

Heat production by sacred lotus flowers depends on ambient temperature, not light cycle

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Abstract

Flowers of the sacred lotus, *Nelumbo nucifera*, maintain receptacle temperature (T_r) between about 30 °C and 36 °C during their 2–4 d sequence of anthesis by increasing the rate of heat production, measured as oxygen consumption (\dot{V}_{O_2}), at low ambient temperature (T_a) at night and reducing it at high T_a during the day. The effects of T_a and photoperiod on \dot{V}_{O_2} were separated by manipulating T_a around outdoor flowers under natural photoperiod or constant darkness. There was no effect of time of day on either T_r or \dot{V}_{O_2} , but both depended on T_a . Rising T_a resulted in an increase in T_r , a brief upward spike in \dot{V}_{O_2} , followed by a long-term decrease in \dot{V}_{O_2} . Decreasing T_a had the opposite effects: a decrease in T_r and a brief depression, followed by a slow rise, in \dot{V}_{O_2} . The two-phase responses to sudden T_a change indicate a rapid, mass-action effect and a slower, regulatory adjustment. Temperature regulation continued in constant darkness for over 3 d.

Key words: Thermoregulation, lotus, photoperiod, temperature, heat production, oxygen consumption, Nelumbonaceae.

Introduction

Flowers of the sacred lotus, *Nelumbo nucifera* Gaertn., are strongly thermogenic (Miyake, 1898) and thermoregulatory (Seymour and Schultze-Motel, 1996). Temperatures inside the flowers generally remain between 30–36 °C during a 2–4 d period, despite changes in environmental temperatures between about 10–45 °C. The relationships between ambient temperature, flower temperature, and respiration rate have been studied in detail (Seymour and

Schultze-Motel, 1998), and a quantitative analysis of heat production and heat loss has been made, using direct and indirect calorimetry (Lamprecht *et al.*, 1998). Thermoregulation begins in the bud, when the petals are tightly closed, continues through a day when the petals open slightly to reveal the stigmas, and ends as the flower opens widely to expose the stamens. The pattern of anthesis is protogynous, and the period of thermoregulation roughly corresponds to receptivity of the carpels to pollination. There is evidence that thermoregulation might be a reward to insect pollinators that would benefit from a high body temperature while remaining in the flower (Seymour and Schultze-Motel, 1997).

Earlier work on the lotus in this laboratory involved simultaneous measurements of temperatures and oxygen consumption rates of flowers in an outdoor pond where ambient conditions were uncontrolled (Seymour and Schultze-Motel, 1996, 1998). Thermoregulation was demonstrated by stability of flower temperatures during naturally occurring cycles of ambient temperature. In addition, there was a reciprocal relationship between ambient temperature and oxygen consumption, which demonstrated that the rate of heat production increased with increased difference between ambient and flower temperature.

Although there seemed to be no relationship between rate of heat production and ambient light levels, there was a possibility that heat production was partly co-ordinated with the light cycle, generally rising at night and decreasing during the day. It is well known that flowering in some thermogenic plants is strongly dependent upon light cycle. For example, the arum lilies, *Sauromatum* and *Arum*, do not bloom or produce heat in constant light or constant dark, but require cycling of both to initiate anthesis (Meeuse and Raskin, 1988). Thermogenesis in the arum lily, *Philodendron selloum*, also depends on light cycle, usually reaching its peak in

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the early evening in Brazil (Gottsberger and Amaral, 1984), but in California there are two patterns of warming of ornamental cultivars, both dependent on time of day (Seymour *et al.*, 1983). Several species of South American *Annona* (Gottsberger, 1989) and the Amazon water lily, *Victoria amazonica* (Prance and Arias, 1975), always appear to warm during the evening hours when the pollinating insects become active.

This study on *Nelumbo* was designed to separate light and temperature as environmental factors that possibly affect thermogenesis. Because of the limited life of cut flowers in the laboratory, intact flowers were studied outdoors under three conditions: (1) natural temperature and light cycles, (2) reversed temperature and constant darkness, (3) reversed temperature and natural light cycle. Temperatures of the flowers and surrounding air were measured to evaluate thermal stability, and rates of oxygen consumption were used as indirect measures of heat production.

