

## Chapter 8

# *Thermal investigation on whole plants and plant tissues*

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### Introduction

Plant thermal analysis – although not comparable in the general attention of the scientific community with those for microorganisms, small animals and animal or human tissues and isolated cells - nevertheless offers a large variety of different aspects. They cover the range from plant tissue over organs to whole plants, from simple thermometry or thermography over combustion and differential scanning calorimetry to adiabatic and isothermal experiments, from germination of seeds to biochemical regulation of heat output and metabolic flare-up at blooming in thermogenic flowers. Moreover, wood as one of the most important plant products opens another field of thermal analysis. All of them will be touched – more or less intensively – in this short survey. But attention will be paid mainly to higher plants to avoid too much broadening.

Plant calorimetry was always assumed to be difficult since plants are poikilothermic living entities with an unfavourable surface to volume ratio. Plants need light of special wavelengths to be photosynthetically active. Metabolic rates are – with a few exceptions – low compared to that of animals or especially microorganisms. Moreover, evaporation with its high degree of energy consumption plays an essential role in the life of plants and may cover all other calorimetric signals if not carefully matched. Table 1 provides a list of heat flow rates from different plants or plant parts, divided into non-thermogenic and thermogenic objects. As a rule, one may expect at least 1 mW g<sup>-1</sup> wet weight (w.w.) for the first group of plants.

If temperature sensing is taken as a genuine part of Thermal Analysis and qualitative statements are allowed, J. B. A. Lamarck was the first to contribute

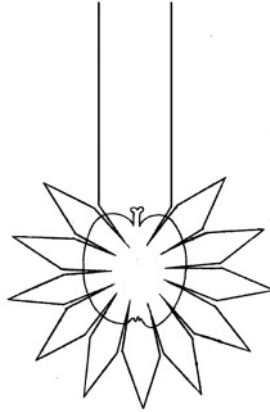
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**Table 1.** Some selected plant tissues, organs and whole plants and their mass specific heat production rates ( $p$ ) per g wet weight (d.w. = dry weight) at ambient temperature  $T_a$  or that of the organ (\*). First part: Non-thermogenic plants: Second part: Thermogenic plants in normal state or during metabolic flare-up. In some cases heat production rates were calculated from  $O_2$  consumption or  $CO_2$  production

Trivial name	Botanical name	$T_a$	$p$	Reference
		°C	mW g <sup>-1</sup> w.w.	
<b>Non-thermogenic plants</b>				
Apple: leaf segment	<i>Malus sp.</i>	23	2.8	[16]
Barley roots	<i>Hordeum vulgare</i>	24	1.4	[50]
Coast redwood, meristem tissue	<i>Sequoia sempervirens</i>	24	0.4 – 1.4	[43]
Coffee roots	<i>Coffea arabica</i>	25	2.4	[56]
Spinach leaves	<i>Spinacia oleracea</i>	25	0.9	[28]
Tomato, undifferentiated cell culture	<i>Lycopersicon esculentum</i>	23	1.7	[98]
<b>Thermogenic plants</b>				
Cuckoo-pint	<i>Arum maculatum</i>	-	385 d.w.	[99]
Eastern skunk cabbage	<i>Symplocarpus foetidus</i>	0	68	[100]
Philodendron	<i>Philodendron selloum (spadix)</i>	5	64	[101]
	<i>(sterile male flowers)</i>	37*	160	[101]
Voodoo lily; appendix	<i>Sauromatum guttatum</i>	26	95 d.w.	[102]
Voodoo lily (whole plant); quiescent	<i>Sauromatum guttatum</i>	25	1	[22]
Voodoo lily (whole plant); flare up	<i>Sauromatum guttatum</i>	25	7	[22]

to thermal investigations on plants [1]. He wrote about several members of the aroid family that the flowering catkin in a special state of ripeness or development is warm to appear burning ('il est chaud au point de paroître brûlant'), and not at all at the temperature of the rest of the plant. *Arum* belongs to the family of *thermogenic plants* that show a metabolic explosion during blooming. Many further thermometric results followed by other authors [2], but always oriented towards temperature, never to energy turnover or heat production.

Presumably the first calorimetric results were published by Rodewald at the end of the 1880<sup>th</sup> [3, 4]. He investigated plant objects of spherical form (apple, kohlrabi, onion) and by distributing a thermopile of 12 or 36 couples over the surface to determine temperature difference between the biological sample and the constant environment (Fig. 1). The corresponding heat flow was calculated with Newton's cooling law after a sophisticated calibration, and rates of evaporation, oxygen consumption and carbon dioxide release were determined simul-



**Fig. 1** Distribution of the thermopile couples along the surface of an apple. In first experiments 12 thermocouples were used, in later ones 36 [3]. Adapted from [3]

taneously. Apples and kohlrabies showed mean metabolic heat losses of 0.13 and 0.37 mW g<sup>-1</sup> w.w., resp. The respiratory quotient (carbon dioxide production divided by oxygen consumption) was practically 1, the heat output per mol O<sub>2</sub> or CO<sub>2</sub> around 440 kJ, near to the value of 455 kJ mol<sup>-1</sup> expected from Thornton's rule [5]. The author supposed that it was the first time to show that energy produced in respiration processes is nearly completely dissipated via heat flow and external work – in this case evaporation of water [3, 4].

Another very early paper on (true) plant calorimetry should be mentioned briefly, Pierce's Dewar-vessel experiments on germinating peas [6]. Within 8 days they heated up from 17 to 56°C, while chemically killed peas kept a temperature of around 15°C. As it became obvious that besides germination fermentation occurred also in the pea sample, peas were sterilized by chemical means before the next run. In this case, temperature rose to about 40°C within 3 days, but no effort was made to calculate the amount of heat released in these experiments.

## WOOD

Wood belongs besides fruits and crops to the most important parts of plants and plays a significant role in the daily life, private as well as in engineering and industry. As the present review shall concentrate on 'living', that means actively growing or at least metabolising plant material, and since a comprehensive, thermal analytically oriented wood survey appeared recently [7], wood shall be neglected in favour of other plant parts.

## Earlier reviews

Periodically, review papers on plant heat production appeared in monographs dedicated to the whole spectrum of biological calorimetry [8–11] or in special is-

sues of scientific journals from the field of Thermal Analysis [12–14]. They inform about instrumental techniques applied for plant investigations [15], some aspects of plant calorimetry [8, 9] or the means to find ‘differences between apples and oranges’ [16, 17]. Special attention should be paid to the comprehensive contribution of Criddle and Hansen to the *Handbook of Thermal Analysis and Calorimetry* [11], which is a true gold mine for historical and modern aspects of plant calorimetry and facilitates the access to this fascinating and growing field.

