, the heat transfer never exceeds 5% of the mean metabolic rate and can be neglected.

3.2. Gradient-layer calorimeter

Heat balance variables were measured on nine cut flowers when they were thermogenic and thermoregulatory, either on the "first day" of anthesis (petals open slightly), or on the day before (petals closed). T_r was generally >30°C at the beginning of the experiments, but the flowers cooled to an equilibrium when placed in the calorimeter at 25°C. Values of T_r were essentially stable during the period of measurement which lasted ca. 4 h; four flowers showed slightly falling T_r, two were constant, and three were slightly rising. The mean T_r of first-day flowers was 5.0°C higher than T_c. Rates of metabolic heat production (Φ_p) of first-day flowers increased with T_r (Fig. 7), but averaged +293 mW (61 CI). Evaporative water loss averaged 7.7 mg min^{-1} (1.2 CI) which corresponds to an evaporative heat loss (Φ_e) of −312 mW (50 CI).

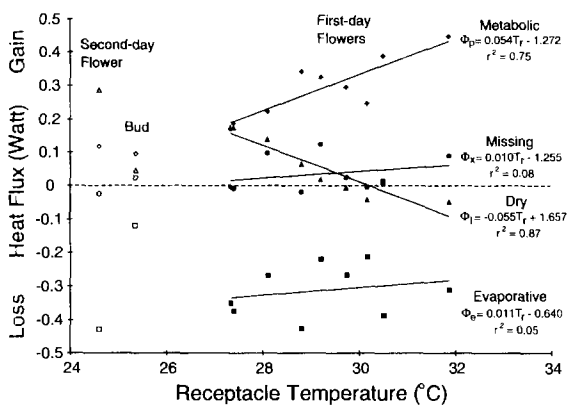


Fig. 7. (◆) Rates of metabolic heat production, and (■) evaporative heat loss, (▲) "dry" heat loss, combining convective, conductive and radiative modes, and (●) the "missing" remainder, in relation to receptacle temperature in lotus flowers. All filled symbols represent thermoregulatory flowers on, or just before, the first day of anthesis, when the petals were slightly open or closed. Open symbols at the left represent a second-day flower with wide open petals and a closed, pre-thermoregulatory bud. Points are means from two-to-three measurement periods of ≈1 h duration from individual flowers, and regression lines are based on these means.

"Dry" heat transfer by a combination of convection, conduction and radiation (Φ_l) averaged +55 mW (58 CI), apparently a heat gain, but not significantly different from zero. Thus, practically all of the heat produced by the flower was lost as evaporation. There was a small, but significant "missing" heat gain (Φ_x) of +36 mW (35 CI) which was unaccounted for by our measurements and considered to be an experimental error. It was not a result of heat storage, because there was no consistent correlation with changes in flower temperature during the course of the measurements, and heat transfer to the air flowing through the calorimeter proved to be negligible.

Closer examination of the data revealed significant correlations between Φ_p and T_r (Fig. 7). Φ_p significantly increased with T_r; this trend was evident both between flowers (Fig. 7) and within individual flowers that either heated or cooled during the measurement period. "Dry" heat loss (Φ_l) was inversely correlated with T_r, being a net heat gain at low temperature and a loss at high temperature. Evaporative heat loss (Φ_e) and the "missing" component (Φ_x) were not significantly related to T_r.

Unreplicated measurements on a pre-thermoregulatory bud and a second-day flower both showed little heat production, and T_r remained near T_c (Fig. 7). Evaporation from the unopened bud was the lowest among all flowers, and that from the wide open flower was highest, reflecting great differences in effective petal surface area. High evaporation of the second-day flower decreased its T_r most, and consequently its "dry" heat gain was the greatest. These results underline the strong influence of evaporation on the calorimeter readings.

