

## Grape berry respiration: Effects of metabolic inhibitors

by

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### Introduction

The developing sultana grape berry takes approximately 100 days to reach maturity (HARRIS, KRIEDEMANN, POSSINGHAM 1968). The growth follows a biphasic pattern associated with an alteration in berry physiology at veraison or "colour change". The first growth period is one of acid accumulation, high rate of oxygen ( $O_2$ ) uptake and a respiratory quotient (R.Q.) value of about 1. During the second growth phase acid dissipation and sugar accumulation is combined with a decreased rate of  $O_2$  uptake and an R.Q. value greater than unity, suggesting a possible shift in respiratory substrate (KRIEDEMANN 1968).

The experiments reported in the present paper were designed to extend these observations on carbon dioxide ( $CO_2$ ) and  $O_2$  exchange to other grape varieties. The effects of certain metabolic inhibitors on the respiratory activity of the developing fruit were also examined in an attempt to delineate some of the biochemical differences between immature and maturing fruit. Observations were made on pigmented and non-pigmented grapes and on seeded and seedless varieties as it was thought that both these characters might quantitatively and/or qualitatively affect the respiration of grape berries.

### Material and Methods

The varieties of *Vitis vinifera* L. selected for study were the pigmented varieties Bastardo (seeded), Black Monukka (seedless), while the non-pigmented varieties were Clare Riesling (seeded) and Sultana (seedless). Potted Bastardo vines were grown in a heated glasshouse at Merbein and measurements were made during the spring of 1967. Berries were taken from field vines of Black Monukka, Clare Riesling and Sultana during the 1968 summer season.

From anthesis to maturity 15 to 20 berries of each variety were collected at weekly intervals. The fruit in each sample was ranked according to fresh weight, and the median berries (usually 6) were used for the measurement of R.Q. and dark respiration ( $O_2$  uptake) rate on intact fruit. The same fruit was used to test the effect of inhibitors on respiration using methods described below. Three berries were used for dry weight measurement, and sufficient material to yield approximately 0.5 g dry weight, was taken for insoluble nitrogen determinations.

Standard Warburg technique was used throughout, the vessels being shaken at 200 oscillations per minute through a stroke of 6 cms at 30° C. R.Q. was calculated from the ratio between  $CO_2$  efflux derived from net change in volume following  $CO_2$  and  $O_2$  exchange with water in the centre well, and the  $O_2$  uptake alone with 20 % potassium hydroxide (KOH) in the centre well to absorb  $CO_2$ . Usually one berry was used per vessel, but where berries were less than 0.5 gms, several berries were used. All Warburg measurements were performed in triplicate.

### Inhibitor studies

After seed removal, berries were cut transversely into 1 mm thick slices. A weight of 0.5 gm per vessel was used and the slices were rinsed for 30 secs in 1.0 mM potassium metabisulphite ( $K_2S_2O_5$ ) to inhibit polyphenyl oxidase. Each vessel contained 2 ml 0.01 M phosphate buffer (pH 6.8) and 0.5 ml of the appropriate inhibitor solution or distilled water in the case of controls. Following ten minutes' infiltration under vacuum, 0.2 ml 20 % KOH was added to the centre well of the Warburg vessels before they were attached to the manometers. Ten minutes were allowed for equilibration and  $O_2$  uptake was followed for the next 60-75 minutes.

Prior infiltration of the metabolic inhibitors such as potassium cyanide was used in preference to tipping solutions from a side arm Warburg vessel in an attempt to minimize distillation of hydrogen cyanide from the main vessel to the centre well. All inhibitor solutions were supplied in this way, as comparisons between tipping and infiltrating of 2,4-dinitrophenol (DNP) yielded essentially similar results.

A number of experiments were conducted to establish that the added inhibitors were exerting their effect on basal metabolism and not simply on the  $O_2$  uptake resulting from increased polyphenol oxidase activity following slicing. In these experiments Sultana tissue showed an increase in respiration ( $O_2$  uptake/g fresh weight) following slicing relative to intact fruit. This was eliminated with  $K_2S_2O_5$  treatment. In the absence of  $K_2S_2O_5$  treatment the slices turned brown, especially those from immature fruit. By contrast slices of the variety Bastardo showed no increase in respiration on slicing and here  $K_2S_2O_5$  exerted no effect on respiration. In the other grape varieties used the respiratory activity of  $K_2S_2O_5$  treated slices was comparable to intact fruit and was sustained for long periods. Accordingly  $K_2S_2O_5$  was used as a standard pretreatment throughout.

