THE PHYSIOLOGY OF SUGAR-CANE

IX.* FACTORS AFFECTING PHOTOSYNTHESIS AND SUGAR STORAGE

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Summary

Photosynthesis in sugar-cane was linearly related to light intensity up to full sunlight when leaf geometry was similar to that of field-grown plants. Individual leaves orientated at right angles to the incident radiation were saturated at about two-thirds full sunlight irrespective of whether the leaves developed in low or high light intensities.

On a short-term basis, the Q_{10} for photosynthesis was $1 \cdot 1$ over the range 8–34°C. Exposure of intact plants to a 17/10°C day and night temperature regime for a prolonged period resulted in cessation of stalk elongation and an initial rise in stored sugar followed by a decline in photosynthetic efficiency, which was slowly reversible when the temperature was raised.

Photosynthetic efficiency appears to be linked to availability of sinks but may also be altered by metabolic imbalance induced by environmental shock. End product repression of photosynthesis by sugars was not the operative control in detached leaves exposed to long light treatments.

I. Introduction

Tropical grasses, including sugar-cane, are highly efficient in their utilization of sunlight (Burr *et al.* 1957; Hesketh and Moss 1963). Dry matter production in sugar-cane increases linearly up to full sunlight (Hesketh and Moss 1963), whereas temperate species become light-saturated at relatively low light intensities.

Studies by Hartt (1963) indicated that photosynthesis in sugar-cane was subject to modulation under conditions in which sucrose accumulated in the leaves. Maximal rates were maintained for only 2 hr in detached leaves at light saturation, and tests with Nujol–decane showed the stomata to be open when photosynthesis declined. When the base of the detached leaf was immersed in 5% sucrose, the sucrose content of the leaf increased and inhibition of photosynthesis increased proportionately. End product control of photosynthesis was suggested previously by Loomis (1949).

This paper reports on factors which influence efficiency and regulation of photosynthesis in intact sugar-cane plants and in detached leaves. The latter experiments do not support Hartt's hypothesis on sucrose control of photosynthesis.

II. METHODS AND MATERIALS

Single eye cuttings of the commercial sugar-cane variety Pindar were established in a 1 : 1 vermiculite–perlite mixture. Plants were grown in air-conditioned glasshouses or artificially illuminated constant-temperature rooms.

(a) Photosynthetic Measurements of Large Numbers of Plants

Carbon dioxide uptake by 42 plants (leaf area index $2 \cdot 7$) was measured on a Beckman infrared gas analyser over the range 320–260 p.p.m. A glasshouse was used as an assimilation chamber, and the sun as a light source. During the measurement of photosynthesis the glasshouse was sealed for a short period and the air flow, humidity, and temperature were maintained by the air-conditioning system. Water loss from a randomly chosen plant was measured over the same period by following its change in weight.

Air flow in the glasshouse (81 ft/min) was adequate to maintain carbon dioxide supply to the leaf surface since increased turbulence induced by continually shaking plants growing at optimum temperature had no significant influence upon dry matter production over a 3-month period.

(b) Photosynthetic Measurements of Four Plants

Four plants were placed in a mobile assimilation chamber (2 by 2 by 11 ft) covered with clear polyester film. A fan mounted in ducting on the back of the chamber maintained an air flow over the leaf surfaces of 73 ft/min. By closing the ducting for a short period the rate of drop in carbon dioxide concentration over the range 320–260 p.p.m. was recorded. Between measurements the ducting was opened and air at controlled temperature from the glasshouse was circulated through the chamber.

Light intensity was measured with an EEL Lightmaster fitted with a visual correction filter and calibrated against a Kipp Thermopile. Normally photosynthetic measurements were made on plants with leaves orientated similarly to those in the field. In some experiments mutual shading was prevented by stretching all the leaves out on a frame. Light intensity was varied by covering the chamber with different grades of Sarlon cloth.