4. Discussion

Compared to data on animal metabolism, data on plant metabolism are rare in the calorimetric literature. Early papers mainly dealt with physico-chemical effects in germinating seeds [31], while only recently has interest been directed at plant tissue metabolism, photosynthesis and determination of plant growth efficiencies by temperature scanning calorimetry [32,33]. This lack of direct calorimetric experiments is probably due to the small metabolic turnover rates in plant tissue and the large disturbing effects of eva-

poration. Thus, most information about plant energy metabolism has been obtained by indirect calorimetry through oxygen consumption and by biochemical methods. Thermogenic flowers are exceptions to the rule of low metabolic heat production in plants. The present study concerns cut lotus flowers in a gradient-layer calorimeter and intact flowers analyzed in situ outdoors. To our knowledge this is the first time that direct calorimetry has been used to measure heat fluxes outside of the laboratory. Moreover, our study involves simultaneous direct and indirect determination of energy turnover in plants, which has been done only a few times previously [16,32,33].

4.1. Differential calorimeter

Rates of heat production of flowers within the differential calorimeter were similar to those measured in transparent hooded flowers at the same temperature in a previous study [18]. At 18° and 30°C, respectively, the flowers produced 0.51 and 0.25 W (Table 1), as compared to 0.65 W and 0.35 W previously. This result indicates that constant darkness inside the calorimeter did not greatly affect the level of heat production over the course of several days. The measurements also indicate that darkness did not affect the flowers' thermoregulatory ability, because receptacle temperatures were identical between this study and the previous one. The thermogenic responses of the flower appear to be strictly related to temperature and not to the light cycle [25].

It might be argued that a calorimetric signal is too slow to analyze the thermoregulatory responses to short-term temperature fluctuations. With a time constant of ca. 8 min, however, our calorimeter was adequately responsive to changes in heat production, as evidenced by the coincidence of direct and indirect recordings (Fig. 6). Another advantage was the low thermal inertia of the whole system, which required only 1 h for stabilization after a water-bath temperature change of 20°C. This was rather quick for a calorimeter of such a large active volume. Thus, we were able to view short-term changes in response to experimental temperature change. However, the results of our first application of the calorimeter to a field situation showed that heat transfer as water vapor is significant and must be taken into account. Future investigations must include measurements of humidity in the gas flowing through the calorimeter to evaluate this factor.

4.2. Gradient-layer calorimeter

The calorimetric experiments performed on cut specimens considerably changed the natural conditions of lotus flowers. Although the dark environment in the calorimeter does not influence thermogenesis or thermoregulation in intact flowers [25], the separation from the stem causes a period of degeneration. While the thermogenic episode lasts for several days in intact flowers, reasonable heat production occurs for only 4–8 h after cutting. This is in agreement with former calorimetric experiments on cut spadices of *Philodendron selloum* which exhibited a thermogenic phase of <2 h, compared to ≈18 h in the intact plant [16]. Exactly why thermogenesis is so short-lived in cut flowers is not known. In *P. selloum* [16], and the voodoo lily, *Sauromatum guttatum* [1], the energy source for thermogenesis appears to be present in the flower before heating and is not supplied from other parts of the plant. However, the American skunk cabbage, *Symplocarpus foetidus*, imports its energy from the rhizome and stops heating when separated from it [5,34].

Heating by cut flowers was different from the general pattern observed in intact flowers in the pond [18]. At a receptacle temperature of 32°C, heat production was ca. 460 mW (Fig. 7), which is similar to the mean of intact flowers at this temperature. However, heat production in cut flowers tended to decrease at lower receptacle temperatures, but increased in intact flowers. The difference may be a result of cutting the flower or it may reside in the thermal history of the flower prior to cutting. Flowers cut during the day were exposed to sunlight and high ambient temperatures which cause a low metabolic rate [17,18]. When these flowers were placed in a cooler, drier calorimeter, their low inherent rate of heat production brought about by field conditions would have been insufficient to maintain high receptacle temperatures, so they quickly cooled until they reached a steady state that depended on the balance between heat production and loss. We often noticed a rapid cooling of the flower when first placed in the calorimeter, but did not quantify it. Increasing heat production, which was expected to occur at lower

receptacle temperatures, may have been prevented by degeneration of the flower as a result of cutting or by insufficient time for recovery. We know that the flowers are very slow to respond to changes in temperature, often requiring hours to adjust [18].

