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MEASUREMENTS OF LEAF TEMPERATURE BY THERMOCOUPLES OR INFRARED THERMOMETRY IN CONNECTION WITH EXCHANGE PHENOMENA AND TEMPERATURE DISTRIBUTION

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I. INTRODUCTION

It appeared to be difficult to obtain light saturation of the photosynthetic apparatus in measurements of photosynthesis of sun and shade leaves of *Acer* and *Populus* at CO_2 saturation. At 20 °C exposure of the leaves to 10^6erg. cm^{-2}. sec^{-1}, even was insufficient. Data from literature also demonstrate that light saturation of the photosynthetic apparatus is not easily reached at CO_2 saturation (GAASTRA, 1959). For maize this even holds in normal air (HESKETH and BAKER, 1967), possibly because the photosynthetic apparatus of maize can be CO_2 saturated already in normal air (PIETERS, unpublished data).

The question arose, whether this light limitation could be caused by the methods used for the measurement of temperature. In the experiments concerned, the leaf temperature was measured with thermocouples and leaf temperature control depended on this measurement. The fact that light-saturation could not be reached, might be explained by a rise of temperature with increasing irradiation of the leaf in spite of the temperature control: then the bending of the photosynthesis versus light curves shifts to higher irradiation levels and maximum photosynthesis increases steadily with irradiation, because of an ever increasing actual leaf temperature.

The applicability of contactive elements for the measurement and control of leaf temperature thus is questioned. Also chemical and chemical-optical methods have been designed and used (PERRIER, 1968, 1971), but we have not tried them because of their complexity and because interference with environmental conditions of the leaf does not seem to be excluded. Infrared thermometry may be the most adequate solution of the problem of leaf temperature measurement and control, because it does not interfere whatsoever with the environment of the leaf. But the application of pyrometric methods neither is without complications of fundamental and practical nature.

In the present study several methods of measuring or controlling leaf temperature were compared with the thermocouple method. It is shown that in spite of apparent light limitation at high irradiation levels, light is not limiting photosynthesis. Physiological evidence is presented for obtaining light saturation in leaves under conditions of more precise temperature control, as e.g. in leaves floating on water or with infrared radiation thermometers. Also temperature distribution over the leaf, as measured with thermocouples deviates strongly from that measured with the infrared camera.

The results demonstrate, that important deviations between the measured leaf temperature and the actual leaf temperature may occur, when thermocouples are used. The existence of systematic errors in the measurement of surface temperatures with thermocouples is fully accepted in technical literature. In the plant physiological literature several studies question the accuracy of the thermocouples (SEYBOLD, 1929; PERRIER, 1968; IDLE, 1968). PIETERS and SCHURER (1973) with leaf models could prove and explain the existence of systematic errors in the determination of surface temperature with thermocouples.

SLATYER (1971) discussed the significance of errors as small as 1 °C in the measurement of leaf temperature for the estimation of transfer resistance of leaves. In this study it is shown that errors of several degrees centrigrade occur.

II. MATERIAL AND METHODS

II.1. *Plant material*

Plants of *Populus euramericana* (Dode) Guinier 'robusta' and *Acer platanoides* L. were cultivated in a subirrigated gravel culture (HOAGLAND A-Z nutrient solution) in the phytotron at 20 °C. The plants were illuminated from three sides. The irradiation was 40.000 erg.cm^{-2}.sec^{-1} (Philips fluorescent tubes, 120 watt).

II.2. *Physiological leaf model with Scenedesmus cells*

The physiological leaf models were produced by evenly spraying a volume of 3 ml of a suspension of *Scenedesmus obtusiusculus* K 22 over a piece of filter paper of 100 cm^2. The density of cells on the filter paper was varied by altering the density of the sprayed cell suspension.

The *Scenedesmus* cells were cultivated in culture tubes in a medium containing 10 mM KNO_3, 2 mM $MgSO_4$ and 15 mM KH_2PO_4 and the necessary trace elements (WESSELIUS, 1973) at 30 °C. Air containing 4% CO_2 was continually bubbled through the culture solution. When the cells in the culture tubes reached a density of about 12 µl packed cell volume per ml, they were harvested, centrifuged, washed with destilled water, and resuspended in tap water.

II.3. *Irradiation of the assimilation chambers*

For the illumination of the leaves in the assimilation chambers Philips HPLR 700 watt mercury lamps or Leitz Prado 500 watt projectors were used. The light intensity could be varied by intercalating gray filters of known density or by varying the feeding voltage of the incandescent lamps. Light intensities were measured with calibrated selenium barrier cells.

II.4. *Measurement of gas exchange*

The measurements of the rates of gas exchange were made with the katharometer. The katharometer and the gas circuit of the assimilation chamber is described by PIETERS (1971). Initially, the measurements were made in an open gas system and the flow rate of the gas through the assimilation chamber could be varied between 40 and 120 l/hr; later on a halfclosed system was used with a direct gas flow rate of 40 to 120 l/hr and a gas flow rate in the recirculation system of about 1000 l/hr (linear air velocity 25 cm/hr).

II.5. *Assimilation chambers*

Three types of assimilation chambers were used: A small one made of plexiglass (inner diameter 6 cm, depth 8 mm) with an open gas flow system. Around the assimilation chamber thermostated water was passing. This chamber was suitable only for the measurement of detached leaf disks with a diameter of about 55 mm. The petiole could be placed in a potometer.

A larger leaf chamber was designed for the measurement of gas exchange rate of leaf parts attached to the plant; the inner dimensions are 100 × 100 × 12 mm^3. The leaf lamina was held in the middle of the assimilation chamber by thin plastic wiring. The air entered and left the chamber via manifolds. This chamber was cooled with a cooling medium passing through the double glass walls. One of these double walls was the lid of the assimilation chamber. To accommodate infared thermometry, this lid could be replaced by a frame closed with polyethylene film (Lupolene H).

The seal between lid and assimilation chamber consisted of a rubber O-ring. The attached petiole fitted air tight in a slot in the aluminium frame of the assimilation chamber with plastic paste (Putty, Artiflex, Holland). When a leaf was too large for the assimilation chamber, it was cut to a square leaf part of 70 × 80 mm^2, attached to the plant via the petiole. Experiments did show that this practice is permitted.