(c) Photosynthetic Measurements of Detached Leaves

Leaf segments 8 in. long were cut under water and the lower ends submerged in a trough of water. The trough was placed on the bottom of a Perspex chamber (9 by 12 by 3 in.) which contained a battery-driven circulating fan. Air, saturated with water vapour, was passed through the chamber to maintain the carbon dioxide concentration at about 320 p.p.m. Photosynthesis was measured by sealing the chamber and recording the rate of drop in carbon dioxide concentration to about 260 p.p.m.

Light was provided by a 400-W Philips HPL lamp in a water-cooled reflector and separated from the chamber by a water filter 0·5 in. thick.

In all experiments the dark respiration rate was measured at the same air temperature as the net photosynthesis measurements. Gross photosynthesis was expressed as the sum of these two measurements. Since the ratio of dark respiration to net photosynthesis was less than 0·18 it is unlikely that a significant error was introduced.

(d) Photosynthetic Measurements of Leaf Disks

An illuminated Warburg apparatus was used to measure oxygen evolution from leaf disks at 30°C. A single disk was immersed in 3 ml of water and the carbon dioxide was supplied by adding 0·4 ml of 3M $KHCO_3$ – 3M K_2CO_3 (85 : 15 v/v) in both side-arm and centre well (Warburg 1963). A constant partial pressure of 4·3% CO_2 (v/v) was maintained by adding 0·5 ml of carbonic anhydrase solution (1 mg per 0·1 ml water) to the side-arm and the centre well prior to sealing the flasks to the manometers.

The light intensity was much lower than for detached leaves. However, the rates of photosynthesis were approximately three times dark respiration apparently because of the high carbon dioxide concentration. Dark respiration was measured before and after photosynthesis by switching off the light and covering the bath with black polythene.

(e) Leaf Resistance Measurements

A porometer similar to that described by Bierhuizen, Slatyer, and Rose (1965) was used to indicate resistance to air flow through a leaf. It was assumed that internal leaf resistance remains constant and that relative magnitudes of stomatal aperture were measured. Values are given as the level of mercury in millimetres after 1 min from starting at 200 mm.

(f) Growth Measurements

Height of stalk to the top visible dewlap was used to follow growth rates in intact stalks.

Upon harvest of the plants all leaves were removed and the areas obtained by exposing them to light-sensitive blue-print paper.

To follow ripening during the experiment on a glasshouse scale, 42 plants were maintained under similar conditions in an adjacent glasshouse. Six plants were sampled at each harvest and these were replaced by six similar plants in order to maintain geometry effects in the glasshouse. Harvested stalks were macerated and sugars extracted by storage in three volumes of 95% ethanol.

In detached leaf experiments leaf sugars were extracted with 70% ethanol and the solutions decolorized with charcoal. Sugar concentrations in the samples were determined after the method of Hatch and Glasziou (1963).

III. Results

(a) Effects of Light and Carbon Dioxide

Normal leaf geometry involves mutual shading of leaves and a considerable proportion of the total leaf area is unfavourably orientated relative to the sun. When photosynthesis was measured on four plants and the leaf area index was 4, the rate of photosynthesis was a linear function of the radiation intensity up to full sunlight (Fig. 1). If the leaves were arranged on a frame in the assimilation chamber, mutual
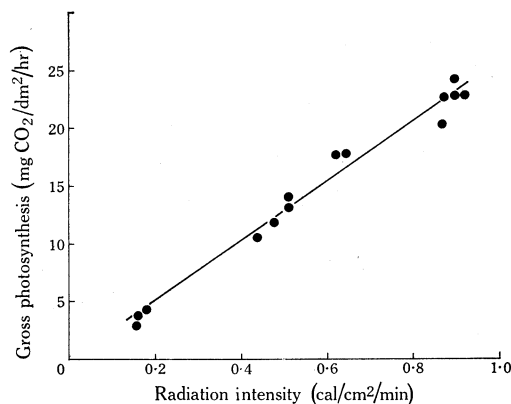

