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Leaf age and photosynthesis in Vitis vinifera L.

by

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Introduction

Leaf development in woody perennials, including the grape vine, follows a well defined sequence of emergence, unfolding, and rapid expansion of the lamina, followed eventually by senescence and abscission. In common with many other species (FREELAND 1952; RICHARDSON 1957; TAZAKI 1959) recently unfolded *Vitis vinifera* leaves have a low level of photosynthesis which increases during subsequent expansion (KRIEDEMANN 1968). In *Vitis vinifera* the chemical nature of CO_2 fixation products also differs according to leaf age (RIBÉREAU-GAYON 1968). The present paper extends these earlier observations on vine leaf age and CO_2 assimilation by studying both the level of photosynthetic activity and the chemical nature of $^{14}CO_2$ fixation products for the variety Sultana. Changes in leaf anatomy and spectral characteristics during leaf development are also described.

Materials and Methods

Rooted cuttings of *Vitis vinifera* cv. Sultana (syn. Sultanina, Thompson Seedless) were grown under shade house conditions (approximately 50% full sun) in large containers of a fertilised potting mixture. Leaf age data were obtained by noting the date of unfolding of terminal leaves on vigorously growing shoots. The photosynthetic activity of leaves was then measured in both the shade house and in a variety of outdoor situations. All of these measurements were pooled for overall analysis of leaf age and photosynthetic activity. The portable equipment used for environmental monitoring and CO_2 assimilation measurements (based upon a Hartmann and Braun URAS-2 infrared CO_2 analyser) is described elsewhere (KRIEDEMANN and SMART 1969).

In the work on ^{14}C fixation products, $^{14}CO_2$ generated from 18 mg $Ba^{14}CO_3$ (specific activity 5 μc/mg) was administered to an intact shoot on a potted vine in the shade house. The entire shoot was enclosed in a transparent nylon sleeve (volume approximately 20 litres) sealed at the terminal end. The basal end of the sleeve was connected to a high-capacity diaphragm pump which recirculated the enclosed atmosphere at 50 litres min^{-1}. Air from the exhaust side of the pump was introduced at a number of points over the length of the shoot through a manifold to improve distribution. A portion of the recirculated air stream was drawn off (1 litre min^{-1}) and passed through a URAS-1 to measure CO_2 concentration. The potted vine carrying the enclosed shoot was mounted on a turntable that could be swung back and forth through 90^0. During administration of the $^{14}CO_2$ the vine was continually moved in this way to encourage even exposure of the leaves to the incident sunlight. During the 20-min feeding period, the CO_2 concentration of the air fell from an initial value of 450 ppm (immediately after release of $^{14}CO_2$) to 120 ppm at the end of the exposure .

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The shoot was excised after administration of the $^{14}CO_2$, and leaf punch samples were taken for ^{14}C assay using a Packard Tricarb Liquid Scintillator. The unsampled portion of the lamina was then killed in boiling ethanol, and the ^{14}C metabolites were extracted and separated using a combination of ion exchange resins and paper chromatography (KRIEDEMANN and BEEVERS 1967). Paper chromatograms were scanned for activity either by cutting up and counting each segment in the scintillator, or by passing intact chromatograms through a Packard Radiochromatogram Scanner. The areas under individual peaks shown on the scan provided a measure of ^{14}C incorporation by the compounds separated on the chromatogram. The identification of unknown solutes was based upon a comparison of running values with those of authentic standards. Running value refers to the degree of migration down the paper compared to the solvent front or to other known solutes.

The chlorophyll content of leaf tissue was determined from the optical density at 645 and 663 nm of a clear 80% acetone extract, following the method outlined by KIRK (1968).

Spectral characteristics of the leaves were studied using an ISCO Model SR Spectroradiometer.

For the anatomical study, specimens were fixed in F. A. A. (Formalin, acetic acid, 50% alcohol, 5 : 5 : 90) dehydrated, and cleared under vacuum in an ethanol-toluene mixture and then embedded in paraffin wax. Sections (10 μm) were cut on a rotary microtome, stained with safranin and fast green, and mounted in Canada balsam.

