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Photosynthesis in vine leaves as a function of light intensity, temperature, and leaf age

by

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Introduction

Although Sultana grape vines are widely distributed and used extensively in horticulture, the general photosynthetic characteristics of the foliage have not been described. The present work was undertaken to provide some information on the effect of light intensity, CO_2 concentration, temperature and leaf age on photosynthesis.

Material and Methods

The experiments used well established rooted cuttings of Sultana clonal material (*Vitis vinifera* c. v. Sultana syn. Sultanina, Thompson seedless) grown in pots in either the glasshouse, or out of doors. Comparisons were also made with well established field vines of the same clonal line. The light intensity in the glasshouse varied between 30 and 50% of the level out of doors.

Photosynthesis and respiration were measured in the laboratory with the aid of a Hartmann & Braun infra red gas analyser (I. R. G. A.), using single attached leaves on potted vines or on detached shoots from field vines (stems recut under water).

Dark respiration was measured in a stream of $-CO_2$ air, while normal air was used in estimating photosynthesis. The leaves were enclosed in a circular cuvette with double walled brass sides and base and double walled glass lid similar to that described by Hardwick *et al.* (1966). The air inside the cuvette was stirred with an electrically driven fan (shaded pole 240 volt a. c. motor). Air temperature inside the cuvette could be regulated from 5° C to 60° C (± .1° C) by circulating water between the double walls from a thermostated water bath. Air and leaf temperatures were measured with a pair of thermistors (STC type F/23) one of which pressed against the lower surface of the enclosed leaf. Air temperature was recorded on the under side of the leaf.

Light was provided from a combination of mercury vapour (HPLR-400 W) and incandescent (internal reflector 300 W) lamps. The light source was mounted above a heat filter (a 2 cm depth of distilled water) and the whole assembly could be hydraulically raised or lowered to vary the intensity incident upon the leaf.

The probe from a Kettering radiometer (Yellow Springs Instrument Co.) was located inside the cuvette to measure incident radiation. The unmasked sensor occupied a plane slightly below that of the leaf. This type of sensor shows a flat response to incident radiation over the wavelength range 250—3300 nm. The incident radiation was expressed as ergs/sec/cm². In the present situation 40 ergs/sec/cm² were equivalent to 1 foot candle (f. c.).

Air was drawn from outside the laboratory and passed through two 50 gallon tanks in series to reduce fluctuations in CO_2 concentration. Humidity sensors were placed in the air stream entering and leaving the cuvette and the increase in relative humidity yielded an estimate of leaf transpiration. Needle valves regulated the flow between 100 and 2000 cc/min (measured on rotameters) and the air stream was dried over CaCl$_2$ before passing into the I. R. G. A. Flow rate was adjusted so that the total CO$_2$ depletion of the air passing over the photosynthesizing leaf never exceeded 50 ppm. The CO$_2$ concentration of the gas entering and leaving the cuvette was recorded on a chart together with traces of leaf and air temperature and the relative humidity of the air entering and leaving the cuvette.

For the determination of the minimum intercellular CO$_2$ concentration (Γ) the air stream leaving the cuvette via the I. R. G. A. was returned to the cuvette through a sealed diaphragm pump. Flow rate in the recirculating system was adjusted to 1500 ml/min.

At the end of each experiment the outline of the leaf was traced and this area determined. Photosynthetic rates are based on the projected area of the lamina and not on the summation of upper and lower leaf surfaces as in some work.

Chlorophyll content was determined by homogenising leaf punches with 80% acetone and measuring the optical density of a clear extract at 645 and 663 nm after the method of MACKINNEY (1941).

Results

(a) Photosynthesis in relation to light intensity

Fig. 1 compares the behaviour of leaves from fully exposed compared with glasshouse grown vines. Photosynthesis was measured at 25° C because this was found to be near the optimum for vine leaves (see later in Results section). Each point in Fig. 1 is the mean from seven separate experiments for both glasshouse and field vines. The photosynthetic activity of leaves from the two situations was comparable up to light intensity of about 10×10^4 ergs/sec/cm^2 i. e. 2,500 f. c. The rate of dark respiration for leaves from fully exposed vines was about twice that for glasshouse grown vine leaves and as a consequence light compensation point was also higher, namely: 125 f. c. compared with 50 f. c.