Fig. 1.—Relationship between the rate of gross photosynthesis and radiation intensity where mutual shading of the leaves occurred. Light intensity was varied by placing shade cloth of different graduated thicknesses around the photosynthesis chamber.

shading was prevented and the leaves became light-saturated at an intensity of $0 \cdot 6$ cal/cm^2/min (Fig. 2). Growing the plants at low light intensity ($0 \cdot 3$ cal/cm^2/min) did not reduce the saturation point.
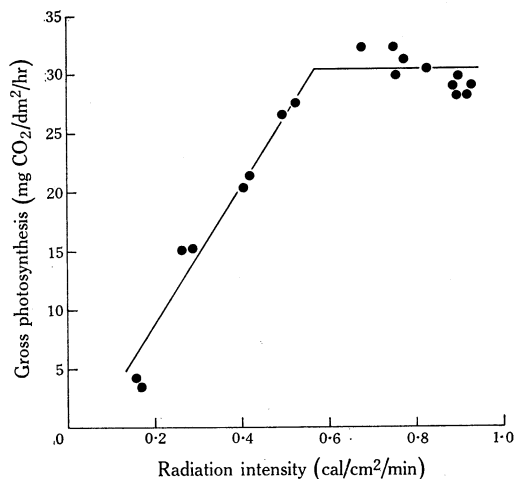

Fig. 2.—Relationship between the rate of gross photosynthesis and radiation intensity where mutual shading of leaves was prevented. Leaves were arranged on a frame so that each leaf received maximum sunlight.

Detached leaves in a closed system gave instantaneous rates of photosynthesis which were proportional to both carbon dioxide concentration and light intensity.

For an open system under steady-state conditions at a light intensity of $0 \cdot 6$ cal/cm^2/min, the rate of photosynthesis in intact plants was approximately doubled by increasing the carbon dioxide concentration from 125 to 237 p.p.m.

(b) Temperature Effects

The effect of temperature on net photosynthesis in mature plants was followed by equilibrating a group of plants at 18, 26, or 34°C for 24 hr prior to measurement (Fig. 3). As this experiment was performed in sunlight and the radiation intensity
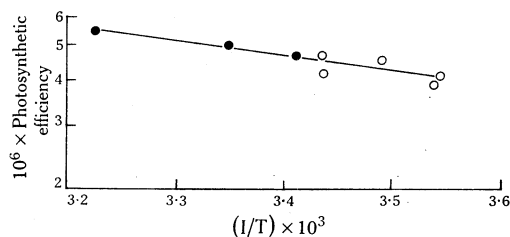

Fig. 3.—Relationship between photosynthetic efficiency (expressed as number of grams of carbohydrate fixed per calorie of visible light radiation) and air temperature over the range 5–34°C. Measurements were made under conditions where individual leaves compete for light and when they had attained their maximum areas. ● One group of plants measured over the range 34–18°C. ○ Another group of plants measured over the range 18–5°C.

varied between measurements the net photosynthesis was expressed per 1 cal/cm^2/min. Such an approach is justified as it has already been demonstrated that, with normal leaf orientation, photosynthesis is a linear function of radiation up to full sunlight (Fig. 1).

A similar group of plants was subjected to air temperatures between 5 and 18°C during a single day. One hour was allowed between each measurement for leaf and air temperature equilibration. During the measurement period leaf temperature rose by about 3 degC and the average temperature during the measurement was taken as effective. The results from this experiment are also plotted in Figure 3 which shows that between 8 and 34°C photosynthesis has a Q_{10} of $1 \cdot 1$.

(c) Leaf Age on Photosynthetic Potential

Under field conditions a mature sugar-cane plant maintains an almost constant number of leaves, usually 8–9. Of these the upper six intercept about 70% of the incident solar radiation and the lower three less than 30% (Table 1).

