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RESEARCH PAPER

# Respiration of thermogenic inflorescences of *Philodendron melinonii*: natural pattern and responses to experimental temperatures

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## Abstract

The patterns of temperature and respiratory changes in the protogynous inflorescences of *Philodendron melinonii* (Araceae) were studied in the field in French Guiana. These are the first respiratory measurements from a member of the large subgenus *Philodendron*, a group previously thought to lack thermoregulatory inflorescences, in contrast to thermoregulatory *Philodendron* species of the subgenus *Meconostigma*. Heating by the male and sterile male florets was strong on the first evening of anthesis when beetles are attracted and the female florets are receptive. Heat production of the inflorescence peaked at ~0.9 W and spadix temperature reached ~39.5 °C, a level somewhat independent of ambient temperature. Thermogenesis continued throughout the night and the next day, but at a lower level, and floral temperatures fell. On the second evening, when pollen was shed, there was a small elevation in respiration and spadix temperature. Responses of cut spadix sections to experimental step changes in ambient temperature resulted in a prompt response in floral temperature and respiration rate in the direction of the change and then a much slower regulatory adjustment in the opposite direction. These responses are consistent with an immediate van 't Hoff effect, followed by up- or down-regulation of thermogenesis. However, the responses required several hours. It is concluded that the male floret tissues possess the same thermoregulatory mechanism of more precise thermoregulatory species, but a combination of small spadix size (that favours heat loss), moderate thermogenic capacity

(that limits heating rate), and slow reaction time (that causes long lags between temperature change and the regulatory response) result in poor thermoregulatory performance during the second day.

Key words: Arum lily, heat production, inflorescence, *Philodendron*, protogynous, regulation, respiration, thermogenesis, van 't Hoff effect.

## Introduction

Floral thermogenesis is common among species of the arum lily family (Araceae) (Mayo *et al.*, 1997). The genus *Philodendron* in particular contains members whose inflorescences are strongly thermogenic (Brattstrom, 1972; Nagy *et al.*, 1972; Gottsberger and Amaral, 1984; Seymour, 1999; Gibernau *et al.*, 1999; Gibernau and Barabé, 2000, 2002; Barabé *et al.*, 2002). Heat is generated by male and sterile male florets on the spadix of the inflorescence. In every case, thermogenesis is associated with the volatilization of floral scents and the attraction of pollinating insects (mainly beetles) that usually reside in a floral chamber for ~24 h. Their residence is required for cross-pollination, because the plants are protogynous, receptive to pollination in the female phase, when the insects arrive on the first evening, and producing pollen on the second evening when they depart.

One aspect of thermogenesis that was first demonstrated in *Philodendron selloum* K. Koch, 1852 is that the florets exhibit temperature regulation, that is they increase respiration and heat production in response to lower environmental temperature and can attain spadix

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temperatures of 39–44 °C in air between 4 °C and 39 °C on the first evening (Nagy *et al.*, 1972). After this initial bout of high temperatures, heat production decreases, but remains somewhat regulated in the range of 25–36 °C until the second evening when heating begins to wane (Seymour, 1999). A similar pattern of continuous heating occurs in *P. solimoense* A.C. Sm., 1939 (Seymour *et al.*, 2003a), but not in *P. acutatum* Schott, 1856, *P. pedatum* (Hook.) Kunth, 1841, *P. melinonii* Brongn. ex Regel, 1874, and *P. squamiferum* Poepp., 1845, all of which show a bimodal pattern of heating with two peaks, a larger one on the first evening and a smaller one on the second, with little evidence of thermogenesis in between (Gibernau *et al.*, 2000; Gibernau and Barabé, 2000, 2002; Barabé *et al.*, 2002). In *P. grandifolium* (Jacq.) Schott, 1829 and *P. imbe* Schott, 1829, heating is weak and apparently unregulated (RS Seymour and P Schultze-Motel, unpublished data). It has been suggested that the differences may lie in the fact that *P. selloum* and *P. solimoense* belong to the subgenus *Meconostigma*, while the others are members of the subgenus *Philodendron* (Cotias-de-Oliveira *et al.*, 1999; Gibernau and Barabé, 2000; Barabé and Lacroix, 2001; Barabé *et al.*, 2002), which apparently is a more derived group (Mayo, 1988).

There is no evidence to indicate whether any member of the large subgenus *Philodendron* is thermoregulatory. Certainly the bimodal heating pattern, with insignificant temperature elevations in the period between thermogenic bouts, suggests that the observed species are not. Not only do these differences have potential phylogenetic significance, they also bear on the question of the role of post-attraction thermogenesis in *P. selloum* and *P. solimoense*. It is generally stated that the role of thermogenesis is to volatilize floral scent, but this does not explain why heating continues after the short period of attraction, why floral chamber temperatures are specifically warmed during the extended period, and why the temperatures are those preferred by many insects. It has been demonstrated, however, that continued heating in *P. solimoense* is a thermal reward for the *Cyclocephala colasi* Endrodi, 1964 beetles that linger in the floral chamber overnight (Seymour *et al.*, 2003a). Heat provided by the inflorescence reduces the need for the endothermic beetles to generate their own heat during activity, and reduces their daily energy requirement several-fold. Because the beetles may spend most of their adult lives in the inflorescences, the reward can be enormous. However, the thermal reward theory is challenged by the fact that *C. colasi* beetles visit both *P. solimoense* that heats continuously and *P. melinonii* that apparently does not. This study therefore determines the conditions in the floral chamber of *P. melinonii* and places them in the context of beetle energy requirements.

The simple explanation for the bimodal pattern of heating in *P. melinonii* is that it is controlled exclusively

by a light cycle-triggered, endogenous pattern, determined by factors related to the co-ordinated functions of the florets and spathe. Because the pattern appears independent of ambient temperature, it would seem that temperature regulation is not involved. However, the possibility exists that the mechanism is present, but overwhelmed by some other factors. Therefore, this study involved exposing spadices to artificial experimental temperatures and measuring the respiratory responses. If respiration rate is inversely related to floral temperature, then the mechanism is present. However, if respiration increases with increasing floret temperature, then this species lacks it.