Light intensity above 15×10^4 ergs/sec/cm^2 elicited little increase in the photosynthetic rate of fully

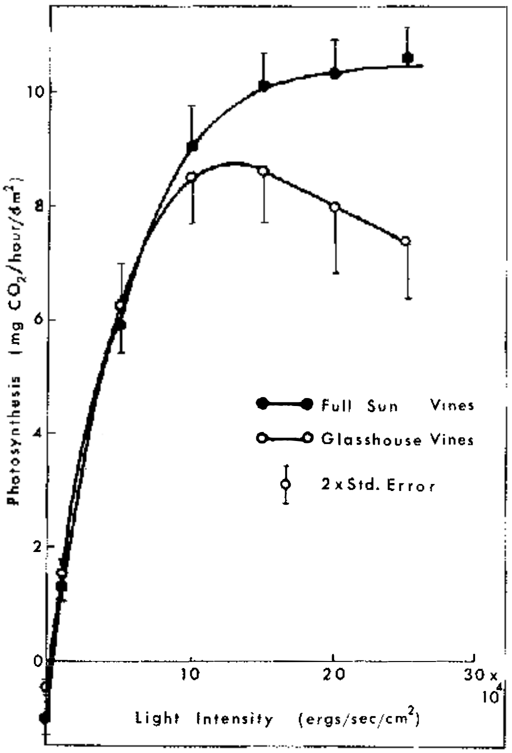

Fig. 1:
Vine leaf photosynthesis in relation to light intensity.
Mean data from single leaves of seven glasshouse grown and seven fully exposed vines. Values above the abscissa show net CO$_2$ fixation, values below the abscissa show dark respiration. Measurements were performed at 25° C in normal air.
40 ergs/sec/cm^2 = 1 f. c.

exposed leaves, but was inhibitory in the case of glasshouse leaves. At the higher light intensity (> 10 × 10⁴ ergs/sec/cm²) the photosynthetic activity of exposed leaves was greater on the basis of both area (Fig. 1) and chlorophyll content. Assimilation number (mg CO_2/h/mg chlorophyll a + b) was 6.54 ± 0.75 and 5.48 ± 0.23 for fully exposed and glasshouse leaves respectively.

The adaptive response of gasshouse leaves to the fully exposed situation is recorded in Fig. 2. Five days after moving a potted plant out of the glasshouse the leaf's photosynthetic rate (area basis) was reduced by more than 50%. Nevertheless the previous inhibitory effect of high light intensity was eliminated. Although the chlorophyll (a + b) content of the leaf showed little change (1.11 to 1.18 mg chlorophyll/dm²), the chlorophyll a/b ratio dropped from 1.91 to 1.40.

This decrease could be related to the fall in assimilation number from 8.60 to 3.72 as well as to the paler appearance of the leaf following exposure to full sunlight. For comparative purposes a potted vine was moved from out of doors into the glasshouse. Photosynthesis and respiration were measured every few days on the same leaves for one month following the relocation but there was no reciprocal change in maximum rates of photosynthesis, saturating light intensity, or dark respiration.

(b) The effect of temperature on leaf photosynthesis

Fig. 3 compares the response of leaves on glasshouse and fully exposed vines to temperature from 15 to 45° C. Measurement commenced at 20° C followed by a reduction to 10 or 15° C and then a stepwise increase and a recheck at 20° C before proceeding to the higher temperatures. Net photosynthesis was measured for about

