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Heat Production in the Voodoo Lily (*Sauromatum guttatum*) as Monitored by Infrared Thermography

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ABSTRACT

The pattern of surface temperatures of the inflorescence of *Sauromatum guttatum* was investigated by using an infrared camera. The male flowers are weakly thermogenic on the first day of inflorescence opening (D-day) as well as on the next day (D + 1), reaching 0.5 to 1°C above ambient temperature. The appendix (the upper sterile part of the inflorescence) is highly thermogenic on D-day, reaching 32°C, and is faintly thermogenic on D + 1, reaching 1°C above ambient temperature. The lower part of the spadix, close to the female flowers, is also thermogenic on D-day and D + 1, reaching a temperature similar to that of the appendix only on D + 1. Salicylic acid does not induce heat production in the lower part of the spadix, as it does in the appendix. Respiration of tissue slices obtained from the appendix shows that the capacity for cyanide-insensitive respiration is present in young and mature appendices. This alternative respiratory pathway is not, however, utilized in young appendix tissue, but is engaged during the maturation of that tissue.

The inflorescence of *Sauromatum guttatum* is a widely used material for the study of heat production in plants. Salicylic acid was recently identified as 'calorigen', the plant hormone that induces heat-production in the inflorescence-appendix of *S. guttatum* (4). The alternative (cyanide-insensitive) oxidase that is required for plant thermogenicity has been purified from appendix mitochondria (2). The production of two of the proteins associated with the alternative oxidase activity is regulated by salicylic acid *in vivo* (3).

Although much is now known about the alternative oxidase and the levels of salicylic acid in various parts of the *Sauromatum* inflorescence, a detailed description of heat production by that inflorescence has not yet been reported. We now present an analysis of heat production during anthesis by using infrared thermography. We also sought to determine whether salicylic acid can trigger heat production in thermogenic parts other than the appendix (the male flowers and the lower part of the spadix). Finally, the presence of the cyanide-insensitive pathway was demonstrated in tissue slices obtained from the appendix.

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MATERIALS AND METHODS

Plant Material

Sauromatum guttatum inflorescences were grown in a growth chamber under 15-h light/9-h dark periods with a photon flux density of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 19°C. The developmental stage of *S. guttatum* was determined retroactively with respect to D-day, the day of inflorescence opening.

Thermal Image-Processing System

A thermographic system previously described was used to obtain surface temperatures of the inflorescence (6). Fifty to 100 images were recorded during the thermogenic response, and images were analyzed only for periods during a change in heat-production pattern. More than 10 inflorescences were examined.

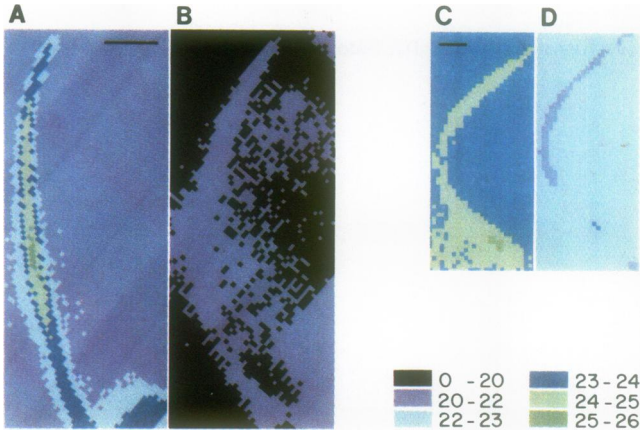

Figure 1. Effect of ambient temperature on heat emission by *Sauromatum* appendix. A and B are images recorded at the beginning of the thermogenic activity for one individual appendix, whereas C and D were recorded at the end of that activity in a second individual. The key on the right side shows the range of temperatures in °C. Each color represents a range from greater than the first value to a temperature equal to the second value. For example, black represents temperatures from above 0°C to 20°C. The shape of the appendix tissue is that of the slender, curved form in the images. Bars represent 3 cm.

