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Thermochimica Acta 394 (2002) 191–204

thermochimica acta

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Energy metabolism of the thermogenic tropical water lily, *Victoria cruziana*

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Received 12 December 2001; received in revised form 18 February 2002; accepted 2 March 2002

Abstract

Energy turnover in the giant water lily, *Victoria cruziana* was determined by indirect calorimetry (oxygen consumption rate) and different kinds of thermometry. Experiments were performed in a greenhouse pond of the Botanical Gardens, Free University of Berlin, Germany at constant water and air temperatures (30 and 24 °C, respectively). Flowers were investigated (i) in situ as a whole floating on the water or elevated a few centimeter above its surface, and (ii) in vitro after cutting and transporting to the laboratory: (a) as a whole or (b) dissected into their single components.

Buds and flowers have heat production rates between 1 and 9 mW g^{-1} wet weight, depending on their state of blooming. Results show that plant structures which are near to the floral chamber exhibit high metabolic rates and temperatures raised by about 10 K compared with outer parts like sepals. Continuous temperature monitoring reveals a time course with two heat dissipation maxima in the first evening/night and the afternoon of the second day of blooming. Growth of leaves is briefly touched.

Keywords: Indirect calorimetry; Metabolism; Thermogenic plants; Thermometry; *Victoria cruziana*

1. Introduction

Plants are supposed to have low metabolic rates compared with animals and due to their unfavourable surface to volume ratio a temperature which is about that of the surroundings. Moreover, evaporation necessary for transport within plants adds a cooling effect which lowers the temperature when leaves are irradiated by sun. This picture is true for the majority of plants in the majority of situations. But there is a group of plants—the thermogenic ones—that show a metabolic flare-up during blooming and as a consequence significantly increased temperatures. This group comprises some aroids like the Lords-and-Ladies *Arum maculatum* and *A. italicum*, the voodoo lily *Sauromatum guttatum* and the American skunk cabbage *Symplocarpus foetidus* or the giant *Amorphophallus titanum*, then the sacred lotus *Nelumbo nucifera* or some cycads and palms [1]. Three evolutionary reasons for the elevated temperatures are discussed in the literature [2]. The observed temperature increases extend from <1 K up to >30 K against ambient and from a few hours to days and some weeks. A few of the thermogenic plants—among them *N. nucifera*, *Philodendron selloum*, *Dracunculus vulgaris* or *S. foetidus*-show a high degree of thermoregulation [3–8], comparable to that of homeothermic mammals and birds.

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0040-6031/02/$ – see front matter
PII: S0040-6031(02)00250-2

Until now, only the easy to breed aroid voodoo lily *S. guttatum*, *P. selloum*, sacred lotus *N. nucifera* and the dragon lily *D. vulgaris* were investigated calorimetrically in an indirect and direct approach [6,9–11] as whole plants in situ, cut for investigations or as samples of their different tissues. Metabolic rates higher than those of other plants or of non-thermogenic tissues of the same plant were found.

The tropical water lilies *Victoria amazonica* and *V. cruziana* belong to the thermogenic plants, but not to the thermoregulating ones. They were discovered 200 years ago and their thermogenecity was reported 50 years later for the first time [12–15]. For more details of their discovery and their triumphant botanical procession to and throughout Europe see the literature [12,15–18]. After several changes the botanical name of this "aquatic monster" and tropical beauty was finally fixed to *V. regia* and more recently to *V. amazonica* which is her scientific name today.

The slightly smaller *V. cruziana*, that is the object of the present investigations, got its name in honour of the Spanish general Santa Cruz. It grows in the cooler areas in Argentina and Paraguay where it is called "Yrupe" that means "water platter". It is not as spiny as *V. amazonica* and thus more easy to handle. *V. cruziana* is even better known by its gargantuan leaves with their stiff upright rims than by its beautiful flowers. The flowers last only 1.5 day with the strongest impression in the late evening of the first day and the next morning so that most greenhouse visitors have only modest chances to see the full beauty and to come near enough to the blossoms to detect their sweet odour of fresh fruit.

The interest in *Victoria* originates from the fact that *Victoria* is a further member of the group of thermogenic plants that were investigated earlier by one of the present authors (I.L.) and that allowed to apply new and rather inorthodox methods of calorimetry [8,19]. Moreover, *Victoria* exhibits a pronounced energy turnover and an interesting time regime during its blooming. As all the present investigations on *V. cruziana* are intended to be pre-experiments for in situ measurements with *V. amazonica* at the Amazon, they were planned to be as simple as possible with small scientific equipment that can be easily shipped to Colombia and also further consumption parts like plastic bottles of different size or canisters for transporting water and shipping chemical substances to be found there.

2. Experimental

2.1. Plant material

All plant material used in the present investigations was obtained from a greenhouse of the Botanical Gardens, Free University of Berlin, in the year 2000 between July and November. A comprehensive description of the experimental conditions and of the instruments used was given in a recent paper on thermometric evaluation of the metabolic flare-up of *V. cruziana* [19]. Therefore, only in brief, the greenhouse pond had a size of 4 m × 16 m and a water depth of 0.40 m. The water was kept at a nominal temperature of 30 °C with a mean value of 29.9 ± 1.0 °C during the growth season. The air temperature had a long term mean of 24.5 ± 1.5 °C with short time maxima in direct sun light near to 50 °C. Nevertheless, no shading facilities were used as the high heat capacity of buds and blossoms had a significant damping effect on tissue temperatures (see also [15]). The relative humidity in the greenhouse was always near to 100%.

Two *V. cruziana* plants were available for the experiments. Although the ambient conditions were rather similar, both developed differently for unknown reasons. Nevertheless, the results were pooled and are presented here together. In total, 61 blossoms of both *V. cruziana* plants were investigated from a late bud state till to the end of the thermogenic phase and the starting of submersion. As the greenhouse was open to the public in a "show character", we were only allowed to investigate the plants in situ and to cut just a few flowers for further laboratory research at the end of the thermogenic phase. Thus, 10 of the 61 blossoms were cut under water and transported to the lab in a bucket with water avoiding entrance of air into the stalk. In parallel to the flowers, leaves of *V. cruziana* were monitored for growth rate, diameter, weight and surface temperatures.

2.2. Thermometry

Thermometry was performed in three different ways: (i) one or two times per day by mercury thermometers (grading 0.1 K) for inside temperatures of buds and blossoms, or (ii) by a non-contact hand-held infrared (IR) thermometer (THI-300, Tasco, Japan; spectral band from 6 to 12 μm) for surface

temperatures of blossoms, leaves and water, or (iii) by a combination of thermoresistors and data loggers to continuously collect data of ambient and blossom temperatures.