Results

1. Photosynthesis and chlorophyll content

Changes in the photosynthetic activity of Sultana leaves as a function of age are shown in Fig. 1. The results are based on individual measurements of 45 leaves along 12 vegetative shoots. The data were grouped into leaf age categories 5—10, 11—16, etc. up to 76 days after the lamina unfolded. The mean rate of photosynthesis was then calculated within each age category. Standard errors are shown for all mean values based on 3 or more observations.

Computor analysis of the data in Fig. 1 revealed that the values closely fitted an inverse polynomial of the form:

$$\frac{x}{y} = B_0 + B_1 x + B_2 x^2,$$

where x = mean leaf age (days),
y = photosynthesis (mg $CO_2 hr^{-1}\ dm^{-2}$),
$B_0 = 2.249$,
$B_1 = 0.0480$,
$B_2 = 0.0011227$.

Photosynthetic activity increased substantially during the first 30—40 days after unfolding. At the end of that period, the leaf had achieved full size (see also MOUNTS 1932). The subsequent decline in photosynthesis was gradual, as shown in Fig. 1. Older leaves (4—5 months) with visual indications of senescence, still achieved photosynthetic rates of 2.5 mg $hr^{-1}dm^{-2}$ in full sunlight. In their field situations, five senescent Sultana leaves at the base of the canopy had a mean assimilation rate, *in situ* of 1.1 mg $hr^{-1}dm^{-2}$.

Fig. 2 indicates the chlorophyll concentration (mg dm^{-2}) and assimilation number (mg $CO_2 hr^{-1}$ per mg chlorophyll) for Sultana leaves referred to in Fig. 1. Standard errors are shown for all mean values based on 3 or more observations.

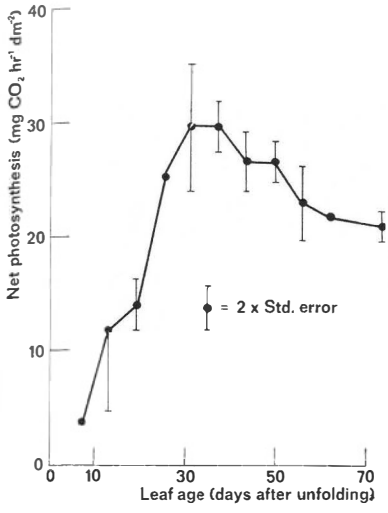

Fig. 1

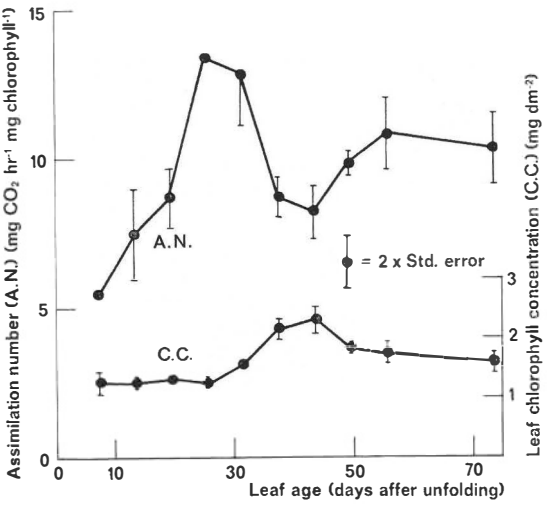

Fig. 2

Fig. 1: The relationship between vine leaf age and photosynthesis.
Fig. 2: Changes in assimilation number and chlorophyll concentration during leaf expansion.

Chlorophyll concentration reached its peak when the leaf achieved maximum photosynthetic activity (area basis). The drop in assimilation number at that stage possibly indicates that chlorophyll concentration was no longer rate limiting for photosynthesis.

2. $^{14}CO_2$ fixation

The level of ^{14}C assimilated by each leaf along a shoot after 20 minutes exposure to $^{14}CO_2$ is indicated in Fig. 3. Peak assimilation occurred near the midpoint of the shoot for well-expanded leaves 30—35 days old. Interpretation of the data in Fig. 3 is complicated by positional effects such as leaf orientation with respect to the incident light, mutual shading, and availability of $^{14}CO_2$; but despite these limitations the relation of leaf age to photosynthesis indicated in Fig. 1 is confirmed.