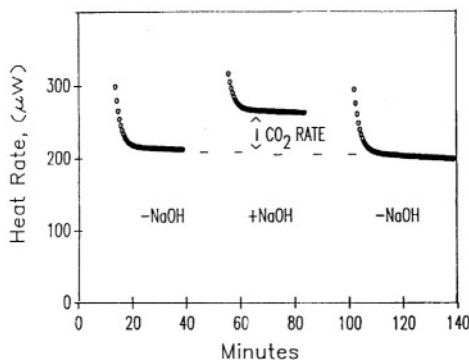
In his 1995 survey of microcalorimetric techniques for plant material investigation Wadsö presented a number of existing instruments and vessels that could be used for this end [15]. They would allow for mere batch experiments in closed ampoules with changing gas atmosphere, for stirring of tissue cultures, for illumination in the sense of photocalorimetry, for gas perfusion and determination of carbon dioxide production. Although multifunctional instrument systems would be ideal tools for more complex analyses and appropriate physiological tissue conditions, the author nevertheless recommended the use of simple static ampoules as the most dependable process monitors [15]. His statement is still valid, but meanwhile he himself presented several sophisticated solutions for advanced tissue studies (see Instrumentation).

In the same special issue of *Thermochimica Acta* Hansen and his colleagues published part 2 of their *Plant calorimetry* [17] that reports about new technical efforts to ameliorate plant calorimetry, e.g. by coupling gas chromatographs or mass spectrometers to traditional instruments. Their work deals with aspects of thermogenic plants (see below) and presents a broad discussion about modelling the relation between growth rate and respiratory variables. Reviews to the stimulating area of thermogenic plants may be found in early surveys of Leick [2, 18], papers of Seymour [19, 20] and the present authors [21–24].

## Instrumentation

It is advantageous to perform simultaneous determinations of the rates of heat and CO<sub>2</sub> production and oxygen consumption in order to get a more complex picture of the underlying plant metabolism. But there are limitations for the application of the corresponding electrodes when the vessels are small, the electrodes not submerged in the solution, the solutions not stirred, or the samples solid. To overcome such problems Criddle *et al.* [25] made threefold experiments: a first run with only the sample in the vessel, the second with an additional container for a CO<sub>2</sub> absorbing base, and the third repeating the first (Fig. 2). The upward shift in the steady heat flow corresponds to the enthalpy shift due to the absorption and neutralization of CO<sub>2</sub>. This is an elegant solution for short term experiments of a few hours, but hardly applicable with changing atmospheres or long-term investigations.

A further step forward to simultaneous determinations were done with a differential heat conduction calorimeter housing three vessels for samples and

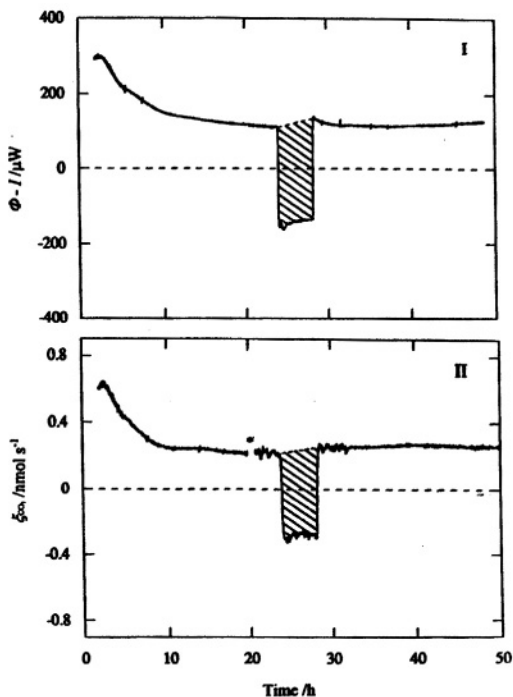


**Fig. 2** Heat production rate of corn meristem tissue without (first and last trace) and with  $\text{CO}_2$  absorber in the calorimetric vessel. With permission from [25]

one as reference. All three sealed 1 ml-reaction vessels were connected with pressure sensors [26]. The pressure signal in the absence of  $\text{CO}_2$  absorption in the vessel renders the difference between oxygen consumption and carbon dioxide production, with  $\text{CO}_2$  absorption, however, it provides the  $\text{O}_2$  consumption rate. The rate of  $\text{CO}_2$  production may then be determined as the difference between both signals and additionally from the shift in the heat flow rate as described in the last paragraph and shown in Fig. 2. The crucial point in such experiments is to know the volume of the head space in the vessels. The functioning of the new setup was tested with redwood meristem tissue [26].

In spite of the successful plant experiments with the ampoule calorimeter, the Lund school of calorimetry developed a gas perfusion instrument for plant tissue under dark conditions [27]: a combination of two twin heat flow calorimeter with 4.5 ml perfusion vessels. The first unit houses the biological sample, the second the  $\text{CO}_2$  absorbing liquid that dissipates  $-97 \text{ kJ mol}^{-1}$   $\text{CO}_2$  heat of solution and neutralization. The gas flow that passes through a prehumidifier and a  $\text{CO}_2$  trap before entering the vessel may be changed during the analysis. Experiments with potato tuber slices of about 1.2 g fresh weight were run over 15 h with maximum heat outputs of 0.7 mW and  $\text{CO}_2$  rates of  $1.8 \text{ nmol s}^{-1}$  and a ratio of  $-450 \text{ kJ mol}^{-1}$   $\text{CO}_2$  [27].

Two years later a photo microcalorimeter system was presented with three twin elements of larger 20 ml volume: unit 1 the sample unit proper, unit 2 the photocalorimetric reference, and unit 3 the  $\text{CO}_2$  absorber with an NaOH solution [28]. This triple instrument was checked with a 130 mg-leaf-tissue sample from spinach and a light flow of about  $2500 \mu\text{W}$  (or  $8 \text{ W m}^{-2}$ ). The metabolically active tissue dissipated  $0.91 \text{ mW g}^{-1}$  (Fig. 3) and rendered a positive ratio of  $+478 \text{ kJ mol}^{-1}$   $\text{CO}_2$  during illumination, nearly identical with the Gibbs energy change of  $\Delta G^0 = +479 \text{ kJ mol}^{-1}$   $\text{CO}_2$  for the formation of glucose from  $\text{CO}_2$  and  $\text{H}_2\text{O}$  at standard conditions [28].



**Fig. 3** Rates of energy expenditure (solid line) and energy uptake (shaded area) (upper part) and of  $\text{CO}_2$  expenditure (solid line) and  $\text{CO}_2$  uptake (shaded area) (lower part) for spinach leaf tissue. During the time of the shaded areas the sample was illuminated. With permission from [28]

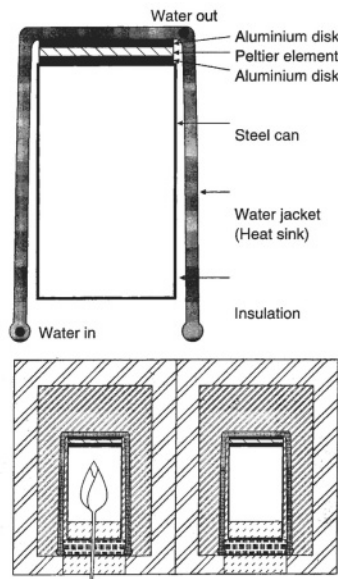
A photocalorimetric method was developed by the Kazan/Russia group on the basis of two differential calorimeter, a Calvet-type and a LKB-type one. The additional module consisted of a 100 W water-cooled lamp, a set of filters to eliminate UV and IR light and to select special wavelengths in the visible range, and a quartz light-guide between the lamp outlet and the calorimetric vessel [29]. This equipment was successfully applied for the determination of energy storage in the unicellular alga *Chlorella* and several agricultural plants [29, 30].