2.3. Data logging

Two types of light (20 g) data loggers (Onset Computer Corporation, Pocasset, MA, USA) were used for continuous temperature monitoring, a one-channel logger with an internal sensor (HOBO Temp, Series 01) and a four-channel version for ambient temperature, humidity, light intensity and a further external signal, e.g. a second temperature (HOBO RH, Temp, Light, External, Series 08). Both worked in a temperature range from −20 to +70 °C with an accuracy of ±0.7 K at 20 °C and a resolution of 0.1 K. The first stores up to 1800 data points (2KB) and the second 8.000 data points (8KB). The stored data could be retrieved to a PC with a special software (BoxCar 3.5) and could be imported to Microsoft Excel for further treatment.

The one-channel HOBOs were so light that they could be placed on a styropore™ plate or on one of the *Victoria* leaves near the flowers. Because of larger movements between leaves and flowers the position of the thermal sensor was not as secure in the second case so that the first was preferred. The floral chamber of the blossom was equipped with two temperature sensors, one horizontally just above the water level, the second vertically through a "tunnel" (Fig. 1, "T") between the paracarpels (Fig. 1, "5"). The indicated temperatures varied slightly but showed an identical graph as function of time. Sometimes, a third reference sensor in a smaller styropore™ block was placed near to the flower on a leaf to monitor the local temperature around the flower which could differ considerably from the mean one of the greenhouse due to direct sun light. It was not possible to place the reference sensor in the stalk of the flower or another part of the plant because the distance between the blossom and the water surface was too small or even negligible.

2.4. Indirect calorimetry

Indirect calorimetry was performed by means of electrolytic oxygen sensors that determine the O_2 concentration in the air (FIGARO GS Oxygen Sensor KE-Series, UNITRONIC, Düsseldorf, Germany). They were screwed to bottles of different volumes from 50 to 1500 ml depending upon the amount of plant tissue available. Moreover, they were attached to a rectangular 10 l canister, for shipping of chemical substances, when the metabolic rates of whole buds or flowers should be determined in the greenhouse pond. The bottom of the canister was sawed off so that the container could be placed as a hood over the flower, floating on the water. To stabilize swimming an inner tube of a children's bicycle was fixed around its middle and counter weights were fastened at the lower rim in the water. In this way, the hood remained upright even at stronger movements of the flower. A scale at the side allowed to evaluate the depth of immersion and thus the active head space of the hood. According to the size of the bud or flower one could change the head space from 1 to 6 l.

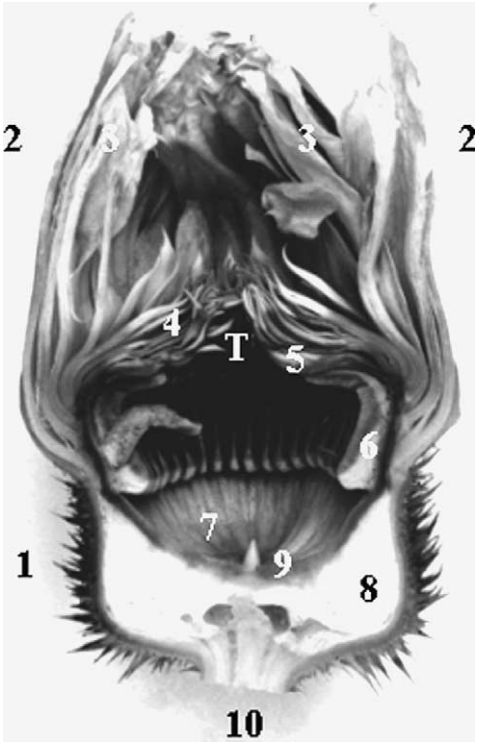

Fig. 1. Cross section of a *Victoria* blossom after its thermogenic episode. (1) Spines; (2) sepals; (3) petals; (4) stamens; (5) paracarpels; (6) carpellary appendages; (7) floral chamber (stigmatic cub); (8) ovaries; (9) floral apex; (10) stem; (T) "tunnel".

The diffusion of dissolved oxygen from the water into the head space can be neglected compared with the metabolic oxygen consumption rate of the plant except for the end of the thermogenic period. An empty hood produced a baseline that was stable for more than half a day so that an influence of the respiration by small fish and by algae in the pond could be excluded. As it was shown earlier that *Victoria* burns carbohydrates during its metabolic flare-up the oxygen consumption rates (in $ml\,O_2\,s^{-1}$) can be transformed into energy rates (W) by multiplying them with a factor of $21.1\,J/(ml\,O_2)$.

2.4.1. Data logging

The oxygen sensors were connected to a four-channel data logger (UNIDAN^{PLUS}, ESYS, Berlin, Germany) with variable amplifications between 1 and 128 times and a resolution of 0.1 mV in these experiments. Up to 4MB of experimental data could be stored with repition rates between 1 s and 24 h. The data were further processed with Microsoft Excel.

3. Results

3.1. Anatomy of Victoria blossoms

The most significant part of the *V. cruziana* blossom is the floral chamber or stigmatic cup (Fig. 1, "7")) that is common for most of the thermogenic flowers and which is protected here by sharp spines (1) against predators. It houses the female part (8) of the blossom and the carpellary appendages (6) that are intended for feeding visiting pollinators. It is firmly closed by the paracarpels (5) that only open twice by an upward bending to form a passage to the chamber, the tunnel (T). The first opening happens during the receptive female state in the evening of day 1 when the strong, appealing scent is produced. At that time, pollen covered pollinators may enter the floral chamber. During the next hours the paracarpels close again till to the next afternoon when the flower turned to its colourful male state and the anthers shed their pollen on the by-passing beetles. During the time interval between the two open states the pollinators are kept warm by the flower to reduce their own metabolism and are fed as reward for their visit.

Above and partly around the floral chamber are the stamen (4) that are the main heat producing organs and that follow the upward movement of the paracarpels. They are enclosed in a manifold of petals (3) and in four sepals (2) that both open in the late afternoon and early evening of the first day. The sepals bend from their upright position through a horizontal plane to a final angle of about 120° downward to the water surface while the petals mainly rest in the horizontal level during the night. Thus, they expose a large central circular disk with an outer rim of intensive pink colour, an intermediate white ring and a smaller pink borderline around the tunnel-altogether an inviting landing platform for pollinators. The final state of the blossom at the end of day 2 is that shown in Fig. 1 when one was allowed to cut the flower.

3.2. Development of plants

Two *V. cruziana* plants occupied most of the space in the pond. Their leaves developed with different growth rates in a sigmoid fashion (see Section 3.2.2.). The temperature differences between the blossoms of the two plants were smaller than the fluctuations during the day so that their results could be pooled. Sixty-one blossoms of *V. cruziana* were monitored during the season 2000. At the end of the thermogenic period 10 of them could be cut and used in the laboratory for further energetic investigations. A corresponding number of leaves was measured during the same time.

