OBSERVATIONS ON GAS EXCHANGE IN THE DEVELOPING SULTANA BERRY

By P. E. KRIEDEMANN*

[*Manuscript received December* 28, 1967]

Summary

In its early phases of growth the grape berry, although lacking stomata, shows measurable photosynthetic activity. There also exists a capacity for dark CO_2 fixation following a period of illumination and a relatively high rate of dark respiration (expressed as O_2 uptake) with a respiratory quotient which is less than unity. Oxygen uptake can be completely arrested by illumination.

The sultana berry assumes a more translucent appearance with an attendant reduction in chlorophyll concentration at and beyond the period of "colour change". Photosynthetic activity is weakened at this time and the capacity for dark CO_2 fixation is reduced. Although respiratory activity falls the respiratory quotient rises to a value greater than unity. This change in the physiological characteristics of berries after the colour change coincides with a phase of decreasing acid content but of vigorous sugar accumulation.

I. INTRODUCTION

The sultana berry requires about 100 days to complete its development from anthesis to maturity, and growth, measured as fresh weight or volume, follows a biphasic pattern with the two phases of approximately equal duration (Coombe 1960). These phases correspond to an initial period of acid build-up followed by the sugar-accumulating phase. During this first period the fruit is characteristically small and green with a hard texture and low pH (2–3). Towards the end of the acid-accumulating phase a growth lag occurs and the berry is said to undergo "colour change" prior to the onset of the period of sugar build-up. During this latter phase the fruit assumes a more translucent appearance, acquires a softer texture, and accumulates sugar (primarily glucose and fructose) with a concurrent, but not quantitatively related, decrease in acid content.

The developing berry almost certainly undergoes major physiological and biochemical changes during its growth. The present paper reports some of these changes. Differences between the immature and maturing fruit with respect to their respiration and CO_2 fixation are described, and the effect of environmental influences on gas exchange is reported.

II. MATERIALS AND METHODS

Experimental material for the developmental study was drawn from potted sultana vines [*Vitis vinifera* cv. Sultana (Thompson Seedless)] grown under heated glasshouse conditions at Merbein during the winter of 1966. In subsequent short-term experiments, which compared

* Division of Horticultural Research, CSIRO, Merbein, Vic. 3505.

immature and maturing berries, field-grown material was sometimes used. Details on the source of material are given for each experiment.

For each sample of the growth study a total of 15 or 16 berries were removed from clusters, ranked according to size, and the 10 median berries used for individual measurements of fresh weight and volume (calculated from dimensions and checked against values derived from fresh weight and specific gravity data). A further subsample was used for dry matter and moisture content determination.

A standard Warburg technique was used for measuring the CO_2 and O_2 flux of excised berries held at 25°C. Oxygen uptake was calculated from manometer readings with 20% potassium hydroxide in the centre well, while CO_2 efflux was derived from the value of the net change in volume following O_2 and CO_2 exchange with water in the centre well. Intact vine fruits were found to maintain their O_2 uptake at an unchanged rate for at least a day following removal from the parent cluster and for the present purposes were ideal experimental material.

Warburg vessels were illuminated as required by four circular fluorescent tubes mounted underneath the water-bath of the apparatus. Independent control of these tubes gave a range of light intensity up to a maximum of $2 \cdot 75 \times 10^4$ ergs/sec/cm^2 at the base of the vessels (YSI Kettering Radiometer measurement).

In an experiment where malate was supplied to tissue slices (reported in Table 2) berries were sliced to a thickness of 2 mm (achieving a similar total fresh weight per vessel) and rinsed for 30 sec in 1 mM potassium metabisulphite, to inhibit polyphenoloxidase activity (Poux 1967) before being placed in a medium of 1 mM Tris–HCl buffer at pH 7·4 in the Warburg vessels. When a stable rate of respiration was established malic acid was tipped from the side-arm to give final concentrations of 0, 1, 10, and 100 m-moles/l. By the end of the experiment the pH had fallen to between 3·5 and 4·0.