An interesting new calorimetric instrument was presented recently by L. Wadsö and Y. Li (see Conclusion) that connects the vessels of two twin isothermal calorimeters by an outside operated valve and each of them to a pressure sensor. This offers the opportunity to study simultaneously calorimetry and Warburg respirometry in a way similar to that used by Criddle, Hansen and their colleagues [25, 26].

In order to investigate whole plants and not just active organs or small samples, Lamprecht and colleagues [22] constructed a single calorimeter with an aluminium cylinder of 9.5 cm inner diameter, 39.5 cm inner height and an active

volume of 2800 ml. The cylinder was connected via 12 Peltier elements ( $4.0 \times 4.0 \text{ cm}^2$ ) to a corresponding rectangular upright cube as heat sink. Three methods of calibration rendered a sensitivity of  $52.8 \text{ mV W}^{-1}$  and a time constant of 13.2 min. The cube was enclosed in an isolating Styrofoam mantle, and the whole setup housed in an air thermostat of an LKB 10700 calorimeter that was expanded on top by a wooden box to provide the necessary height. This calorimeter was designed to house a complete voodoo lily from the bulb to the tip of the appendix (about 170 g w.w.). Due to the enormous heat output during the metabolic flare-up of more than 1 W and a calorimetric signal of 60 mV or more, it is possible to run this calorimeter in a single and not in the usual twin mode with convenient baseline stability. A perspex cover of the calorimeter proper allowed for illumination during the experiments.

A completely different way was followed by the group of Roger Seymour in Adelaide [31]. To broaden their metabolic investigations of thermogenic flowers and especially of the sacred lotus *Nelumbo nucifera* they designed a light and portable twin heat flow calorimeter that could be used outside in the lotus pond of the Adelaide Botanical Gardens. The idea was to have an inverted calorimeter open at the bottom that could be put over the blossom from the top and be closed by foam stoppers around the stalk. The calorimetric vessels proper were inverted thin-walled tinned steel cans with an inner diameter of 80 mm and a height of 145 mm. They were



**Fig. 4** Two cross-sections through one calorimetric unit (top) and the complete differential setup (bottom) of the lotus calorimeter designed in Adelaide. The Styrofoam insulation and the three foam stoppers are indicated. With permission from [31]

placed in two double-walled styrene wine coolers (100 mm diameter and 185 mm height). 3-mm aluminium plates were glued to the wine cooler inside bottom for better heat conduction. The coolers were equipped with water in- and outlets and connected to a refrigerated, thermostatic water bath and served as heat sinks for the cans. A Peltier element (40x40x4 mm<sup>3</sup>) was placed between cans and coolers in tight contacts to both surfaces. The whole set-up is seen in Fig. 4. This calorimeter had a sensitivity of about 25 mV W<sup>-1</sup> and a time constant of 476 s for a drop to 1/e of the initial signal. After changing the working temperature from 10 to 30°C baseline stability was achieved after 1 hour. The baseline fluctuated less than 0.5 mV during 24 h in the 50 mV range that was used throughout the flower investigations. Such a stability was good enough for the normal biological fluctuations. The calorimeter was constructed in such a manner that simultaneous respiratory investigations could be performed without disturbing the calorimeter and that the heat flux due to air flow could be calculated from temperature differences.

## Heat production in plant tissue

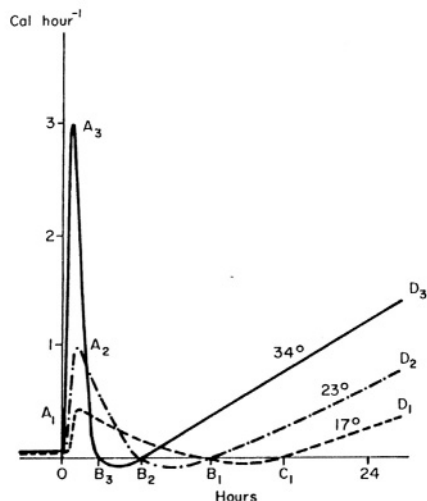
Investigating plant tissues and seeds in a calorimeter, a *caveat* is necessary: except for tissue cultures, samples are not sterile and microbial growth may strongly interfere in the obtained heat dissipation. An exponentially growing heat output during experiments with plant tissues is a clear sign of microbial contamination. The danger can be minimized by surface sterilization with diluted hypochlorite, addition of microbial inhibitors and use of solutions that do not support bacterial growth [16].

### EARLIER OBSERVATIONS

Early calorimetric plant investigation often concerned germination of seeds. Prat [8, 9] described Calvet-calorimeter experiments with 1 g of wheat grains of a resting metabolism very near to zero. After addition of 1 ml water three distinct phases became visible in the first hours: a mere physicochemical effect with a rapid increase of heat flow due to water uptake and swelling of the grains and a drop back under the zero line ('dead phase') followed by a steady increase of heat flow due to the biological thermogenesis (Fig. 5). These phases are typical for germination, but they can be changed by drying the seeds prior to the experiment (augmentation of the first peak), illumination of the germinating plants and by variation of the gas mixture in the head space. Similar germination experiments were already performed on various seeds in the 1880ties by G. Bonnier with a Berthelot calorimeter and a thermocalorimeter after Regnault [32] (Figs. 6a, b). These classical observations were confirmed recently when studying germination and root elongation in quinoa (*Chenopodium quinoa*) seeds and looking for the presence of water transporting channels (aquaporines) [33].

In the 60<sup>th</sup> and 70<sup>th</sup> of the last century Zholkevitch and his colleagues in Moscow made intensive investigations of plant metabolism by means of a





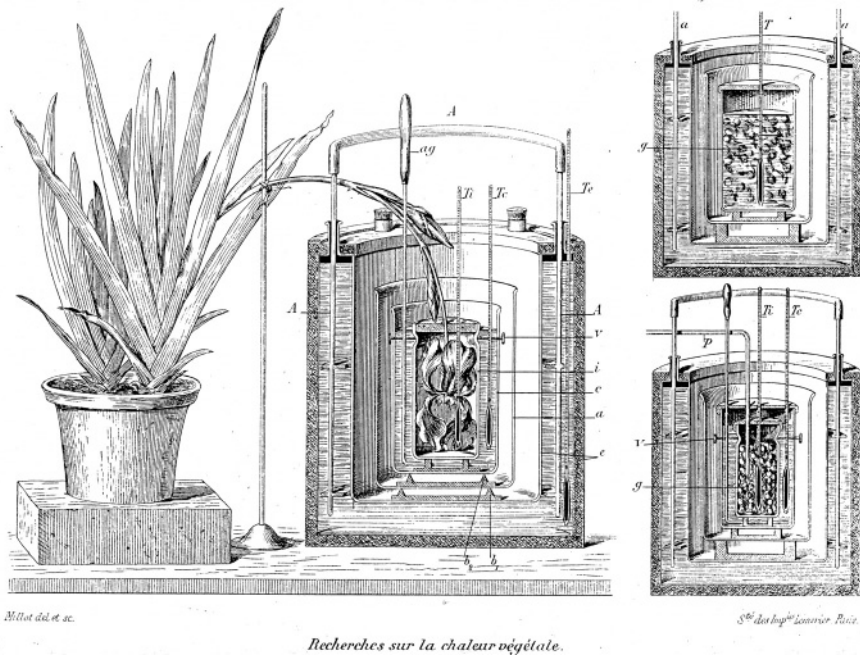
**Fig. 5** Course of thermogenesis in 32 wheat grains (~ 1 g) at 24°C in a Calvet calorimeter with the three phases described in the text. Adapted from [9]

Calvet microcalorimeter and a conventional Warburg apparatus that remained unnoticed by western calorimetrists [34–38]. The aim of their experiments was to compare energy liberation as result of respiration with heat dissipation to the surrounding in various plant and plant tissue reactions: (i) pollination of isolated pistils of winter rye directly in the calorimetric vessel including swelling of pollen and rapid increase of energy turnover rate [34]; (ii) cellular redistribution of energy short time before elongation or division [35]; (iii) establishing an intracellular energy balance in plant tissue under varying conditions of water supply [36]; (iv) the relation between respiration and heat production in slightly withering plants [37]; and (v) low temperature influence on the energy metabolism in cucumber leaf tissue [38].