For the measurement of photosynthesis of leaves floating upside down on water, a glass vessel, comparable to a small gas washing bottle, was used. The leaf was illuminated from below. The vessel was flushed with a continuous air stream. The air entered the vessel via the glass joint through a thin glass tube piercing the leaf disk through a hole punched in the middle of it, and was lead into the small amount of water, on which the leaf was held floating by the air bubbles. The air left the vessel via a side arm to the gas analyser.

II.6. *Leaf temperature measurement*

II.6.1. Thermocouples. Thermocouples were made by soldering together the stripped and cleaned Cu and Co wires of Honeywell thermocouple wire of a diameter of 0.1 mm. The junctions were made as short as possible. The reference junction was held at a defined temperature. One to nine junctions were pressed carefully against the leaf surface. The output of the thermocouples was recorded on a 1 mV recorder (sensitivity 40 μV/°C). If more than one thermocouple was used, mean temperature was calculated and temperature control was based on this average temperature.

II.6.2. Infrared thermometry. The thermal infrared radiation of the leaf in the closed assimilation chamber could be measured only via the infrared transmitting polyethylene film sealing the assimilation chamber. This creates difficulties in the calculation of leaf temperature, which will be discussed separately (SCHURER and PIETERS, in preparation).

Point thermometers. These infrared thermometers measure the average temperature of a certain leaf area. The diameter of the measured spot depends on aperture of the used apparatus and distance to the object. The instruments used were the 'STOUTJESDIJK' radiometer (STOUTJESDIJK, 1966) and the infrared thermometer PRT4 of Barnes engineering.

Infrared cameras. The instruments used are the Aga Thermovision (model 652) from Aga, Lidingö, Sweden and the Sems thermographe (model TS 405) from Société Européenne de Matériels Spéciaux, Clamart, France. It was found necessary to test the equipment before use. The thermopicture of the Aga Thermovision (old model) could be distorted by temperature gradients in the room, being out of focus of the camera and this influenced the accuracy of the instrument. One should be aware also of the fact that infrared radiation, emitted by the projectors illuminating the leaf in the assimilation chamber, and transmitted by the assimilation chamber assembly and the leaf, may interfere with the measurement of leaf temperature.

II.6.3. Leaf temperature control. At first control of leaf temperature was attempted by rightly choosing the temperature of the cooling medium passing through the double walls of the assimilation chamber, so that the measured mean leaf temperature was kept as constant as possible. It will be shown in a separate article that differences in temperature between leaf and cooling medium are the basis of the systematic errors in the measurement of leaf temperature with thermocouples (PIETERS and SCHURER, 1973). Leaf temperature was adjusted in the same way, when the Aga thermovision was used for temperature measurement. This is probably disadvantageous, because the temperature gradient over the leaf is affected by changing illumination and mean temperature was estimated visually from the thermopicture. The measurements with the infrared point thermometer (PRT4) were made under more favourable conditions, because a new system of automatic temperature control was used: the infrared temperature compensation method (PIETERS, 1972), by which the temperature gradient was only weakly influenced by the illumination of the leaf. Furthermore, the optical properties of the assimilation chamber were greatly improved and the properties of the PRT4 are ideal for this type of work. The temperature control of the leaf was also much improved by the application of a recirculation system of the air through the assimilation chamber (linear air speed 25 cm/sec.).

III. RESULTS

III.1. *Photosynthesis-light curves of attached leaves of Populus and Acer measured in the assimilation chamber at constant temperature, as measured with thermocouples, and at CO_2-saturation*

Fig. 1 represents a photosynthesis - light curve as generally observed. It appears, that CO_2-saturated photosynthesis of a *Populus* leaf is light limited, even in intensities up to 7.10^5 erg.cm^{-2}.sec^{-1}. Analoguous data can be found in literature (GAASTRA, 1959; HESKETH and BAKER, 1967); data on photosynthesis of leaves at CO_2-saturation, however, are relatively scarce.

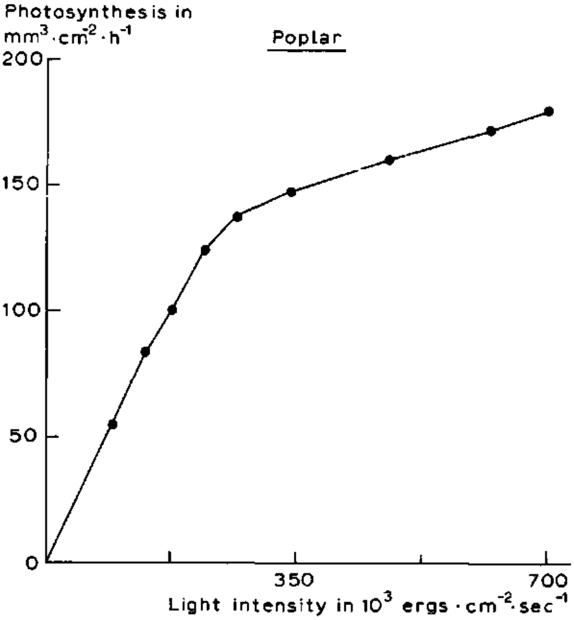


FIG. 1. Photosynthesis-light intensity curve of a poplar leaf, attached to the plant, measured in the assimilation chamber and demonstrating apparent light limitation. Airflow between 40 and 120 l.hr^{-1}. Temperature 20°C as controlled by thermocouples. CO_2-concentration of the air : 5‰. Illumination with HPLR 700 (Philips).

The amount of air passing through the assimilation chamber ranged from 40 to 120 l/hr in this type of experiments. Leaf temperature, regulated by thermocouples, was held at 20°C by changing the temperature of the double wall of the assimilation chamber.

III.2. *Light saturation of CO_2-saturated photosynthesis in spite of apparent light limitation*

Especially in the case of sun leaves, which contain a large amount of chlorophyll per unit area, it may be suggested, that the abaxial side of the leaf has not been light saturated at the applied irradiation intensities, because of the weakening of the light during its passage through the leaf. To test this hypothesis, some experiments were performed with *Scenedesmus obtusiusculus* K 22, sprayed on filter paper to form physiological leaf models of varying thickness, and also some with attached leaves in the assimilation chamber, illuminated from both sides to diminish the influence of light weakening in the leaf.