Materials and methods

Nelumbo nucifera was studied in a large outdoor pond at the Adelaide Botanic Gardens, during January and February, 1997. The sacred lotus, *Nelumbo nucifera nucifera*, and the American lotus, *N. n. lutea*, are considered subspecies by Borsch and Barthlott (1996). The plants and conditions were the same as previously described (Seymour and Schultze-Motel, 1996, 1998). Measurements of oxygen consumption were made with a 4-channel flow-through system that operated continuously. Briefly, a constant-flow pump drew atmospheric air through a hood covering the flower, and sent it through a mass flowmeter that was vented to the atmosphere. Subsamples of the flows through three hoods and one reference channel of atmospheric air were drawn into a paramagnetic oxygen analyser. Temperatures, flow rates and oxygen levels were recorded at 2 min intervals with a Grant Squirrel data logger, but the complete cycle of four channels required 24 min, because the oxygen analyser dwelt on each channel for 6 min to ensure a stable reading. Computations of oxygen consumption were standard for flow-through respirometry and were appropriate to the system (Withers, 1977).

For the present experiments, the hood over the flower was equipped with a temperature-controlled water jacket, constructed from a clear, styrene, double-walled bucket designed to keep a wine bottle cool (10 cm i.d., 20 cm deep; Decor, Australia). The space between the walls was sealed and fitted with inlet and outlet ports that provided an even pattern of circulation. Water was provided by a refrigerated water bath (Julabo model F20-HC; 2550 W), through two, 6 m, 1 cm i.d. hoses, insulated with 1 cm thick foam rubber sleeves. The hood was inverted over the flower and supported by wooden stakes driven into the bottom of the pond. The opening was sealed around the petiole except for a small hole that allowed entry of air. Thermocouples provided temperatures of the hood interior and the flower receptacle. One hood incorporated elements for direct calorimetry as part of another study (Lamprecht *et al.*, 1998). It was surrounded by opaque insulation and its opening was sealed with 6 cm thick foam rubber which eliminated light. Another hood was translucent and insulated outside by 6 mm thick polyethylene bubble-pack sheeting, and the opening was

sealed with clear plastic cling wrap. Although this hood permitted entry of light, a glass-domed solarimeter showed that total intensity was reduced to 45% of outside insolation.

Flower buds were chosen on the day before anticipated opening and measurements were made continuously during the thermogenic period. Experimental flowers were exposed to a reverse temperature regime. The water bath was set at 15 °C at about 07.00 h and to 30 °C at about 19.00 h. When possible, records from experimental flowers in jacketed hoods were compared to control flowers in translucent hoods without jackets that were measured simultaneously under the natural temperature and light regime.

Results

During the normal flowering sequence in translucent hoods without the water jacket, hood temperature (T_h) decreased at night and increased during the day (Fig. 1). During the first two nights of thermoregulation, receptacle temperature (T_r) tended to remain between 30–35 °C, except that there was often a dip in the early evening. The T_h increased during the day, and T_r tended to be slightly above it. Oxygen consumption rates (\dot{V}_{O_2}) increased during the night and decreased during the day,

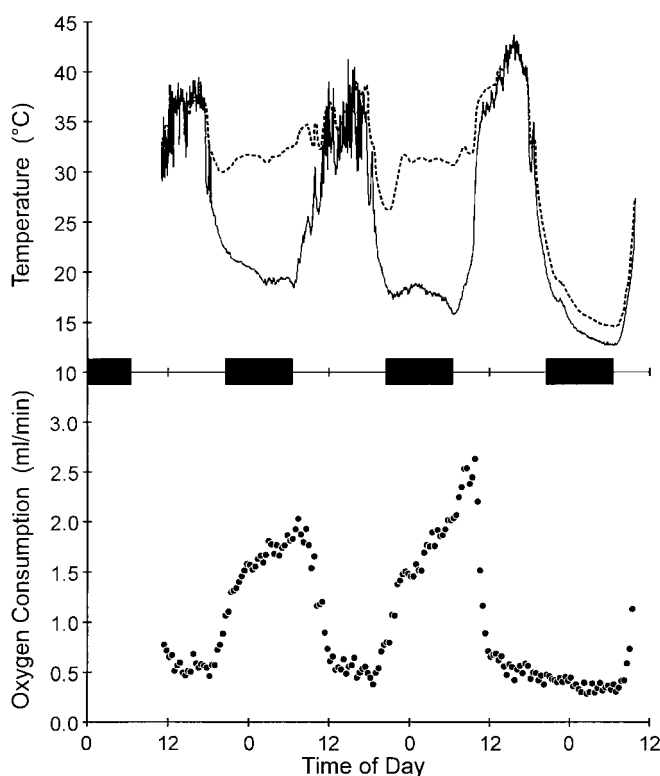


Fig. 1. Normal pattern of temperature regulation in a sacred lotus flower under the natural photoperiod and temperature regime. Temperatures inside the respirometry hood (solid line) and the receptacle (dashed line) and oxygen consumption (dots) are given in relation to time. Night (dark bars) is defined as the period between local sunset and sunrise. Temperature regulation is apparent at night during the first two cycles, and it disappears after the flower opens widely on the third day.