The chemical distribution of ^{14}C incorporated by the leaves referred to in Fig. 3, is indicated in Table 1. The levels of ^{14}C incorporated by amino acids, organic acids, and ether soluble materials declined with leaf expansion. Serine and aspartic acid accounted for most of the amino acid radioactivity. Irrespective of leaf age, malic acid, and citric acid to a lesser degree, were the principal ^{14}C organic acids. The higher level of ^{14}C activity in the organic acid fraction from the younger leaves is attributable to the presence of ^{14}C tartrate. This metabolite did not incorporate a detectable amount of ^{14}C in leaves more than 23 days old; Ribéreau-Gayon (1968) has reported a similar finding.

Irrespective of leaf age, ^{14}C sugars accounted for a major portion of the incorporated label. Sucrose was primarily responsible for this incorporation, although the level of ^{14}C-labelled oligosaccharides increased with leaf age. Fig. 4 shows the distribution of ^{14}C among the sugars in the neutral fraction from leaves at three developmental stages:
(a) rapid expansion, (b) full size, (c) early senescence. Identification of the stachyose peaks in Fig. 4 is based on Rf values, and is therefore tentative. The presence of an

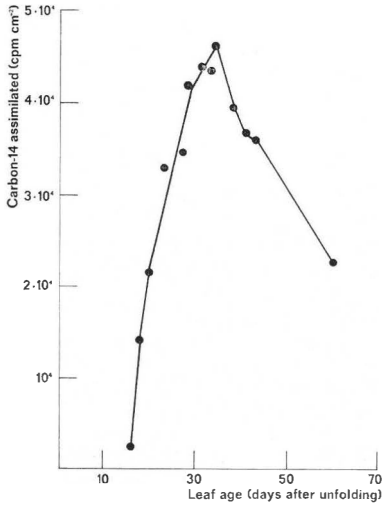

Fig. 3

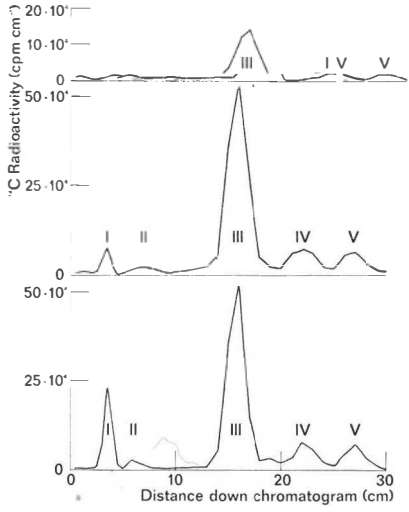

Fig. 4

Fig. 3: The level of $^{14}CO_2$ fixation as a function of leaf age.
Fig. 4: The occurrence of ^{14}C labelled sugars in chromatographed extracts from leaves at three development stages.
a (above): Young leaf, b (middle): mature leaf, c (below): senescing leaf. I: Stachyose, II: raffinose, III: sucrose, IV: glucose, V: fructose.

adjacent raffinose peak was however confirmed by the formation of labelled melibiose and fructose following invertase hydrolysis.

The ethanol-insoluble residue referred to in Table 1 includes ^{14}C proteins and ^{14}C polysaccharides. No attempt was made to separate these compounds, which would be synthesized at different relative rates according to leaf age; nevertheless the higher level of ^{14}C incorporated subsequent to 41 days is probably attributable to increased ^{14}C starch formation.

Table 1

The effect of leaf age on $^{14}CO_2$ fixation products. (Data are expressed as percentages of counts in each leaf sample)

Leaf age (days)	Amino acids	Organic acids	Sugars	Ethanol insoluble residue	Ether soluble
16	7.6	12.1	30.2	33.6	16.6
18	6.6	11.6	35.4	38.9	7.0
20	3.2	11.2	38.9	42.0	4.9
23	3.4	7.1	46.3	40.0	3.2
27	3.4	7.6	54.2	34.6	0.6
28	3.4	3.4	51.9	41.3	0.4
31	2.8	3.0	66.5	27.6	0.4
33	2.7	3.2	73.7	20.3	0.3
38	2.6	1.8	66.3	29.2	0.1
41	2.2	1.9	81.5	14.0	0.3
43	2.1	1.5	74.0	22.2	0.1
60	3.5	2.6	63.4	30.4	0.1