III.2.1. Photosynthesis of physiological leaf models, composed of Scenedesmus cells on filter paper, as measured in the assimilation chamber. With a small vaporisator, *Scenedesmus* cells were evenly distributed over a piece of moistened filter paper (see methods). A part of this physiological leaf model (⌀ 6 cm) was placed against one of the cooled, thin plexiglass walls of the small assimilation chamber. Due to the close contact with the cooled wall, the temperature control of the leaf model may be considered to be sufficiently precise. The amount of chlorophyll per unit area and light absorption

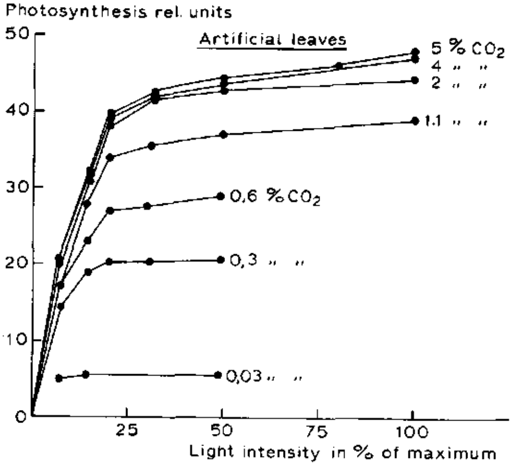


FIG. 2. Photosynthesis – light intensity curves at a number of CO_2-concentrations of the air of a physiological leaf model, consisting of *Scenedesmus* cells on filterpaper, fitted against one of the walls of the small assimilation chamber. A high level of light saturation is found. Photosynthesis is CO_2-saturated above 2% CO_2 in the ambient air. Temperature 20°C.

could be varied by varying the amount of cells brought onto the paper. In the densiest packing of cells, the leaf models did contain about 8 mg chlorophyll per dm^2 and are comparable to the darkest sun leaves. The results of a number of photosynthesis measurements are reproduced in fig. 2. The photosynthetic rates of these artificial leaves are also comparable to natural leaves. It may be concluded, that photosynthesis of *Scenedesmus* cells, measured under the conditions described, behaves in a classical way and that light and CO_2-saturation could be obtained.

It may be expected that a linear relationship exists between the amount of chlorophyll per dm^2 and the maximum rate of photosynthesis at light and CO_2 saturation, if starting from one and the same culture of cells. According to fig. 3 and 4 this situation seems to be approximated, and it thus may be stated that photosynthesis also of the densiest leaf model still was light saturated.

III.2.2. Rate of photosynthesis of attached leaves of *Acer* in the assimilation chamber, illuminated from both sides. When indeed light weakening in the object should be a cause of the absence of light saturation of the photosynthetic apparatus, it may be expected that illumination from both sides with half the intensity and at constant temperature, would give a higher rate of photosynthesis. From table I it can be concluded, that this is not the case. This means that the leaf is either fully light limited or fully light saturated. Because in the conditions used the Q_{10} of photosynthesis is known to amount to 2.2, it is clear that the leaf is fully light saturated.

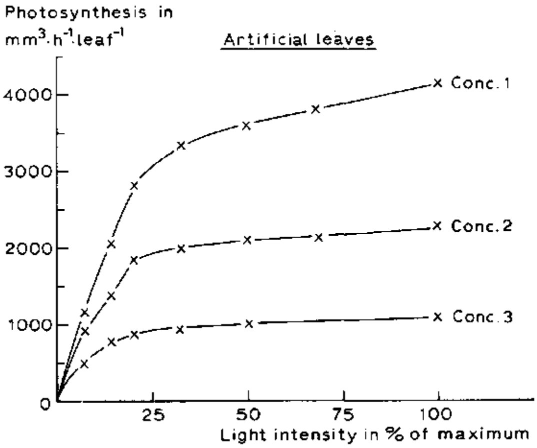


FIG. 3. Photosynthesis-light intensity curves of artificial leaves of various densities, showing that especially at the lower concentrations the degree of light saturation is reasonable. CO_2-concentration 5%. Temperature 30°C.

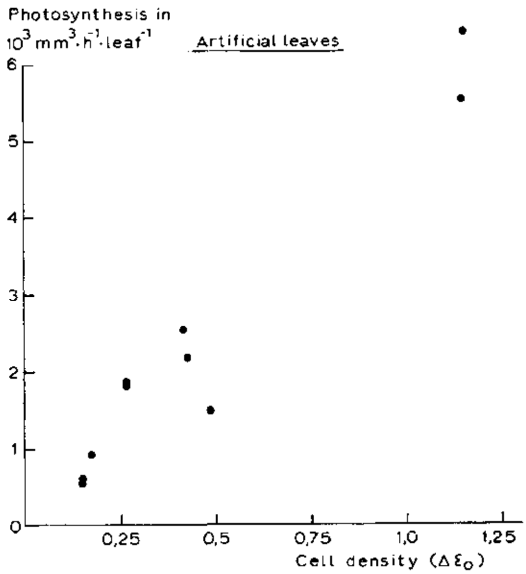


FIG. 4. The relation between cell density on the physiological leaf model (*Scenedesmus*, on filterpaper), estimated by the chlorophyll concentration.cm^{-2} after SHIBATA (1955), and photosynthetic rate in mm^3.h^{-1}.leaf^{-1} at saturating light intensity. The linearity of the relationship suggests that also the highest density can be light saturated. CO_2-content of the air : 5%. Temperature : 20°C. Area approximately 25 cm^2.

III.2.3. Conclusion. The presented data show that in spite of the apparent light limitation, as shown in fig. 1, light does not limit photosynthesis. This supports the hypothesis that systematic errors in the measurement of leaf temperature with thermocouples disturb the relation between irradiation and photosynthetic rate of the leaf.