3.2.1. Development of blossoms

Buds of *Victoria* blossoms and those of leaves develop under water and slowly emerge at the water surface within 1 week. The further development occurs slightly above the water surface following a strict protocol of blooming [15,20–24]. The thermogenic episode starts in the late afternoon of the first day when the dark green–brown sepals open and show the pure white petals. In this first thermogenic phase, *Victoria* disperses a fruity fragrance like pineapple or fruit salad as a result of increased temperature in the floral chamber. Temperature differences between 5 and 10 K against ambient were typical for the present investigation.

3.2.2. Blossom mass as function of the season

The gardeners needed the blossoms of plant 2 for seed production by artificial pollination. Thus, only blossoms of plant 1 could be cut in a limited number

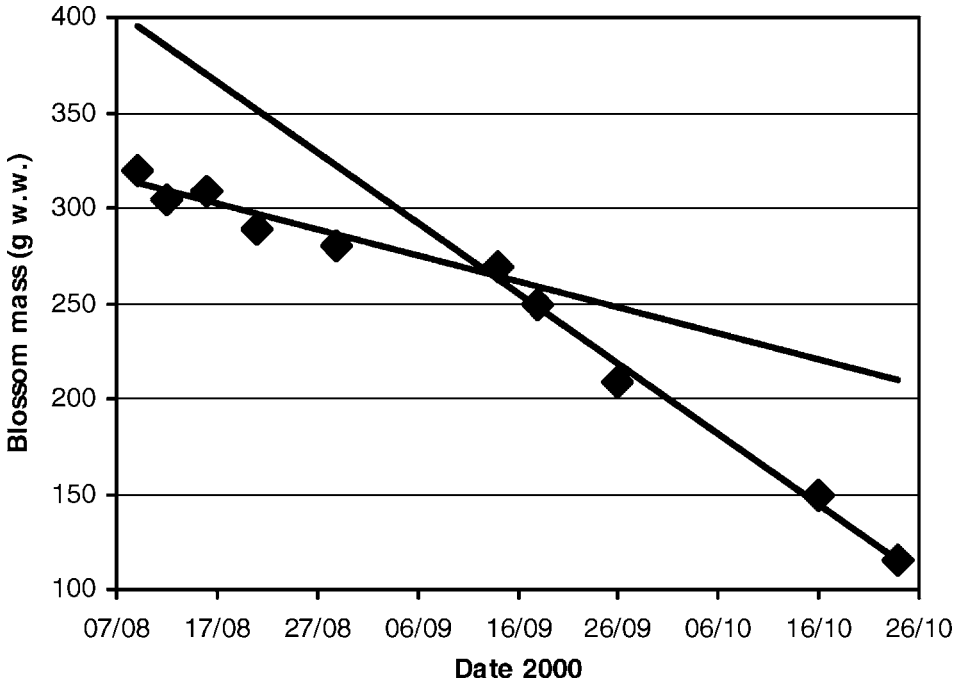

Fig. 2. Change of blossom mass (plant 1) during the observation season in the year 2000. Mass is given as gram wet weight.

for further laboratory experiments. It became clear that their size decreased during progressing season, at first slowly and then rapidly. But as mass specific metabolic data were of interest, masses of the 11 cut samples were used as a "calibration curve" for the rest 50 that had to remain in the pond.

The biphasic slope of the mass decrease is shown in Fig. 2. After a slow loss of weight during high summer (and therefore strong light fluxes), a dramatic reduction started in the middle of September bringing the blossom mass to one-third at the end of the season. The use of this curve to estimate the unknown masses of uncut flowers includes considerable errors but it is the only possible approach in a greenhouse with show character.

3.2.3. *Development of Victoria leaves*

The huge leaves of *V. amazonica* and *V. cruziana* belong to the most impressive parts of the plant especially when they cover the whole surface of a lake or of the pond in the greenhouse. Their diameter is largest during the summer season (in northern Europe) and decreases to the end of the season due to lack of sun light. Fig. 3 exhibits the extraordinary size increase for two leaves of each of the two *V. cruziana* plants of the present paper. The reasons for the large difference in growth are unknown but it remained throughout the season. Leaves of both plants reach their final size (plant 1: 1.8 m^2; plant 2: 0.8 m^2) after about 10 days with an extended logarithmic phase. Maximum growth rate amounts to 1 m^2 within 2.6 days or 62 h. These values transform to 3800 cm^2 per day or 160 $cm^2\ h^{-1}$. At the turning-point around 0.7 m^2, the leaf diameter is 90 cm, 1 day later already 120 cm with a surface of 1.1 m^2. The values of plant 1 are less dramatic, but still astonishing. In any case, the growth in the pond is so quick that older leaves have to be taken out from the pond every fortnight to give space to the active younger ones.

As the main interest of these investigations concentrated on *Victoria* blossoms, only a few leaves were measured during the season. Mass specific metabolic rates were found between 0.05 and 0.35 $mW\ g^{-1}$ wet weight that correspond to 0.5 $W\ m^{-2}$ of leaf. Assuming a final size of 155 cm (Fig. 3) and an area of about 2 m^2, the mean size during the whole growth episode

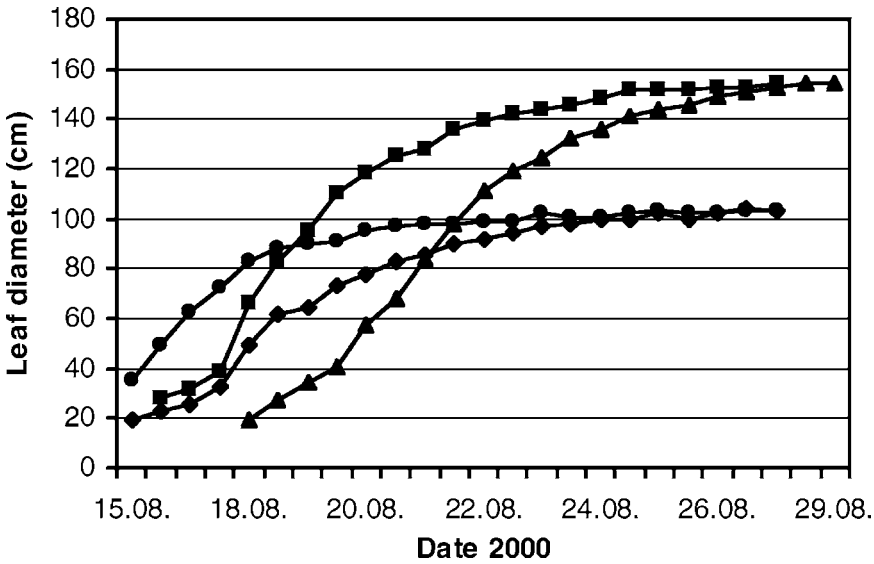

Fig. 3. Growth of two leaves of *V. cruziana*, plant 1 (lower curves) and plant 2 (upper curves), in a greenhouse pond at 30 and 24 °C mean water and air temperature, respectively.

equals 1 m^2. Thus, the mean metabolic rate of a leaf is comparable to that during the flare-up of a blossom.