## Materials and methods

### Plant material

Specimens of *P. melinonii* were studied at Petit Saut, French Guiana, in June–July 2007. Eight plants had been removed from the forest and replanted at ground level at the Laboratoire Environnement 6–7 years earlier. Two other plants were located near the edge of the Sinnamary River, just below the dam. In total, 31 inflorescences were investigated. In all cases, floral linear dimensions were measured with a millimetre ruler, and the mass of the spadix and severed florets measured to 2 mg with a Tanita model 1210 balance.

### Intact field inflorescences

Thermometry and respirometry were conducted on intact inflorescences outdoors. Six inflorescences were fitted with individual temperature loggers (Hobo Temp S08-563-9000 equipped with 40 cm leads and H08-001-02 with short leads; Onset Computer Corporation, Bourne, MA, USA), with the thermistors in the centre of the spadix in the male or sterile male section, or in the floral chamber. Another logger registered air temperature adjacent to the inflorescence. The loggers and inflorescences were shaded from direct sunlight with cardboard ~10 cm away.

Ten inflorescences were used for respirometry. The system recorded temperatures with insulated, copper–constantan thermocouples inserted into the centre of the spadix within the male floret section, in the sterile male floret section, in the floral chamber adjacent to the female florets, and in the air outside of the spathe. After placement of the thermocouples, the entire inflorescence was covered with a hood made from a 330 ml plastic water bottle and a cling-wrap skirt. The skirt was incompletely sealed with a string around the base of the inflorescence to admit air, and the hood was shaded with cardboard. Air from the hood was pumped through a liquid water trap with a Gilair 3 air sampling pump (Sensidyne, Clearwater, FL, USA), through a pressure buffer into a mass flowmeter (1 l min<sup>-1</sup>, Mass Trak model 822, Sierra Instruments, Inc., Monterey, CA, USA), calibrated with a bubble flowmeter (Gilibrator, Sensidyne), and then vented to atmosphere. A subsample of this excurrent gas was sampled with a solenoid valve assembly every 30 min, for 6 min, and diverted into a CO<sub>2</sub> analyser (model 280, David Bishop Instruments Leamington Spa, Warks, UK). The analyser was calibrated with CO<sub>2</sub>-free air and a precision 0.49% CO<sub>2</sub> in N<sub>2</sub> mixture. Samples from the hood were measured with reference to samples of humidified ambient air flushed through the system for 6 min every 30 min. Because of the long periods of measurement, water vapour could not be practically absorbed, but floral samples were compared with ambient air under the assumption that the vapour density was the same in all samples. Rates of

CO<sub>2</sub> production ( $\dot{M}_{CO_2}$ ) were calculated from air flow rate ( $\dot{M}_E$ ) and fractional difference in CO<sub>2</sub> ( $\Delta F_{CO_2}$ ) according to:  $\dot{M}_{CO_2} = \dot{M}_E \times \Delta F_{CO_2}$ .

### Cut floral parts

To determine whether thermoregulatory responses occurred in male floral tissue, respirometry was also carried out on pieces of the spadix in the laboratory. Sections of the spadix with male and sterile male florets, 20–40 mm long, were cut from the inflorescence with a wet knife and the cut end put immediately into a thin layer of water in the inverted lid of a 40 ml HDPE vial. A hypodermic needle thermocouple pierced the lid and entered 15 mm into the centre of the section from below. Two PVC tubes also penetrated the lid to provide a flow-through system when the vial was closed. A second thermocouple was taped to the outside of the vial to provide ambient temperature. The vial was covered with a custom-made, 0.6 l volume, insulated, constant temperature (CT) cabinet, with Peltier-element control system, similar to that described earlier (Seymour, 2004), but air-cooled rather than water-cooled. The walls of the cabinet admitted very little light. Respirometry was carried out as described above, except that it used a 500 ml min<sup>-1</sup> mass flowmeter and involved a more sensitive CO<sub>2</sub> analyser (LI-820, Li-Cor Inc., Lincoln, NE, USA). The air flow rate was ~250 ml min<sup>-1</sup> to ensure that the CO<sub>2</sub> level was within the range of the analyser. The analyser output was recorded at 5 s intervals with the Grant logger. The protocol was to place the tissue sample (2.4–6.0 g of spadix, either the male tip or the sterile male cylinder) in the chamber at a temperature similar to the ambient temperature of the inflorescence when cut. Respiration was measured continuously as the chamber temperatures were altered in steps of a selected variety of magnitudes and directions, and at different times of the flowering cycle.

Units of respiration rate are given in the commonly used, mass-specific units (nmol s<sup>-1</sup> g<sup>-1</sup>), where wet mass is for florets only, not the entire spadix, because respiration of the stalk of *P. selloum* is negligible (Nagy *et al.*, 1972). It should be remembered that mass-specific values do not eliminate the effect of mass (Packard and Boardman, 1999). If the reader requires truly comparable, mass-independent values, the units should be multiplied by mass<sup>0.33</sup>, assuming that the underlying allometric relationship is proportional to mass<sup>0.67</sup>. The range in spadix mass was so low in this study, however, that mass-specific values are acceptable. Statistics include means and 95% confidence intervals. Least squares linear regressions are described by number of inflorescences (*n*), coefficients of determination (*R*<sup>2</sup>), standard errors of the slope (SE<sub>b</sub>), and probabilities (*P*). Two-tailed *t* tests were used where appropriate. Excel and StatisticXL software (statisticxl.com) were used for the analyses.