The [1-^{14}C]malate administered to tissue slices was obtained from the Radiochemical Centre at Amersham, U.K. The isotope was subsequently assayed in a Packard liquid scintillator. The scintillation fluid consisted of 350 ml toluene, 350 ml dioxane, 210 ml methanol, 73 g naphthalene, 4·52 g PPO (2,5-diphenyloxazole), and 0·078 g POPOP [1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene].

For acid determination berries were ground in acid-washed sand and titrated with 0·05N NaOH using phenolphthalein as an indicator. The "sugar" content of berries was measured on the juice from crushed fruits using a refractometer. Moisture contents were based on the water lost from tissues after 3 days at 80°C in an oven with forced draught.

III. Results

(a) *Berry Growth and Related Physiological Changes*

The increase in berry volume and dry weight during growth follows a diauxic pattern. There is a characteristic lag in volume increase at the onset of the sugar-accumulating phase. It is at this point that the berry assumes a more translucent appearance and is said to undergo colour change. Present observations on berry growth and sugar accumulation showed general agreement with those of Coombe (1960). Berry respiration (O_2 and CO_2 exchange) also demonstrated certain changes over this period. Two phases of development were clearly evident. The respiratory quotient (R.Q.) showed a distinct shift at about 6 weeks after anthesis, i.e. at the time of colour change, and more than doubled (from 0·9 to 1·9) in 7 days. Oxygen uptake (dark respiration) fell steadily during this first growth phase from 110 μl O_2/g fresh weight/hr 2 weeks after anthesis to 20 μl O_2/g fresh weight/hr at colour change. There was no further change in dark respiration during the second growth phase.

(*b*) *Respiratory Quotient in Relation to Age and Temperature*

The dependence of R.Q. upon both age and temperature is demonstrated in Table 1. Two berries were used per Warburg vessel and the experiment was performed in duplicate. The experimental material was allowed to equilibrate at the higher temperatures for at least 1 hr before measurements of gas exchange were undertaken.

TABLE 1

RESPIRATORY QUOTIENT AND OXYGEN UPTAKE RATE IN RELATION TO TEMPERATURE AND BERRY DEVELOPMENT

Two berries were used per vessel and the experiment was performed in duplicate. Temperature equilibration continued for at least 1 hr at each level before gas exchange measurements were commenced

Temperature (°C)	Immature Berries		Mature Berries	
	O_2 Uptake (μl/vessel/hr)	Respiratory Quotient	O_2 Uptake (μl/vessel/hr)	Respiratory Quotient
25	28	1·13	23	1·24
35	80	1·22	70	2·25
45	150	1·94	115	4·06

Immature berries show only a slight rise in R.Q. with increased temperature compared with the more mature fruit. Confining this comparison to their behaviour at 25 and 35°C, it is clear that temperature can elicit a response in mature berries of which immature fruits are incapable. The particularly high R.Q. of the larger berries at 45°C must be regarded with suspicion. It can be partly attributed to fermentation induced at this high temperature because, in subsequent experiments where the vessels were flushed with oxygen for 15 min prior to respiratory measurement at each temperature, the 45°C value was restored to that originally measured at 35°C. Oxygen flushing, however, had no effect on R.Q. up to 35°C.

(*c*) *Malic Acid Metabolism*

One conspicuous feature of the developing sultana berry following colour change is the loss of endogenous malic acid (Harris, Kriedemann, and Possingham 1968). In this connection, the present data demonstrate a difference between immature and mature berries in their ability to metabolize added malic acid. This relates to the previous observations on R.Q. The total volume of gas evolved in the 95 min following the addition of malic acid is recorded in Table 2. Immature and mature tissues differ in their ability to respond to added malic acid. Gas evolution was enhanced by this addition only with mature tissue. This effect of added malic acid was repeated using 0·1M potassium malate also in 1 mM Tris–HCl buffer at pH 7·4. Immature tissue failed to respond to this addition while slices from mature berries increased CO_2 evolution by 50% compared with controls.