3.3. Temperature course during blooming

The stimulated metabolic turnover leads to a temperature elevation of the blossom, mainly in the floral chamber that is well insulated by the plant tissue from its surrounding. Usually, the tunnel formed by the stamens and the carpellary appendages is closed so that the insulation also occurs on the top part of the chamber. Temperature differences of up to or even >10 K against ambient are observed two times during the flare-up (Fig. 4). The first one occurs in the late evening of the first day and coincides well with the transition point around 19:30 h from low to high

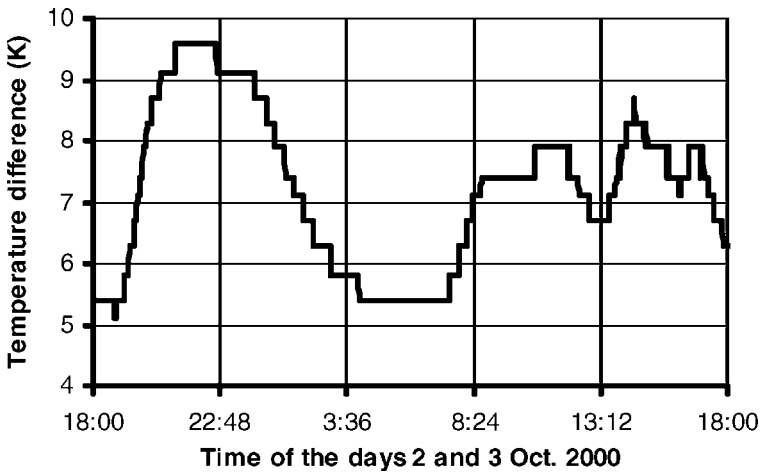

Fig. 4. Temperature difference between the floral chamber and the ambient air during the blooming period of a *Victoria* flower in the pond as a function of time. Flower no. 35, plant 1, mass approximately 190 g wet weight.

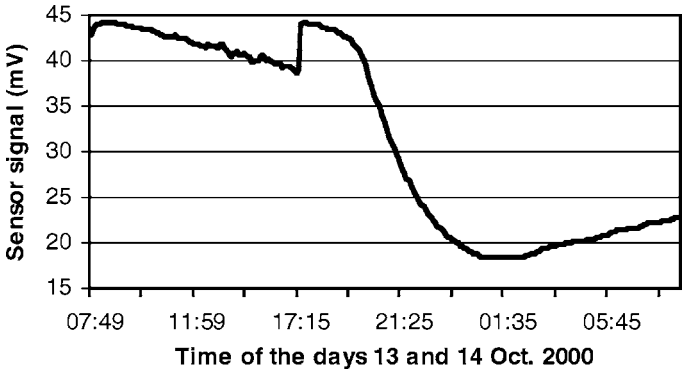

Fig. 5. Oxygen tension under the floating hood during the metabolic flare-up of a *V. cruziana* blossom (no. 47 of plant 2, about 150 g wet weight) on the 13 and 14 October 2000. In the morning, the bud was still closed with slightly visible white petals. In the afternoon (around 16:20 h), the hood was ventilated and the oxygen tension returned to saturation (around 44.5 mV). The transition to a highly increased metabolism and the opening of the blossom occurred at 19:21 h. The maximum oxygen consumption rate equalled 0.84 W or 5.59 mW g^{-1} wet weight.

metabolic rates (see later). The second maximum appears in the morning or at noon of the second day.

As the flower is usually a few centimeters above the water surface, it loses its heat to the fluctuating ambient air. At the end of the second day, the flower is actively drawn back to and later on into the water and experiences there a constant temperature of 30 °C. Due to this plant behavior, it is not possible to get information about the "normal" blossom temperature when it is not in heat. Further details on temperature observations with *Victoria* buds, blossoms and leaves were published recently [19].

3.4. Metabolic flare-up of a Victoria blossom

The blooming period of a *Victoria* blossom takes about 2 days beginning during the first morning with a still firmly closed green bud just above the water surface. During the morning and the early afternoon the outer leaves (sepals) open slowly showing the white petals underneath. The rate of respiration is nearly constant during this time. The situation changes significantly with dusk: the sepals open downwards beyond the horizontal level, the petals follow and the upper part of the stamen becomes visible. The central tunnel is still closed but opens in the next hours, a sweet fragrance becomes detectable and the early white of the blossoms changes to pink. Highest temperature differences of about 10 K are detected in this period. In the morning, the tunnel is again closed, the blossom is coloured, the temperature difference lower and the smell less intensive. The real metabolic flare-up is over.

This typical time course is depicted in Fig. 5 which presents a continuous observation of about 24 h. The only experimental artefact in the slope occurs around 4:00 p.m. when the hood was ventilated to provide enough oxygen for the following flare-up. There is a 12-fold increase of respiration rate from the smooth, nearly constant decline after the ventilation to the maximum turnover in the first hours of night. The point of sharp inflection is typical for the flare-up and appears—more or less pronounced—in most experiments. No significant connection with the time of the year could be found. In 13 determinations, the median occurred at 19:33 h, the mean at 19:35 h with an S.D. of 29 min. The increase in oxygen tension after the minimum at 1:00 a.m. is due to the diffusion of oxygen dissolved in water to the head space in the hood and has no biological reason.