## Results

### Intact field inflorescences

The spadix of *P. melinonii* was white and consisted of three floral types (Fig. 1). The top comprised fertile male florets of ~59% of the spadix length (Table 1). These were associated with the production of a red, resinous fluid that was extruded in several droplets around the lower part of the fertile male zone during the afternoon of the second day. This fluid could be seen on the first day in cut spadices, under the fertile males. The fertile males produced copious pollen strings on the evening of the second day. Below the fertile males was a band of sterile

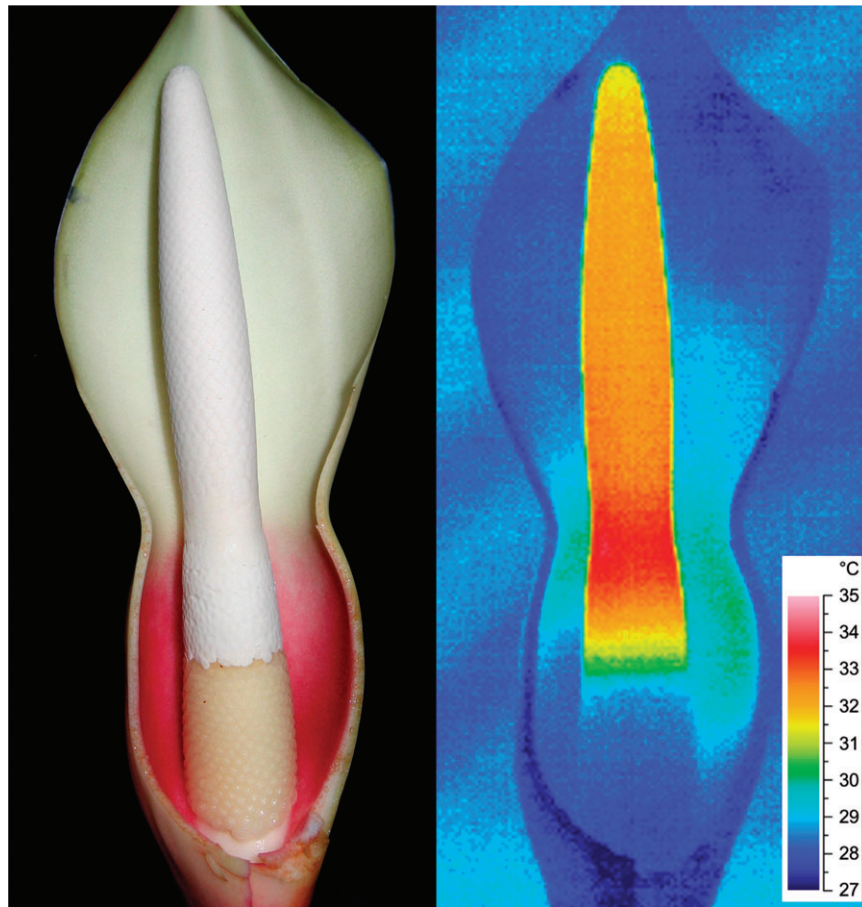
males, ~14% of the spadix length. These did not produce resin or pollen. Below them was a band of female florets, with a strongly angled base, such that the mean length was ~27% of spadix length. Outside of the spadix was a thick, greenish white spathe that opened initially in the period between dawn and noon on the first day, revealing a creamy white interior. The spathe was constricted at the level above the sterile male band, but enlarged below it to form the floral chamber containing the female florets and the sterile males. The dimensions of the floral chamber were ~60 mm high and 8 mm between the spathe and florets when the spathe was open. It remained open from the first day until the evening of the second day when it closed tightly around the spadix, eliminating the floral chamber entirely.

There were no significant differences (*t*-tests; *P* > 0.05) between exposed inflorescences instrumented with temperature loggers and those enclosed in respirometry hoods with respect to spadix mass, floral mass, first day maximum spadix temperature, and difference between maximum spadix and air (or hood) temperature. Therefore, temperature data from uncovered and covered inflorescences were combined (Tables 1, 2).

A major bout of thermogenesis occurred on the evening of the first day, all inflorescences beginning a steep increase in male spadix temperature between 18.00 h and 18.52 h, and peaking between 19.12 h and 20.01 h (Table 2; Fig. 2A). This was mirrored by a sharp increase in respiration (Fig. 2B). Then thermogenesis gradually decreased, although it was somewhat arbitrary to define an 'ending' time, because significant heating continued throughout the night and the following day. On the evening of the second day, there was a minor increase in respiration, peaking rather precisely between 18.49 h and 19.20 h (Fig. 2B). The peak maximum temperature occurred significantly later on the first day (19.29 h) than the second day (19.05 h) (*t*-test; *P* < 0.001).

Thermocouples and thermal camera images revealed that both the male and sterile male florets were thermogenic (Figs. 1, 2). There was no evidence of thermogenicity in the female florets. The pattern of temperature increase, however, was different in the fertile and sterile males (Fig. 2). Heating began simultaneously, but fertile males reached a slightly higher temperature than sterile males and declined more quickly during the night. Thus, depending on the time, one or the other could have a higher temperature.

Floral chamber temperatures averaged 26.5 ± 0.5 °C in 11 inflorescences during the 24 h period from 18.30 h on the first day. This temperature was affected by warming of the sterile male flowers and by buffering against ambient air changes due to the insulation effects of the fibrous material that surrounded the base of each inflorescence (Fig. 2). In hooded inflorescences, this material had to be removed. In unhooded inflorescences, floral chamber



**Fig. 1.** Inflorescence of *Philodendron melinonii* photographed with cameras for the visible and infrared spectra. The side of the lower spathe is removed to reveal the floral chamber, with female florets at the bottom of the spadix and sterile male florets above them. Protruding above the pink chamber is the spadix with fertile male florets and open spathe. The photographs were taken at about 19:00 on the first day of opening, when the surface temperature of the sterile males was higher than that of the males, while the female florets were not thermogenic. The thermal image was made with an Avio TVS-500 infrared camera (Nippon Avionics, Ltd, Tokyo, Japan).

temperature was  $25.8 \pm 1.2$  °C and  $2.7 \pm 1.4$  °C higher than air during the 12 h overnight from 18.30 h. The minimum chamber temperature was 1.9 °C higher than the minimum air temperature at night and the maximum chamber temperature was 6.5 °C lower than the maximum air temperature during the day.