III.3. *Leaf temperature distribution*

III.3.1. General. The observations of the preceeding chapter suggest that the temperature readings of the thermocouples do not represent the temperature of the surface to which they are attached under the conditions prevailing in the assimilation chamber. Therefore attention was directed to alternative methods

TABLE I Photosynthesis of sycamore leaves (in rel. units), measured in the assimilation chamber, illuminated from one side with 100% light or from two sides with 50% light, to show that light weakening in the leaf is not an important factor in the phenomenon of apparent light limitation. CO_2-concentration : 0.5%. Temperature 20°C.

Illumination		Photosynthesis	
		leaf 1	leaf 2
front	100%	80	60
back	100%	77	51
front + back:	50% + 50%	79	60

of temperature measurement. An interesting method is pyrometry. In recent years the technology of pyrometry is greatly improved and pyrometric temperature measurement is possible in the physiological temperature region with sufficient resolution with respect to temperature and topography. Sometimes, however, such instruments as the infrared temperature image cameras may produce distorted temperature pictures. However, the results obtained with such cameras about temperature distributions over a leaf, were so different from the results obtained with thermocouples, that a description appears justified.

III.3.2. Temperature distribution over a leaf as measured with thermocouples. Table II depicts temperature distributions over a leaf in the assimilation chamber as measured with thermocouples. In the dark the temperature distribution is rather even, but as soon as the light has been switched on, the temperature distribution becomes very irregular. Displacement of each thermocouple over a small distance creates again a different picture of temperature distribution. On the basis of such observations one may assume, that strong temperature differences exists over short distances on the leaf surface. Furthermore, the edges of the illuminated leaves appear to be cooler (Table II). The same type of temperature distributions can be found in non-physiological leaf models, made of copper plate, the true temperature of which is easily measured

TABLE II Temperature distributions (in °C) over poplar leaves, attached to the plant, in the assimilation chamber, in the dark and the light, as measured with thermocouples. Each square represents a square leaf disc.

	in dark				in 500.10^3 erg.cm^{-2}.sec^{-1}		
a	13.6	13.6	13.6	b	16.6	16.1	15.5
	13.7	13.6	13.7		15.0	17.2	14.2
	13.9	14.9	14.0		13.1	14.4	14.6
c	26.7	26.8	26.7	d	27.5	26.9	26.0
	26.1	26.1	26.3		25.6	28.1	25.6
	26.3	26.6	26.8		24.0	25.6	25.0

TABLE III Actual (internally measured) temperature distribution over a non-physiological leaf model (square copper plate), placed vertically in the assimilation chamber. The direction of the airflow, indicated by the arrow, and the speed of the airflow influence the temperature gradient. Linear air velocity maximally 25 cm.sec^{-1}. Irradiation 700.10^3 erg.cm^{-2}.sec^{-1}. Temperature of the double wall 12°C.

	32.3	32.7		34.5	34.8		36.6	37.3
	31.5			36.9			35.9	
30.5	30.8		37.3	37.2		35.0	35.3	
airspeed 1150 l/h, ↑			1150 l/h, ↓			600 l/h, ↑		
tem. gradient 1.5°C			−2.7°C			1.4°C		

TABLE IV Temperature distribution over a non-physiological leaf model (square copperplate) irradiated in the assimilation chamber. A small shift of the externally applied thermocouples, creates another apparent temperature distribution. The difference of the externally (apparent) and internally (actual) measured mean temperature is indicated. Temperature of the double wall 12 °C.

				Thermocouples somewhat shifted		
	26.7	28.5	29.0	27.1	28.4	29.1
	30.8	26.3	29.1	29.8	31.3	27.0
	24.3	25.4	25.5	27.5	25.1	25.7
mean apparent temp.			27.3		27.9	
mean actual temp.			34.1		34.0	
△ temp.			-6.8		-6.1	

by putting thermocouples in transversal holes in the copperplate. In this way data are obtained by which the true temperature distribution in the copper plate (Table III) can be compared with the temperature distribution as measured with externally applied thermocouples (Table IV). Two conclusions are obvious: a. thermocouples are underestimating the actual temperature; b. the magnitude of the error differs from place to place. It can be shown that the mean error depends on the temperature difference between leaf and incoming air (PIETERS and SCHURER, 1973).

RASCHKE (1956) presented a wellknown temperature distribution over a *Canna* leaf, as measured by thermocouples (fig. 5). Also in this case a rather large irregularity in the temperature distribution has been established.

III.3.3. Temperature distribution over a leaf as measured with the thermal image camera. – Temperature distribution over the leaf lamina – A fully different picture of temperature distribution over the leaf is observed with an infrared temperature camera. In the assimilation chamber, the temperature distribution is regular and resembles that of the leaf model. Under an irradiation load of 7 × 10^5 ergs.cm^{-2}.sec^{-1}, a temperature gradient of 2.5 °C/

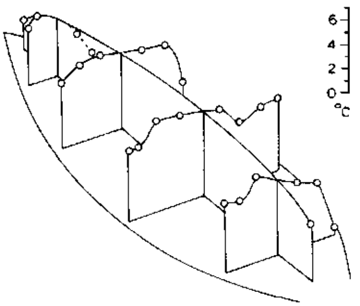


FIG. 5. Temperature distribution over a leaf of *Canna indica* (14.5 × 34.0 cm) in the afternoon. Mean temperature difference leaf – air 5.5 °C. Air speed 3.28 m.sec^{-1}. Irradiation balance : S = 0.972 / 2 cm^2.min. (From K. RASCHKE, 1956).

10 cm is established in the direction of the airstream. If the illumination is performed with a Prado 500 projector a concentrical temperature distribution is superimposed over this gradient. With a super high pressure mercury lamp, the light distribution is smoother and resulted in a more even temperature distribution.