Kreshek and coworkers [39] analysed the heat production rate during elongation of *Avena* coleoptiles in an adiabatic solution calorimeter and determined rates around 1.2 mW g<sup>-1</sup>. Comparison with respiration measurements ensured that oxidative metabolism accounted for essentially all energy changes in the cell. Moreover, it was found that the auxin indole-3-acetic acid stimulated the elongation by a factor of 10, but heat production rate by only 25 %. This is consistent with earlier findings that this auxin influences the aerobic respiration, inducing a significant increase at lower concentrations and a decrease at higher ones [39].

#### GENERAL OBSERVATIONS

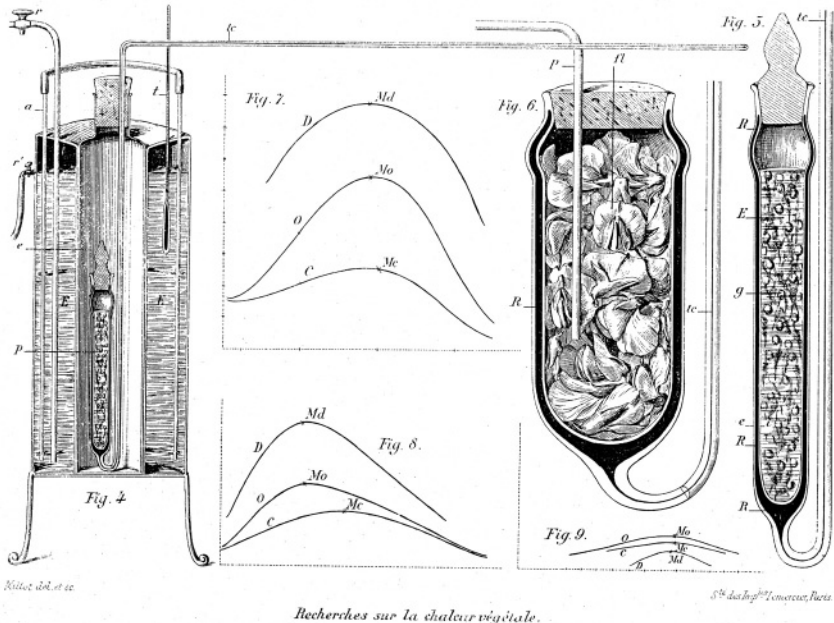
Due to the limited space in this review, only a few examples of plant calorimetry will be presented here in a condensed form. More details can be easily found in



**Fig. 6 a)** Investigation of plant heat production by means of a modified Berthelot calorimeter in the beginning of the 1890<sup>th</sup> by G. Bonnier [32]. The heat produced by the flower in vessel (i) leads to an increase of the air temperature ( $T_i$ ) and of the heat sink ( $T_c$ ). A ring-like stirrer (ag) enables a homogeneous water temperature in the heat sink (c). The external water mantle (e) with another ring-like stirrer (A) and thermometer ( $T_e$ ) protects the calorimeter proper against disturbances. The two smaller pictures show similar or slightly modified experiments with germinating peas in water (top, right) and in air (bottom, right). Adapted with pleasure from [32]

the cited references. A background discussion about kinetics of plant growth and metabolism [40], about the general influence of the alternative oxidative phosphorylation pathway on heat generation [41] and on four methods to evaluate the enthalpy change during anabolism [42] may serve as a stimulating introduction to the field.

It could be shown by heat-flow calorimetry that the integrated growth rate of the coast redwood (*Sequoia sempervirens*) is correlated to the dark metabolic heat rate [43]. This observation may provide a chance to discriminate clones with a high growth rate. Calorimetrically determined dark respiration of the coastal Douglas-fir (*Pseudotsuga menziesii*) was used to evaluate its drought hardiness in 3-years-old seedlings from different families. Heat and  $\text{CO}_2$  production rates measured from 20 to 50°C together with Arrhenius plots rendered significant differences between drought sensitive and drought hardy families [44]. Increased heat production rates were observed calorimetrically after



**Fig. 6 b)** Investigations of plant heat production by means of a thermocalorimeter Regnault for peas in water (left and inner part enlarged: right) and for blossoms (half-right). The black areas (R) correspond to the “thermometer” mercury that expands due to heat transfer and shifts its meniscus along the horizontal capillary (tc). The graphs in the center show heat production rates as function of time determined directly (D) or calculated from oxygen consumption (O) and carbon dioxide production (C). Adapted with pleasure from [32]

wounding of carnation (*Dianthus caryophyllus* L.) shoot tips [45] as to be expected from the intensive thermodynamic discussion of regenerative processes by Zotin [46] and shown for several groups of animals from worms to lizards [47]. Corresponding results with strongly increased heat dissipation (up to six fold) were reported for sliced potato tubers (*Solanum tuberosum* L.) [48].

HEAT PRODUCTION UNDER STRESS

In conquering the surface of the Earth plants had to learn how to cope with hostile environments. Temperatures and high salt concentration in the soil belong to the most prominent stress factors. In many cases, plants became highly adapted to such conditions and only react metabolically to changes in both parameters. Here, calorimetry offers the possibility to detect subtle degrees of adaptation. In a more general paper Smith and colleagues dealt with questions of metabolism, photosynthesis and growth of different plants under stresses by temperature and salt [49]. In another paper the heat output of barley (*Hordeum vulgare* L.) root tips un-

der salinity stress demonstrated two levels of inhibition with increasing NaCl concentration and a cooperative reaction responsible for the decrease in metabolism and nutrient uptake at a high salinity [50]. Connected with such observations are the changes in the rate of heat output on ion balance shifts seen in excised roots of wheat (*Triticum spec.*) seedlings growing in CaCl<sub>2</sub> [51]. Two ion transporters were applied rendering significant varieties in the energy metabolism of the roots. Cerulin is known to specifically block the fatty-acid synthesis and thus should provoke a decrease in heat production and oxygen consumption rates. This could be confirmed calorimetrically with excised wheat roots [52].

### HEAT PRODUCTION AND TEMPERATURE

Seedlings from five populations of the big sagebrush (*Artemisia tridentata*) growing in three different locations were investigated among others for heat and CO<sub>2</sub> production in relation to temperature [53]. Adaptation to their site of origin and significant stress when seedlings were transplanted to other sites were confirmed, by monitoring changes in heat output and growth. High temperatures from 30 to 40°C meant high stress, low values (5 to 10°C) were without stress.