First-day maximum spadix temperature ( $T_{\max}$ ) in uncovered and hooded inflorescences was related to contemporaneous ambient temperature ( $T_a$ ) over a range of 23.6–31.0 °C according to:  $T_{\max} = 0.33T_a + 30.5$  (Fig. 3). The slope was not significantly different from zero ( $n=14$ ;  $R^2=0.20$ ;  $SE_b=0.19$ ;  $P=0.11$ ), but was significantly less than unity. Thus the difference between  $T_{\max}$  and  $T_a$  increased significantly with decreasing  $T_a$  according to:  $T_{\max} - T_a = -0.67T_a + 30.5$  ( $n=14$ ;  $R^2=0.50$ ;  $SE_b=0.19$ ;  $P=0.005$ ). One would expect that heat production would therefore increase with decreasing  $T_a$ . However, this could not be demonstrated statistically ( $P=0.53$ ) as the ambient temperature range within the hooded respirometry inflorescences was too narrow (26.4–31.0 °C).

**Table 1.** Morphometrics of *P. melinonii* inflorescences in relation to floret type

Total mass includes the pith supporting the florets; floret mass has pith removed. Statistics are means, 95% confidence intervals (CI) and number of inflorescences.

		Range	Mean	95% CI	<i>n</i>
All males	Length (mm)	86–125	103.3	5.7	17
	Mean width (mm)	14–17	15.0	0.4	17
	Total mass (g)	7.7–12.1	14.6	0.9	17
Fertile males	Length (mm)	63–99	84.2	5.7	13
	Florets (g)	5.3–8.1	6.8	0.4	13
Sterile males	Length (mm)	14–30	20.8	2.7	13
	Florets (g)	1.5–3.9	2.7	0.4	13
Females	Length (mm)	27–54	38.2	3.6	17
	Total mass (g)	7.8–15.9	11.0	1.0	17

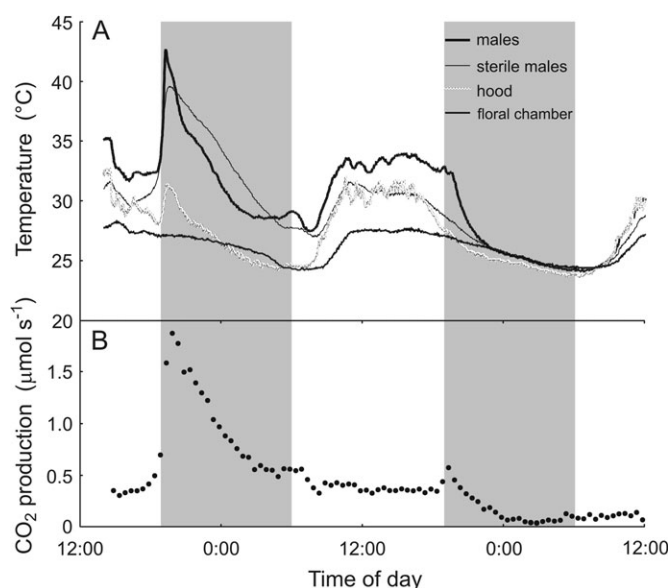
Similar to maximum spadix temperatures, the maximum rates of CO<sub>2</sub> production ( $\dot{M}_{CO_{2max}}$ ) occurred significantly later on the first day (19.42 h) than the second day (19.09 h) ( $t$ -test;  $P=0.001$ ). There were no significant relationships of total mass of either male and sterile male florets

**Table 2.** Pattern of temperature changes and respiration during the 2 d sequence of anthesis

'First day' refers to the major episode of thermogenesis in the first evening. 'Second day' refers to the second episode on the next evening.

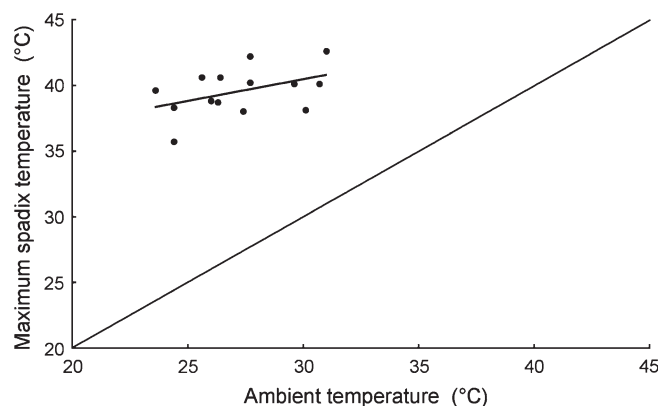
$T_{\max}$ =maximum spadix temperature,  $T_a$ =ambient temperature,  $\dot{M}CO_{2\max}$ =maximum rate of  $CO_2$  production of whole inflorescence, time=time of day. Mass-specific respiration rates refer to floret mass, not whole spadix mass. Statistics as in Table 1.

Stage	Variable	Units	Mean	95% CI	<i>n</i>
First day	Time of onset	h:min	18:36	0:08	11
	Time at $T_{\max}$	h:min	19:29	0:08	15
	Time at finish	h:min	21:09	4:10	15
	$T_{\max}$	°C	39.5	0.9	15
	$T_a$ at $T_{\max}$	°C	27.2	1.3	14
	$T_{\max}-T_a$	°C	12.3	1.2	14
	Time at $\dot{M}CO_{2\max}$	h:min	19:42	0:12	10
	$\dot{M}CO_{2\max}$	nmol s <sup>-1</sup>	1880	250	10
	$\dot{M}CO_{2\max}$	nmol s <sup>-1</sup> g <sup>-1</sup>	200	28	10
	Second day	Time at $T_{\max}$	h:min	19:05	0:06
$T_{\max}$		°C	31.9	0.5	13
$T_a$ at $T_{\max}$		°C	26.0	1.0	13
$T_{\max}-T_a$		°C	5.8	1.0	13
Time at $\dot{M}CO_{2\max}$		h:min	19:09	0:10	10
$\dot{M}CO_{2\max}$		nmol s <sup>-1</sup>	710	70	10
$\dot{M}CO_{2\max}$		nmol s <sup>-1</sup> g <sup>-1</sup>	77	11	10
Total $\dot{M}CO_2$	mmol	62.6	7.6	10	
Total heat	kJ	29.6	3.6	10	



**Fig. 2.** (A) Temperatures of the fertile male and sterile male parts of the spadix, the floral chamber and ambient air in the hood surrounding an inflorescence of *Philodendron melinonii* during the 2 d sequence of anthesis. (B) Rates of  $CO_2$  production from the whole inflorescence. Shading indicates the period between dusk and dawn.