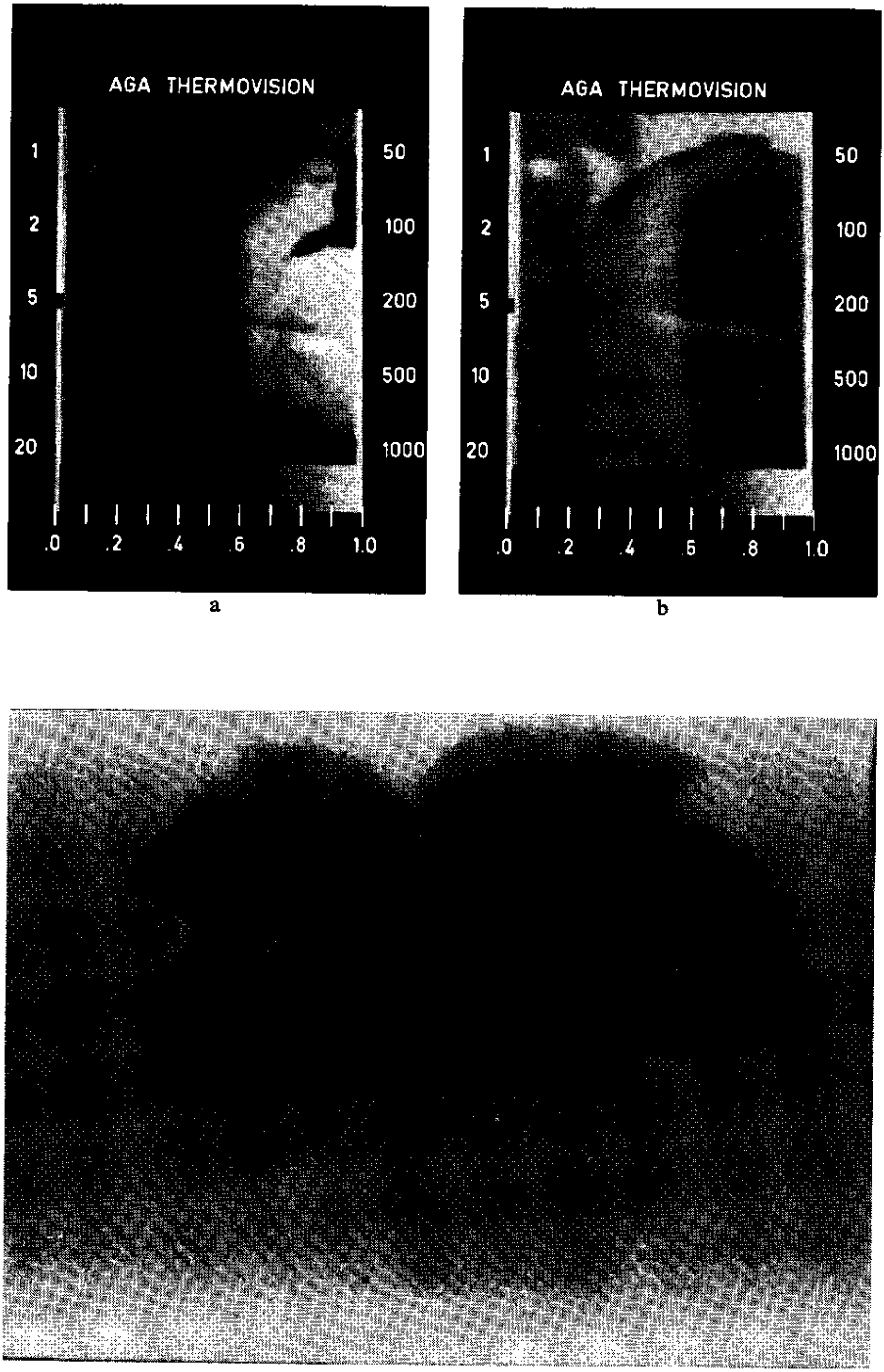
Leaves outside the assimilation chamber show a regular temperature distribution. Temperature differences normally were not larger than 1 °C in the dark and 5 °C in the light, dependent on local illumination intensity (Plate I, a, b, c). Naturally thermal inertia of the veins is greater than that of the lamina. Anything that created relatively fast temperature changes, like illumination, shadowing, drafts of air of varying temperature made the vein system show up brighter or darker (warmer or cooler) than the lamina in the thermal picture, within seconds. When the leaf was in steady thermal equilibrium with the environment, the veins faded in the picture. The edges of the leaves were not distinguishable from the middle portion by different temperatures, opposite to what is described in literature (RASCHKE, 1956: see also fig. 5; HOFMANN, 1955).

It may be stressed that smooth temperature distributions only reflect the evenness of the conditions to which the leaf is exposed. Because of the high resistance to heat transfer on the leaf blade, sharp temperature transitions may occur, e.g. as a consequence of partial illumination (Plate Ia) and by differences in stomatal opening (plate Ib and c). In the last two figures, the stomatal opening was induced by a preceeding illumination of 20 minutes duration. A stay of 10 minutes in the dark is sufficient for the establishment of a new thermal equilibrium, in which stomatal opening is showing up by differences in temperature. These temperature differences have an order of magnitude of a half degree Celsius under still air conditions.

– Temperature distribution from the front to the back of the leaf – PALLAS e.a. (1967) mentions the existence of temperature differences between the adaxial and abaxial sides of leaves of the cottonplant, *Gossypium hirsutum* L. and tries to correlate these differences with stomatal opening, irradiation, transpiration and air movement.

Because the existence and magnitude of these gradients are bound to irradiation of the leaf, they can provide a partial explanation why light saturation of photosynthesis is not reached. With the infrared camera the possibility was given to test the occurrence of temperature gradients across the leaf under high irradiation load. A picture of the experimental set-up is given on Plate II. It is possible to examine the front and the back of a leaf at the same time: the front directly and the back via a mirror. The metal surface mirror used, reflects infrared radiation for more than 99%. Two objects were simultaneously in the picture: the leaf and a cylindrical radiator of known temperature (not in plate II).