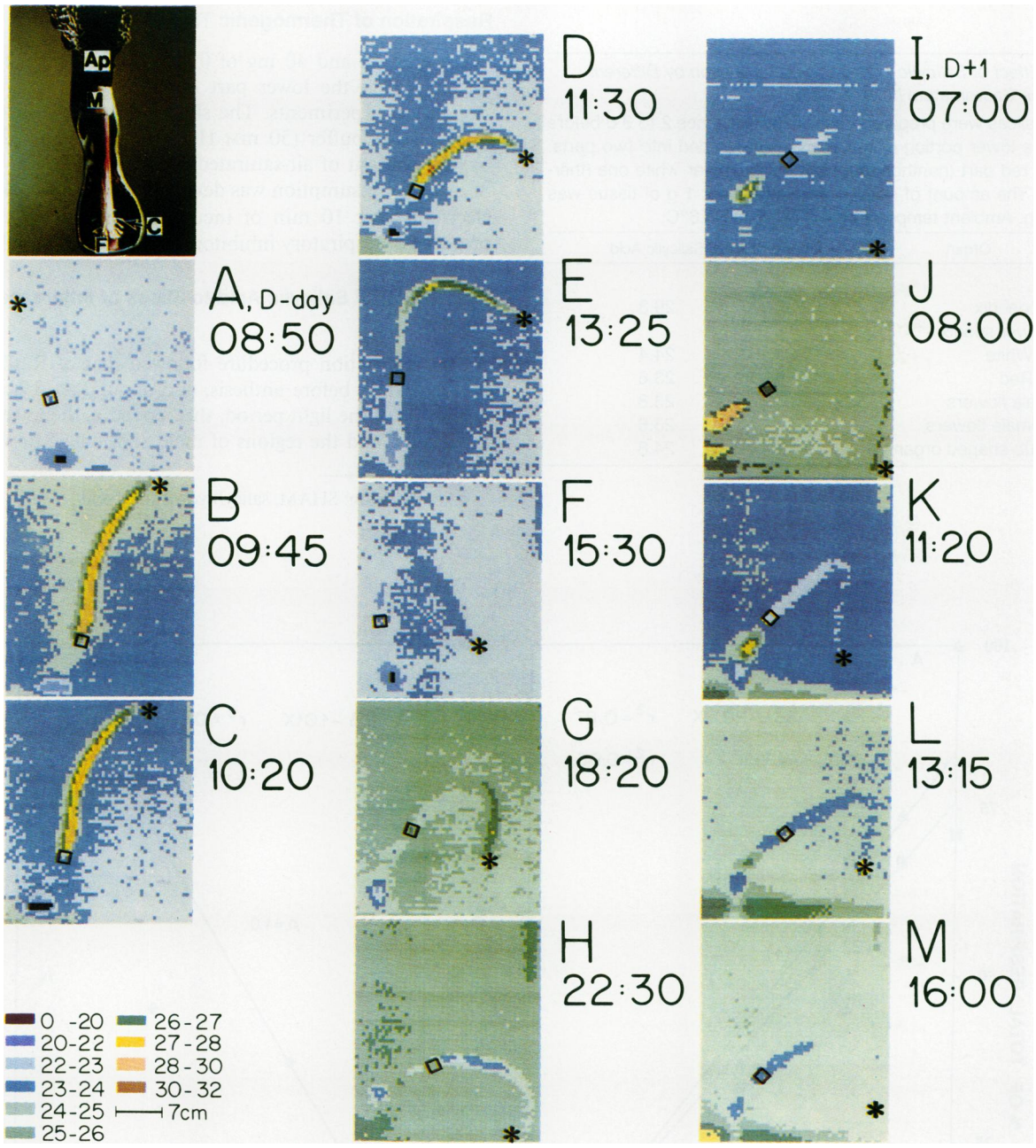

Figure 2. Time sequence of surface temperatures of the *Sauromatum* inflorescence. Thermal images recorded during the thermogenic activity of one individual inflorescence. The time of day is indicated next to each image. The size scale is for the thermographic images only; it does not apply to the photograph of the inflorescence, the first picture in the series. Information on the color key as in Figure 1. Ap, appendix; M, male flowers; F, female flowers; C, club-shaped organs. An asterisk (*) indicates the tip of the appendix.

Table I. *Effect of Salicylic Acid on Heat Production by Different Parts of the* Sauromatum *Inflorescence*

Tissue slices were prepared from an inflorescence 2 to 3 d before D-day. The lower portion of the spadix was divided into two parts: the upper red part (nonthermogenic) and the lower white one (thermogenic). The amount of salicylic acid added per 1 g of tissue was 0.5 to 1 μg. Ambient temperature was 23.6 to 24.8°C.

Organ	Control	Salicylic Acid
	°C	
Appendix	24.0	29.3
Lower spadix		
White	24.1	24.4
Red	23.6	23.6
Male flowers	23.8	23.8
Female flowers	23.6	23.6
Club-shaped organs	24.1	24.8

Respiration of Thermogenic Tissue

Between 10 and 40 mg of 0.5 mm tissue slices from the appendix and the lower part of the spadix were used for respiration experiments. The slices were placed in 4 mL of fully aerated buffer (50 mM Hepes [pH 6.6]) at 22°C. The oxygen content of air-saturated water was 237 μM. The rate of oxygen consumption was determined with a Clark oxygen electrode after 10 min of incubation in the buffer in the presence of respiratory inhibitors KCN and/or SHAM[2] (6).

