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Oxygen-dependence of metabolic heat production in the appendix tissue of the voodoo lily (*Sauromatum guttatum* Schott)

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Abstract

Scanning electron microscopy revealed abundant stomates in the epidermis of the appendix tissue of voodoo lily (*Sauromatum guttatum* Schott). The stomates were closed before and after the day of flowering climacteric (D-day), but were fully open at the time of peak metabolic activity. The rate of metabolic heat production, as determined by microcalorimetry, was completely dependent on oxygen availability. The highest heat rates were obtained from D-Day appendix tissue in 100% oxygen, the smallest bits of tissue having the highest rates (9100 μW mg^{-1} dry wt.). Metabolic rates were lower for larger pieces of tissue or for tissue in air or nitrogen compared with oxygen. Even a thin film of water on the tissue surface reduced the respiratory rate. Metabolism of the D-day appendix had a Q_{10} of 2.5.

Keywords: Metabolic heat; O_2 dependence; SEM; Stomates; *Sauromatum guttatum*

1. Introduction

Sauromatum guttatum Schott (voodoo lily) is native to the foothills of Kashmir. The monsoon weather pattern of that area allows the plant to produce lush vegetative growth and storage of starch in an underground corm during the wet season. In the dry season, the vegetative tissue dies and the corm can be removed from the soil. Even without soil or water, a spadix covered with a spathe begins to grow at the expense of starch in the corm. One morning the spathe peels back, revealing a dark purple appendix with clusters of male and female flowers at its base. The appendix produces an odor reminiscent of fresh cow dung. Due to a high respiration rate, the spadix can reach temperatures as high as 15°C above ambient [1]. Salicylic acid produced in the male flowers is the chemical messenger that initiates these events [2]. Prior to D-day, appendix cells are packed with starch, most of which is burned during the respiratory burst. Respiration before and after D-day is energy-conserving, while that during D-Day is not ADP-dependent and largely involves the alternative path of respiration [3,4]. On D-day the female flowers are receptive of pollination. Heat produced in the appendix volatilizes indoles, amines and ammonia which attract pollinating insects [5]. Many accounts of respiration in Aroids have been published [1,6]. Peak appendix temperature has been

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measured with thermometers [7], thermocouples, heat-sensitive dyes, infra-red radiation [8], and heat rates with calorimeters [9,10] although lack of thermal equilibration in one case severely compromised the results [11]. Considerable variation exists between the various measurements of both respiration and heat production. The appendix tissue of the voodoo lily at anthesis is of significant interest because it is generally conceded to have one of the highest metabolic rates of any plant tissue. The purpose of this study is to determine the reasons for the measurement discrepancies by measuring the metabolic heat rate of the appendix tissue under various conditions.

2. Materials and methods

Sauromatum guttatum Schott (Araceae) corms grown in the greenhouses of the University of Washington were shipped to Provo, free of soil. Eighteen corms were placed directly on a shelf (no soil or water) in a growth chamber under 15 h light/9 h dark periods with a photon flux density of 50 μmol m^{-2} s^{-1}. Daily each corm was weighed and the height measured from the bottom of the corms (tabletop) to the tip of the spathe and spadix. The spathe and spadix remained intact and attached to the corm except for small amounts of tissue used in calorimetry.

Slices of appendix tissue were cut on a sliding microtome. Tissue was weighed at the conclusion of a run (fresh weight) and after drying overnight at 60°C in a vacuum oven (dry weight). The day of maximum heat and odor production is called D-day according to the convention established many years ago by Meeuse [12]. Age of tissue before and after flowering is designated as D−2, D−1, D+1, D+2, etc.

Appendix epidermal tissue at various stages was preserved by an osmium/liquid carbon dioxide treatment, and photographed with a scanning electron microscope. The number of stomates in a given area of the appendix surface was counted in several scanning electron micrographs to obtain stomatal density (Fig. 3-1). At higher magnification (Fig. 3-2–3.4) stomates from each stage were measured for length and width with the width/length ratio, an index to the degree of stomatal opening. A Hart Scientific model 7707 differential scanning microcalorimeter with four ampules, each with a total volume of 1 ml was used to measure metabolic heat rates [13]. One ampule (empty) is a reference blank and the other three contain samples. The calorimeter was run in the isothermal mode at 25°C. After a thermal equilibration period of 20–30 min, the heat rate from an ampule is solely due to metabolic activity. Specific metabolic heat rate is typically expressed as micro-watts (μW) per milligram dry weight. Each determination required 40–60 min. Baseline values were obtained and corrections made for each ampule and each position with empty ampules. An appendix tissue slice, 1 mm thick, was placed in each ampule. Slices from some of the appendices were larger in diameter than the ampule, so half and quarter slices and even smaller pieces were used. For atmospheres other then air, ampules were flushed three times with oxygen or nitrogen and sealed while inside a plastic bag.