With the radiator of known radiation temperature it was possible to check whether the temperatures measured directly and via the mirror were the same and, indeed, no difference in temperature could be detected. Therefore, it seems justified to compare the leaf temperatures measured directly and via the mirror.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a

b

c

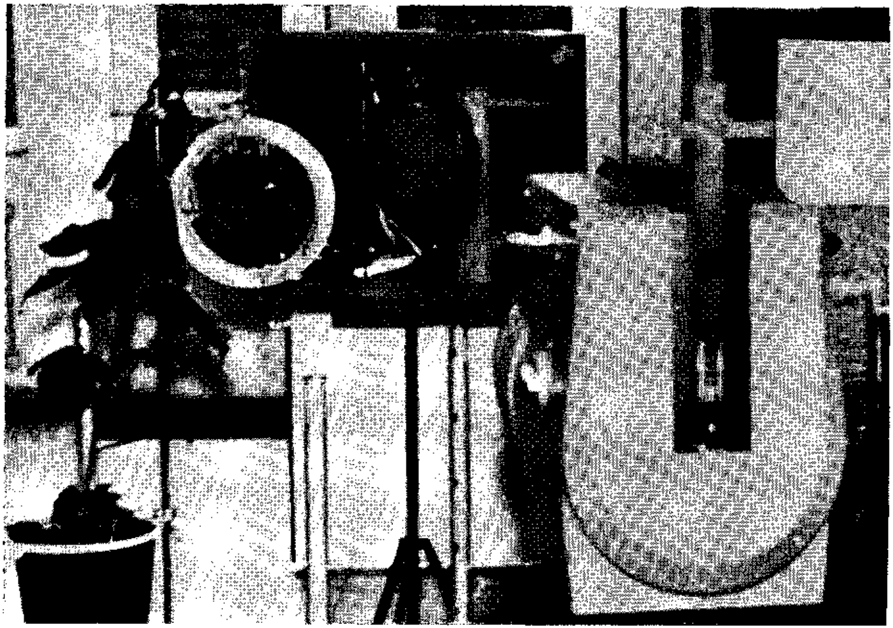

PLATE II Experimental set-up to measure the surface temperature from the upper and lower side of a leaf (directly and via the mirror) with the infrared temperature image camera (AGA thermovision).

a. front of sycamore leaf, seen directly (in metal frame).
b. lower side of the leaf, seen indirectly via the metal surface mirror.
c. AGA – thermovision camera.

With the aid of a lens the discharge tube of a super high pressure mercury lamp (Philips SP 500) was focussed on the leaf lamina, so that an oblong light spot of high intensity (10^6 ergs.cm^{-2}.sec^{-1}) was formed on the leaf. The temperature rise was considerable and facilitated to measure leaf temperature directly and via the mirror on exactly the same spot. The temperature difference between front and back of the leaf at that illuminated spot appeared to be less than 0.1 °C.

PLATE I Temperature distribution over a sycamore leaf as observed with the temperature image camera (bright = warm, dark = cool).

a. Leaf partly illuminated. The illuminated part is warmer. Note that the veins are cooler (thermal inertia). (AGA thermovision).
b. After illumination the same leaf was left 10 minutes in the dark. The previously illuminated part is now cooler owing to opening of the stomata. Temperature difference about 0.5 °C.
c. Temperature distribution over a leaf in the dark. The cooler parts have been illuminated previously. The sharp temperature differences demonstrate the high resistance to heat transfer in the leaf lamina and the local reaction of stomata on light. (Sems thermograph).

III.3.4. Conclusion. It is obvious that under certain conditions in the assimilation chamber, surface temperatures, measured with thermocouples have little to do with the actual leaf temperature. Under even conditions the temperature distribution over a leaf is smooth. No measurable temperature differences between the front and the back of *Populus* leaves occur, even under a high irradiation load, as is expected also from physical viewpoint. The results of PALLAS e.a. (1967) thus are not confirmed.

III.4. *The relation of the quality of leaf temperature control and the degree of apparent light limitation of the photosynthetic apparatus*

III.4.1. Introduction. Assuming for the moment that the absence of light saturation of the photosynthetic apparatus of leaves, when photosynthesis is measured with control of leaf temperature by thermocouples (fig. 1 and table V), is due to a light dependent underestimation of leaf temperature, it may be expected that any measure taken to assure a better control of the temperature of the leaf, will lead to a lower degree of apparent light limitation. In the previously described experiments with *Scenedesmus* cells on filter paper, it was assumed that the thermal contact between waterbath and *Scenedesmus* cells via a thin plastic wall and moist filter paper is good and thus a low degree of apparent light limitation was expected and indeed found.

A comparable type of experiment has been made with leaves floating on water. Also in this case the degree of apparent light limitation is clearly lowered. In a last type of experiments, which will be discussed, the temperature control of the leaf in the assimilation chamber was done with different types of infrared thermometers.

III.4.2. Photosynthesis of leaves floating on water. Using leaves or large leaf disks floating on water, kept at constant temperature, it was necessary to bubble air under the leaf to prevent it from sinking under the water level. The resulting continuous movement of the leaf disk, caused a certain irregularity in the irradiation of the leaf and decreased the thermal contact between leaf and water. Tiny water droplets originating from the air bubbles and dropping on the leaf (where they evaporated quickly) may have caused an increase of the noise level in recording photosynthesis. The high degree of light saturation found (fig. 6, table VI) conforms the idea that the apparent light limitation decreases by a better temperature control, at least if photosynthesis is CO_2-saturated. In the assimilation chamber leaves of Populus normally are CO_2-saturated at a CO_2-concentration of about 0.15% or lower. It was expected

TABLE V Quotients of photosynthesis in 50% and 100% light intensity of attached leaves of *Populus euramericana* 'robusta' in the assimilation chamber at CO_2-saturation. Leaf temperature 20 °C, as measured with the thermocouples. Maximum irradiation 700.10^3 ergs.cm^{-2}.sec^{-1}

0.82	0.81	0.84	0.82	0.85	
0.88	0.75	0.80	0.83	0.76	mean : 0.81

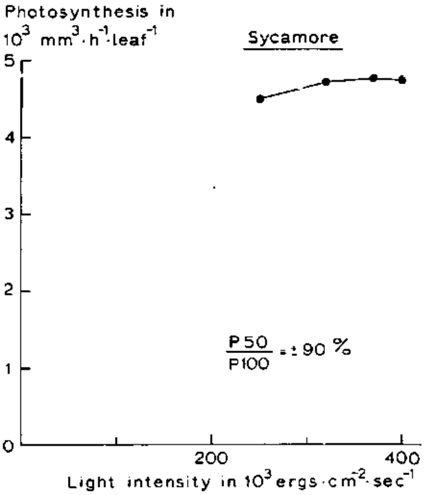

FIG. 6. Light saturation of the photosynthetic apparatus of a sycamore leaf floating on water. CO_2-concentration : 5%. Temperature 20°C. Leaf area approximately 20 cm^2.