( $M_{m+sm}$ ) with maximum first-day  $\dot{M}CO_2$  ( $\dot{M}CO_2 = 0.088M_{m+sm} + 1.04$ ;  $R^2 = 0.076$ ;  $SE_b = 1.09$ ;  $P = 0.44$ ) or with maximum difference between peak spadix temperature and air ( $P = 0.60$ ).



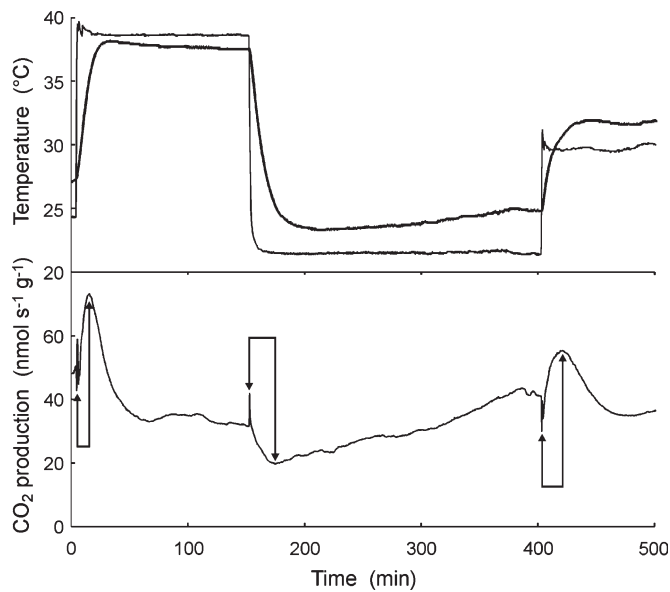
**Fig. 3.** Relationship between maximum spadix temperature and ambient (air or hood) temperature in *P. melinonii*.

In terms of heat production, the mean maximum rate was 0.88 W on the first day and 0.34 W on the second, assuming 21 J of heat per ml of  $CO_2$  produced. The total amount of heat produced during the 48 h period encompassing the two heating episodes averaged 29.6 kJ; however, the amount ( $H_{\text{tot}}$ , kJ) was related to combined male and sterile male floral mass ( $M_f$ , g) according to  $H_{\text{tot}} = 2.84M_f + 2.65$  ( $n = 10$ ;  $R^2 = 0.39$ ;  $SE_b = 1.26$ ;  $P = 0.05$ ). There was no significant relationship between  $\dot{M}CO_{2\max}$  and the maximum difference between spadix and hood temperatures:  $T_s - T_h = -3.17\dot{M}CO_{2\max} + 17.3$  ( $n = 8$ ;  $R^2 = 0.38$ ;  $SE_b = 1.79$ ;  $P = 0.13$ ).

#### Cut floral parts

Individual sections of spadix cut from the inflorescence and measured in the miniature CT cabinet showed reproducible responses for periods up to 14 h. In general, a step change in ambient temperature was followed by changes in floret temperature and respiration rate, first in the same direction and then in the opposite direction toward a plateau (Fig. 4). If the step change was up, then floral temperature and respiration rate increased, peaked, and then decreased. Downward step changes caused the opposite effects. The initial changes in respiration rate are ascribed to the van 't Hoff ( $Q_{10}$ ) effect, in which an increase in tissue temperature increases metabolic rate, and vice versa. The reversals are termed 'inhibition' in response to high temperature and 'activation' in response to low temperature, because they are thought to demonstrate biochemical regulation.

Male florets and sterile male florets reacted similarly. In four inflorescences tested during the major thermogenesis event on the evening of the first day (between 18.59 h and 1.31 h), all exhibited inhibition, but not activation. In eight inflorescences tested after the major bout of thermogenesis on the first evening (i.e. after 02.00 h), however, all showed both inhibition and activation. The responses could be demonstrated as late as 21.30 h on the evening of the second day. Thus the responses lasted at least 19 h.

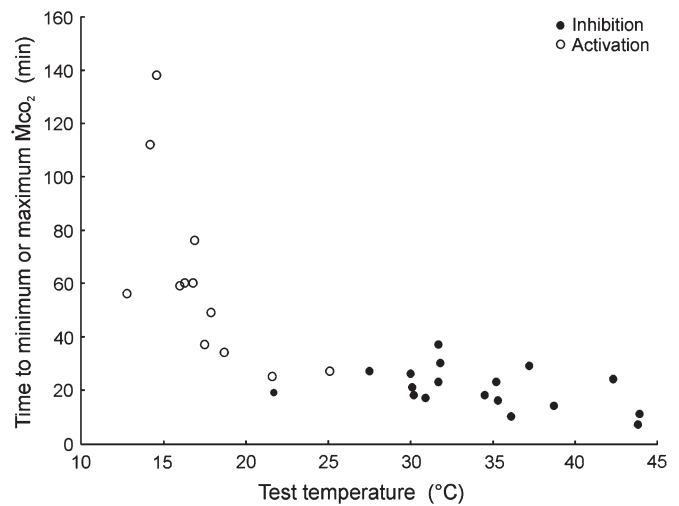


**Fig. 4.** Example of the laboratory experiment involving the effects of selected ambient temperatures (thin line, above) on floral temperature (thick line, above) and respiration rates (below) of male florets measured during the second day. In this case, the section of a spadix included 3.28 g of florets. The respiratory responses are an initial van 't Hoff effect in the direction of the temperature change, followed by a slower inhibition or activation in the opposite direction. The timings of the responses are quantified by arrows that delimit the period between the temperature change and the change in direction of the trace (zenith or nadir).