3. Results

Average values with standard error bars for height and weight of 18 corms are shown in Fig. 1. The decrease in combined corm and spathe and spadix weight was due to water loss and metabolism and was slow at first but near D-day was much more rapid, amounting to 50 g per day. By contrast the growth rate in the height of the spathe and spadix was constant until near the time of flowering [9]. Growth of the spathe and spadix occurs largely by transfer of material from the corm, but events associated with flowering take a much larger toll of corm biomass. Fig. 2 indicates a good correlation (R=0.85) between weight loss and spathe and spadix growth. Prior to flowering the newly formed appendix cells are packed with starch, which is largely gone by D+1 [12].

Scanning electron micrographs of the voodoo appendix epidermis at different flowering stages revealed that stomates occur in a regular pattern (Fig. 3-1) and at a very high density (3.3×10^6 stomates cm^{-2}). In fact, no plant tissue to our knowledge has as many stomates per unit area as does the voodoo lily appendix [14,15]. Measurements of length and width of the apertures were made on 300 stomates from each stage (D-1, D-day, and D+1). Stomata from the voodoo appendix epidermis are about twice as long (12–15 μm) as bean leaf stomata (7 μm) but much shorter than oat leaf stomata (38 μm). Based on the

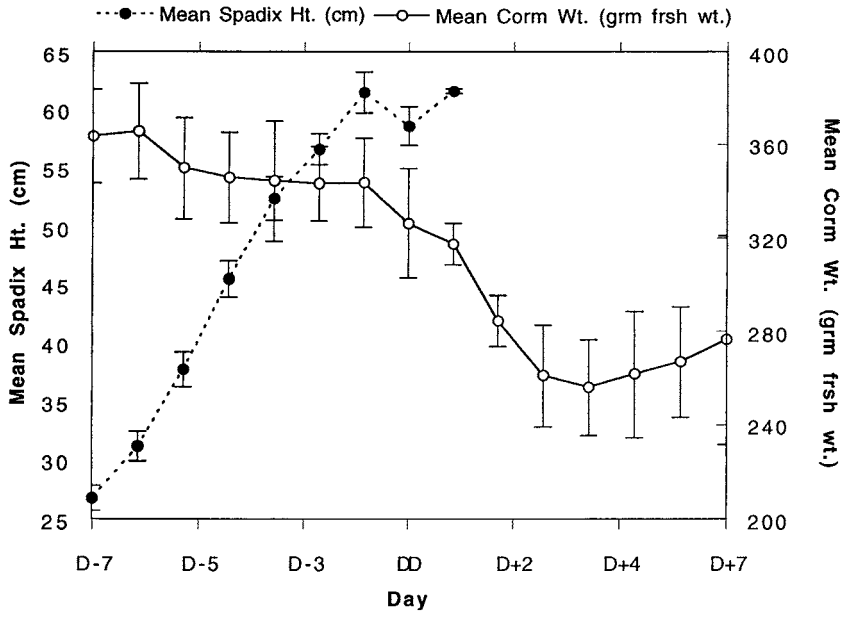

Fig. 1. Growth of spadix and loss of corm fresh weight with time. Spadix height was measured from the bottom of the corm. D-day is the time of respiratory climacteric and maximum heat production. D−2, D−1, D+1, D+2, etc. are days before and days after flowering. Vertical bars represent +one standard deviation.

width/length ratio in the SEM images, stomata were classified as closed, partly open, or open. The stomata were open on D-day but closed on D−1 and D+1 (Fig. 3 and Table 1).

Appendix tissue on D-day (Table 2) had a much higher metabolic heat rate in oxygen than in air or nitrogen. Therefore all subsequent experiments were run in ampules flushed and filled with 100% oxygen.