Application of Salicylic Acid to Slices of Inflorescence Tissue

The application procedure followed that of Raskin *et al.* (4). Two days before anthesis, and about 1 to 3 h after the beginning of the light period, the appendix, the lower part of the spadix, and the regions of male and female flowers were

[2] Abbreviation: SHAM, salicylhydroxamic acid.

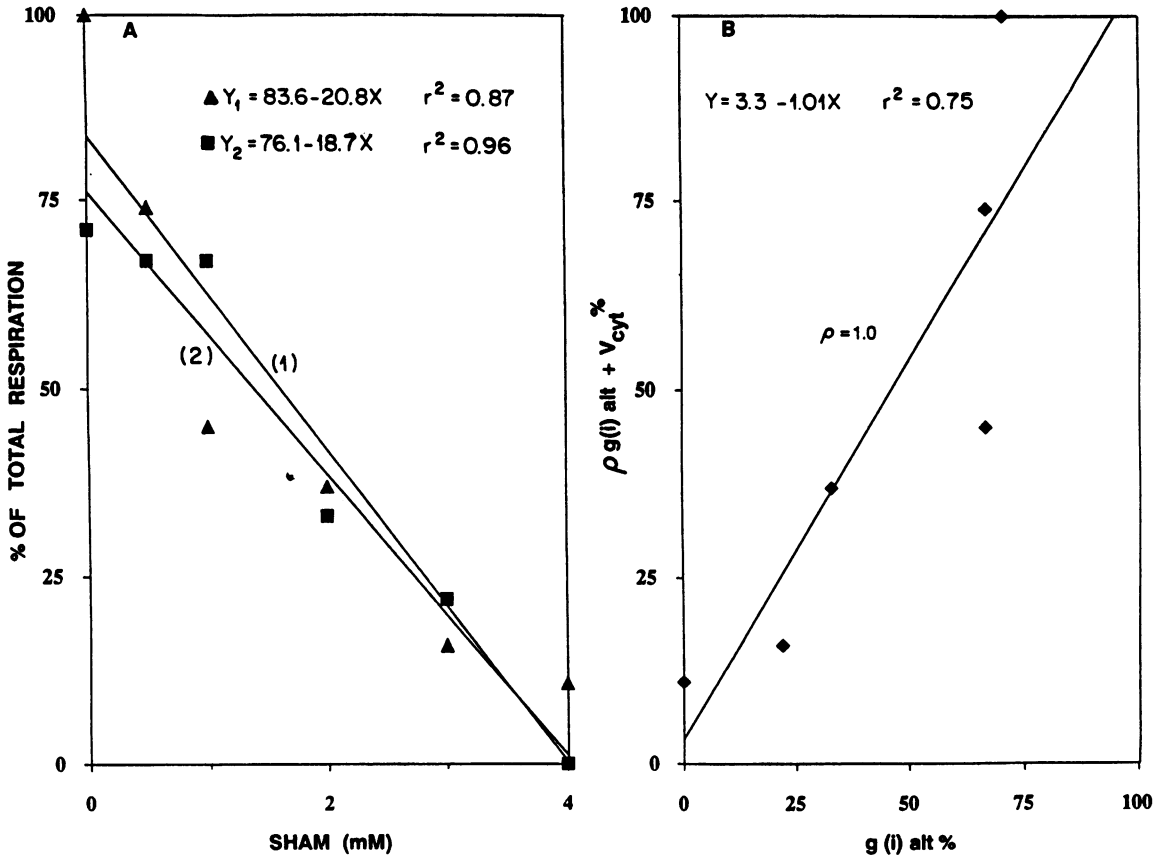

Figure 3. Titration of oxygen-uptake for slices of *S. guttatum* appendix during thermogenic activity. Panel A shows the rate of oxygen uptake at different concentrations of SHAM in the absence (line 1) and presence (line 2) of 1 mM KCN. Total respiration rate for 10 tissue slices in the absence of any inhibitor was 1.4 ± 0.3 μmol O_2/min/g fresh weight. In panel B the values from line 2 are plotted *versus* those from line 1. The data were obtained from one appendix and each datum represents one single experiment. *r*, Correlation coefficient; ρ, ratio of activity/capacity for the alternative pathway; *g(i)* alt, maximal contribution of the alternative pathway at a given concentration of SHAM; V_{cyt}, contribution of the Cyt pathway to respiration.