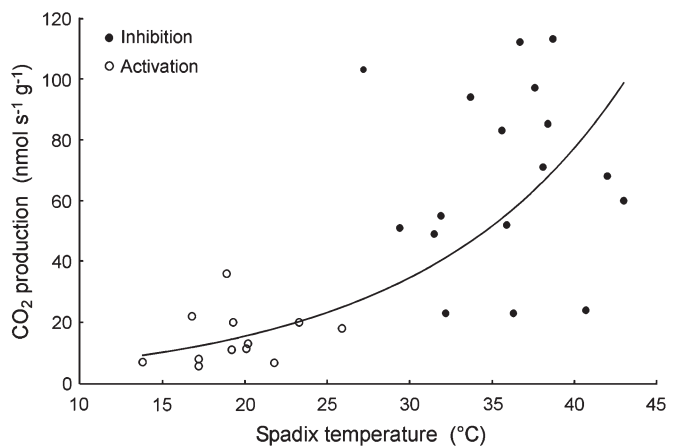
The temporal characteristics of inhibition and activation were quantified by measuring the time between the temperature change and the maximum deviation of the initial van 't Hoff respiratory response (see arrows in Fig. 4). Inhibition was relatively fast and occurred even more quickly in response to step changes to higher temperatures in 12 inflorescences (Fig. 5). Activation was slower, especially at the lowest test temperatures, where the van 't Hoff effect could predominate over 1 h.

$\dot{M}CO_2$  was measured at the zenith rate during rising temperature and at the nadir rate during falling temperature, and the values were related to floret temperature at the time (Fig. 6). These points are transients, dominated by the van 't Hoff effect and before regulatory adjustments took over. Higher spadix temperature resulted in exponentially increasing respiration. Despite great variability at the higher temperatures, the exponential equation for the data,  $\dot{M}CO_2 = 0.003e^{0.0811T_s}$  is significant ( $R^2 = 0.60$ ), and indicates a  $Q_{10}$  of 2.3.

The time-course of inhibition and activation was measured at selected temperatures in five inflorescences by waiting for the spadix temperature and  $\dot{M}CO_2$  to stabilize. Similar to the time to zeniths and nadirs, the time to equilibrium depended on both the magnitude of the step change and the absolute ambient temperature. In response to rising temperatures, equilibrium was reached in a mean of  $129 \pm 34$  min at values within the range of

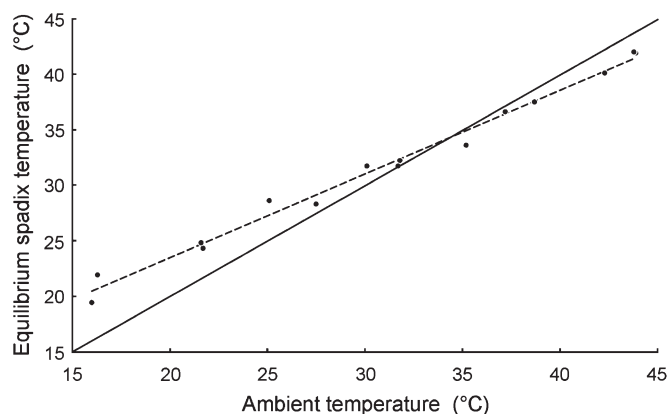


**Fig. 5.** Duration of initial response to a step change to a different test temperature in male or sterile male florets of *P. melinonii*. Time is measured from the point of ambient temperature change to the zenith or nadir of the  $\dot{M}CO_2$  trace (see Fig. 4). The time to the zenith due to rising ambient temperature is a measure of the speed of inhibition (filled circles) and the time to the nadir due to falling temperature measures activation (open circles).

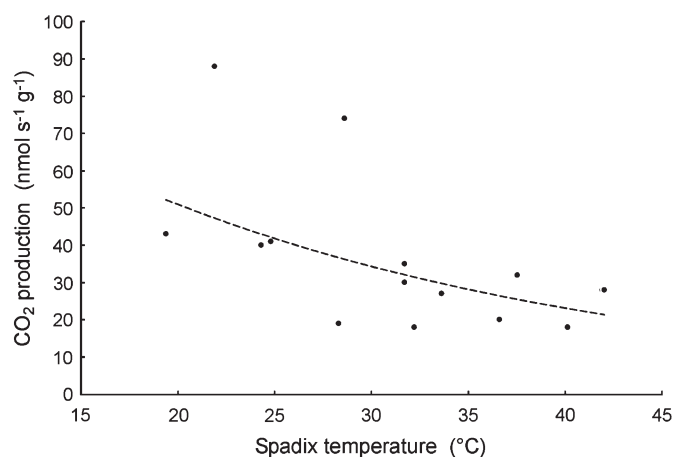


**Fig. 6.** Transient respiration rate of cut male or sterile male sections of the spadix in relation to temperature of the section at the time when the van 't Hoff effect was overtaken by the regulatory adjustment (zenith or nadir in Fig. 4). Responses to rising ambient temperature resulted in inhibition (filled circles) and falling temperatures resulted in activation (open circles).

21.7–43.9 °C. After falling temperatures, equilibrium required  $258 \pm 113$  min to stabilize between 14.2 and 25.1 °C. Spadix temperature stabilized higher than ambient temperature at low temperatures, and vice versa (Fig. 7). The equation for the regression was:  $T_s = 0.75 T_a + 8.4$ . The slope of this line was significantly lower than 1.0 ( $R^2 = 0.99$ ;  $SE_b = 0.02$ ;  $P < 0.001$ ). Equilibrium  $\dot{M}CO_2$  decreased significantly at higher spadix temperatures (Fig. 8). An exponential equation  $\dot{M}CO_2 = 0.112e^{-0.0396T_s}$  ( $R^2 = 0.35$ ) provides an estimate of the  $Q_{10}$  of 0.7, which quantifies the negative effect of temperature.



**Fig. 7.** Temperatures of cut sections of spadix with male or sterile male florets after completion of activation or inhibition in relation to ambient temperature. The equation for the dashed linear regression is in the text. The slope of solid line is 1.0.