Gross potential photosynthetic rates (with 95% confidence limits) of leaves 0–6 and 7–9 were $31 \cdot 1 \pm 3 \cdot 2$ and $20 \cdot 1 \pm 0 \cdot 8$ mg CO_2/dm^2/hr, respectively. Leaves were measured separately while attached to the plant. They were spread out on a frame so that each leaf received maximum sunlight. These results showed that the rate of the younger leaves was about $1 \cdot 5$ times greater than that of the older leaves.

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<div align="center">TABLE 1</div>

<div align="center">RADIATION INTENSITY FALLING ON LEAVES OF A SUGAR-CANE PLANT</div>

The average radiation intensity (with 95% confidence limits) falling on the upper surfaces of individual leaves of plants in a closed crop is given. Radiation intensity was measured at 15 positions along each leaf of six plants taken at random in the field. During the course of these measurements average solar radiation was $1 \cdot 15 \pm 0 \cdot 04$ cal/cm^2/min

Leaf No.	Mean Total Radiation Intensity over Whole Leaf (cal/cm^2/min)	Leaf No.	Mean Total Radiation Intensity over Whole Leaf (cal/cm^2/min)
0*	$0 \cdot 61 \pm 0 \cdot 10$	5	$0 \cdot 39 \pm 0 \cdot 20$
1	$0 \cdot 62 \pm 0 \cdot 10$	6	$0 \cdot 32 \pm 0 \cdot 16$
2	$0 \cdot 57 \pm 0 \cdot 08$	7	$0 \cdot 17 \pm 0 \cdot 06$
3	$0 \cdot 46 \pm 0 \cdot 18$	8	$0 \cdot 04 \pm 0 \cdot 02$
4	$0 \cdot 49 \pm 0 \cdot 24$	9	$0 \cdot 13 \pm 0 \cdot 10$

* Leaf 0 is the spindle (unfolded leaves).

<div align="center">TABLE 2</div>

<div align="center">RELATIONSHIP BETWEEN STALK GROWTH AND PHOTOSYNTHESIS OF WHOLE SUGAR-CANE PLANTS</div>

Carbon dioxide uptake was measured on 42 whole plants as a unit in full sunlight. Six-month-old plants were brought into the greenhouses and equilibrated at 24/19°C for 1 month prior to the commencement of measurements. Transpiration rates were measured on randomly chosen plants by measuring the water loss over a 5-hr period. Relative humidity in each greenhouse was maintained at 70–75%. Measurements were made on days when there was little or no cloud cover

Time (days)	Day/Night Temperatures (°C)	Stalk Growth Rate (cm/day)	Total Sugars (% fresh wt.)	$10^5 \times$ Photosynthetic Efficiency*	Transpiration Rate per Plant (g water lost/hr)
0	24/19	—	6·8†; 11·1‡	1·15	131
5	20/12	—	—	—	—
13	20/12	—	—	1·08	101
31	20/12	1·0	—	0·76	83
33	17/10	—	—	—	—
35	17/10	0·1	8·9†; 11·8‡	0·69	83
55	17/10	0·1	10·4†; 12·8‡	0·44	55
67	24/19	—	—	—	—
69	24/19	0·2	—	0·34	108
70	24/19	—	—	0·51	106
80	24/19	0	—	0·72	103
81	24/19	0	10·4†; 12·8‡	0·76	98
97	24/19	1·1	—	0·85	96
121	24/19	0·7	11·7†; 13·9‡	0·80	137

* No. of grams of carbohydrate fixed per calorie of visible light radiation.

† Sugar from top 100 cm of stalk.

‡ Sugar from remainder of stalk.

(d) Effect of Sugar Concentration in Stalks on Rates of Photosynthesis in Intact Plants

Immature expanding internodes of growing cane normally have low sugar levels. A check to internode elongation such as can be induced by temperature shock may be accompanied by a sharp rise in the sugar content (Glasziou *et al.* 1965).