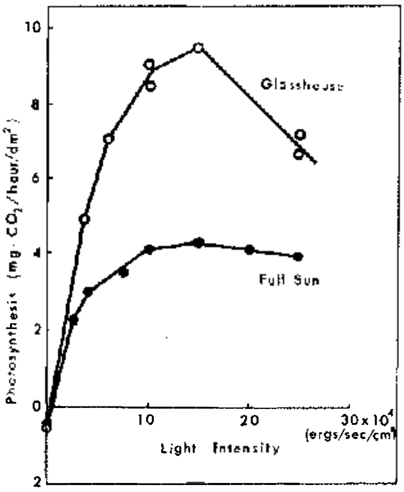

Fig. 2

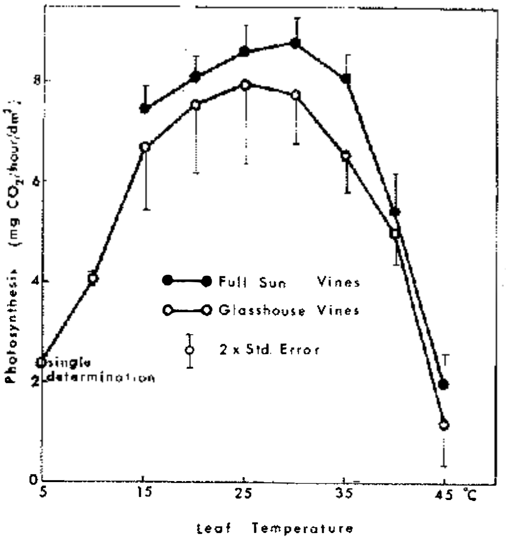

Fig. 3

Fig. 2: The change in leaf photosynthetic activity 5 days after transferring a glasshouse grown vine into full sunlight.
Other details as in Fig. 1. O before transfer. ● 5 days after transfer.

Fig. 3: The effect of leaf temperature on net photosynthesis.
Mean data from single leaves on four glasshouse grown and seven fully exposed vines. Measurements performed at light intensity of 15×14⁴ ergs/sec/cm² in normal air.

an hour after the leaf had come to equilibrium with new experimental conditions. The data shown are means taken from 4 or 7 separate experiments on glasshouse and fully exposed leaves respectively. The optimum temperature for net photosynthesis was 25° C for the leaves on glasshouse vines and 30° C for the leaves on fully exposed vines. The rate of assimilation declined rapidly above 35° C reaching zero between 45 and 50° C. Provided leaf hydrature was maintained, leaves could be safely held for up to 3 hours at 48 - 49° C. If the leaf temperature exceeded 50° C a rapid efflux of CO_2 occurred and the lamina quickly developed discoloured regions.

The optimum temperatures for net photosynthesis recorded in Fig. 3 were the same irrespective of whether photosynthesis was measured in response to increasing or decreasing temperature between 5 and 40° C. Furthermore, there was no indication of leaf adaptation to temperature following exposure to 40° C for two consecutive days.

(c) The effect of CO_2 concentration on photosynthesis

For fully expanded leaves on glasshouse grown vines, photosynthesis responded linearly to increasing CO_2 concentration up to 300 ppm (higher concentrations not tested) at a light intensity of 24×10^4 ergs/sec/cm² and leaf temperature of 28.5° C. The plot of photosynthesis as a function of CO_2 concentration intersected the abscissa at 25 ppm CO_2.

(d) Leaf age and net photosynthesis

Table 1 shows some relevant data for field grown Sultana vines. Leaves at six positions along five currently growing shoots were examined towards the end of the growing season (March-April 1968). The mean data are shown in Table 1. At leaf position 1 the lamina was soft and translucent while at position 6 the mature and

Table 1

Physiological characteristics of vine leaves as a function of age

Leaf position***	1	2	3	4	5	6
Leaf area (cm²/leaf)	11	16	50	103	133	139
Chlorophyll content (mg chlr. a + b/dm²	0.46	0.55	0.75	1.13	1.47	1.75
Chlorophyll $\frac{a}{b}$ ratio	1.46	1.48	1.48	1.43	1.40	1.35
Transmittance of incident radiation (as % of incident level 15.10⁴ ergs/sec/cm²)	35	33	21	17	15	10
Respiration (dark CO_2 evolution mg/h/dm²)	4.1	1.9	2.0	1.4	1.7	0.8
Net photosynthesis * (mg CO_2/h/dm²)	4.85**	1.30	4.16	7.09	9.86	6.96
Assimilation number (mg CO_2 fixed/mg chlr./h)	—	3.29	5.73	6.29	6.20	4.76
CO_2 Compensation point (Γ)	306	265	202	68	61	50

* Experimental conditions: Light intensity 15×10^4 ergs/sec/cm², leaf temperature 25° C, CO_2 concentration c. 300 ppm.