mirroring the changes in T_h , but lagging behind it. There was often a peak in \dot{V}_{O_2} at sunrise, when T_h and T_r were rising rapidly because of solar input. This pattern was evident in three flowers in the present study and 19 flowers studied previously (Seymour and Schultze-Motel, 1998).

Five flowers measured in constant darkness showed reproducible responses to enforced temperature changes (Fig. 2). When T_h was raised, T_r also rose and showed a sharp overshoot before stabilizing near 34 °C. Often coincidental with the overshoot was an immediate spike in \dot{V}_{O_2} before it too decreased to a lower level. When T_h decreased, T_r also decreased, always showing a pronounced undershoot before recovering to temperatures above 30 °C. The depth and duration of the undershoot trough increased in older flowers. There was often a small transient decrease in \dot{V}_{O_2} during the T_r undershoot, not always caught during the 24 min sampling cycle, but apparent on the third day in Fig. 2. Then there was a gradual rise in \dot{V}_{O_2} during the period of cold exposure. Heat production by the flower influenced T_h , as evidenced by the small peak at the onset of the warm phase and instability during the cold phase.

Receptacle temperatures in four water-jacketed flowers on a reverse temperature cycle also tended to remain between 30–35 °C, except when T_h was changing rapidly. Figure 3 shows an heuristic example, containing an

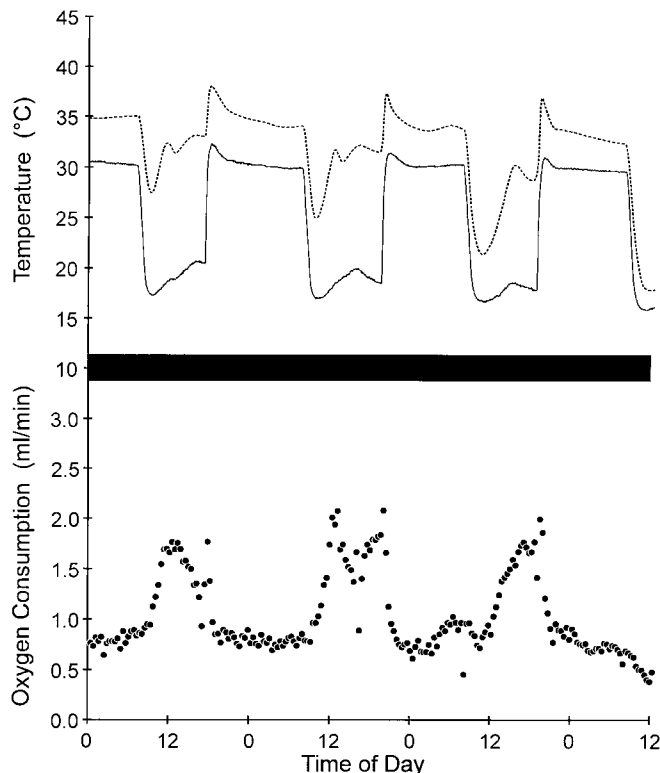


Fig. 2. Pattern of temperature regulation of a lotus flower under reversed temperature cycles and constant darkness. Symbols as in Fig. 1.