3. Leaf anatomy

Anatomical changes during leaf expansion and subsequent senescence are illustrated in Fig. 5. Sections from a leaf undergoing rapid expansion (Fig. 5 a) revealed tightly packed palisade and mesophyll tissues with little evidence of intercellular spaces. In fully expanded leaves (Fig. 5 b), transverse sections indicated a less compressed assemblage of cells, with readily discernible cellular outlines and greater incidence of intercellular spaces. There was further development of intercellular spaces in the senescent leaf (Fig. 5 c), and an overall increase in leaf thickness. Irrespective of leaf age, crystalline inclusions were of common occurrence.

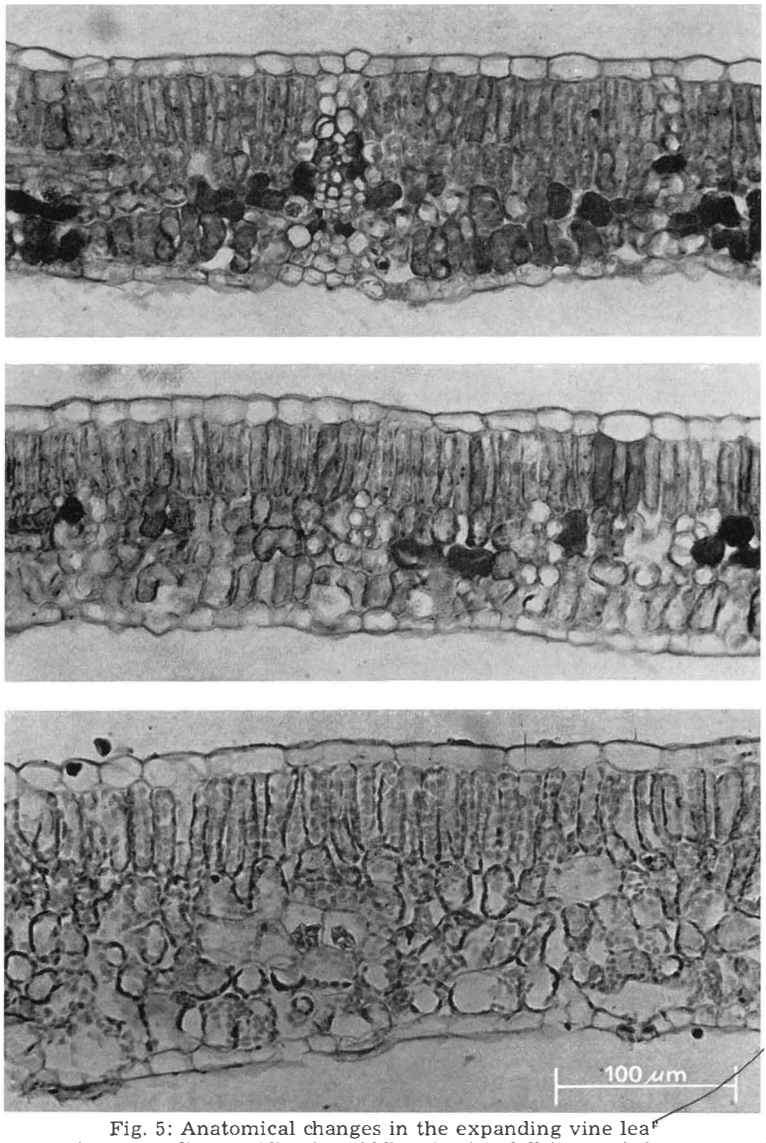

Fig. 5: Anatomical changes in the expanding vine leaf
a (above): Lamina expanding rapidly; b (middle): lamina fullsize; c (below): senescing leaf.

4. Spectral characteristics

The recently unfolded and rapidly expanding vine leaf has a glossy surface, and translucent appearance, which contrasts with the appearance of mature foliage. This visual difference prompted a comparison of spectral properties of the two leaf stages. Representative data are shown in Fig. 6. Measurements were made in the laboratory under a pair of Philips 400 W HPLR mercury vapor lamps mounted above 2 cm of distilled water, which acted as a heat filter.