The balance between heat production measured indirectly by respirometry and heat loss measured directly indicates that a steady state exists and that heat loss is not a byproduct of biosynthetic activity within the flower. Thus, there is no evidence of biochemical conservation of energy and we conclude that the function of heat production is to warm the flower. Lack of conservation of energy was also demonstrated by direct and indirect calorimetry in the thermogenic arum lily, *Philodendron selloum* [16]. It is not known whether, for any thermogenic plant, the heat-producing respiratory pathways bypass ATP formation or create ATP and hydrolyze it immediately. Both possibilities exist in thermogenic arum lilies that employ a branched respiratory pathway leading to cytochrome oxidase and an alternate oxidase [1], and both branches occur in the lotus [14].

4.3. Comparison of differential and gradient-layer calorimetry

Enclosing a flower in a respirometry hood or calorimeter can greatly influence the heat budget, mainly by affecting ambient water vapor density and, consequently, the evaporation rate. Because completely dry air entered the gradient-layer calorimeter, it might be thought that evaporation was promoted. However, at the prevailing flow rate of 900 ml min^{-1} and evaporation rate of 7.7 mg min^{-1}, the air inside the calorimeter held 8.55 mg l^{-1}, or a relative humidity of 37%. This level is within the 30–70% range as recorded by our weather station associated with field respirometry. Given that the absolute air convection rates would be expected to be high around free flowers outdoors, evaporation rate in the gradient-layer calorimeter would be expected to be normal or even low.

A flow of atmospheric air at ca. 400 ml min^{-1} through the differential calorimeter inhibited evaporation even more. Evaporative heat loss was up to 33% of the heat produced in the differential calorimeter (Table 1), compared to practically 100% in the gradient-layer calorimeter. In fact, the air in the differential calorimeter often exceeded saturation at water-jacket temperatures of 15°C so that condensation on the walls occurred. We have seen condensation on the insides of transparent hoods and have recorded large differences in temperature between hooded and unhooded flowers in the field [18]. At ambient temperatures >35°C, hooded flowers can be 7°C higher than unhooded ones, the difference due mainly to evaporation. To achieve conditions closer to those experienced by the natural flower, therefore, it would be necessary to pass dry air through the instrument at a considerably higher rate than was used in our experiments.

Acknowledgements

This study was supported by the Free University of Berlin, the Australian Research Council (RSS), and the Deutsche Forschungsgemeinschaft (PSM). We appreciate a travel grant from the University of Adelaide under the Distinguished Visiting Scholar scheme. We thank Ted Hammel for loaning his calorimeter, the staff at the Adelaide Botanic Garden for permission to work there, and Helen Vanderwoude, Jennifer Cutten, Heidi Walters and Farid Moussavi-Harami for technical assistance.

References

[1] B.J.D. Meeuse, I. Raskin, Sexual reproduction in the arum lily family, with emphasis on thermogenicity, Sex. Plant Reprod. 1 (1988) 3–15.
[2] R.S. Seymour, Plants that warm themselves, Sci. Amer. 276(3) (1997) 90–95.
[3] R.S. Seymour, P. Schultze-Motel, Heat-producing flowers, submitted.
[4] K.A. Nagy, D.K. Odell, R.S. Seymour, Temperature regulation by the inflorescence of *Philodendron*, Science 178 (1972) 1195–1197.
[5] R.M. Knutson, Heat production and temperature regulation in eastern skunk cabbage, Science 186 (1974) 746–747.
[6] J.B. de Lamarck, Flore Françoise. 2nd Ed., Vol. 3, Agasse, Paris, 1794.
[7] G. Gottsberger, A. Amaral, Pollination strategies in Brazilian *Philodendron* species, Ber. Deutsch. Bot. Ges. 97 (1984) 391–410.
[8] C.A. Schroeder, Temperature elevation in palm inflorescences, Principes 22 (1978) 26–29.
[9] G. Gottsberger, Flowers and beetles in the South American tropics, Bot. Acta 103 (1990) 360–365.

[10] C. Listabarth, Pollination of *Bactris* by *Phyllotrox* and *Epurea*. Implications of the palm breeding beetles on pollination at the community level, Biotropica 28 (1996) 69–81.

[11] W. Tang, Heat production in cycad cones, Bot. Gaz. 148 (1987) 165–174.

[12] H. Skubatz, W. Tang, B.J.D. Meeuse, Oscillatory heat-production in the male cones of cycads, J. Exp. Bot. 44 (1993) 489–492.