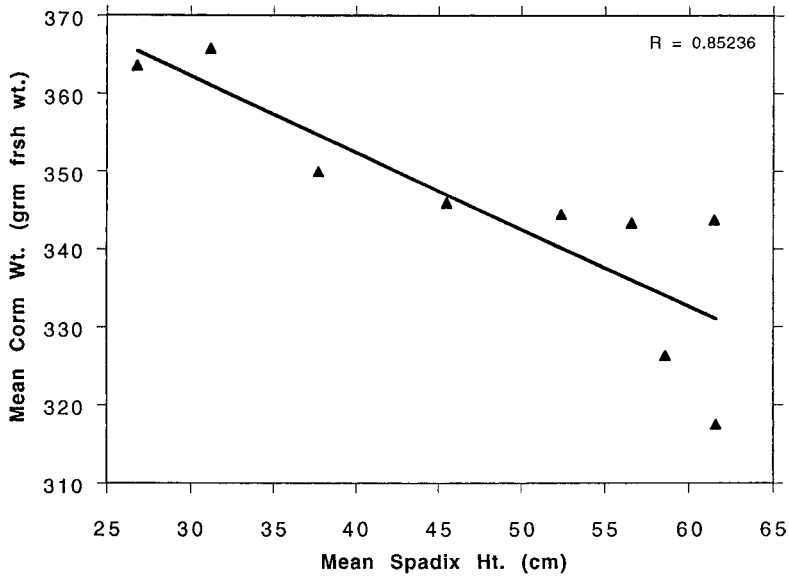

Fig. 2. Relationship between corm weight and spadix height.

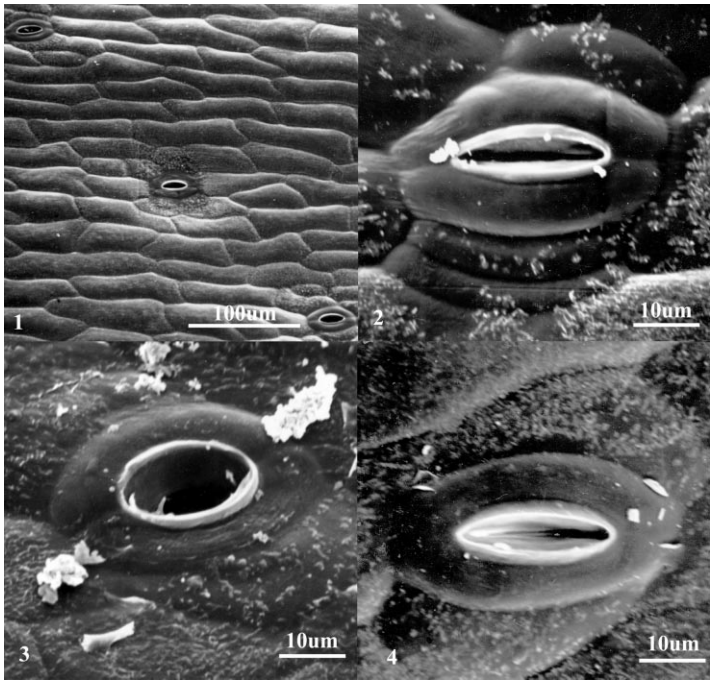

Fig. 3. Scanning electron micrographs of *Sauromatum guttatum* epidermis. Bar is 100 μm in 3−1 and 10 μm in Fig. 3-2–3.4. Fig. 3-1 is D-day, low magnification (×250). Fig. 3-2 is D−1 (×1500). Fig. 3-3 is D-day (×1500). Fig. 3-4 is D+1 (×1500).