Further evidence for a difference in malate decarboxylase activity was provided by a similar experiment where 0·5 μCi of [1-^{14}C] malate was added from the side-arm of the Warburg vessel to give a final concentration of 0·1M potassium malate in the main vessel. The $^{14}CO_2$ liberated by 0·8 g of tissue after 2 hr from the unbuffered aqueous medium (final pH 4·5–5·5) was collected on KOH-impregnated paper in the centre well, and counted in the liquid scintillator. Both immature and mature tissues produced $^{14}CO_2$ but the mean count rates for three replicates were 2600 and 9200 counts/min respectively. This same experiment also demonstrated that respiration (O_2 uptake) was unaffected by the malate addition which rules out any possibility that the increase in gas evolution referred to in Table 2 is complicated by a changed rate in O_2 uptake following malate addition.

TABLE 2

CARBON DIOXIDE EVOLUTION BY SLICES FROM IMMATURE AND MATURE BERRIES IN RESPONSE TO ADDED MALATE

Either 1 g fresh weight immature berry slices or 2 g fresh weight mature berry slices were used per vessel. Assay temperature was 35°C, and duration of measurement was 95 min

Final Malate Concn. (mM)	CO_2 Evolved (μl/g fresh wt. tissue)	
	Immature Berry Slices	Mature Berry Slices
0	5·3	17·3
1	1·1	19·2
10	1·1	22·9
100	2·9	26·5

(*d*) *Photosynthesis and CO_2 Fixation by the Berry*

The gas exchange of illuminated compared with darkened berries was studied at weekly intervals between anthesis and maturity using the intact berries whose growth has been described. The rate of photosynthesis was measured at a light intensity of $2{\cdot}75 \times 10^4$ ergs/sec/cm^2 and is expressed in Figure 1 as the volume of oxygen produced during the first 30 min of illumination. The reduction in gas pressure when the lights were turned off (dark CO_2 fixation) has been expressed as a volume change over the first 30 min of the dark cycle. The data in Figure 2 show that unilluminated control vessels (water in the centre well) showed little net change in volume during the course of measurements such as these. On this basis no attempt was made to distinguish between dark CO_2 fixation and respiratory CO_2 evolution during the first 30 min of the dark cycle.

Further criteria can be cited to support the conclusion that this negative pressure change following illumination is due to CO_2 fixation — no pressure change

occurred when the air in the Warburg vessel was thoroughly flushed out with CO_2-free gas; conversely, the pressure decrease was enhanced when the air in the vessel was enriched with CO_2. Furthermore, from the direct assay of the CO_2 concentration in the effluent air stream from illuminated and subsequently darkened fruit (using an infrared gas analyser) it was obvious that CO_2 uptake continued during the dark period following illumination. Figure 1 demonstrates the change in photosynthesis and dark CO_2 fixation rates as the berry develops. The capacity of the immature berry for dark CO_2 fixation exceeds its photosynthetic rate, but this capacity falls to a minimum at colour change, with some resurgence of activity later on.

Fig. 1

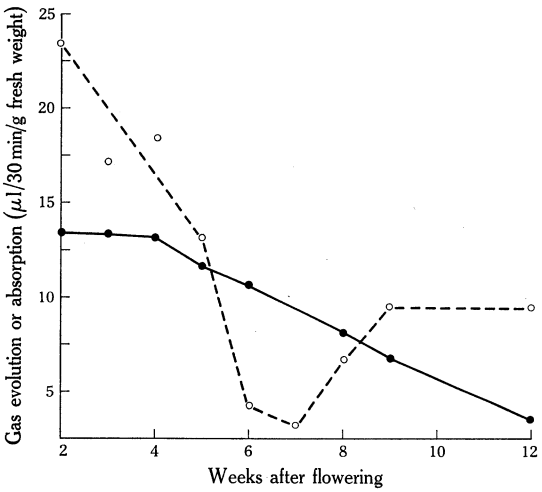

Fig. 2

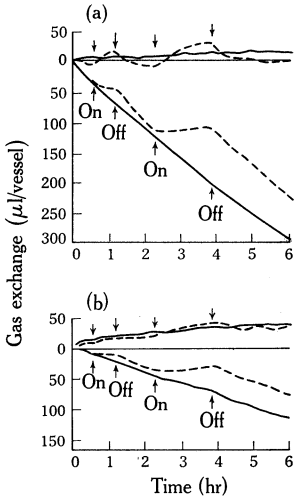

Fig. 1.—Photosynthesis (●) and dark CO_2 fixation (○) in the developing sultana berry. Photosynthesis measured at a light intensity of $2 \cdot 75 \times 10^4$ ergs/sec/cm^2. The ordinate shows volume of gas either evolved as O_2 from photosynthesis over the first 30 min of illumination, or fixed as CO_2 during the first 30 min of the dark cycle. Each point is the mean from duplicate or triplicate determinations.