** CO_2 was evolved at rate indicated; no net fixation occurred.

*** Leaves numbered basipetally, No. 1 = terminal leaf. Positions 1 to 6 do not represent consecutive leaves, chlr. = chlorophyll.

hardened leaf had developed bronzed and damaged areas over its lamina following weeks of exposure in the field. Positions 2 to 4 represent leaves over a range of developmental states between these two extremes. Vine leaves take about 40 days to reach full expansion (Mounts 1932) so that the leaves from position 1 to 5 would have developed over the previous 6 – 7 weeks. Between positions 3 and 4 the leaves would have completed their phase of rapid expansion and having lost their glossy surface and translucent appearance would have entered the hardening phase of their development.

Leaf area, chlorophyll content, and the absorption of incident radiation all increased with age. There was an obvious break in chlorophyll content between positions 3 and 4 although the ratio of chlorophyll a/b remained essentially unchanged.

Physiological characteristics also showed a general drift with leaf age. The rate of dark respiration showed an overall decline with increasing age while net photosynthesis (leaf area basis) rose to a maximum at position 5 with a subsequent decrease. The pattern of change in assimilation number was comparable. The smallest leaves examined (position 1) showed no net fixation of atmospheric CO_2 under the present experimental conditions (light intensity 15 10^4 ergs/sec/cm², leaf temperature 25° C, CO_2 concentration c. 300 ppm). CO_2 compensation point (Γ) for these very young leaves was at or above normal atmospheric CO_2 concentration and this could relate to their failure to show net photosynthesis. Values for Γ showed an overall decline with increasing leaf age but an obvious break occurred between positions 3 and 4.

(e) Intermittent light

An experiment was performed where a mature sultana leaf was enclosed in the cuvette which was then masked except for a circular hole 3 cm diameter above the leaf. This aperture allowed illumination of ca. 8.5% of the area of the leaf. Light flashes at an incident intensity of 15 × 10^4 ergs/sec/cm² and of 0.02 sec duration were then provided. The loss of respiratory CO_2 from the leaf was offset when the frequency of the flashes approached 6 per second. Light compensation for the whole leaf was thus achieved by illuminating only 8.5% of its surface for the equivalent of 8.4% of the time. Continuous illumination of this exposed portion of the lamina enabled a photosynthetic rate equivalent to 25% of the rate when the entire lamina was illuminated.

Discussion

(a) Light intensity and rates of photosynthesis

The comparisons drawn between leaves from glasshouse and fully exposed vines show differences in light compensation point, response to light intensity, light saturated rates of photosynthesis, and assimilation number. The direction of these differences is in general agreement with other comparisons between sun and shade leaves quoted in the literature (see Björkman and Holmgren 1963 and literature cited therein).

(b) Intermittent light

Although the photosynthetic activity of both glasshouse grown and fully exposed leaves was light saturated at an intensity considerably below full sunlight (2,500 – 3,000 f. c. compared with 10—12,000 f. c. for full sun) these measurements were performed on leaves whose entire lamina was exposed at right angles to the incident light. Under field conditions however, a large proportion of leaves within the vine canopy experience only intermittent illumination over part of their surface. The experiment described in section (e) of Results suggested an efficient utilisation of partial and intermittent illumination. This could be of importance in the field situation where lower leaves within the canopy receive occasional sun flecks in an otherwise poorly illuminated zone. Light intensity within a dense canopy commonly fell to 10 f. c. (Merbein measurements in mid-summer).