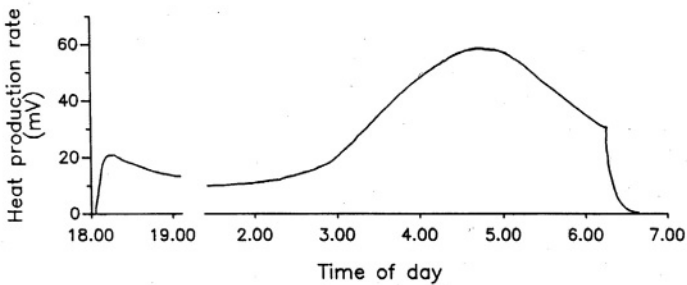
Chilling-sensitive plants can already be injured by temperatures significantly above the freezing point (5 to 15°C). When they are brought back from low to higher temperatures they show a respiratory burst, which can be easily detected by microcalorimetry [54]. Heat output – connected with the alternative pathway – of leaf discs increased up to 98 % after chilling in sensitive species, but only up to 22 % in resistant plants [55]. Coffee seedlings (*Coffea arabica* L.) belong to the sensitive group and are hindered in growth by temperatures below 15°C, closely correlated with the reduction of heat production rates. An Arrhenius plot of the heat rate revealed a break in the line at 15°C, sign of a metabolic transition at this temperature [56]. Further chilling connected experiments are described for soybean (*Glycine max*) cultivar leaves [57], for husk tomato (*Physalis ixocarpa*) leaves [58] and for dormant vegetative apple (*Malus sp.*) buds [59]. Seeds from different populations of the cold-desert subshrub winterfat (*Eurotia lanata*) germinated at temperatures between 0 and 20°C. Heat and CO<sub>2</sub> production rates were determined at the same temperatures rendering metabolic efficiencies and specific growth rates. It became obvious that the seedlings' responses reflected the climate at the site of their origin [60].

### HEAT PRODUCTION OF THERMOGENIC PLANTS

True thermogenic plants that produce heat for their own sake and not as by-product of usual metabolic activity are found in several plant families, for instance in Araceae (arum lilies), Nymphaeaceae (water lilies) and Nelumbonaceae (true lotus) (see e.g. [19, 20, 23, 61]). The specific heat output of flowers of such plants can reach that of a hovering hummingbird or raise the inflorescence temperature

35 K above that of the environment [19]. Some of them are even able to regulate their temperature (in the sense of warm-blooded animals) for hours or days [62]. Several reasons for this astonishing phenomenon are presented in the literature: in a first place the volatilisation of odour molecules to attract insect pollinators and the provision of a heated shelter for these insects as well as the possibility to bloom at low temperature and to protect sensitive parts in the flowers. The biochemical background of the metabolic burst is (i) a shift from the normal to a cyanide-insensitive pathway rendering only one third of the ATP amount obtained by the usual phosphorylation and dissipating the residual energy as heat and (ii) even more important a strongly increased respiration rate [63].

Most of the thermogenic plants are large and soil-bound so that inflorescences as place of heat production have to be cut and investigated separately from the rest of the plant. Moreover, these organs are usually so large that only few calorimeters are large enough to house a whole inflorescence. Thus, they must be used as tissues slices. Problems accompanying slicing of plant tissue (and thus minimizing diffusion barriers for oxygen) are arrestingly described for voodoo lily (*Sauromatum guttatum*) tissue showing a heat output of  $174.7 \text{ mW g}^{-1}$  dry weight for a 10 mg sample, but  $9097.1 \text{ mW g}^{-1}$  (!) for a 0.1 mg piece [64]. The voodoo lily is the only member of the thermogenic family that is able to flower just from the corm without soil and water, predestined for a 'whole-body' calorimetry. In a special plant calorimeter described above complete voodoo lilies were investigated. Figure 7 shows the metabolic burst of a 155 g lily in the early morning hours of D-day with a maximum calorimetric signal of 60 mV or 1020 mW, rendering  $6.8 \text{ mW g}^{-1}$  w.w. for the whole plant, but about  $100 \text{ mW g}^{-1}$  for the metabolically active appendix. Integration of heat output over a 4.5-h period comes to 11.6 kJ, the oxygen consumption in the same period to 11.4 kJ. This underlines that the flare-up is carried by a strongly increased respiration intensity [22]. The pre-history of flowering in the voodoo lily was investigated calorimetrically for the five days preceding the metabolic burst [65]. The authors used tissue slices



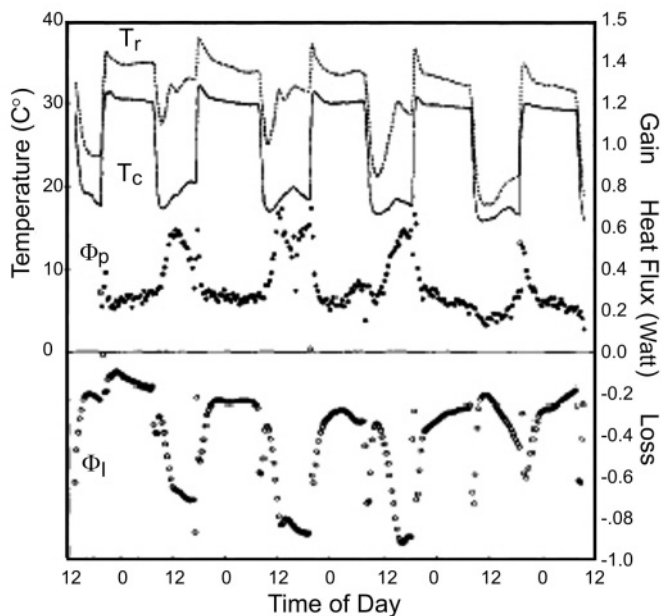
**Fig. 7** Anthesis of a voodoo lily (*S. guttatum*) of 155 g wet weight in a 3-liter heat flow calorimeter. The decrease in heat production after 5:00 h is an artefact due to the decreasing oxygen concentration in the calorimeter. With permission from [22].

from three parts of the appendix and determined the influence of salicylic acid as natural inducer of heat production and of the head space atmosphere. The same authors detected an oscillatory behaviour of the heat production in the thermogenic male cones of two cycad species (palm ferns) with maximum amplitudes of 14 to 18 mW g<sup>-1</sup> w.w. and confirmed the presence of the alternative respiratory pathway [66].

The first true calorespirometric investigations on plants with a combination of a gradient layer calorimeter of the Benzinger/Kitzinger type and an external gas analyser concerned the severed spadix of *Philodendron selloum*, a member of the arum family [67, 68]. Its spadix warms up to 38 to 46°C independent of the ambient temperature changing between 4 and 39°C. It indicates that this organ enforced its metabolism when the temperature decreased. While heating endured for several hours under normal conditions, it reduced to 1 to 2 hours for cut samples. Nevertheless, they obtained the same maximum respiratory heat production around 50 mW g<sup>-1</sup> w.w. Additionally, bomb calorimetry was applied to determine the heat content of cut sterile male florets (the main seat of heat production with a maximum rate of 150 mW g<sup>-1</sup> w.w.) before, during and after the metabolic burst.