The metabolic turnover of *Victoria* buds and blossoms was determined as respiration rate under the floating hood. Some of the experiments took <1 h, other followed the whole flare-up period during 2 days. As there is a large scatter in the rates not due to different age of the blossoms all data were pooled and are depicted as power or as mass-specific-power histograms in Fig. 6a and b. Fig. 6a presents a his-

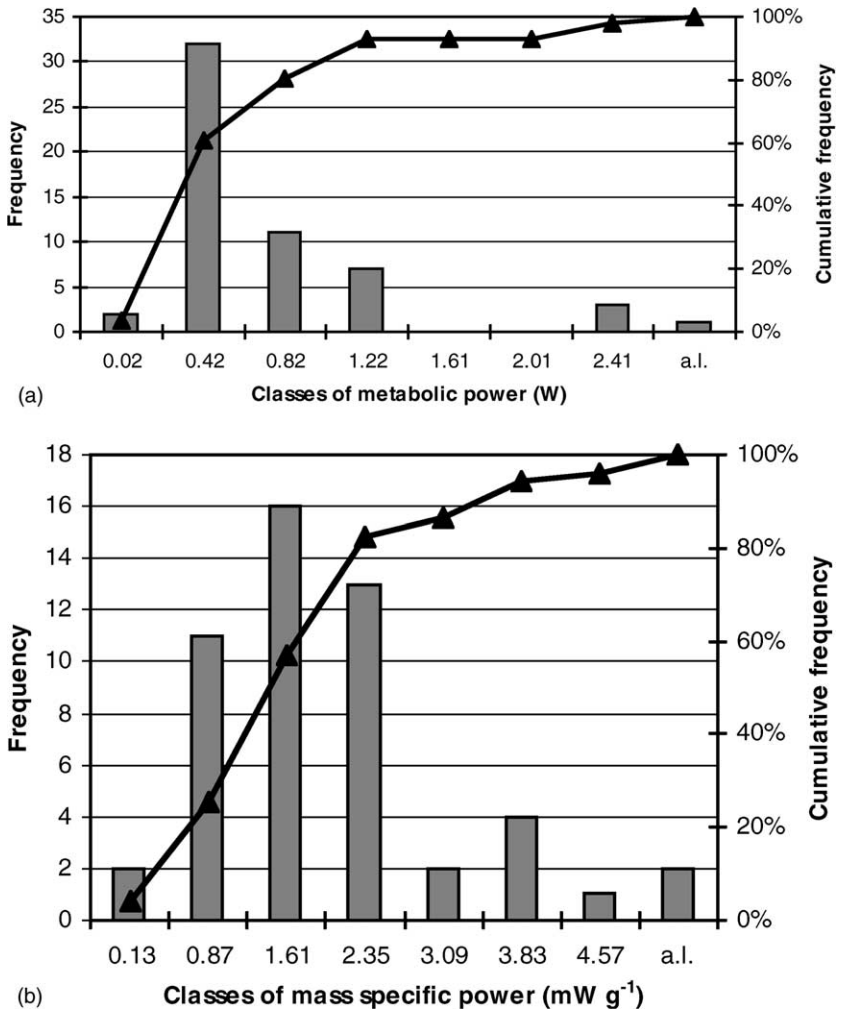

Fig. 6. Power distribution histograms of the investigated *Victoria* blossoms during the season 2000, presented as the number of blossoms in the given class (bars) and as cumulative frequency of the classes. (a) Metabolic power per blossom (W) for 56 samples, (b) mass specific metabolic power (in mW g^{-1} wet weight) for 52 samples excluding the four highest data of (a) (for further details see text).

togram of the observed metabolic turnover rates of all 56 *Victoria* buds and blossoms investigated by indirect calorimetry. The oxygen consumption rates (in ml h^{-1}) were transformed to power units (W). The distribution has a mean of 0.497 W with a very large standard deviation of 0.586 W and a median of 0.276 W. The two empty classes indicate that the four highest values may be omitted as artefacts. Then mean value, S.D. and median of the 52 remaining samples change to 0.386, 0.285 and 0.264, respectively.

Fig. 6b shows the mass specific rates (in mW g^{-1} wet weight) for these 52 blossoms. Again, the large scatter remains with a mean of 1.71, an S.D. of 1.33 and a median of 1.37 mW g^{-1}. These data indicate that sample size is not responsible for the spread values.

3.5. Metabolic turnover of different blossom tissues and of leaves

After cutting *Victoria* blossoms in the pond, they were transported to the laboratory and monitored as

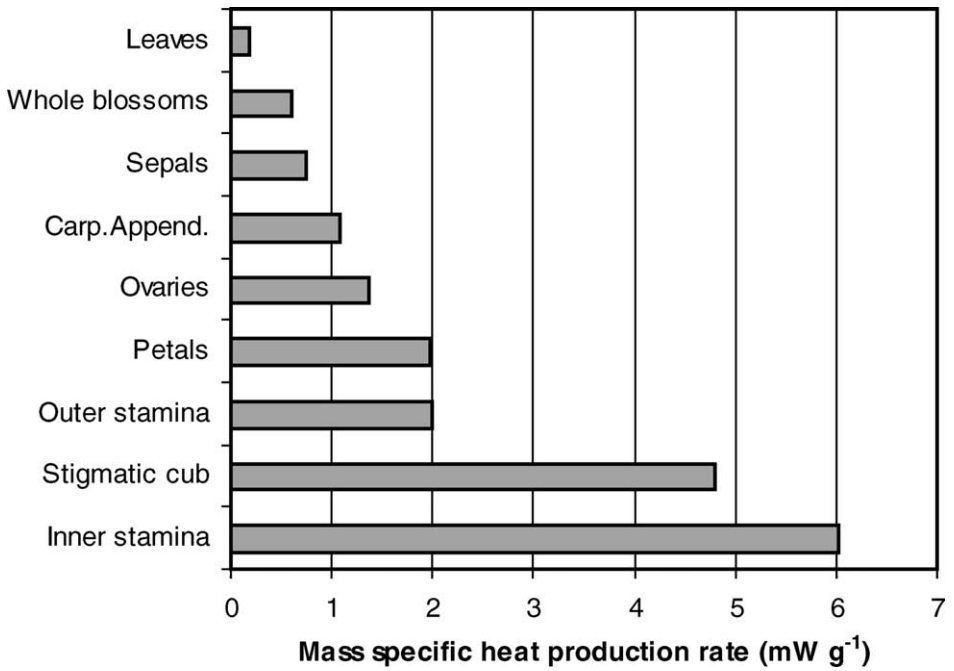

Fig. 7. Mass specific heat production rates of the different organs of a *Victoria* blossom determined by indirect calorimetry in the laboratory. The values are given per gram wet weight.

a whole for their respiration rates and later on dissected into different tissues. These were investigated and compared in their mass specific metabolic rates by means of indirect calorimetry. Flasks for respiration measurements varied from 50 to 250 ml, tissue mass from a few grams to 20–30 g wet weight. For all the data presented later, it should be kept in mind that they were obtained at the end of the individual metabolic flare-up and thus they are for sure smaller than in the active tissue during peak heat production.

Fig. 7 compares mass specific heat production rates of several *Victoria* tissues with those of whole blossoms and leaves. One observes a factor of about 30 between leaves and inner stamina. It seems reasonable that the metabolic power in sepals, as the external border of the flower, is smaller than in the numerous internal petals that show an increase in specific rates with approaching the center. Again the outer stamina are less active than the inner ones. Specially high rates are found in the tissue surrounding the floral chamber (stigmatic cub, inner stamina) where the pollinators have to be effective. All the data presented here concern heat production rates per gram wet weight. The distribution given in Fig. 7 changes slightly with the transition to values per gram dry weight as—for example—the density of carpellary appendages is about two-fold higher than those of other tissues.