Fig. 2.—Effect of light on the gas exchange of immature (*a*) and mature (*b*) berries. Lines below the abscissa indicate O_2 uptake (KOH in the centre well). Lines above the abscissa show the net effect of CO_2 and O_2 exchange. —— Gas exchange in continuous darkness. – – – Gas exchange over periods of light and darkness; arrows indicate light on and off. The immature and mature berries had a mean fresh weight of 0·62 and 1·02 g/berry respectively with an acid titre of 11·9 and 6·1 ml 0·05N NaOH/g fresh weight.

The immature berry shows a low rate of photosynthesis which is even further reduced during development. This very minor photosynthetic activity was not encouraged by CO_2 enrichment of the air in the vessel using bicarbonate buffer. Leaf disks punched from the parent vine and exposed to the same conditions evolved 3·5 ml O_2/hr/g fresh weight compared with the maximum observed for berries of about 50 μl O_2/hr/g fresh weight; i.e. a difference of almost two orders of magnitude.

This age effect on gas exchange characteristics is again demonstrated in Figure 2 which shows the data from a single experiment comparing the behaviour of immature (week 5) and maturing (week 10) fruits. This experiment involved two sequential light–dark cycles and was performed in duplicate. One vessel of each pair was wrapped in aluminium foil to show gas exchange in continuous darkness, while the other was unwrapped and received the maximum light intensity of $2 \cdot 75 \times 10^4$ ergs/sec/cm^2.

Considering the net O_2 and CO_2 exchange of illuminated compared with continuously darkened berries (upper plots in Fig. 2), only the immature fruits show a readily measurable oxygen evolution in response to illumination. During the dark period immediately after illumination the immature fruits display CO_2 fixation while in mature fruits, fixation is barely detectable. As the berries approach full maturity their CO_2-fixing capacity is partly regained.

(e) *Respiration in Light and in Darkness*

The data in Figure 2 also demonstrate an effect of light on O_2 exchange which was observed in over 20 comparable experiments on berries although vine leaf disks did not demonstrate this phenomenon. The O_2 uptake of fruit which were twice illuminated is compared with those continually darkened. In both immature and mature berries, O_2 uptake is immediately arrested when the light is turned on, and recommences within seconds of its being turned off again. A similar phenomenon has been reported recently by Kowallik and Gaffron (1967) for a yellow mutant of *Chlorella* where O_2 uptake was completely arrested by blue light.

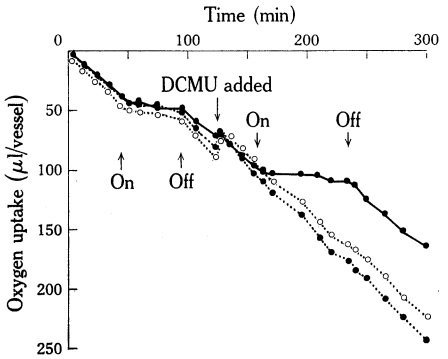

Fig. 3.—Effect of DCMU on the oxygen exchange of illuminated berries. ●——● Control. O····O, ●····● Duplicate runs with DCMU ($0 \cdot 2 \times 10^{-4}$M) added. Each trace represents a single Warburg vessel containing five small berries with a total fresh weight of 0·24–0·27 g/vessel. Arrows indicate light on and off.