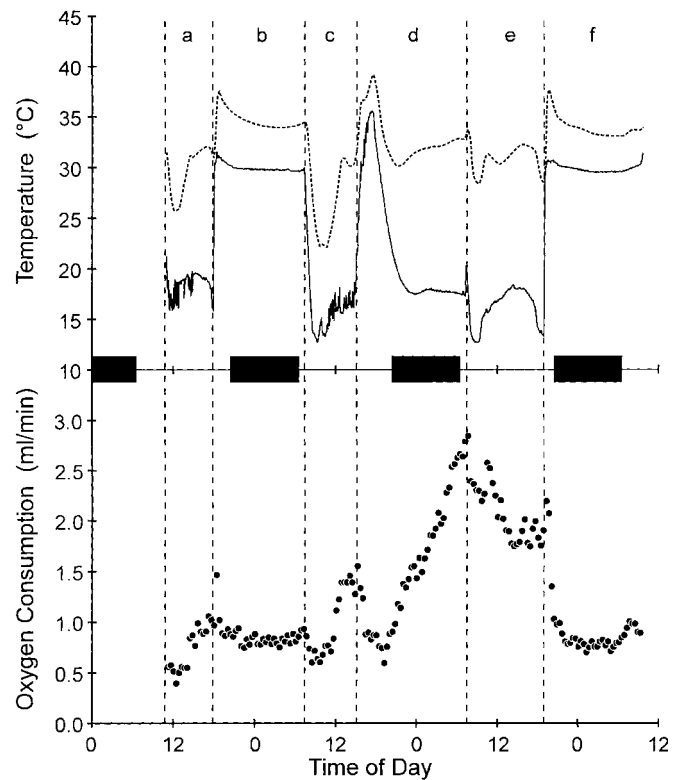


Fig. 3. Pattern of temperature regulation of a lotus flower under manipulated hood temperatures and natural photoperiod. The data were taken simultaneously with those in Fig. 1. Lettered periods are discussed in the text. Symbols as in Fig. 1.

unplanned perturbation. Instruments were attached to an unopened bud on the day before anticipated opening (a). \dot{V}_{O_2} was low and falling, similar to the control flower that was measured at the same time (Fig. 1). Exposed quickly to a cold temperature, flower temperature dipped to 26 °C, resulting in an immediate dip in \dot{V}_{O_2} to 0.5 ml min⁻¹, followed by increasing \dot{V}_{O_2} to 1.0 ml min⁻¹, and recovery of T_r to above 30 °C. Low \dot{V}_{O_2} during this period was associated with tightly closed petals that retained the heat within the bud. When the water bath was set at 30 °C at about 18.00 h, it produced a rise in T_r , briefly overshooting to 37 °C before stabilizing at about 34 °C throughout the night (b). The overshoot was immediately coupled with a brief spike of \dot{V}_{O_2} to 1.5 ml min⁻¹, followed by stabilization at about 0.8 ml min⁻¹. At about 07.00 h the water bath was set at 15 °C, causing a large drop in T_r to about 22 °C (c). Again, there was a dip in \dot{V}_{O_2} , followed by a steady rise during the day. At about 15.00 h, the power supply to the water bath failed and T_h began to rise (d). This initially pushed up T_r and decreased \dot{V}_{O_2} , but in the evening the unregulated T_h decreased to about 17 °C, followed by a decrease in T_r to 30 °C. A smooth rise in \dot{V}_{O_2} to 2.8 ml min⁻¹ and T_r to 33 °C continued throughout the night. In the morning when power was restored

to the water bath, a quick drop in T_h was followed by T_r and \dot{V}_{O_2} (e). For reasons unknown, T_h was unstable between 13–18 °C during the day, but T_r remained between 28–31 °C, while \dot{V}_{O_2} mirrored T_h . \dot{V}_{O_2} during period e was higher than that in period a, because the petals had opened partially, releasing heat from the receptacle into the hood and requiring greater heat production to keep the receptacle warm. At 19.00 h the water bath was returned to 30 °C (f) and the subsequent pattern was similar to that in period (b).

In summary, changes in T_h appeared to have two effects on T_r and \dot{V}_{O_2} . First there was an immediate effect, changes in T_h resulting in changes in T_r and \dot{V}_{O_2} the same direction. Then there was a delayed effect, increased T_r resulting in decreasing \dot{V}_{O_2} , followed by a reduction in T_r , and vice versa. There was no detectable effect of time of day on either T_r or \dot{V}_{O_2} .

After initial regulatory temperature adjustments to temperature change, T_r and T_h tended to stabilize in the water-jacketed hoods. For comparison of these three treatments, and to show the reproducibility of the responses, data for flowers during this period of stability were selected (Table 1). During cold and warm exposure, T_h averaged about 18 °C and 30 °C, while T_r averaged about 32 °C and 34 °C, and \dot{V}_{O_2} averaged 1.9 ml min⁻¹ and 0.9 ml min⁻¹, respectively. Temperature elevations and respiratory rates of water-jacketed flowers, in both darkness and reverse-cycle, were similar to values taken within the ranges of 17–19 °C and 29–31 °C in a larger study of the lotus under natural light cycles (Table 1).