TABLE VI Quotients of photosynthesis in 50% and 100% light intensity of leaf discs of *Populus euramericana* 'robusta', floating on water at CO_2-saturation. Temperature 20°C. Maximum irradiation 500.10^3 ergs.cm^{-2}.sec^{-1}.

1.01	0.91	0.97	0.93	0.98	
0.90	1.02				mean : 0.96

that for leaves floating on water an increase in diffusion resistance for CO_2 would occur, because only one side was left for diffusion of CO_2. To check this, the photosynthesis of a leaf, one or two sides covered with vaseline as compared to normal, was measured in the assimilation chamber. Only a small decrease of photosynthesis was found, if the vaseline was applied to one side of the leaf (table VII). Fig. 7 shows that CO_2 limitation of a leaf, floating on water in the glass vessel could be eliminated only by increasing the CO_2-content of the ambient air to 2–4%.

It may be concluded that photosynthesis is always CO_2-saturated at a CO_2 concentration of 5%.

TABLE VII Photosynthetic rate of poplar leaves, attached to the plant, at light- and CO_2-saturation, with one or two sides covered with vaseline as compared to normal.

number of sides covered	photosynthetic rate in relative units		
	leaf 1	leaf 2	leaf 3
0	54.0	37.0	50.0
1	46.5	36.0	46.0
2	11.0		

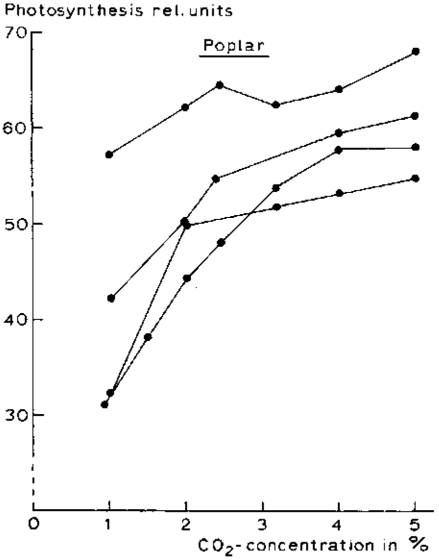

FIG. 7. Relation of light saturated photosynthesis of poplar leaves, floating on water, and CO_2-concentration. Temperature 20°C. Light intensity 300. 10^3 ergs.cm^{-2}.sec.$^{-1}$.

III.4.3. Control of leaf temperature in the assimilation chamber with infrared thermometers. Table VIII shows that a higher degree of light saturation of CO_2-saturated photosynthesis can be reached, when the control of leaf temperature is based on measurements with an infrared thermal picture camera than when this is done with thermocouples (see table V). It may be due to the general difficulties encountered in the used apparatus with the measurement of temperatures, and to the unfavourable optical properties of the assimilation chamber for infrared thermometry that full light saturation has not been reached. Furthermore, the air speed in the assimilation chamber was low (3 cm/hr) causing large temperature gradients over the leaf in the direction of the air stream and thus also difficulties in the estimation of mean leaf temperature.

These disadvantages were eliminated in the measurements of CO_2-saturated photosynthesis of attached leaves in the assimilation chamber by the introduction of improved optical conditions, increased air speed (half closed system) and a new type of temperature control, viz., the infrared compensation system (see methods). Under the conditions of our experiments this way of temperature control based on measurements with the infrared point thermometer, may have been nearly optimal, and the photosynthesis – light curves demonstrated a nearly 100% light saturation (fig. 8).

TABLE VIII Quotients of photosynthesis in 60% and 100% light intensity of attached poplar leaves in the assimilation chamber at CO_2-saturation. Leaf temperature 20°C, as measured with the AGA-thermovision. Maximum irradiation 700.10^3 $ergs.cm^{-2}.sec^{-1}$.

0.99	0.98	0.91	0.84	0.99	mean : 0.94

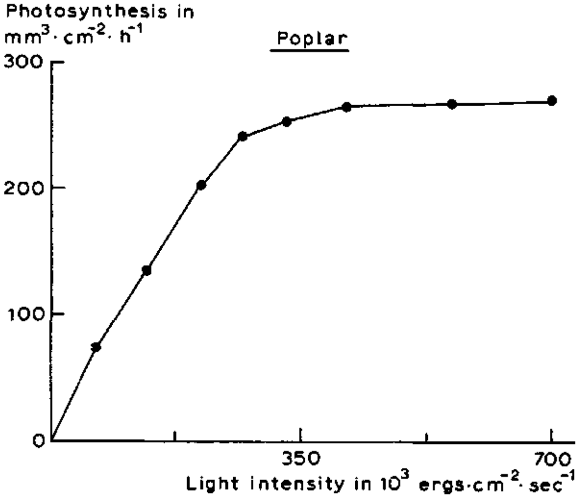

FIG. 8. Photosynthesis – light intensity curve of an attached poplar leaf in the assimilation chamber, at CO_2-saturation, demonstrating full light saturation. Linear air velocity 30 cm. sec^{-1}. Temperature control with the infrared point thermometer (PRT4) and the infrared temperature compensation system. Leaf temperature 21 °C.

IV. DISCUSSION ON SYSTEMATIC ERRORS IN LEAF TEMPERATURE MEASUREMENT

It has been suggested that thermocouples are measuring a rather arbitrary temperature in the temperature gradient from leaf surface to air (cf. IDLE, 1968). SEYBOLD arrived at the same conclusion already in 1929. PERRIER (1968, 1971) states that he has been trying other methods of surface temperature measurement, because quite a number of errors are adhering to the use of thermocouples: contact resistance, turbulence of the air, radiation and wounding of the leaf. In the technical literature thermocouple errors are generally accepted as important.

Accepting the idea that thermocouples are measuring a rather arbitrary temperature in the gradient leaf-air, the outcome can be predicted in general terms. The error of the thermocouple will increase by:

a. a poor thermal contact between junction and leaf. The irreproducibility of thermal contact explains the large differences in temperature observable from spot to spot over the leaf surface.

b. an increase of the actual temperature gradient leaf – air, e.g. by increasing irradiation.

c. an increase of the steepness of this gradient by increased wind speed and turbulence.