### Insoluble nitrogen

Tissue residue following 80 % acetone extraction was assayed for insoluble nitrogen using standard micro Kjeldahl procedures.

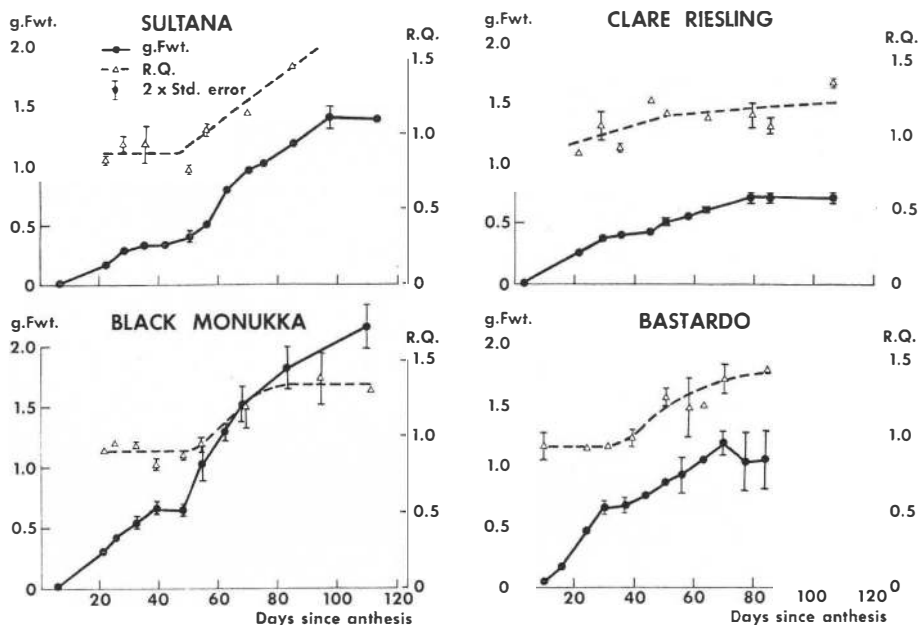
## Results

### a) Growth and respiratory quotient (Figs. 1-4)

In all four varieties, two growth phases were apparent and were separated by a distinct lag in fresh weight occurring approximately 40 days after flowering. With the exception of Clare Riesling, resumption of growth following the lag phase coincided with an increase in R.Q. to a value greater than unity. In the case of the varieties Bastardo and Black Monukka the change in R.Q. occurred at the time of pigment development.

### b) Respiration Rate (Oxygen Uptake) (Figs. 5-8)

All varieties showed a decline in their rate of  $O_2$  uptake, expressed on a fresh weight basis, during their development. The decline was greater during the first growth period. Irrespective of the presence or absence of seeds or pigment, the initial level of respiration was about 280-300  $\mu l O_2/hr/g$  fresh weight and this rate fell to 20-40  $\mu l O_2/hr/g$  fresh weight towards berry maturity.



Figs. 1 - 4: Changes in fresh weight and respiratory quotient during grape berry development.

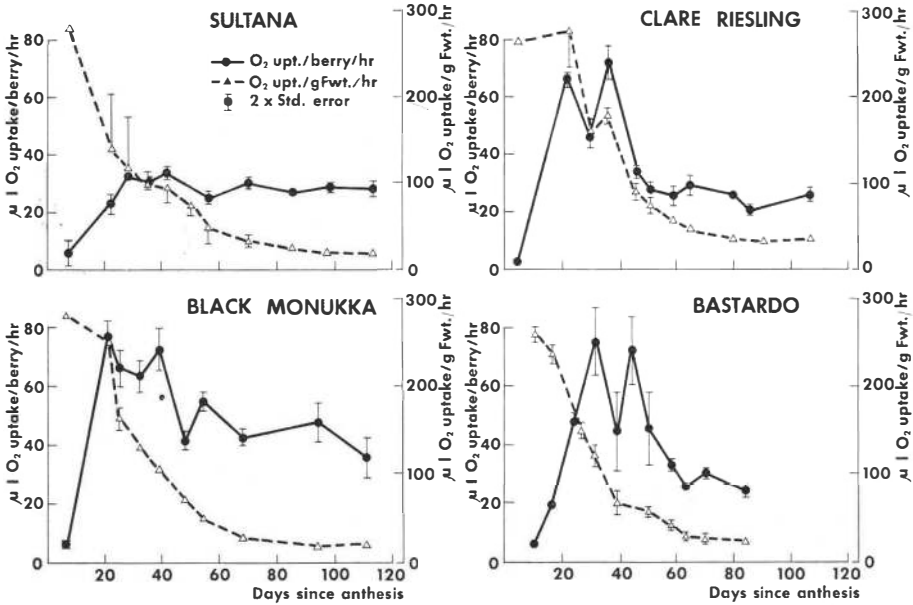
—●— Fresh Weight (g per berry). --△-- Respiratory Quotient.  $2 \times$  Std. Error (Vertical bar shown where its length exceeds the dimension of the symbol showing the mean value.)