3.6. Electrical calibration of heat production

An autumn blossom of plant 1 was cut and transported to the laboratory. It was placed in a can partly filled with water in such a way that its distance to the water surface was approximately the same as before in the pond. Room and greenhouse temperatures were about the same. An electrical resistor of 15 mm length, 5 mm diameter and maximum of 2 W power was placed inside the floral chamber. At that time of development, the outer blossom leaves (sepals) were approximately horizontal, the petals upright and the tunnel completely closed. Two temperature sensors were inserted into the flower as usual, one from the top and one from the side, both connected to HOBO data loggers for continuous registration. A constant voltage

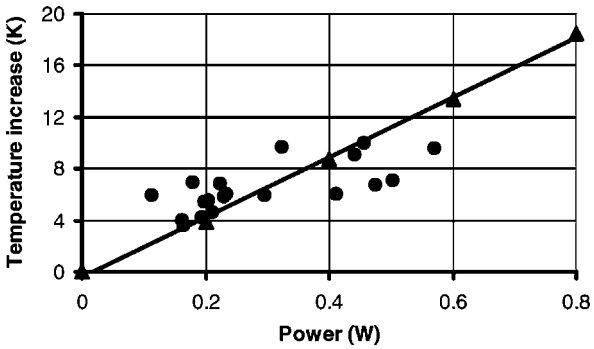

Fig. 8. Electrical calibration of heat production in a *Victoria* blossom and comparison with the metabolic data. The triangles (▲) concern the electrical heating, the dots (●) the metabolic one by indirect calorimetry. For further information see text.

source (Gossen, Model 1; Erlangen, Germany) supplied the calibration power growing from 0 to 0.8 W. Additionally, surface temperatures were monitored by an IR detector. At any given power, a stable thermal state of constant temperature was obtained within 0.5 h. The temperature increase, as a function of the time, exhibited by the internal sensors looked like a typical calorimeter calibration curve with a rounded step-wise function (not shown).

Fig. 8 presents the observed difference between the blossom and the environment due to the electrical heating of the floral chamber. As temperature elevations of >10 K were observed in the greenhouse, the electrical power was extended to 0.8 W rendering a difference of about 18.5 K (more than ever observed in nature). The full dots show experimental results from the pond where the heat production rate was determined as oxygen consumption rate inside the hood. It becomes clear from this linear graph that temperature differences render a first approximation to true metabolic data.

4. Discussion

Significantly increased temperatures in flowers of some plants were first described by Lamarck in 1778 [25] for several members of the *Arum* family. Meanwhile, thermogenic plants were detected in five further families, including palms, cycads and water lilies. Some review papers render a survey of this interesting group of plants, their activities, alternative metabolism, highest temperatures, even thermoregulating facilities and the supposed reasons for their behaviour [2,3,22,26]. Among the most interesting and spectacular ones is the giant tropical water lily *V. amazonica* and her smaller sister, *V. cruziana*, that is presented in this paper. Both are known since about 200 years, their temperature increase described 150 years ago under greenhouse conditions [12–14] and 65 years ago in the natural habitat [21]. Some more background information is given by the present authors in a recent paper [19], a set of more than 30 fotos of the unfolding of a *Victoria* blossom was detected in the internet [27].

The time course of the thermogenic period of *Victoria* was explained by the first greenhouse observers [12–14] and only later confirmed in the Amazon [21]. It could be shown that the temperature increase starts already in the buds, at least 9 h before the blossom opens and comes to its metabolic climax [14,20]. This climax occurs early in the first night, well in parallel with the activity period of the most frequent pollinators of *Victoria*. The opening is triggered by the afternoon decrease in light intensity but not by weather [15]. In the tropical homeland of this water lily day length does not change during the year so that a flare-up between 17:30 and 18:30 h can be predicted throughout the year [15] while this time shows a tendency to be later in summer and earlier in autumn in middle Europe. Seymour and Schultze-Motel showed for other thermogenic plants that the heat production is largest when the female floral parts are most receptive [28]. Then, temperature is highest and scent production strongest [28]. In contrast to the ugly stench of many other, specially aroid plants the fragrance of *Victoria* is sweet and pleasant, reminding of fresh fruits. At the end of this first active period, pollinators are trapped in the floral chamber where they have to stay till to the next afternoon when the tunnel opens again and releases the visitors now covered with the male pollen of their host flower.

This thermogenic episode of about 36 h is intermediate in length compared with *A. italicum* (about 1 h [29]), male cones of some cycads (a few hours [30]), *Philodendron* (18–24 h [5]), dragon lily (<2 days [11], sacred lotus (2–4 days [7]) and skunk cabbage (2 weeks or more [4,31]).

As shown in Fig. 2, time of the year and thus length of the day influence the development of

blossoms, but it is also observed for leaves (not presented). The latter remain smaller at the end of the season with less pronounced rims at their periphery. Blossom masses decrease from >300 g wet weight in the high season to only 100 g of the last flowers. But this decrease in size does not influence the general form of the time course of heat production and temperature rise during the investigation period (see also Figs. 4 and 5).

Dividing the mass of a grown up leaf by its surface area renders a total area density of 0.34 g cm^{-2}. If one uses just the tissue pieces between the pronounced rips, it decreases to only 0.14 g cm^{-2}. This shows that the leaf is rather fragile and mainly strengthened by its spin-web like underneath structure. The well-known pictures of a child or even an adult man standing on a *Victoria* leaf are only possible if their weight is evenly distributed over the whole surface by a wooden or perpex plate. A rough calculation for a mature leaf of plant 2 (diameter 150 cm) renders a total volume of 53 l of air trapped between the radial and circular rips. That means that a corresponding weight of a person can be carried by such a leaf.

Temperature data for *V. cruziana* blossoms are sparce in the literature but supposed to be similar to those of *V. amazonica* that will be discussed in the following. The first information about a maximum increase of 6 K against ambient was presented in 1850/52 [12], a corresponding value from a greenhouse in Hamburg [13], but already 10.4 K from the botanical gardens in Berlin and Marburg [14,20]. Decker [21] was the first to show such temperature rises (11–14 K) for flowers in their natural surroundings of the Amazon and not under glass. In the present investigations, temperature differences of up to 11 K were observed in greenhouse *V. cruziana*.

The usual temperature profile of both *V. amazonica* and *V. cruziana* shows two maxima, a higher one in the first night, a further smaller one at noon or in the afternoon of the second day (Fig. 4). But due to external influences, both maxima may be equally strong or even inverted in their order. Temperature differences found during our investigations or cited in the literature never exceeded 15 K so that *Victoria* belongs to the group of pronounced thermogenic plants but remains less active than e.g. the Eastern skunk cabbage (35 K [3,4,31]), sacred lotus (>20 K [7,8]) or *Philodendron* (<20 K [5,31]).