For a number of reasons the present effect of light on O_2 exchange in grape berry is thought to be photosynthetic in nature. Firstly, O_2 uptake resumes its original dark rate as soon as the light goes off. There was no maintenance of the suppressed rate of uptake as might be expected with indirect effects on respiration. Secondly, if the outer chlorophyll-containing tissues of the berry are peeled away, O_2 uptake is unaffected by illumination. A third observation supporting this interpretation of light effects appears in Figure 3 which illustrates the effects of the photosynthetic inhibitor DCMU [3-(3,4-chlorophenyl)-1,1-dimethylurea] on O_2 exchange. Five small field-grown sultana berries (total fruit weight 0·24–0·27 g) were placed intact into 2 ml of water in each of 12 Warburg vessels. Figure 3 shows three of the relevant plots of O_2 uptake. The remaining vessels comprised additional

DCMU concentrations and various pretreatments. The first cycle of light and darkness indicated that each system was capable of arresting O_2 uptake in the light and of resuming their normal dark rate immediately afterwards. DCMU was subsequently added from the side-arm of two vessels to give a final DCMU concentration of $0{\cdot}2\times10^{-4}$M in the main vessel. Distilled water was tipped from the side-arm of the control vessel. DCMU at $0{\cdot}2\times10^{-6}$M was also used. The high concentration of inhibitor, which had no effect on dark respiration, completely offset the previously observed effect of light on O_2 uptake. The influence of DCMU at $0{\cdot}2\times10^{-6}$M was measurable but far less pronounced.

These effects of light on O_2 exchange can be extended to include effects of light on respiratory CO_2 output measured with an infrared gas analyser in a stream of CO_2-free air. Six berries (mean fresh weight 0·93 g/berry) were given two sequential dark–light cycles using a flow rate of 100 ml/min. In the dark they showed CO_2 evolution equivalent to 0·39 mg/hr. Upon illumination ($12{\cdot}4\times10^4$ ergs/sec/cm^2) respiratory CO_2 production fell to 47% of the dark rate, i.e. 53% of the CO_2 normally given off in dark respiration was refixed in the light.

(*f*) *Diurnal Fluctuations in the Acid Titre of Immature and Mature Berries*

Although the grape berry is capable of dark CO_2 fixation and stores substantial amounts of organic acids (principally malic and tartaric acids) it does not display a diurnal fluctuation in acid titre characteristic of plants with the crassulacean type of acid metabolism.

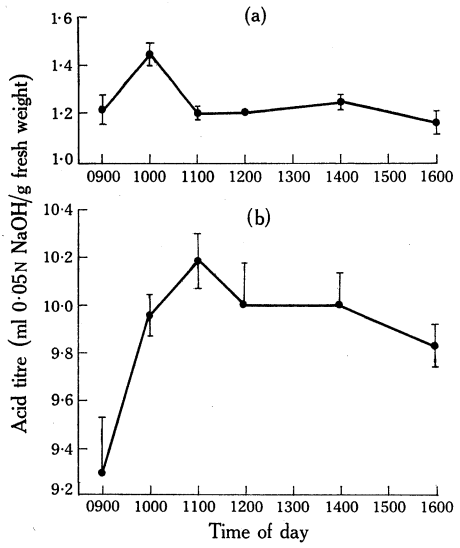

Fig. 4.—Changes in acid titre of glasshouse-grown mature (*a*) and immature (*b*) sultana berries during one day. Data are based on four replicate titrations using three berries per sample. Bars indicate 2× standard errors.

A series of measurements on immature and mature berries from a number of varieties revealed no major change in acid titre over the course of a day. Nevertheless, there was a suggestion of increased titre following illumination for immature berries of both Sultana and the pigmented variety Bastardo. The results for Sultana are shown in Figure 4. A single cluster of both immature and maturing berries was wrapped in aluminium foil overnight and at 9 a.m. the following morning the cover

was removed and the first sample of 12 berries was taken. On five subsequent occasions over the course of that day (August 5, 1966) berries were removed and titrated against 0·05N sodium hydroxide with phenolphthalein as indicator. The values given in Figure 4 are the mean titre from four replicate determinations using three berries per sample.

The rise in acid titre of the maturing berries following illumination is of doubtful significance while the more substantial change for the immature fruit seems slight when compared to the extreme fluctuation (50% change — see Bonner 1950, p. 154) in acid content that occurs in the Crassulaceae.

IV. Discussion

The existing literature on vine physiology describes the ontogenetic development of the berry in terms of morphology (Coombe 1960), some aspects of its physiology (Ribereau-Gayon and Peynaud 1960), together with numerous reports covering the effects of age or environmental conditions or both on its metabolism. The present data will be discussed within this framework.