Even a thin film of water (Table 3) diminished metabolic heat rate by 13% due to slower diffusion of oxygen through liquid water as compared to the gas phase. Metabolic heat production was very sensitive to temperature, with a Q_{10} of 2.5 (Table 4). Metabolic heat rate of the voodoo appendix tissue (1 mm thick slices) was measured during the 4 days before and 3 days after flowering (Table 5). Metabolic activity began to increase on the afternoon of D−1. The respiratory peak was reached between 9 and 10 am

Table 1
Stomatal opening with flowering stage of *Sauromatum guttatum* appendix tissue from scanning electron micrographs

	Percent of stomates		
	Open	Partly open	Closed
D−1	2	14	84
D−day	88	0	12
D+1	4	28	68

Table 2
Metabolic heat production of 1 mm thick slices of D-day *Sauromatum* appendix tissue in atmospheres of air, oxygen and nitrogen

Atmosphere	μW mg^{-1} Dry wt.
Air	59.7
Oxygen	138.6
Nitrogen	40.0

Table 3
Metabolic heat produced by a 1 mm slice of D-day *Sauromatum* appendix tissue in 100% oxygen with and without a thin film of water

μW mg^{-1} Dry wt.	
Slice	243.9
Slice+4 μl H_2O	212.7

Table 4
Metabolic heat produced by a 1 mm thick slice of D-day *Sauromatum* appendix tissue in oxygen at 15 and 25°C

μW mg^{-1} Dry wt.			
	25°C	15°C	Q_{10}
D−day	434.8	174.8	2.5

Table 5
Metabolic heat produced by 1 mm thick slices of *Sauromatum* appendix tissue in oxygen at different flowering stages

Flowering stage		$\mu W\ mg^{-1}$ Dry wt.
D−4		43.2
D−3		42.6
D−2		48.5
D−1	Morning	42.3
	Afternoon	64.8
D−day	Morning	425.0
	Afternoon	207.0
D+1		63.6
D+2		48.0
D+3		49.7

on D-day, then gradually declined. D+1 tissue was still roughly comparable in metabolic activity to D−1 afternoon tissue; however, by D+2 had further decreased to levels comparable to those from D-tissue.

Different metabolic heat rates were obtained from the D-day appendix tissue—even when taken from the same appendix, same part of the appendix, same time of day, etc. (see Tables 2–5). We were satisfied that metabolism reached a maximum on the morning of D-day, but what was the correct value? In early experiments, using relatively large amounts of tissue, the heat rate dropped precipitously after a short time, indicating a lack of oxygen. For that reason we consistently used 1 mm thick slices. However, due to differences in appendix diameter, sometimes we used whole slices, sometimes part of a slice. Since oxygen seemed to limit the metabolic rate (Table 2), a systematic study was done using ever smaller bits of tissue to increase the surface-to-volume ratio. The smallest sample used was 2 mg fresh weight, which became 0.1 mg dry wt., and gave a metabolic heat rate of 9100 $\mu W\ mg^{-1}$ dry wt. (Table 6). This is equivalent to almost 38 calories per gram of tissue per second.

Table 6
Metabolic heat production as a function of sample size for D-day *Sauromatum guttatum* appendix tissue

Mg dry wt. of sample	$\mu W\ mg^{-1}$ Dry wt.
0.1	9097.1
0.8	822.4
1.8	554.8
2.7	488.0
2.8	401.5
2.9	384.0
3.2	402.2
3.9	351.0
6.5	243.9
10.0	174.7

4. Discussion

Voodoo lily appendix tissue has a very high density of stomates that are fully open at the time of peak metabolic activity, but are closed before and after that time. Thus, stomates appear to exist for the transport of oxygen to cells of the appendix. Since metabolic heat rate appears to depend on the surface-to-volume ratio (Table 6), oxygen diffusion rate into the cells limits respiration rate. Eastern skunk cabbage (*Symplocarpus foetidus*) flowers in February and March with the heat from the inflorescences melting the surrounding snow. Temperature control (mechanism unknown) has been claimed for skunk cabbage [16] and other species, including *Sauromatum guttatum* [6]. However, Knutson [16] reported that the heat produced by skunk cabbage inflorescences varied directly with the spadix weight—implying that heat production was limited by oxygen diffusion, which our work has confirmed. In fact, our results show (Table 4) a positive relationship between metabolic heat rate and ambient temperature, as would be expected in ectothermic organisms. While general agreement exists for a high rate of peak respiration on D-day, the published values differ 10-fold [4,6,9]. Differences in sample size are one probable cause for differences in respiration rates. Since oxygen diffusion in water is much slower than in air, metabolism of isolated mitochondria as usually measured [3] is oxygen-limited. Thus tissue slices for the voodoo lily appendix in air or oxygen give a much more accurate picture of metabolic capacity.

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