Discussion

These experiments demonstrate that the thermoregulatory responses of the sacred lotus depend on temperature and are not influenced by photoperiod. Flowers on the reversed

temperature cycle ultimately increased their heat production when hood temperature was lowered during the day, both in constant darkness (Fig. 2) and in natural photoperiod (Fig. 3). Although photoperiod does not influence thermoregulation, it probably affects flower development and the onset of thermogenesis as it does in arum lilies (Buggeln *et al.*, 1971). Photoperiodic initiation of flowering can begin as much as 2 d before an arum lily inflorescence opens (Meeuse and Raskin, 1988). The experiments in this study began on the day before the petals opened for the first time, and the flowers were already thermoregulating (Seymour and Schultze-Motel, 1996, 1998).

Interestingly, thermoregulation continued in constant darkness for over 3 d (Fig. 2), showing that exposure of the flower to photoperiodic changes is not necessary to maintain the responses. Because only the flowers were kept in darkness, however, it is possible that continued photoperiodic exposure of the rest of the plant could have influenced the results. The sequence of petal opening and closing is strongly correlated with light cycle in the Asian lotus subspecies (Miyake, 1898) and the American subspecies (Sohmer, 1977; Schneider and Buchanan, 1980), but it was not possible to observe whether the petals continued to move in the dark in this study. It is well known that cyclic movements of petals continue with an endogenous rhythm in other flowers in constant darkness (Sweeney, 1979).

The two-phase responses to sudden ambient temperature change indicate a rapid, mass-action effect and a slower, regulatory adjustment. For example, raising hood temperature resulted in immediate increases in receptacle temperature and rate of oxygen consumption (Figs 2, 3). This response was apparent in control flowers, when morning sunlight quickly raised hood temperature, resulting in a spike in oxygen consumption (Fig. 1; Seymour and Schultze-Motel, 1996, 1998). The brief, inappropriate rise

Table 1. Temperatures of receptacle and rates of oxygen consumption (\dot{V}_{O_2}) of lotus flowers in natural and reversed light cycles, and in constant darkness

Data were chosen when hood temperatures were stable, i.e. during the period of temperature regulation and several hours after a hood temperature change. Means, 95% confidence intervals and sample sizes are given for cool and warm environments.

Light cycle		Temperature		\dot{V}_{O_2} (ml min ⁻¹)	Temperature		\dot{V}_{O_2} (ml min ⁻¹)
		Hood	Receptacle		Hood	Receptacle	
Natural	Mean	17.0	29.7	1.9	37.5	42.5	0.6
	CI	1.1	4.6	0.8	5.6	7.2	0.1
	n	3	3	3	2	2	2
Natural ^a	Mean	17–19	30.5	1.9	29–31	35.8	1.1
	CI	–	0.8	0.2	–	0.5	0.3
	n	18	18	18	14	14	14
Darkness	Mean	18.8	32.2	1.9	30.8	34.6	0.9
	CI	2.1	1.3	0.1	0.7	0.5	0.1
	n	5	5	5	5	5	5
Reverse	Mean	17.5	31.5	1.8	29.5	34.0	0.9
	CI	2.3	1.3	0.3	0.6	0.0	0.1
	n	4	4	2	4	4	2

^aData from Seymour and Schultze-Motel (1998).

in heat production was probably due to van't Hoff's Q_{10} generalization that biochemical reactions roughly double with a 10 °C temperature rise. However, the full Q_{10} effect of temperature was obscured by the slower development of an indirect thermoregulatory mechanism that decreases heat production. Fast and slow components are also apparent after quick decreases in ambient temperature.

The slow regulatory component is probably responsible for a marked hysteresis curve on axes relating rate of heat production to temperature of lotus flowers (Seymour and Schultze-Motel, 1998). Heat production is slow to rise in the evening, and slow to fall in the morning, when flower temperature is changing rapidly. Sluggish regulation is also apparent in the oscillations of flower temperature after a quick decrease in ambient temperature (Fig. 2). These oscillations, often of decreasing amplitude, suggest that the rate of heat production is too high as flower temperature approaches its thermostatic 'equilibrium-point', and an overshoot results. The overshoot, in turn, reduces heat production too much, resulting in a smaller temperature undershoot. The oscillation eventually disappears as the flower finds its equilibrium-point. It is felt that the sluggishness of the responses indicates that temperature regulation occurs by changes in concentrations of putative biochemicals that affect the rate of heat production, rather than changes in the structure of enzyme systems.

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