The alteration in berry physiology at the time of the lag phase is marked by an abrupt change in R.Q. The value of approximately 1·5 routinely obtained during the latter phase of development agrees with earlier observations (Genevois 1938). Saulnier-Blache (1963) suggests that a sudden increase in R.Q. at this stage of berry development is partly a consequence of a temporary drop in the rate of O_2 uptake. The present data lend some support to this suggestion, but regardless of the rate at which the berry consumes O_2, the relative rate of CO_2 evolution must have increased for the R.Q. to have changed so abruptly, and this suggests a change in respiratory substrate.

The change in metabolism which underlies this alteration of R.Q. was also evident in the response of slices from maturing berries to added malic acid. A similar situation exists for apples (Spencer 1965) where tissue slices taken from post-climacteric fruit showed a sharp increase in CO_2 evolution following the addition of malic acid, while slices from pre-climacteric fruit did not. This difference was thought to be related to the increase in R.Q. at the climacteric in apples, and it was suggested that the added malate was being decarboxylated by the malate dehydrogenase with an attendant reduction of $NADP^+$ to NADPH according to the equation:

$$\text{L-Malic acid} + NADP^+ \xrightleftharpoons[\text{malate dehydrogenase}]{} NADPH + H^+ + \text{pyruvic acid} + CO_2.$$

It is not known how NADPH is reoxidized, although it is presumed to be by an internal process. The pyruvate is subject to further decarboxylation (Spencer 1965).

There is a further point of similarity between the response of tissue slices from maturing sultana and that of post-climacteric apples to the addition of malate. As reported by Neal and Hulme (1958) for apple fruits, the present work also demonstrated that under anaerobic conditions (Warburg vessels flushed with nitrogen) the addition of malate did not produce any sharp increase in the rate of CO_2 evolution.

The suggestion of Neal and Hulme (1958) that during the climacteric some malate is diverted from the Krebs cycle to the malate dehydrogenase (decarboxylating) and pyruvate decarboxylase pathways might therefore apply to grapes during their sugar-accumulating phase.

General observations indicate that elevated temperatures favour acid dissipation (Amerine 1956; Radler 1965) and are also responsible for elevating R.Q. A previous interpretation of this temperature effect on R.Q. (Gerber, cited by Genevois 1938) was that the decarboxylation of malic acid commenced as temperatures approached 30°C while above 35°C the metabolism of tartaric acid became possible. In the present work, flushing vessels with oxygen suppressed R.Q. above 35°C, suggesting some degree of fermentation at these higher temperatures. Earlier interpretations must therefore be viewed in the light of this observation. Fleshy fruits with possibly low internal oxygen tension are known to embody this characteristic of fermentation at elevated temperatures (Beevers 1961, and literature cited therein). The relevance of these findings to field conditions can be gauged by temperature measurements made with thermocouples on maturing berries of exposed clusters growing at Merbein. On occasional days when ambient air temperature attained 38°C, the internal temperature of berries reached 40–42°C. Presumably the berry under such conditions would be fermenting to some extent. The effects of accumulation of fermentation end-products on fruit quality are not known.

The photosynthetic activity of the berry is known from CO_2 analysis to be slight (Geisler and Radler 1963) but nonetheless measurable. Thus the grape berry holds a feature in common with many other fruits (see Bean, Porter, and Barr 1963) in that local photosynthesis cannot account for observed growth. The present experiments extend the observations of Geisler and Radler (1963) by demonstrating that the rate of dark CO_2 fixation can exceed initial rates of photosynthesis during the preceding light phase. The level of light intensity used for these experiments would, however, approximate to only 5% of full sunlight, and under field conditions exposed bunches of sultanas would have a much greater prospect of showing significant photosynthetic gain. Nevertheless, a considerable part of the sultana crop is obscured from the sun by the overhead leaf canopy and in this situation is frequently exposed to only 1% of the incident sunlight (field measurements at Merbein). In such a situation it is conceivable that the rate of berry photosynthesis would be of the same order as that measured in the laboratory despite the relatively low intensity and incomplete illumination of the berry surface provided in the Warburg apparatus.