The metabolic flare-up shown in Fig. 5 as a rapid decrease in oxygen tension under the floating hood occurs in the odoriferous, pistillate (female) stage of the blossom [26]. It may endure from 1 h (*Arum*) to several weeks (skunk cabbage, see earlier). It is connected with high production rates of heat, fragrance and carbon dioxide. It could be shown that heat is not attractant per se but it may be a reward for pollinators entrapped in the floral chamber during a cold night. Carbon dioxide is supposed to be attractive for carrion flies in other thermogenic plants because CO_2 is a main metabolic product in microbial decomposition of feces or similar organic material.

Only the combination of intensive metabolic rate and large size allows the establishment of plant temperatures higher than that of the environment [3]. As Seymour and Schultze-Motel pointed out thermogenic flowers are usually large to reduce the surface to volume ratio and to decrease heat loss [28]. *Victoria* follows the further rule that blossom morphology is advantageous for pollinators when there is a large floral chamber, many carpels exist, visitors find edible parts of the blossom (e.g. carpellary appendages) and when a large platform appeals for landing. Moreover, the comfortable temperature of the floral chamber serves as a further reward.

The electrical calibration of heat production—presented in Section 3.6 and in Fig. 8—renders a first rough approximation to real heat dissipation. Stronger deviations appear on both sides of 0.27 W. At higher activities, the temperature differences remain smaller than those for calibration. In this part of the floral development, the blossoms are fully opened and the tunnel not closed so that an enforced heat exchange is possible. On the other hand, lower metabolic activities correspond to early development with young tightly closed buds with a stronger thermal insulation. Thus, the observed temperatures lie above the trend line of the graph. This explanation is plausible since the highest positive deviations originate from early stage buds, but it could not be proved experimentally as flowers were cut only in their final state and electrical calibration in the greenhouse atmosphere of 100% relative humidity appeared not to be wise.

Turnover rates—determined from oxygen consumption and transformed to thermal power units—show elevated energy consumption during the thermogenic episode of *V. cruziana*. Although the mean of 0.497 W

per blossom seems low compared with other members of the thermogenic family, top values of more than 1 W are observed. The low thermal mean may be explained by the fact that only few flowers could be followed through their flare-up while most were monitored only for 1 h in the morning and the afternoon to let the beautiful blossoms be exposed to the greenhouse visitors during the main time of the day. Due to the large amount of metabolically ineffective tissue in the blossom the mean mass specific power is only 1.7 mW g^{-1} with maximum values of 5 mW g^{-1}. Figures like e.g. 29 and 8.4 mW g^{-1} are presented in literature for lotus [32] and *Philodendron* [33], respectively.

The blossoms of thermogenic plants do not warm up homogeneously but contain special organs that are mainly responsible for the increased heat production. In *S. foetidus* spadices of 4.5 and 2.1 g corresponding mass specific heat production rates of 68.4 and 132 mW g^{-1} were observed, respectively, and up to 170 mW g^{-1} in their florets [4,31]. A spadix of *P. selloum* weighing 164 g exhibited 29.8 mW g^{-1}, its sterile male florets 175 mW g^{-1} [5,33], a *D. vulgaris* appendix (47.2 g) 34.1 mW g^{-1}, its male florets 53.5 mW g^{-1} [11], the *N. nucifera* receptacle 66 mW g^{-1} [32]. Top values are cited for *A. maculatum* appendices with 237 mW g^{-1} and for their florets with 400 mW g^{-1} [34]. Compared with such values the data presented in Fig. 7 seem minimal. But they show similar ranking like in other flowers that the male parts (stamina) and the stigmatic cub have the highest specific rates. Again it has to be pointed out that the present values were obtained at the end of the thermogenic episode and not at the climax as the other ones cited previously. Thus, the intended investigations at Amazon with a plenty of flowers and the possibility to cut them in all stages are demanding and fascinating. Nevertheless, the present results are already in agreement with old observations of Caspary [14] and Knoch [20] who found that stamina are the most active parts of the *Victoria* blossom, followed by petals and the stigmatic cub but who could not obtain any energy values.

Carpellary appendages are often told to be the most active tissue [20]. This statement does not coincide with the present results. The late moment of cutting may be responsible for the observed low turnover because most of the available energy was used up before. Or it might be that the published high intensity was a misinterpretation when touching the wall of the floral chamber through the tunnel. Besides, it was shown that the level of salicylic acid (calorigen, see later) was high in tissues of *Arum*, *Philodendron* and cycads, but below detection in *Victoria* carpellary appendages [35].

Establishing an energy balance for a complete flower from its different tissues or comparing the mass specific heat production rates of intact blossoms or spadices with that of special parts of them, e.g. the sterile male florets, one should keep in mind that in the latter oxygen diffusion restrictions are often removed so that metabolic rates become extremely high. It was shown recently by means of direct calorimetry for appendix samples of the thermogenic voodoo lily that a decrease in mass to 1% of the initial mass provoked a 50-fold increase in mass specific heat production rate [36].

V. cruziana blossoms with mass specific heat production up to 9.4 W kg^{-1} exhibit metabolic rates 10 times that of resting man (about 1 W kg^{-1}). Several authors pointed to the fact that other thermogenic flowers equalled in their activity small mammals or birds of the same size, e.g. shrews or humming birds [2,3] and that sterile male florets of *Philodendron* are as active as brown fat tissue of hibernators [2]. It is well known that the latter switch on their increased heat production by uncoupling ATP production from oxidative phosphorylation so that no energy is stored in chemical form but all dissipated as heat. Thermogenic plants use an other trick: they shut down the usual cytochrome pathway and thus force electrons to flow through an “alternative, cyanid-resistant” electron transport chain that produces only one third of the normal amount of ATP [26,37–39]. This alternative pathway is typical for plant mitochondria under normal conditions and not as active as the cytochrome pathway so that a high ATP against a low heat production is typical for plant tissue. But the alternative pathway might be stimulated actively by the plant sending a calorigen called messenger substance to the thermogenic tissue [40]. Calorigen turned out to be salicylic acid [41] which is not only active in thermogenic plants but also in other plant tissue [42]. It also stimulated heat production in voodoo lily tissue in vitro [43,44].