Table 2 shows that the decline in stalk growth and increase in sugar content induced by moving plants to low temperatures (17°C day/10°C night) was accompanied by a slow but marked fall in photosynthetic efficiency and transpiration. On return to a 24°C day/19°C night regime the rate of transpiration rose more quickly to the initial level than photosynthetic efficiency, showing that stomatal resistance was not an important factor in the adaptation of photosynthesis. Since there was no significant change in the leaf canopy due to emergence of new leaves during the first 2 weeks at the higher temperature, the partial recovery of photosynthetic efficiency was due to reversible changes in the old leaves.
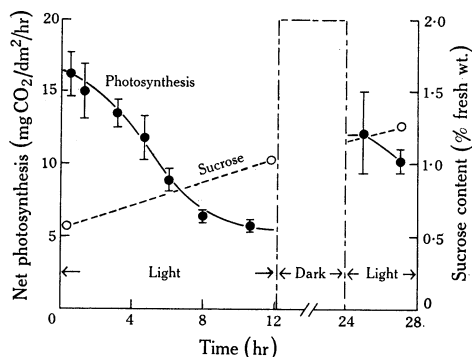

Fig. 4.—Relationship between net photosynthetic rates (with 95% confidence limits) and the total sugar contents of detached cane leaves subjected to long light treatment followed by a period of darkness. Photosynthetic measurements were made with one surface of each leaf segment fully exposed to the artificial light source (0·35 cal/cm^2/min). Midribs of leaves are not included in sucrose determinations.

(e) Effect of Sugar Concentration on Photosynthesis in Detached Leaves and Leaf Disks

Prolonged exposure of detached leaves to artificial light caused an increase in sucrose and reducing sugar contents and a concomitant reduction in photosynthesis as reported by Hartt (1963). However, after a 12-hr dark period the rate of photosynthesis increased but there was no decline in the levels of either sucrose or reducing sugars (Fig. 4). In this type of experiment it is difficult to avoid some water stress when high light intensities are used. By maintaining very high humidity in the leaf chamber it was sometimes possible to obtain almost constant rates of photosynthesis in continuous light over a 24-hr period. When the rate of photosynthesis fell, it was initially accompanied by a rise in stomatal resistance (Fig. 5). In many experiments it was possible to restore the initial rate of photosynthesis by treating the leaves with ammonia vapour to induce stomatal opening (Ketellapper 1963). After prolonged exposure to light complete reversal was not always attained by ammonia treatment (Fig. 5). However, it cannot be concluded that the irreversible component was due to product inhibition of photosynthesis since damage to the photosynthetic apparatus may occur in detached leaves because of aging or mild water stress.

When leaf disks were placed in $0·4M$ sucrose, glucose, or mannitol solutions in Warburg vessels and exposed to light for 22 hr, there was no significant effect of the sugars on photosynthesis as measured by oxygen evolution (Table 3). In an experiment to check the validity of comparing photosynthetic oxygen evolution and
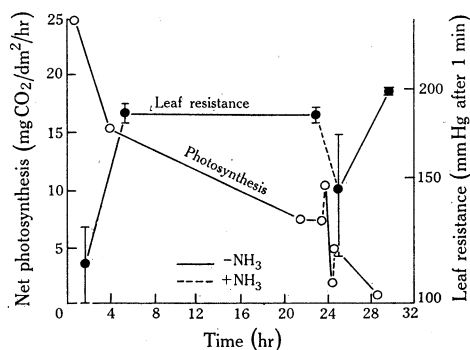

Fig. 5.—Relationship between net photosynthesis and leaf resistances (with 95% confidence limits) as measured with a porometer on detached cane leaves in continuous light. One surface of each leaf segment was exposed uniformly to the artificial light source $(0·47 \text{ cal/cm}^2/\text{min})$.

carbon dioxide uptake, $^{14}CO_2$ was supplied at constant partial pressure using labelled bicarbonate buffer and carbonic anhydrase in the side-arm of the manometer vessel, and total carbon fixation determined by combusting the leaf sample. The ratio of oxygen evolution to carbon dioxide fixation was 1.