Both leaves had uniformly high absorption of radiant energy between 400 and 525 nm. Nevertheless, the immature leaf showed a high transmission of wavelengths corresponding to green and red light. The increased energy absorption by the mature leaf corresponds to an increase in chlorophyll concentration from 1.25 to 1.83 mg dm^{-2}. The translucent appearance of young Sultana foliage probably depends therefore on a lower absorption of wavelengths corresponding to green light.

Discussion

In common with other higher plant leaves (FREELAND 1952; RICHARDSON 1957; TAZAKI 1959) grape vine foliage achieved maximum photosynthetic activity when the leaves reached full size. During the initial expansion phase the chlorophyll concentration remained the same. As a consequence, the assimilation number increased parallel to photosynthesis expressed on a leaf area basis. Clearly then, some factor other than chlorophyll concentration is responsible for the increased photosynthetic activity when leaves first reached full expansion. Spectral characteristics did change during the course of leaf expansion; and although the mature leaf showed an increased absorption of incident radiation, this was largely confined to energy in the 525—600 nm wavelength range which is probably of least consequence for photosynthetic activity (HOOVER 1937).

Anatomical changes during leaf expansion could contribute to the increase in photosynthesis. The densely packed palisade and mesophyll tissues of the rapidly expanding leaf (Fig. 5 a), as compared with the more open structure of mature or senescent foliage, may offer greater physical impedance to CO_2. A higher incidence of intercellular spaces, coupled with the reduction in internal CO_2 concentration which occurs when a vine leaf achieves full size (KRIEDEMANN 1968) ,woud encourage an inward flux of CO_2. This change should be evident as reduced mesophyll resistance.

The photosynthetic rates reported here are comparabe to those recorded by KRIEDEMANN and SMART (1969) for field grown Sultana grapes and for the wine variety Black Shiraz (syn. Petite Sirah). In all cases, CO_2 assimilation was measured under

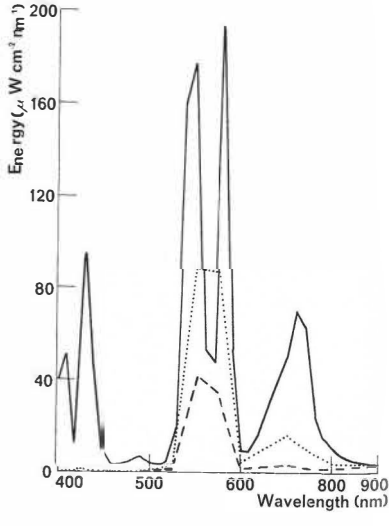

Fig. 6: Spectral characteristics of immature and mature Sultana leaves. Light soucre was 2 Philips HPLR 400 W mercury vapour lamps. ——— Lamp emission, — — mature leaf, ... expanding leaf.

a light intensity which saturated the photosynthetic apparatus and at leaf temperatures favourable for photosynthesis. Laboratory measurements (KRIEDEMANN 1968) on glasshouse-grown Sultanas and on excised field shoots taken towards the end of the growing season, yielded lower values for photosynthesis. The differences have been attributed to lowered vegetative vigour in these vines, and imply that the demand for photosynthate can influence the current rate of CO_2 assimilation in the vine, as has been reported for a number of other species (see NEALES and INCOLL 1968 and literature cited therein).

Summary

The photosynthetic activity of individual vine leaves was measured on potted plants grown out doors, using both infrared CO_2 analysis and $^{14}CO_2$ fixation techniques. Peak photosynthetic activity occurred at the time when the leaf became fully expanded, approximately 40 days after unfolding. Thereafter, photosynthesis declined gradually.

The increase in photosynthesis during leaf expansion was not attributable to increased chlorophyll concentration, altered spectral characteristics, or to any outstanding anatomical change. Nevertheless, a sensecent leaf was readily distinguished anatomically from an expanding or mature leaf.

Irrespective of leaf age, sucrose was the major $^{14}CO_2$ fixation product. Oligosaccharides also incorporated label, and accounted for a higher proportion of the ^{14}C fixation products in older leaves. Regardless of leaf age, malic and citric acids became labelled, but the formation of ^{14}C tartaric acid was restricted to the rapidly expanding foliage. The amino compounds serine and aspartic acid showed some incorporation of label in all leaves examined.

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