When respiration was expressed on a per berry basis ( $\mu\text{l O}_2/\text{hr}/\text{berry}$ ) the pattern of change with development differed between the varieties. With Clare Riesling, Bastardo and Black Monukka respiratory activity per berry increased for approximately 40 days after flowering, then subsequently declined. In the seedless variety Sultana respiration per berry showed only a small initial rise and remained virtually unchanged after 30 days.

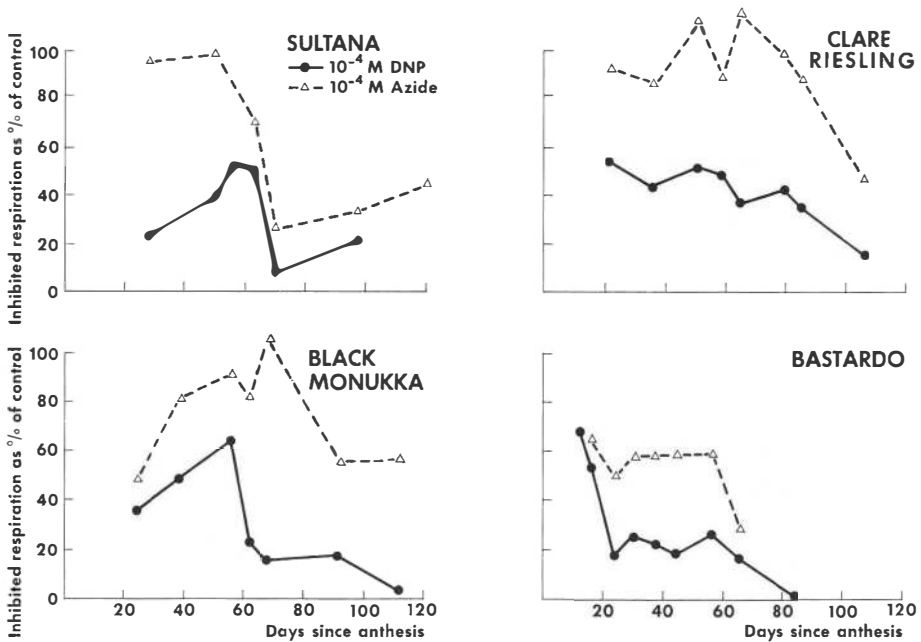
### c) Respiratory inhibitors (Figs. 9—12)

Figures 9-12 show the rate of  $\text{O}_2$  uptake of tissue slices in the presence of inhibitors as a percentage of the rates of corresponding control slices. The data were derived from the slopes of plotted Warburg measurements and represent the relative rates of  $\text{O}_2$  uptake by inhibitor treated tissue slices. These relative values, shown in Figs. 9-12, were based on pooled data from usually triplicate measurements, and precise statistical analysis of them was not possible. However it is considered that they enable broad comparisons to be made of the effects of respiratory inhibitors over the course of berry development.

1) 2,4-dinitrophenol: Berry slices infiltrated in  $1 \times 10^{-4}\text{M}$  DNP showed a reduction in  $\text{O}_2$  uptake regardless of their stage of development. In a separate series of experiments, Sultana and Bastardo berry slices were exposed to a range of DNP concentrations ( $1 \times 10^{-7}\text{M}$  to  $1 \times 10^{-3}\text{M}$ ) but at no stage was the rate of  $\text{O}_2$  uptake sti-



Figs. 5 - 8: Respiratory changes in the developing grape berry.  
 —●—  $\text{O}_2$  uptake ( $\mu\text{l O}_2$  per berry hour). -- $\Delta$ --  $\text{O}_2$  uptake ( $\mu\text{l O}_2$  per fresh weight per hour).  
 $2 \times$  Std. Error (Vertical bar shown where its length exceeds the dimension of the symbol showing the mean value).