(c) Temperature and photosynthesis

The effects of temperature on photosynthesis are variable. BIERHUIZEN and SLATYER (1964) showed no significant temperature effect on cotton over the 30°—40° C range while WALDRON *et al.* (1967) obtained a Q10 = 1.1 for sugar cane leaves between 8° C and 30° C. By contrast other reports MITCHELL (1936), WENT (1950), PHARIS and WOODS (1960), ORMROD (1961) and WARREN-WILSON (1966) give optimum temperatures in the vicinity of 20° C for a wide variety of plants. The optimum temperature for vine leaf photosynthesis obtained in the present work (25° C for glasshouse grown plants) coincides with the optimum temperature for vine growth in artificially lit cabinets quoted by BUTTROSE (1968).

In the present location, where summertime leaf temperatures are frequently of the order of 40° C and above, it is significant that Sultana leaves withstand this high temperature and remain productive. The rate of photosynthesis showed no substantial decline until leaf temperature exceeded 35° C and even at 40° C the leaves were still photosynthetically active. Nevertheless the light intensity required to saturate photosynthesis was no higher at 40° C than a 25° C.

Tissue damage did not occur under the present experimental conditions until the leaf temperature rose to 50° C or above, provided leaf hydrature was maintained. The damage which can occur under field conditions at air temperatures around 45° C is therefore more likely due to leaf desiccation than to heat injury per se. At elevated temperature leaf transpiration becomes excessive and the demand for water by the foliage can outstrip the supply from the root system.

(d) Leaf age

In general the photosynthetic activity of most leaves rises to a peak at or about the time full size is attained, and they maintain this rate for varying lengths of time, depending on leaf ontogeny, before decreasing again towards senescence (RICHARDSON 1957 and literature cited therein). The vine leaves examined fit this general pattern and the eventual decrease in photosynthetic activity beyond a certain point agrees with the observation of STOEV *et al.* (1966) that recently formed leaves are more active photosynthetically than the oldest leaves on the vine. The present data extend the observations of STOEV *et al.* by showing a decline in leaf photosynthesis towards the tip of the shoot.

Young and rapidly expanding terminal leaves on the vine shoot show strong import of ^{14}C labelled photosynthate from other parts of the shoot in order to maintain their growth (HALE and WEAVER 1962) and at this early stage are not photosynthetically self sufficient. Although these young leaves (area: 11cm^2) were incapable of showing a net fixation of atmospheric CO_2 under the present experimental conditions, such leaves were still capable of fixing respiratory CO_2 in CO_2-free air or in normal air. The photosynthetic apparatus was therefore operative. The commencement of net photosynthesis at position 2 (leaf area: 16 cm^2) did not correspond to a major increase in leaf chlorophyll but rather to a drop in CO_2 compensation point (Γ) to a level substantially below the concentration of CO_2 in air.

The oldest leaves examined were credited with a mean photosynthetic rate of 6.96 mg CO_2/h/dm^2 but it was not shown in the results that this was a maximum stable rate of assimilation following a steady rise over the first 2 – 3 hours of illumination in the laboratory. If under field conditions this same feature occurs, it would tend to reduce the net contribution made by these older leaves compared to younger leaves which achieved their maximum rate of photosynthesis within a few minutes.

Summary

Photosynthesis and respiration were measured in the laboratory with an infra red CO_2 analyser using attached leaves on Sultana vines growing either in the glasshouse or out of doors. At light saturation (2,500 – 3,000 f. c.) the photosynthetic rates approximated 8.5 and 10.5 mg CO_2/h/dm^2 with light compensation points of 50 and 125 f. c. respectively. The optimum temperature for photosynthesis was between 25 and 30^0 C.

Age effects were examined using shoots from well established field vines. Small but rapidly expanding terminal leaves showed no net photosynthesis but had a high rate of dark respiration and a CO_2 compensation point above 300 ppm. With further expansion the leaves showed a steady increase in photosynthetic activity with a corresponding decline in dark respiration and CO_2 compensation point. The rate of photosynthesis again declined with the onset of senescence.

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