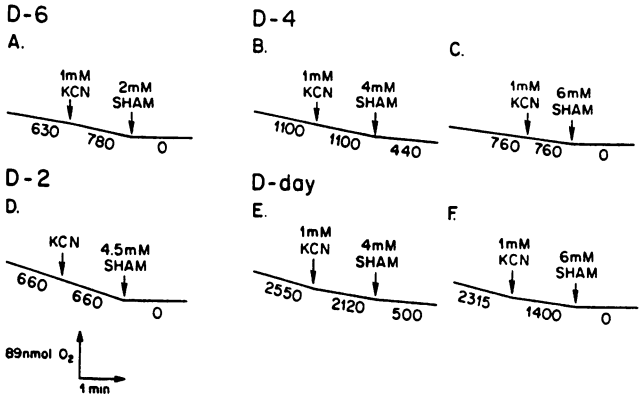

Figure 4. Assessment of the capacity of the alternative pathway in slices of *S. guttatum* appendix. The arrows indicate the point at which the inhibitors were added. Thirty-three mg of tissue were used in the experiment at 6 d before D-day (D − 6); 28 mg in D − 4; 58 mg in D − 2; 10 and 13 mg, respectively, in D-day experiments. The numbers below the lines indicate the respiration rate in nmol O_2/min/g fresh weight.

sliced transversely into discs of about 1 g. Five or 10 μL of 0.7 mM salicylic acid were pipetted on top of each section so that the acid concentration was 0.5 to 1 μg/g fresh weight. Water was pipetted onto the control sections. The sections were placed on a moist piece of filter paper and incubated overnight in a growth chamber at 19°C. The next morning, 4 h after the beginning of the light period and at the peak of heat production, surface temperatures were determined with the aid of a copper constantan thermocouple.

RESULTS

Thermographic Detection of Heat Production

Infrared thermography was used to demonstrate the effect of ambient temperature on heat emission from *Sauromatum* appendix. Figure 1A shows a thermogenic appendix at the beginning of heat-production; its surface temperature reaches 25 to 26°C. However, when placed in a colder environment (about 17°C), the temperature of the appendix reaches no more than 22°C (Fig. 1B). When the ambient temperature declines from 23 to 22°C (Fig. 1, C–D) the emission of heat from the appendix surface also declines: its temperature drops from 24 to 20°C. This suggests that a temperature of 22 to 25°C is best for detecting thermogenic activity.

The time course of the changes in surface temperature of the inflorescence of *S. guttatum* is depicted in Figure 2. Early on D-day morning, around 8:50, the male flowers (circumscribed by a square) are thermogenic (Fig. 2A). Later in the morning, the entire appendix heats up at the same time, and the male flowers are still thermogenic. The tip of the appendix (indicated by an asterisk) is at 26 to 27°C, while the rest of the appendix reaches 30°C (Fig. 2, B and C). Around 11:30, the surface temperature of the appendix reaches a peak, and the male flowers have begun to cool (Fig. 2D). The appendix temperature then slowly declines with the upper part cooling less rapidly than the lower part (Fig. 2, E and F), and the male flowers and lower part of the spadix (near the female flowers) start heating up (Fig. 2G). At 22:30, the male flowers and the lower part of the spadix are warmer than the appendix, which is now colder than ambient (Fig. 2H). The next morning (D + 1), the male flowers and the lower part of the appendix are still thermogenic (Fig. 2, I–J). The upper part of the appendix is also weakly thermogenic on D + 1 (Fig. 2J). Later during the day, surface temperature slowly declines (Fig. 2, K–M).

Induction of Heat Production by Salicylic Acid

Salicylic acid induces heat production in *Sauromatum* appendix but it does not cause the release of heat by the lower part of the spadix (Table I). We could not detect a significant increase in heat production by that part in experiments carried out with five different inflorescences. Since the increase in temperature of the male flowers is small, the effect of salicylic acid on heat production by these flowers could not be assessed.

Table II. *Respiration Rates of* S. guttatum *Appendix Slices during Development*

Total respiration is v_t. The capacity of the Cyt pathway is v_{cyt}. The capacity of the alternative pathway is V_{alt}. The activity of the alternative pathway is v_{alt}. No residual respiration was detected. SHAM concentration was between 3 and 13 mM depending on the appendix age. The data represent the mean ± SD of four to eight determinations done on one appendix at a certain stage of development, and the mean of two determinations done on the lower (thermogenic) part of one spadix.