Figs. 9 - 12: Respiration rate (oxygen uptake), expressed as a percentage of the control rate, in the presence of 2,4-dinitrophenol ( $10^{-4}$  molar) or sodium azide ( $10^{-4}$  molar).

mulated. This is in contrast to a number of climacteric fruit where DNP causes a stimulation in the preclimacteric stage.

It is considered that the change in DNP sensitivity of Sultana and Black Monukka tissue slices after the lag phase is due to an altered metabolism rather than to increased tissue permeability. In both immature and mature tissue slices,  $10^{-6}$ M was found to be threshold concentration for DNP effects (i.e. the minimum concentration at which effects on respiratory rate were first evident) and increased permeability in the mature tissue slices would have resulted in a lower threshold concentration.

2) Sodium azide: This inhibitor was either without detectable effect, or else caused some measure of inhibition which varied according to berry developmental stage and variety. Tissue slices taken from immature fruit of the unpigmented varieties Sultana and Clare Riesling were virtually unaffected by azide, while all varieties showed inhibition as they approached maturity. This is particularly evident in Fig. 13 which compares immature and mature Sultana berries. Here the increased sensitivity in slices from mature berries could have resulted from their increased permeability to azide. This is suggested by their general decline in  $O_2$  uptake with increasing azide concentrations that occurs in mature fruit compared to the more abrupt cut-off above  $10^{-5}$ M azide, which takes place with immature berry slices.

3) Potassium cyanide (Fig. 14): The effect of cyanide on respiration was tested only with the variety Sultana. Slices from immature berries showed a stimulation in  $O_2$  uptake in concentrations of  $10^{-8}$ — $10^{-6}$ M cyanide, while higher concentrations ( $10^{-3}$  and  $10^{-2}$ M) reduced respiration.

By contrast, slices taken from mature Sultana berries showed no respiratory stimulation at any cyanide concentration. The inhibitor was either without effect, or else caused inhibitions at higher concentrations.

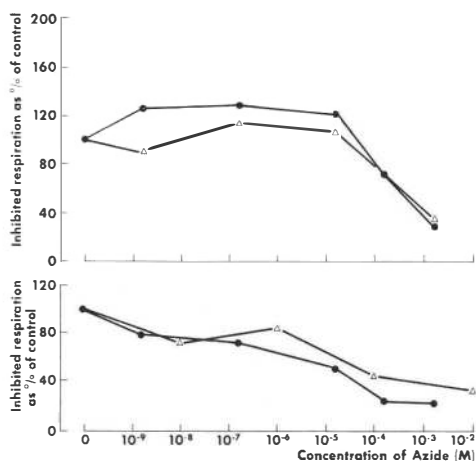


Fig. 13

Fig. 13: The effect of sodium azide concentration on respiration (oxygen uptake), expressed as a percentage of the control rate of tissue slices taken from immature (20-30 days after anthesis, above) or mature (80-90 days after anthesis, below) Sultana berries. (Duplicate measurements ●, Δ).

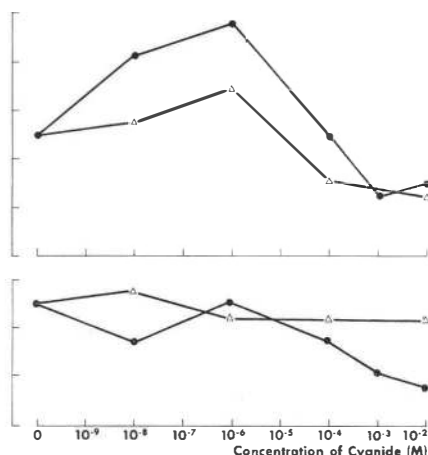


Fig. 14

Fig. 14: The effect of potassium cyanide concentration on respiration (oxygen uptake), expressed as a percentage of the control rate, of tissue slices taken from immature (20-30 days after anthesis, above) or mature (80-90 days after anthesis, below) Sultana berries. (Duplicate measurements ●, Δ).

## d) Berry nitrogen levels (Table 1)

Over the period of berry development that was examined, the total amount of insoluble nitrogen per fruit rose by a factor of four in the two varieties examined. Insoluble nitrogen (expressed as mg/100g fresh weight) showed no appreciable change. The nitrogen data were obtained from duplicate measurements made on sub-samples drawn from bulked material. It is suggested that the fluctuations shown in mg insoluble nitrogen content per 100g fresh weight for the variety Black Monukka, are not significant.