TABLE 3

EFFECT OF CONTINUOUS LIGHT AND SUGARS ON PHOTOSYNTHESIS OF LEAF DISKS
IMMERSED IN WATER

Disks were immersed in water in Warburg flasks with carbon dioxide supplied in the gaseous phase from carbonate–bicarbonate buffer and carbonic anhydrase in the side-arm and centre well. One surface of each disk was fully exposed to the light source

Treatment	Initial Rate of Gross Photosynthesis ($\mu l\ O_2/hr$)	Rate of Gross Photosynthesis after Exposure to Light for 22 hr ($\mu l\ O_2/hr$)	Percentage Inhibition of Gross Photosynthesis
Control	52	39	25
$0·4M$ sucrose	78	59	24
$0·4M$ glucose	69	54	22
$0·4M$ mannitol	55	41	25

IV. DISCUSSION

The sugar-cane crop has the ability to utilize full sunlight for photosynthesis. Individual leaves saturate at about $0·6 \text{ cal/cm}^2/\text{min}$ which is also the average light intensity intercepted over the surfaces of the top leaves of the crop in full sunlight.

Leaves which develop at relatively low light intensities photosynthesize at full efficiency in full sunlight. Each stalk produces a new leaf about every 10 days (Glasziou *et al.* 1965) and an older leaf senesces leaving a constant number of eight to nine leaves per stalk. The major portion of the incident light is intercepted by the upper six leaves. The lower leaves which are commencing to senesce nevertheless retain about 60% of their earlier photosynthetic potential.

Short-term temperature fluctuations scarcely affect the photosynthetic process which has a Q_{10} of about $1 \cdot 1$ over the range 8–34°C. Sugar-cane is grown in the moist tropics and subtropics where breeding and selection has also been concentrated. It is not surprising then that dry matter production over an extended period tends to be independent of temperature in the 20–30°C range (van Dillewijn 1952; Glasziou *et al.* 1965). Long-term treatments at temperatures below 20°C affect both the partitioning of photosynthate between sugar storage and stalk elongation (Glasziou *et al.* 1965) and the efficiency of photosynthesis (Table 2). Temperatures of 17–18°C appear to be particularly favourable for partitioning photosynthate into sugar storage and for accumulation of high levels of sucrose. However, the lower temperatures used in the current work lead to a fairly rapid decline in photosynthetic efficiency and high sugar levels were not attained. Since adverse effects of low temperature on stalk growth and sugar movement and storage could conceivably lead to sucrose accumulation in the leaf, Hartt's (1963) hypothesis on end-product inhibition of photosynthesis appeared to provide a rational interpretation of these observations.

The possible operation of a control mechanism was indicated by the reversibility of the low temperature effect on photosynthetic efficiency (Table 2). Since water loss and photosynthesis were out of phase during the recovery period at high temperature, the control mechanism for intact plants was not via changing stomatal resistance. Contrary to Hartt's results, experiments on detached leaves showed that inhibition of photosynthesis after extended periods in light was always correlated with increased stomatal resistance and unrelated to the sucrose content of the leaf. We were also unable to inhibit photosynthesis by exposing leaf sections immersed in sucrose solutions in Warburg flasks to 22 hr of light.

The present evidence is inadequate to distinguish between a model based on negative feedback from sinks to source for the regulation of photosynthesis in sugar-cane, and an alternative model based on effects of temperature shock on the induction of metabolic imbalances which differentially alter rates of photosynthesis, stalk elongation, and sugar storage. However, the results clearly show that the reversible loss in photosynthetic efficiency due to low temperature shock to intact plants is not mediated by changes in stomatal resistance, and is presumably a biochemical phenomenon.

V. References

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