Direct and indirect calorimetric field experiments on flowers of the sacred lotus *Nelumbo nucifera* were performed in an outdoor pond of the Adelaide Botanical Gardens [31] by means of the light, transportable differential calorimeter already described above. Its adjustable water-bath heat-sink allowed to deceive the flower and to simulate a cold environment during day and a warm at night. Thus, it could be shown that heat production in lotus flowers depends on the ambient temperature and not on the light cycle [69]. The flowers kept their temperature rather constant at 30.7 and 34.2°C at mean set calorimeter values of 18.4 and 30.4 °C, respectively. The maximum heat production dropped from 0.51 W (60 mW g<sup>-1</sup> w.w.) to 0.25 W (30 mW g<sup>-1</sup>) at high temperatures. Figure 8 presents the outcome of a five-day experiment in the lotus pond with a reversed daily temperature regime of the calorimeter (15°C cool during the day, 30°C warm at night).  $T_r$  and  $T_c$  represent the receptacle and the calorimeter temperatures. Neglecting the over- and undershoots, one observes plateau phases in both half-days. The receptacle was always warmer than the calorimeter, but at a decreasing difference with time. Longer periods of constant metabolic heat production rates  $\Phi_p$  (upper part of the figure) and of dry heat loss  $\Phi_1$  (lower part) are seen in the graph interrupted by metabolic bursts when the calorimeter temperature was lowered. During these periods the differences between  $T_r$  and  $T_c$  amounted to 12.3°C, in the other time only 3.8°C – a clear sign of the stated thermoregulation.

In addition to the field investigations, cut flowers were placed in the same gradient layer calorimeter arrangement as used before for the *Philodendron* spadix [67, 68]. An energy calculation showed that the metabolic heat produc-



**Fig. 8** Graph of a 5-days outside experiment on a flower of the sacred lotus *Nelumbo nucifera*. The right ordinate gives gains (upwards) and loses (downwards) of the metabolic heat production  $\Phi_p$ , calculated from the oxygen consumption rate and for the dry heat loss  $\Phi_l$ . Due to heat take-up in the 6 m long hose from the water thermostat to the calorimeter and the warming by the flower the mean low calorimeter temperature was 18.4 °C at a thermostat setting of 15°C. For more information see text). With permission from [31]

tion was almost completely balanced by evaporative heat loss and that there was no conservation of energy in metabolic processes during thermogenesis.

This lotus field research was – to our knowledge – the first outdoor application of direct calorimetry to plants while there were already a number of indirect field investigations on several aroids including *Philodendron* and eastern skunk cabbage or on the tropical water lily *Victoria cruziana*.

## OTHER TECHNIQUES

Thermal analysis – in the title of this monograph – means not only just isothermal microcalorimetry, but also other techniques like combustion calorimetry, differential thermal analysis/differential scanning calorimetry, thermogravimetry or infrared false color thermography. But as the daily routine and the main interest of the authors focus on microcalorimetry, these other fields will be touched more marginally saying nothing about their importance in thermal analysis. Including some papers in this review enables the deeper-interested reader

to track the activities of the chosen authors and to get access to related fields by their cited references.

### COMBUSTION CALORIMETRY (CC)

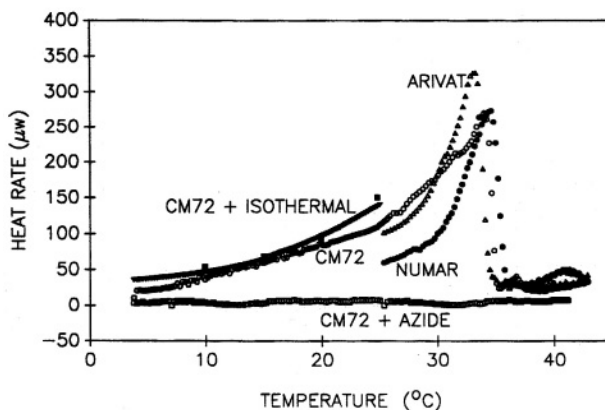
Combustion calorimetry is one of the oldest techniques in Thermal Analysis dating back to Black, Crawford, Lavoisier, and Count Rumford; in its modern form to Berthelot. In its earliest application energy content of wood played an essential role. Later on, other plant material like leaves, fruits and roots were enclosed and CC is nowadays an important tool in ecological energy balances. Recently, a contribution by one of the present authors (I. L.) appeared in the *Handbook of Thermal Analysis and Calorimetry* [70] covering the whole field from instrumentation (see also [71]), sample preparation and experimental approaches to applications in different areas including plant material. Therefore, this section may be kept short. Meanwhile, further papers were published from the Santiago group around Prof. Nunez Regueira dealing with caloric values and flammability of living forest species or forest waste biomass and the risk of wildfires in Galicia [72–76] from which a risk index map of this area emerged [77]. Wild fires represent an enormous problem in Spain as they devastated thousands of square kilometre forest and bush land in the last 40 years with a value of billions of Euro.

### DIFFERENTIAL SCANNING CALORIMETRY (DSC)

In a review on biologic applications of DSC [12] plants played an important role with questions concerning the state of water in plant cells, of cold resistance, supercooling, freezing cryo-protection and dehydration, but also of wood, its components and fungal degradation and finally of ancient plant material like papyri or fig-tree bark for historical paper production. More information on DSC used in wood analysis may be found in a handbook survey [7].

While in the ‘classical’ DSC fresh or dried milligram samples of plant material are investigated in the range from room temperature to about 600°C, with scanning rates of several K min<sup>-1</sup> and in air as well as in inert atmosphere, a modern alternative for plant investigation was opened following an inseminating paper by Sturtevant and coworkers [78] on heat production of murine macrophages in a strongly reduced temperature range and at low scan rates. This technique is frequently applied today in groups of plant calorimetrists [79]. Some examples were already cited in the ‘chilling’ paragraph [57–59] and only one shall follow concerning the temperature dependence of tissue metabolism from barley (*Hordeum vulgare* L.) roots [80]. Instead of a stepwise increase of temperature as in true isothermal experiments the sample was slowly scanned (2.4 to 3.5 K h<sup>-1</sup>) from 5 to 45°C (Fig. 9). This rate was sufficiently slow so that the tissue metabolism occurred at a steady, quasi-isothermal state at each time. This is underlined by the 4 square points at the curve ‘CM72 + ISOTHERMAL’ that result from isothermal calorimetry at 5 K different temperatures. Smooth