**Fig. 8.** Equilibrium respiration rate of cut male or sterile male sections of the spadix in relation to spadix temperature after completion of inhibition or activation. The equation for the regression line is in the text.

## Discussion

### *Intact field inflorescences*

The natural pattern of spadix temperature rise and floral respiration rate in *P. melinonii* is characterized by a major bout of thermogenesis on the evening of the first day, in association with powerful scent production, receptivity of the female florets to pollination, and arrival of pollen-bearing insects (Gibernau *et al.*, 2000). After this, thermogenesis continues at a lower level until the next evening, when there is a smaller episode of heating associated with pollen production and departure of the insects. This pattern is essentially the same in other species of *Philodendron* of the subgenus *Philodendron* including *P. acutatum* and *P. pedatum* (Gibernau and Barabé, 2000). However, members of the subgenus *Meconostigma*, including *P. selloum* (Seymour, 1999; Seymour *et al.*, 1983)

and *P. solimoense* (Gibernau *et al.*, 1999; Gibernau and Barabé, 2000; Seymour *et al.*, 2003a), have a major heating episode during the first evening, but it is followed by a long period of moderate thermogenesis that is maintained until the following evening, when it begins to die away without a second thermogenic peak. In *P. selloum*, thermogenesis is inversely dependent on ambient temperature, a phenomenon termed temperature regulation, because it tends to stabilize floral temperature (Nagy *et al.*, 1972; Seymour, 1999). The maximum temperature of the first-day thermogenic episode is regulated at  $\sim 40$  °C, while the following 'plateau stage' temperature is regulated at  $\sim 32$  °C (Seymour, 1999).

Peak spadix temperatures in field *P. melinonii* reached a mean of 39.5 °C (Table 2), values similar to those of *P. selloum*, 39.6 °C (Seymour, 1999) and *P. solimoense*, 38.4 °C (Gibernau and Barabé, 2000). The difference is that *P. selloum* is able to achieve these temperatures at ambient temperatures down to  $\sim 4$  °C (Nagy *et al.*, 1972), while *P. melinonii* begins to fail at temperatures just below 20 °C (present study), which is related to the difficulty in warming a smaller spadix. The spadix of *P. melinonii* (total mass=25.6 g, including 11.0 g of female zone; Table 1) is much smaller than that of *P. selloum* (124 g; Seymour *et al.*, 1983) or *P. solimoense* (98.6 g, unpublished data). At an ambient temperature of 27.2 °C, the mass-specific metabolic rate of *P. melinonii* is 200 nmol s<sup>-1</sup> g<sup>-1</sup>. The maximum rate recorded from cut inflorescences in the first evening is 260 nmol s<sup>-1</sup> g<sup>-1</sup>. These values are somewhat lower than previous measurements from other *Philodendron*. The maximum oxygen consumption rates of *P. selloum* sterile male florets reach 360 nmol s<sup>-1</sup> g<sup>-1</sup> (Nagy *et al.*, 1972; Seymour *et al.*, 1983), which converts to a CO<sub>2</sub> production rate of 300 nmol s<sup>-1</sup> g<sup>-1</sup>, assuming a respiratory exchange ratio for a mainly lipid substrate (MCO<sub>2</sub>/MO<sub>2</sub>) of 0.83 (Walker *et al.*, 1983; Seymour *et al.*, 1984). The physics of heat exchange limits the maximum temperature elevation in smaller spadices, even if the mass-specific heat production rate is the same (Gibernau *et al.*, 2005). There is no physical barrier to small spadices producing large temperature elevations if they have sufficient thermogenic capacity and ambient temperatures are sufficiently low. For example, 4.7 g spadices of skunk cabbage *Symplocarpus foetidus* [L.] Salisb. ex Nutt. can achieve a 33 °C elevation above an ambient temperature of  $-10$  °C (Seymour, 2004), but it takes high mass-specific metabolic rates. For example, maximum rates of male and bisexual florets of other small aroids can reach 820 nmol s<sup>-1</sup> g<sup>-1</sup> (Seymour *et al.*, 2003b). By comparison, the rates in *P. melinonii* are relatively low.

It is clear from the present results that the maximum spadix temperature is somewhat regulated in *P. melinonii*, because it was not significantly related to ambient temperature in uncovered and hooded individuals



(Fig. 3). The precision of temperature regulation can be gauged by the slope of the relationship of spadix temperature to ambient temperature, with perfect regulation having a slope approaching zero, and no regulation having a slope of unity. The slope of the present data, although not significantly different from zero, was 0.33 (Fig. 3). This compares with first-day *P. selloum* that shows a slope of 0.145 for intact inflorescences (Seymour, 1999) and 0.179 for severed ones (Nagy *et al.*, 1972). Slopes in other thermoregulatory species range from 0.09 to 0.59 (Seymour, 2004). Thus *P. melinonii* falls mid-way within a spectrum of thermoregulatory precision.

It was not possible to detect an increase in respiration with decreasing ambient temperature in intact inflorescences in the field, because the range of ambient temperature of respirometry inflorescences was narrow. However, respiration in four inflorescences cut and measured on the first night was clearly inhibited by high temperatures. When exposed to high temperature, respiration increased, peaked, and reversed at a mean spadix temperature of 38.4 ( $\pm 3.7$ ) °C, which is similar to the mean of 39.5 °C in the field (Table 2).

After the main episode of thermogenesis on the first evening, the difference between spadix and ambient temperature declines and the respiration rate decreases until the next evening (Fig. 2). Spadix temperature clearly falls during the night, and there is little evidence from field inflorescences that a thermoregulated 'plateau' stage exists, as it does in *P. selloum* (Seymour, 1999). Nevertheless, evidence from cut inflorescences indicates that some thermoregulatory ability exists throughout the 2 d sequence.