### Discussion

Previous studies on respiration, respiratory quotient (R.Q.) and substrate utilisation in the developing Sultana (KRIEDEMANN 1968 and literature cited therein) have suggested that there is a major change in berry metabolism at the lag phase. The present data confirm with other varieties the previously reported increase in R.Q. at that time and also provide information on the change in respiratory sensitivity to various metabolic inhibitors.

No consistent differences in respiration occurred between seeded and seedless, and between pigmented and non-pigmented varieties. It would appear that the influence of pigment and seed development on respiratory activity are small compared with the influence of other factors that alter during berry development, such as cell size. In other plant systems undergoing rapid cell enlargement, such as the developing pea root, cell expansion is not simply a process of inflation. The protein content per cell increases, and respiratory rate rises to a maximum at about the time the cell achieves full size (HEYES and BROWN 1965). By contrast the grape berry shows peak respiration on a per berry basis at the commencement of the phase of rapid cell expansion and in most varieties declines thereafter. On a per unit fresh weight basis and on a unit protein basis, respiratory activity declines almost continuously from anthesis to full maturity over which time cell enlargement continues to take place. During this period of declining respiratory activity the berry undergoes considerable biochemical differentiation, especially after the lag phase, when it accumulates a wide range of flavour compounds, softens, and takes on the characteristics of a fruit.

In general the respiration of mature berries was more sensitive to inhibitors than that of immature berries. In this connection cyanide and azide sensitivity have been used as a criterion for the operation of metal containing terminal oxidases (BEEVERS 1961), and on the basis of azide sensitivity all varieties examined would be expected to contain such systems especially during the second growth phase. However, the possibility of alternative terminal oxidase systems, especially in immature Sultanas, must be considered because both cyanide and possibly azide at concentrations below  $1 \times 10^{-5}M$  caused some stimulation in  $O_2$  uptake (Figs. 13, 14).

HALE (1968) has suggested that the second growth phase (post lag phase development) in grapes is analogous to a prolonged senescence and corresponds to post-climacteric changes in other fruits. In the present experiments differences in respiratory activity between immature and mature grapes in terms of  $O_2$  uptake rate, R.Q., and sensitivity to cyanide azide and DNP were recorded, but the second growth phase was not associated with a climacteric respiratory rise in the generally accepted sense. As well many climacteric fruits show a respiratory stimulation in response to DNP during their preclimacteric phase

Table 1

The Nitrogen Content<sup>1)</sup> of Grape Berries during Development

	Sultana							
Days after flowering	35	42	50	63	70	85	95	113
Acetone Insoluble N								
: mg/100g Fresh Wt	71	58	70	57	59	55	61	67
: mg/berry	.24	.20	.30	.39	.66	.64	.76	.89
	Black Monukka							
Days after flowering	21	32	39	48	54	62	68	91
Acetone Insoluble N								
: mg/100g Fresh Wt	88	47	52	60	48	42	44	44
: mg/berry	.23	.24	.35	.33	.49	.47	.63	.93

<sup>1)</sup> Data are based on duplicate determinations using sub-samples drawn from bulked material.

(SPENCER 1965). By contrast, grape tissue slices showed a reduction in O<sub>2</sub> uptake following DNP addition, irrespective of developmental stage.

### Summary

The rate of O<sub>2</sub> uptake, respiratory quotient and the effects of metabolic inhibitors on respiration were followed throughout berry development of 4 varieties of *Vitis vinifera* L. No obvious differences in respiratory characteristics were found between seeded and seedless and between pigmented and nonpigmented grapes.

Physiological differences between immature and maturing grape berries involve altered rates of respiration and a changed sensitivity to metabolic inhibitors. With development in all varieties there was a decline in O<sub>2</sub> uptake on a fresh weight basis while R.Q. values rose to greater than unity. Dinitrophenol failed to stimulate O<sub>2</sub> uptake at any stage and the inhibition of O<sub>2</sub> uptake caused by azide or DNP was more severe in mature fruit than in immature fruit. Cyanide stimulated O<sub>2</sub> uptake in tissue slices from immature Sultana berries but inhibited O<sub>2</sub> uptake in slices from more developed fruit.

These data, coupled with the absence of any major alteration in the level of insoluble nitrogen per unit fresh weight of berry suggest that respiration is both quantitatively and qualitatively different in immature compared with maturing grape berries.

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