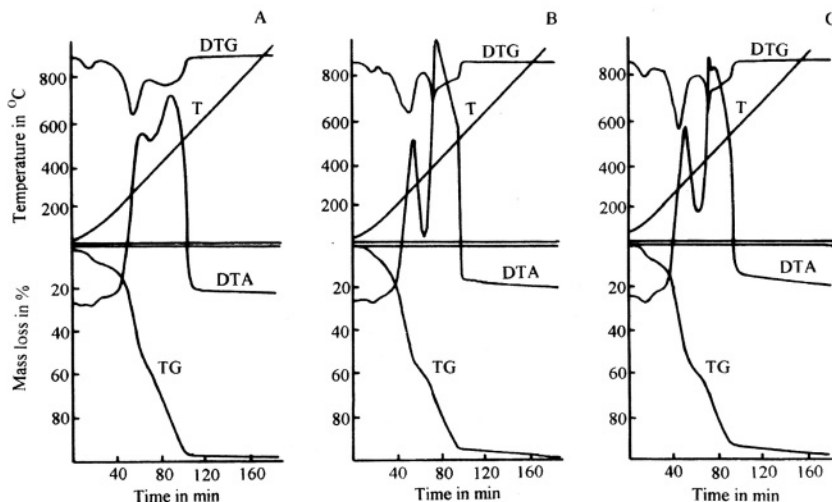
**Fig. 9** DSC thermograms of 3 barley cultivars from 5 to 45°C (CM72) and 25 to 45°C (Arivat, Numar). The graph (CM72 + isothermal) compares the DSC thermogram with 4 isothermally obtained data points (■) in 5 K steps. With permission from [80]

curves result for all barley cultivars up to about 30°C where a threefold increase in activation energy becomes obvious. While this change was reversible, the next one at 34°C lead to an irreversible decrease of the heat rate to nearly zero. In the whole DSC thermogram from 5 to above 30°C no physical state changes of membranes or proteins became visible although sensitivity was high enough to show them [80].

### THERMOGRAVIMETRY

Thermogravimetry alone or coupled with mass spectrometry is not so often applied to plant tissue. In thermogravimetric experiments the weight loss of the sample is determined during a heating up program with usually constant heating rates of a few  $\text{K min}^{-1}$ . The obtained signals show, as a function of time (or temperature), the increase in temperature (T), the relative weight loss in percent (TG, taking the initial weight as 100%, thus starting with a weight loss of 0 %), the first time derivative of this loss (DTG) and the differential thermal analysis signal (DTA) as the temperature difference between the sample and the reference crucible. By convention, exothermic peaks point upward, endothermic ones downward.

Commercial raw plant drugs, material consisting of leaves, flowers, roots, rhizomes and bark, were heated up to 900°C at a rate of  $5 \text{ K min}^{-1}$  and the obtained DTA, TG and DTG curves were analysed [81, 82]. Moreover, the non-metallic and metallic elements in the samples were determined. It became obvious in the majority of cases that samples taken from the same plant species render similar results within the five kinds of plant material listed above: Fig. 10 shows a typical set of curves (T: sample temperature) for three different flowers. Only one further

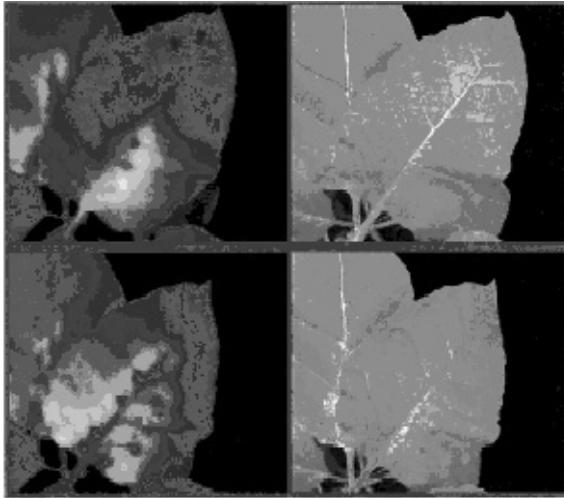


**Fig. 10** DTA, T, TG and DTG curves obtained during thermal decomposition of three flowers. A: Scotch heather (*Calluna vulgaris*); B: coltsfoot (*Tussilago farfara* L.); C: yarrow (*Achilla millefolium* L.). Sample size about 100 mg, heating rate  $5 \text{ K min}^{-1}$ . The DTA curve consists of one weak endothermic and two pronounced exothermic peaks. With permission from [82]

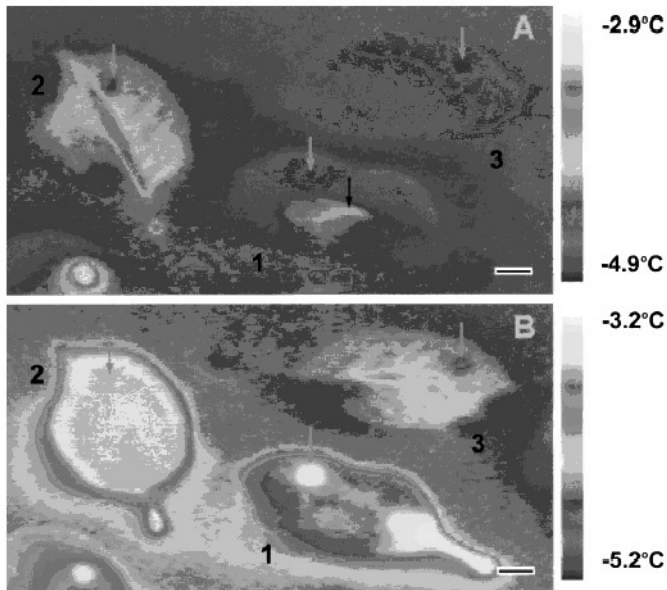
example of plant thermogravimetry shall be presented here, a coupling of a modified thermobalance with a mass spectrometer [83] for the investigation of two herbaceous plants with high biomass production potential. Small samples of a few mg were heated from 20 to  $900^\circ\text{C}$  with a rate of  $20 \text{ K min}^{-1}$ . This is slow enough to resolve the different steps of temperature dependent biomass decomposition: starting with moisture evolution and followed by decomposition of hemicellulose and cellulose. The simultaneous mass spectrometry data provide information about the low molecular weight volatile products of pyrolysis as function of temperature and thus together with the DTG curves also information about the underlying degradation mechanism. They are essential for the understanding and optimisation of different biomass conversion techniques [83].

### IR THERMOGRAPHY

Infrared false-colour thermography [84] is well introduced in various fields of industry, research and medicine (including veterinarian), but only recently more intensively used for surface investigations of plants. Thermographic cameras collect near infrared radiation in the wavelength range from about 5 to  $15 \mu\text{m}$ , has a typical sensitivity of  $0.1 \text{ K}$  at  $30^\circ\text{C}$  and may discriminate between points of 2 mm distance and  $0.2 \text{ K}$  temperature difference. The detected IR radiation is proportional to the temperature distribution over the surface of the object. The camera transforms this IR picture into a false-colour picture in the visible range;



**Fig. 11** Thermographic and visual imaging of cell death (yellow parts) in bacterio-opsin tobacco 32 h (upper two pictures) and 40h (lower two) after first detection of a thermal effect. The maximum temperature difference amounts to 0.6 K. With permission from [85] (See colour section, p. 347).