Finally, it is interesting to ask for the reasons that some plants show such an extreme, but similar thermal behavior under completely different

environmental conditions. Skunk cabbage remains active down to temperatures far below zero [4,31], lotus experiences day temperatures <40 °C and cold nights around or <10 °C [7], *Victoria* only modest to high tropical values [15,21]. Of course, it seems clear before-hand that all plant activities should be connected with pollination and survival. The first observation that often leads to the detection of the plants is the intensive vaporisation of odours, very often unpleasant for the human nose, sometimes sweet and interesting. They shall attract pollinators when visual clues are not sufficient or not detectable during night. Moreover, these stenches are far-reaching hints for pollinating insects. The second reason sounds more egoistic: protecting sensitive parts of the blossom against freezing and allowing for proper development of reproductive structures even at unfavorable temperatures, both by heating the floral chamber. The third reason may be a by-product of the second one: providing a warm shelter for pollinators at night. Beetles usually become cool and inactive during the night and need considerable amounts of energy to actively warm up in the morning and to keep their body temperature during the day. Floral chamber temperatures are approximately the same as those of active beetles so that the entrapped pollinators do not consume own stored energy but feed on attractive plant tissue, e.g. carpellary appendages in *Victoria*. It was shown that food consumption rates of the beetles in the floral chamber might be reduced to 2.5% of the normal values if they would have been left to their own [28]. Pollinators stay active in the chamber during the time of imprisonment, including copulation, and guarantee for an intensive pollination of the receptive female parts of the blossom.

Acknowledgements

We acknowledge with pleasure the technical support that was given to us by Mrs. Gudrun Welge, Berlin; and Alexandra Torres Sanchez, Bogota; the help with the manuscript by Mr. Assegid Garedew, the permit of our investigations by the Botanical Garden/Botanical Museum of the Free University of Berlin, especially by Prof. W. Greuter and Dr. B. Leuenberger, and last not least the friendly acceptance and cooperation in the Victoria House by H. Wilke, Ch. Schrader, M. Schmidt and K. Siedel.

References

[1] G. Gottsberger, Naturwiss. Rdsch. 39 (1986) 350.
[2] R.S. Seymour, Sci. Am. 3 (1997) 91.
[3] R.M. Knutson, Nat. Hist. 88 (1979) 42.
[4] R.M. Knutson, Science 186 (1974) 746.
[5] R.S. Seymour, G.A. Bartholomew, M.C. Banhart, Planta 157 (1983) 336.
[6] R.S. Seymour, Thermochim. Acta 193 (1991) 91.
[7] R.S. Seymour, P. Schultze-Motel, Nature 383 (1996) 305.
[8] I. Lamprecht, R.S. Seymour, P. Schultze-Motel, Thermochim. Acta 309 (1998) 5.
[9] I. Lamprecht, K. Drong, B. Schaarschmidt, G. Welge, Thermochim. Acta 187 (1991) 33.
[10] I. Lamprecht, B. Schaarschmidt, ThermoMed. 7 (1991) 75.
[11] R.S. Seymour, P. Schultze-Motel, Proc. R. Soc. Lond. B 266 (1999) 1975.
[12] J.E. Planchon, Fl. Serres Jard. Eur. 6 (1850) 193–249; J.E. Planchon, Fl. Serres Jard. Eur. 7 (1852) 25–49.
[13] E. Otto, J. Hooker's, Bot. Kew Garden Misc. 4 (1852) 62.
[14] R. Caspary, Monatsber. Königl. Preuss. Akad. Wiss. Berlin (1855) 711.
[15] G.T. Prance, J.R. Arias, Acta Amazon. 5 (1975) 5.
[16] R. Caspary, Bonplandia 3 (1855) 175.
[17] G.T. Prance, A.E. Prance, J.R. Arias, Citacia Cultura 27 (12) (1975) 293.
[18] J.J. Valla, D.R. Cirino, Darwiniana 17 (1972) 477.
[19] I. Lamprecht, E. Schmolz, S. Hilsberg, S. Schlegel, Thermochim. Acta 382 (2002) 199.
[20] E. Knoch, Biblioth. Bot. 9 (47) (1889) 1.
[21] J.S. Decker, Aspectos Biológicos da Flora Brasileira, Rotermund Co., Sao Leopoldo, Brasil, 1936, p. 50
[22] B.J.D. Meeuse, E.L. Schneider, Isr. J. Bot. 28 (1979/1980) 65.
[23] F. Lehmann, Ber. 29. Vers. Deutsch. Naturf. Ärzte Wiesbaden (1852).
[24] M. Klotzsch, Monatsber. Berl. Akad. (1852) 547.
[25] C. Lamarck, Flore Françoise ou Description Succinte de Toutes les Plantes, Sec. Edition, Tome, 3 H. Agasse, Paris, 1778.
[26] B.J.D. Meeuse, I. Raskin, Sex. Plant Reprod. 1 (1988) 3.
[27] http://www.h2olily.com/first.html.
[28] R.S. Seymour, P. Schultze-Motel, Endeavour 21 (1997) 125.
[29] H. Skubatz, T.A. Nelson, A.M. Dong, B.J.D. Meeuse, A.J. Bendich, Planta 182 (1990) 432.
[30] H. Skubatz, W. Tang, B.J.D. Meeuse, J. Exp. Bot. 44 (1993) 489.
[31] R.S. Seymour, A.J. Blaylock, J. Exp. Bot. 50 (1999) 1525.
[32] R.S. Seymour, P. Schultze-Motel, Phil. Trans. R. Soc. Lond. B 353 (1998) 935.
[33] R.S. Seymour, J. Exp. Bot. 50 (1999) 845.
[34] P.C. Lance, Plant Sci. Lett. 2 (1974) 165.
[35] I. Raskin, H. Skubatz, W. Tang, B.J.D. Meeuse, Annu. Bot. 66 (1990) 369.
[36] C.M. Lytle, B.N. Smith, M.S. Hopkin, L.D. Hansen, R.S. Criddle, Thermochim. Acta 349 (2000) 135.

[37] I.M. Möller, A. Berczi, L.H.W. van der Plas, H. Lambers, Physiol. Plant. 72 (1988) 642.
[38] T.E. Elthon, R.L. Nickels, I. McIntosh, Planta 180 (1989) 82.
[39] H. Skubatz, P.S. Williamson, E.L. Schneider, B.J.D. Meeuse, J. Exp. Bot. 41 (1990) 1335.
[40] A.W.H. van Herk, Kon. Akad. Wet. Amsterdam, Proc. Sci. 40 (1937) 709.
[41] I. Raskin, I.M. Turner, W.R. Melander, Proc. Natl. Acad. Sci. U.S.A. 88 (1989) 2214.
[42] D. Van Der Straeten, L. Chaerle, G. Sharkov, H. Lambers, M. Van Montagu, Planta 196 (1995) 412.
[43] I. Raskin, A. Ehmann, W.R. Melander, B.J.D. Meeuse, Science 237 (1987) 1601.
[44] H. Skubatz, B.J.D. Meeuse, J. Exp. Bot. 44 (1993) 493.