#### Cut floral parts

It was initially surprising that the florets on cut segments of the spadix remained viable and responsive for so long after cutting. Respiration of inflorescences and isolated florets of *P. selloum* decreased to very low levels within 2 h after being cut from the plant (Seymour *et al.*, 1983). The longevity in the present case may be due to maintaining moisture in the tissue by keeping the cut surface wet, which was not previously done.

The biphasic pattern of respiratory responses to step changes in ambient temperature (Fig. 4) are consistent with biochemical regulation. A rise in air temperature warms the florets and causes an immediate increase in respiration rate, as one would expect. In this case, however, another effect slowly overcomes this one and causes respiration to peak and then decline. This inhibition causes spadix temperature to decrease. Conversely, lowering ambient temperature cools the tissue and immediately causes respiration to decrease, but, after some time, the trend reverses and respiration rises. It is clear that both inhibition and activation are reversible, as the responses continued for up to six step changes in

opposite directions in a single specimen (not shown). The initial responses of the flowers are consistent with the well-known van 't Hoff effect, which tends to double the rate with each 10 °C rise in temperature. Transient respiration measured at the inflections early in the response indicates a  $Q_{10}$  of 2.3 (Fig. 6). However, the subsequent adjustments change respiration rate in the opposite direction and at equilibrium cause heat production to be higher at lower temperatures, such that the  $Q_{10}$  is 0.7 (Fig. 8).

This pattern of response to experimental step changes in ambient temperature has been demonstrated in *P. selloum* (Seymour *et al.*, 1983), the sacred lotus *Nelumbo nucifera* Gaertn. (Seymour *et al.*, 1998), and skunk cabbage *S. foetidus* (Seymour, 2004), which are all thermoregulatory species. The dynamics of respiratory changes in the latter species have been successfully modelled, assuming lags due to thermal inertia, the van 't Hoff effect, and the activity of a putative rate-limiting enzyme (Seymour, 2004).

The time-course of changes depends primarily on how quickly enzyme activity can change. In *P. melinonii*, the van 't Hoff effect was reversed in ~20–30 min at temperatures above 20 °C (Fig. 5), but it required on average 2–4 h to reach a new equilibrium after a step change. At temperatures below 20 °C, the reversal could require >2 h and the entire adjustment >7 h. In contrast, skunk cabbage is faster, overcoming the van 't Hoff effect in 38 min, and the entire adjustment in 68–88 min, on average (Ito *et al.*, 2004; Seymour, 2004). Responses of the sacred lotus are also slow, requiring 4–8 h to complete (Seymour *et al.*, 1998), resulting in a pronounced hysteresis in flowers in the field (Seymour and Schultze-Motel, 1998).

Inhibition of respiration in *P. melinonii* in response to rises in experimental temperature was apparently quicker than activation by lowering the temperature (Fig. 5). This is the case for intact sacred lotus subjected to step changes in air temperature (Seymour *et al.*, 1998) and in outdoor flowers naturally inhibited by rising temperature in the morning and activated at night (Seymour and Schultze-Motel, 1998). There is also a similar tendency for inhibition to be faster in skunk cabbage (Seymour, 2004), but the biochemical significance of the difference is not known.

Experiments on cut spadices in the CT cabinet indicate the existence of a thermoregulatory mechanism in second-day inflorescences (Fig. 8), but they do not demonstrate very effective thermoregulation, and the results are not useful in interpreting the data from intact inflorescences in the field. Although the temperature of the cut spadices moved in the appropriate direction for temperature regulation (Fig. 7), the slope of the relationship between spadix and ambient temperature (0.75) indicates very poor regulation. This is not surprising in retrospect, as conditions in the CT cabinet made it difficult for the

tissue to regulate; a high air-flow rate (chamber volume replaced every 10 s) around a small part of the spadix without the protection of the spathe no doubt enhanced convective and evaporative heat losses.

### Ecological significance

The function of the main bout of thermogenesis of *P. selloum* on the first evening is clearly related to the production of floral scent that is attractive to scarab beetles (Gottsberger and Silberbauer-Gottsberger, 1991). Heating of the spadix may enhance the production of volatiles, but may also help disperse them by creating free convection of heated, less dense air that rises away from the inflorescence. To maximize these effects, the surface of the spadix should be as warm as possible. However, there is the potential problem that too much heat could damage the fertile male florets or be repulsive to beetles that generally avoid temperatures above 42 °C (Seymour, 1999). Regulation of maximum spadix temperature to ~40 °C on the first night may be related to these limitations, but there are no investigations of the effects of high temperature on either the production and viability of pollen or the behaviour of beetles.

Thermogenesis after the main bout of the attraction phase can be explained as an energy reward to insect visitors (Seymour and Schultze-Motel, 1997). Many species of beetle produce enough heat in their thoraxes to raise their body temperature significantly, but the energy cost can be enormous, even if the ambient temperature is only slightly below preferred body temperature (Heinrich, 1993). Thus raising the ambient temperature of active beetles only a little can save them considerable energy. For example, in French Guiana, the 28 °C floral chamber of *P. solimoense* is only 3.4–5.0 °C above ambient, but reduces the nocturnal energy requirements of active endothermic *C. colasi* beetles by a factor of 2.0–4.8 (Seymour *et al.*, 2003a). The same species of beetle is a primary pollinator of *P. melinonii* (Gibernau *et al.*, 2000). In the present experiments, mean floral chamber temperature was 25.8 °C and ambient temperature was 23.1 °C during the night. At these temperatures, the energy required for beetle activity in the flower would be ~50% of that outside, according to equations in Seymour *et al.* (2003a). It is worth noting three observations that are consistent with an energy reward role of thermogenesis in *P. melinonii*. First, the band of sterile male florets exists below the constriction in the spathe, where they would warm the floral chamber (Fig. 1). Secondly, thermogenesis by the sterile males is several hours more protracted than that by the fertile males (Fig. 2). Thirdly, the reported measurements of floral chamber temperature were taken adjacent to female florets that are not appreciably thermogenic (Fig. 1), but the beetles climb the spadix to reach the sterile males, which they eat. It is

somewhat warmer higher in the chamber, especially if the beetles are clinging to the thermogenic sterile male florets.

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