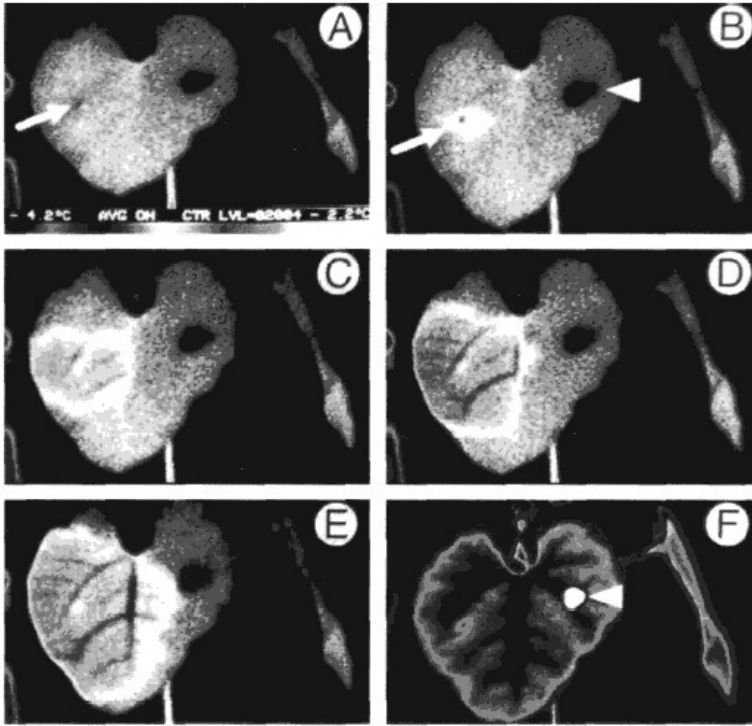
**Fig. 12** Holly leaves (*Ilex* sp.) during freezing shown in false colours of a 2 K temperature range. Picture B was taken about 3 min after A. The pale blue to whitish areas (A,B) indicate an initial exothermic effect of low intensity, the yellish colours (B) a second stronger exothermic effect. Green arrows point to water droplets put on the leaves before cooling started. With permission from [90] (See colour section, p. 347).

colours or grey intensities can be freely chosen to render an optimal resolution for the interesting temperature range. In recent years increasing, but still small numbers of papers were published concerning plant IR thermography dedicated to freezing and ice nucleation, infections, leaf energy balances, metabolic flare-up in thermogenic flowers and seeding quality assessment. A few shall be presented in this survey.

Chaerle and Van Der Straeten recently published a paper that could render the title for this chapter on thermography: 'Seeing is believing: imaging techniques to monitor plant health' [85]. It deals with the IR observation of stress-induced changes in plants before the human eye can detect them, with screening for mutants of increased stress tolerance and with the application of such results for plant engineering [85]. In the same sense, the presymptomatic thermographic visualization of plant destruction by tobacco mosaic virus infection was demonstrated by the same authors [86, 87]. Already 8 h before the visual detection lesions were clearly visible in the IR pictures as 'hot spots', 0.3 to 0.4 K warmer than the surrounding (Fig. 11). These spots were colocalized with later on formed lesions due to an accumulation of salicylic acid, a compound that is known to stimulate heat production in thermogenic plants (see below), to induce thermogenicity in non-thermogenic leaves [88] and to serve as a signal against pathogens [89].

One of the major general plant stresses is freezing, an effect of enormous importance for wild plants as well as for crops and thus for agriculture also. Freezing in plants is accompanied by an (exothermic) heat release and temperature increase of up to 1 K that can easily be detected by thermography. A number of papers (e.g. see [90–93]) appeared dedicated to nucleation of ice and its propagation, mainly in plant leaves and buds (Fig. 12). Figure 13 shows ice nucleation and propagation in a bean leaf induced by a 2  $\mu$ l droplet of a *Pseudomonas syringae* solution (left) and a drop of deionised water (right) (A). Since the water drop loses heat by evaporation it is cooler than the leaf and outside the chosen temperature scale (2 K) at the lower end (black). The droplet with the 'Ice<sup>+</sup> bacteria' freezes first (B), gains a higher temperature outside the temperature range at the upper end (white) due to the exothermic heat of freezing and serves as centre for ice nucleation throughout the leaf (B to E). At the end, when the freezing of the leaf and the exothermic process came to an end, the leaf turned blue (in false colours), only then the water drop started to freeze and to obtain heat and a higher temperature (change from black to white).

A specially interesting field for IR thermography is that of the already mentioned thermogenic plants, a group of plants mainly consisting of species of the arum lily family and a few water lilies. Hanna Skubatz and colleagues presented an impressive collection of IR pictures of eight members of the *Arum* lily family, the temperature distribution along these plants (mainly in their appendices) and respiration data from the most thermogenic tissues [94]. The same authors thermographically followed the metabolic burst of the voodoo lily (*Sauromatum*



**Fig. 13** Ice nucleation and propagation in a bean leaf shown by false-colour thermography. The temperature range was chosen 2 K. Black and white parts are out of range at the lower and the upper end, resp. For further explanations see text. With permission from [91] (See colour section, p. 348).



**Fig. 14** Thermogenic active evening flower of the giant water lily *V. cruziana* in false colour. At air and water temperatures of 24.0 and 31.0°C, resp., the centre of the blossom shows a temperature from 30.9 to 33.5, significantly above the air temperature. The white area in the left upper corner represents the arm of the investigator. With permission from [23] (See colour section, p. 348).

*guttatum*) [95], as was done in the same year by one of the present authors (I. L.) [21, 22]. Similar *Arum* investigations on the temporal and spatial distribution of heat production were dedicated to the Lords and Ladies (*Arum maculatum*) showing two main centres of metabolic activity, the appendix and the male florets [96].

In the course of thermoanalytical greenhouse investigations on the giant tropical water lily *Victoria cruziana*, IR thermography was applied intensively, for flowers as well as for the structure of the huge floating leaves [23, 24, 97]. *V. cruziana* and *V. amazonica* increase their flower temperature up to about 10 K above ambient and release an attractive sweet odour of pineapple or fruit salad. The most thermogenic tissue is found in the inner stamina that are not directly visible from the outside. Thus, the infrared picture (Fig. 14) shows the hottest part (33.5°C) around the 'tunnel' that leads through the paracarpels into the floral chamber and that was closed in the moment when the picture was taken. As the flower was 10 cm above the water, the temperature difference against air (24.0°C) and not against water (31.0°C) counts.

## Conclusion

In recent years, plant calorimetry has finished its sleeping beauty dormancy that lasted more or less continuously since the beginning of last century and realizes quite a number of scientifically attractive princes around it. They appeared with new armours and weapons and are eager to dedicate their life to her beauty. New sophisticated calorimeter allow investigations that were excluded before, combination with other highly specific instruments renders more information than gathered earlier by calorimetry alone, rigid and flexible light guides illuminate the otherwise dark vessels and open the field to photosynthesis experiments, infrared thermography provides a means to determine temperature distributions without injuring or even only touching the object. Imagination and technical skill are asked for further developments in plant calorimetry that will for sure come.

A special issue of *Thermochemica Acta* in 2004 will be dedicated to the outcomes of the XIII<sup>th</sup> Conference of the International Society for Biological Calorimetry *Energetics of Adaptation and Development – From Molecular Mechanisms to Clinical Practice* that took place in Würzburg / Germany 2003. Many contributions deal with photocalorimetry and its application to plant systems as well as with conventional heat flow and combustion calorimetry for plant cells. Among other, topics like *A new calorespirometric instrument, Calorespirometric ratios and metabolic efficiency, Energy processes in model plant cells, Heat production and respiration of wheat roots, Calorimetric studies of vegetable tissue wounding, Life zones for key plant species can be predicted with calorespirometry and temperature measurements, Photo-Bio-Calorimetry of Chlorella vulgaris growth, Energetic evaluation of forest formations by bomb calorimetry* will be approached. This issue is recommended for further information.

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