It will be clear that an increase in linear air velocity not only steepens the temperature gradient leaf – air and thus increases the temperature error, but also lowers the temperature difference between leaf and air, because of increased convection and eventually latent heat exchange and thus tends to lower the error. The resulting error is difficult to predict without a full knowledge of the terms of the heat balance of leaf and thermocouple. With a non-transpiring leaf model PIETERS and SCHURER (1973) estimated the thermocouple error to be roughly 25% of the temperature difference between the leaf and the double wall of the assimilation chamber under the conditions existing in the assimilation chamber used. This estimated value is enough to explain the shape of the photosynthesis – light curve, as shown in fig. 1, because in that case the difference in temperature between leaf (30°C) and waterbath (0°C) has been about 30°C at the highest illumination level, hence the error has been 7.5°C and in the region of supposed light saturation about 3.5°C. The influence of transpiration has not been taken into account. The same order of magnitude has been found by direct measurement with the 'STOUTJESDIJK' radiometer.

Increase of the linear velocity of the air in the assimilation chamber resulted in a much better control of the assimilation chamber climate. With a linear air-velocity of about 30 cm/sec, the difference in temperature between leaf and double wall has become about 8°C at an irradiation of 7 × 10^5 ergs/cm^2sec, so that the thermocouple error is now estimated to be 2.0°C. Under these conditions, the infrared point thermometer (BARNESS, PRT4) measured a leaf temperature of 20.7°C while the thermocouple at the same time measured 19.3°C. Hence, the thermocouple error is slightly less than predicted. In the dark, the PRT4 measured a leaf temperature of 16.6°C and the thermocouple one of 16.4°C; thus, as expected, a reasonable agreement between the two methods.

The occurrence of systematic errors in the measurement of leaf temperature and the complications encountered with the estimation of mean leaf temperature as a consequence of temperature distribution may exert an important influence on the calculated values of transfer resistances of the leaf, as discussed by SLATYER (1971). Furthermore one may well assume that the thermocouples deliver more information on the temperature gradient between leaf and air than over the leaf temperature itself. Observations that the edges of a leaf are normally cooler than the middle (HOFMANN, 1955; RASCHKE, 1956) require confirmation. The measurement of respiration in light and in darkness may also be influenced by errors in the temperature measurement, especially when the air velocities used are low.

V. SUMMARY

The experiments discussed in this paper lead to the following conclusions.

1. The light limitation of CO_2-saturated photosynthesis of leaves in the assimilation chamber observed at high irradiation levels, often is only apparent.
2. Application of better methods of temperature measurement and control, like infrared thermometry tends to remove this apparent light limitation, and in some cases nearly full light saturation is observed.
3. The application of contacting elements, like thermocouples, for the measurement and control of leaf temperature is inadequate.
4. The temperature distribution over the leaf lamina as measured by thermocouples deviates from the thermopicture of such a leaf, produced by an infrared temperature image camera.
5. Opposite to what is measured with thermocouples, there is no important temperature gradient across the leaf in air, even under a high irradiation load.

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LITERATURE

GAASTRA, P. (1959) Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. Meded. Landbouwhogeschool Wageningen, Netherlands *59* (13); 1–68

HESKETH, J. and BAKER, D. (1967) Light and carbon assimilation by plant communities. Crop Science *7*: 285–293

HOFMANN, G. (1955) Die Thermodynamik der Taubildung. Ber. des Deutschen Wetterdienstes 3 nr. 18, Bad Kissingen, Germany 45 pp.

IDLE, D. B. (1968) The measurement of apparent surface temperature. In: The measurement of environmental factors in terrestrial ecology. Ed. R. M. WADSWORTH, Blackwell Scientific Publications, Oxford and Edinburgh.

PALLAS, J. E., B. E. MICHEL and D. G. HARRIS (1967) Photosynthesis, transpiration, leaf temperature and stomatal activity of cotton plants under varying water potentials. Plant physiology 42: 76–88

PERRIER, A. (1968) Contribution à l'étude des échanges thermiques en biologie végétale. Revue Gen. de Thermique VII, no. 79–80: 721–740.

PERRIER, A. (1971) Leaf temperature measurement. In: Plant photosynthetic production, Manual of methods. (Z. ŠESTÁK, J. ČATSKÝ and P. G. JARVIS, ed.) Junk, The Hague.

PIETERS, G. A. (1971) Katharometric (diaferometric) determination of carbon dioxide, oxygen and water vapour. In: Plant photosynthetic production, Manual of methods. (Z. ŠESTÁK, J. ČATSKÝ and P. G. JARVIS, ed.) pp. 177–188, Junk, The Hague.

PIETERS, G. A. (1972) A new method for controlling leaf temperatures in assimilation chambers used for measurement of gas exchange phenomena. Meded. Landbouwhogeschool Wageningen, Netherlands 72–32,

PIETERS, G. A. and SCHURER, K. (1973) Leaf Temperature measurement. I. Thermocouples (in preparation).

RASCHKE, K. (1956) Uber die physikalischen beziehungen zwischen Warme übergangs zahl, Strahlungs Austausch, Temperatur und Transpiration eines Blattes. Planta *48*: 200–238.

SCHURER, K. and PIETERS, G. A. Leaf Temperature measurement. II Infrared thermometry (in preparation)

SEYBOLD, A. (1929) Die physikalischen Komponente der pflanzlichen Transpiration. Monographien aus dem Gesamtgebiet der wissenschaftlichen Botanik II

SHIBATA, K. (1955) Spectral measurements of the absorbtion and reflection of translucent material. Carnegie Inst. of Washington. Annual report 1955–1956, 252–256.

SLATYER, R. O. (1971) Effect of errors in measuring leaf temperature and ambient gas concentration on calculated resistances to CO_2 and water vapour exchanges in plant leaves. Plant Physiol. *47*: 269–274.

STOUTJESDIJK, Ph. (1966) On the measurement of the radiant temperature of vegetation surfaces and leaves. Wentia *15*: 191–202.

WESSELIUS, J. C. (1973) Influence of external factors on the energy conversion and productivity of *Scenedesmus* sp. in mass culture. Meded. Landbouwhogeschool, Wageningen, Netherlands.