[13] G.T. Prance, J.R. Arias, A study of the floral biology of *Victoria amazonica* (Poepp.) Sowerby (Nymphaeaceae), Acta Amazonica 5 (1975) 109–139.

[14] H. Skubatz, P.S. Williamson, E.L. Schneider, B.J.D. Meeuse, Cyanide-insensitive respiration in thermogenic flowers of *Victoria* and *Nelumbo*, J. Exp. Bot. 41 (1990) 1335–1339.

[15] K. Miyake, Some physiological observations on *Nelumbo nucifera*, Gaertn. Bot. Mag. Tokyo 12 (1898) 112–117.

[16] R.S. Seymour, G.A. Bartholomew, M.C. Barnhart, Respiration and heat production by the inflorescence of *Philodendron selloum* Koch, Planta 157 (1983) 336–343.

[17] R.S. Seymour, P. Schultze-Motel, Thermoregulating lotus flowers, Nature 383 (1996) 305.

[18] R.S. Seymour, P. Schultze-Motel, Physiological temperature regulation by flowers of the sacred lotus, submitted.

[19] R.S. Seymour, Analysis of heat production in a thermogenic arum lily, *Philodendron selloum*, by three calorimetric methods, Thermochim. Acta 193 (1991) 91–97.

[20] I. Lamprecht, K. Drong, B. Schaarschmidt, G. Welge, Some like it hot. Calorimetric investigations of voodoo lilies, Thermochim. Acta 187 (1991) 33–40.

[21] H. Skubatz, B.J.D. Meeuse, Energy loss in tissue slices of the inflorescence of *Sauromatum guttatum* (Schott) analysed by microcalorimetry, J. Exp. Bot. 44 (1993) 493–499.

[22] B. Heinrich, The Hot-Blooded Insects. Strategies and Mechanisms of Thermoregulation, Harvard University Press, Cambridge, MA, 1993.

[23] T. Borsch, W. Barthlott, Classification and distribution of the genus *Nelumbo* Adans. (Nelumbonaceae), Beitr. Biol. Pflanzen 68 (1996) 421–450.

[24] E.L. Schneider, J.D. Buchanan, Morphological studies of the Nymphaeaceae. XI. The floral biology of *Nelumbo pentapetala*, Amer. J. Bot. 67 (1980) 182–193.

[25] R.S. Seymour, P. Schultze-Motel, I. Lamprecht, Heat production by sacred lotus flowers depends on ambient temperature, not light cycle, submitted.

[26] T.H. Benzinger, C. Kitzinger, Direct calorimetry by means of the gradient principle, Rev. Sci. Instr. 20 (1949) 849–860.

[27] L.R. Prouty, M.J. Barrett, J.D. Hardy, A simple calorimeter for the simultaneous determination of heat loss and heat production in animals, Rev. Sci. Instr. 20 (1949) 357–363.

[28] R.W. Lawton, L.R. Prouty, J.D. Hardy, A calorimeter for rapid determination of heat loss and heat production in laboratory animals, Rev. Sci. Instr. 25 (1954) 370–377.

[29] H.T. Hammel, J.D. Hardy, A gradient type of calorimeter for measurement of thermoregulatory responses in the dog. In: C.M. Herzfeld, J.D. Hardy (Eds.), Temperature. Its Measurement and Control in Science and Industry, Vol. 3, Part 3, Biology and Medicine, Reinhold, New York, 1963, pp. 31–42.

[30] W. Wieser, Bioenergetik. Energietransformationen bei Organismen, Georg Thieme, Stuttgart, 1986.

[31] E. Calvet, H. Prat, Microcalorimétrie. Applications physico-chimiques et biologiques, Masson, Paris, 1956.

[32] R.S. Criddle, R.W. Breidenbach, D.R. Rank, M.S. Hopkin, L.D. Hansen, Simultaneous calorimetric and respirometric measurements on plant tissues, Thermochim. Acta 172 (1990) 213–221.

[33] R.S. Criddle, A.J. Fontana, D.R. Rank, D. Paige, L.D. Hansen, R.W. Breidenbach, Simultaneous measurement of metabolic heat rate, CO_2 production, and O_2 consumption by microcalorimetry, Analyt. Biochem. 194 (1991) 413–417.

[34] R.M. Knutson, Plants in heat, Nat. Hist. 88 (1979) 42–47.