	Age	v_t	v_{cyt}	V_{alt}	v_{alt}	v_{alt}/V_{alt}
			μmol O_2/min/g fresh weight			
Appendix	D−30	0.3 ± 0.1	0.3 ± 0.02	0.3	0.0	0.0
	D−15	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.03 ± 0.03	0.08
	D−6	0.6 ± 0.2	0.3 ± 0.1	0.7 ± 0.3	0.2 ± 0.1	0.2
	D−4	0.8 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	0.1 ± 0.1	0.1
	D−2	0.8 ± 0.2	0.4 ± 0.2	0.9 ± 0.2	0.3 ± 0.1	0.3
	D−1	1.7 ± 0.3	1.1 ± 0.1	1.2 ± 0.3	0.7 ± 0.2	0.5
	D-day	1.3 ± 0.2	0.2 ± 0.2	1.2 ± 0.1	1.0 ± 0.2	0.8
	D + 1	1.1 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6
	D + 2	1.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.4 ± 0.1	0.5
Lower spadix	D-day	0.5 ± 0.01	0.1	0.4	0.4	1.0

Tissue Respiration

The effect of SHAM on oxygen-uptake by fresh slices obtained from D-day appendix was determined in the presence and absence of KCN (Fig. 3A). Regression of the values obtained yield two straight lines with 95% confidence intervals for the two slopes: 20.8 ± 11 for line 1 and 18.6 ± 5 for line 2. This indicates that the slopes of the two lines are not significantly different. Furthermore, the graphs show that SHAM does not cause any side effects and only mitochondrial respiration contributes to the oxygen uptake by the appendix. Figure 3B shows that the ratio of activity/capacity of the alternative pathway in these slices is not significantly different from 1.0 ($P > 0.8$).

The presence of the alternative pathway in appendices at different stages of development was determined in two experiments with two different concentrations of SHAM for each individual appendix (Fig. 4, B and C; E and F). This pathway was present in all the tissue slices regardless of their stage of development and its capacity increased from D − 6 to D-day from 630 to 2550 nmol O_2/min/g fresh weight (Fig. 4, A and F). A 30% difference was observed in respiration rates of different slices obtained from one appendix (Fig. 4, B and C). The activities of both the alternative and the Cyt pathways increase during development (Table II). The activity of the Cyt pathway, however, is restricted on D-day (0.2 μmol O_2/min/g fresh weight). After D-day, the activity of the Cyt pathway increases while that of the alternative pathway decreases. The alternative pathway is also present in the lower part of the spadix.

We conclude that the capacity of the alternative and the Cyt pathways increases during the development of the inflorescence. The alternative pathway is present in the tissue in an inactive form and its activity gradually increases until it reaches a maximum on D-day. While the alternative pathway is fully operative on D-day, the Cyt pathway is restricted.

DISCUSSION

It was shown previously that the 'metabolic explosion' in *Sauromatum* is triggered by salicylic acid (4). The levels of salicylic acid were determined in the various parts of the inflorescence: appendix, male and female flowers, and the lower part of the spadix (5). It was shown that the level of salicylic aid in the appendix as well as in the male flowers is high in the evening, one day before D-day, suggesting that salicyclic acid indirectly triggers heat-production on D-day, and indeed, 1 d later both parts were thermogenic. Since salicylic acid was undetectable in both parts on D + 1, the occurrence of a second heating period (Fig. 2J) cannot be caused by salicylic acid. Furthermore, the level of salicylic acid is higher in the male flowers than in the appendix (5), and yet the appendix is much warmer than the male flowers. This suggests that there is no correlation between the level of salicylic acid and the amount of heat produced by a certain organ. The fact that the salicylic acid level in the lower part of the spadix is high on D-day evening (some 10–14 h before maximum heat production) when this part is already thermogenic indicates that salicylic acid may not be the trigger of heat production in this part of the spadix. Another indication that the release of heat from the lower part of the spadix involves different chemical signals is the fact that heat production by the lower part of the spadix could not be triggered by salicylic acid. Salicylic acid is only one of the two biologically active compounds found in the original preparation of 'calorigen' (1). The second compound could conceivably be the trigger of heat production in the lower part of the spadix.

The respiration of appendix slices is different from that of the whole organ. A high rate of oxygen uptake (1.7 μmol O_2/min/g fresh weight) was observed with slices obtained from the appendix on D − 1, while the whole appendix respired at a much lower rate (0.48 μmol O_2/g fresh weight; Table III, line 2 in ref. 7) at this time. The restriction of electron flow through the Cyt pathway on D-day was also observed in mitochondrial preparations (3), and it again suggests that when the Cyt pathway is restricted the activity of the alternative pathway increases.

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