The phenomenon of CO_2 fixation and acid metabolism in grape berries has been the subject of extensive investigations by other workers (Hale 1962; Kliewer 1964, 1965, 1966; Kliewer and Schultz 1964) and the effects of light, darkness, temperature, and state of development on the fate of ^{14}C-fixation products have already been well documented. Drawert and Steffan (1966) provide data which show that the berry fixes $^{14}CO_2$ predominantly into organic acids in darkness but into sugars under illumination. Accordingly they postulated the existence of a diurnal acid rhythm analogous to that found in crassulacean plants. Present data (Fig. 4) do not support this contention for either immature or mature sultana berries. Comparable observations made for the pigmented varieties Bastardo and Tempranillo were in agreement.

There are additional grounds for regarding the grape berry as a non-crassulacean type of organ. In the present work, Sultana berry respiration (O_2 uptake) showed no response when the atmosphere in the Warburg vessel was flushed out with pure oxygen. This behaviour was observed in three separate experiments where R.Q. was being measured at 25, 35, and 45°C in normal and in O_2-enriched atmospheres. Plants which demonstrate the classical crassulacean-type metabolism commonly show a significant increase in respiratory activity in oxygen (Beevers, Stiller, and Butt 1966).

V. Acknowledgments

The expert technical assistance of Mrs. E. Törökfalvy and a gift of DCMU from Dr. L. T. Evans, Division of Plant Industry, CSIRO, is gratefully acknowledged. Thanks are also due to my colleagues for their criticism of the manuscript.

VI. References

Amerine, M. A. (1956*a*).—*Wines Vines* **37**, 27–32.

Amerine, M. A. (1956*b*).—*Wines Vines* **37**, 53–5.

Bean, R. C., Porter, G. G., and Barr, Barbara K. (1963).—*Pl. Physiol., Lancaster* **38**, 285–90.

Beevers, H. (1961).—"Respiratory Metabolism in Plants." (Row Peterson: Evanston and New York.)

Beevers, H., Stiller, M. L., and Butt, V. S. (1966).—In "Plant Physiology". Vol. IVB, pp. 119–262. (Ed. F. C. Stewart.) (Academic Press, Inc.: London and New York.)

Bonner, J. (1950).—"Plant Biochemistry." (Academic Press, Inc.: London and New York.)

Coombe, B. G. (1960).—*Pl. Physiol., Lancaster* **35**, 241–50.

Drawert, F., and Steffan, H. (1966).—*Vitis* **5**, 377–84.

Geisler, G., and Radler, F. (1963).—*Ber. dt. bot. Ges.* **76**, 112–19.

Genevois, L. (1938).—*Revue Vitic.* **88**, 103–10, 121–5.

Hale, C. R. (1962).—*Nature, Lond.* **195**, 917–18.

Harris, June, Kriedemann, P. E., and Possingham, J. V. (1968).—*Vitis*. (In press.)

Kliewer, W. M. (1964).—*Pl. Physiol., Lancaster* **39**, 869–80.

Kliewer, W. M. (1965).—*Am. J. Enol. Vitic.* **16**, 92–100.

Kliewer, W. M. (1966).—*Pl. Physiol., Lancaster* **41**, 923–31.

Kliewer, W. M., and Schultz, H. B. (1964).—*Am. J. Enol. Vitic.* **15**, 119–29.

Kowallik, W., and Gaffron, H. (1967).—*Nature, Lond.* **215**, 1038–40.

Neal, G. E., and Hulme, A. C. (1958).—*J. exp. Bot.* **9**, 142–57.

Poux, C. (1967).—*Annls Nutr. Aliment.* B **21**, 205–22.

Radler, F. (1965).—*Am. J. Enol. Vitic.* **16**, 38–41.

Ribereau-Gayon, J., and Peynaud, E. (1960).—"Traite d'Oenoligie." Vol. 1. (Cl. Beranger: Paris and Liège.)

Saulnier-Blache, P. (1963).—*Annls Physiol. vég., Paris* **5**, 217–28.

Spencer, Mary (1965).—In "Plant Biochemistry". Ch. 30. (Eds. J. Bonner and J. E. Varner.) (Academic Press, Inc.: London and New York.)