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Anatomical aspects of grape berry development

by

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Introduction

The growth of fruits such as apple (18), orange (3), pear (4), and avocado (22) has been examined at both the whole fruit and cellular level. For these fruits it has been possible to assess the contribution made by cell division and cell enlargement towards fruit development. Some aspects of the growth and development of fruits of *Vitis vinifera* varieties have been described by Coombe (8) who provided suggestions but no quantitative data on growth at the cell level.

The present investigations were undertaken to assemble quantitative data on the growth of the sultana grape in terms of cell number and cell size. The effects of some genetic and environmental factors on berry growth at the cellular level were also examined.

Material and Methods

Experimental material and growth conditions: The grapes used in this study were from *Vitis vinifera* (cv. sultana) vines grown in three different situations. Grapes from three-year-old vines grown in 2-gallon tins of fertilized soil in a glasshouse at CSIRO Merbein, Victoria, were examined initially. Subsequently fruit from four-year-old field-grown vines at CSIRO Merbein, together with fruit from well established vines grown in the vicinity of Adelaide, South Australia, was studied. In addition, grapes from an established clonal line at Merbein, known to produce small fruits, were examined.

Glasshouse-grown berries developing out of season were sampled from September 9, 1966 (two days after the flowers were in full bloom) until December 5, 1966. The daily range of temperature was narrow, with a mean maximum of 29° C and minimum of 20° C. The average daylength was 13 hours.

Field-grown sultanas from Merbein and Adelaide were studied during the commercial ripening period. Average daylength was 14 hours in both cases. Merbein sampling was made from November 12, 1966 until February 13, 1967, with a mean daily maximum over the growing period of 31° C and minimum of 18° C. Adelaide berries were sampled from November 21, 1966 until February 27, 1967. The mean daily maximum was 26° C and 16° C minimum. Further climatic details are available in Bulletin No. 1 of the Commonwealth Bureau of Meteorology.

Berry sampling and measurement techniques: In the field experiment at Merbein two selected bunches were divided into longitudinal sectors to facilitate sampling. Each week from the stage of full bloom of the flowers to the stage of full fruit maturity 10 berries per bunch were removed over the length of each sector. The glasshouse vine bore a single bunch from which 15 berries were sampled each week using this system.

Following harvest the pedicels were sliced off level with the surface of the fruit, and length-breadth dimensions taken. The berries were ranked according to

their fresh weight, and extreme sized berries were discarded. Five median sized fruits were taken for pericarp cell counts. The remaining fruits were used for measuring dry weigth, % moisture, refractive index, acid titre, and for wax embedding.

Overall berry dimensions were measured to the nearest 0.1 mm using dial calipers. Percentage moisture was obtained after drying the material to a constant weight at 80° C in an oven with forced draught. The refractive index of expressed juice was measured with a hand refractometer calibrated for percent sucrose. These values were used to derive specific gravity (Table from: WILEY: "Principles and practice of agricultural analysis" 1914).

The sugar data for the Adelaide fruit were based on chemical analysis following chromatographic separation (10). Specimens for wax embedding were fixed in F.A.A. [formalin, acetic acid, 50% alcohol (5:5:90)], dehydrated, and cleared under vacuum in either an ethanol toluene mixture or in tertiary butyl alcohol, and embedded in paraffin wax (M. P. 60° C) containing ceresin. Sections (6—20 μ) were cut on a rotary microtome, stained with safranin and fast green and mounted in Canada balsam. The wax embedded specimens were used for counting the number of pericarp cells across a transverse section, observing the occurrence of mitotic figures and estimating the percentage of the berry's volume occupied by the pericarp tissue.

The percentage of the berry represented by pericarp was determindet as follows: An L. S. image of the whole mount was projected onto a ground glass screen 9×7 cm and the outlines of individual tissues traced. The tissues' close resemblance to prolate spheroids (as seen in Fig. 4 and 5) permitted volume calculations from length and breadth measurements. Pericarp volume was expressed as a percentage of the total volume of embedded fruits and used to estimate the volume of pericarp in berries sampled for maceration.

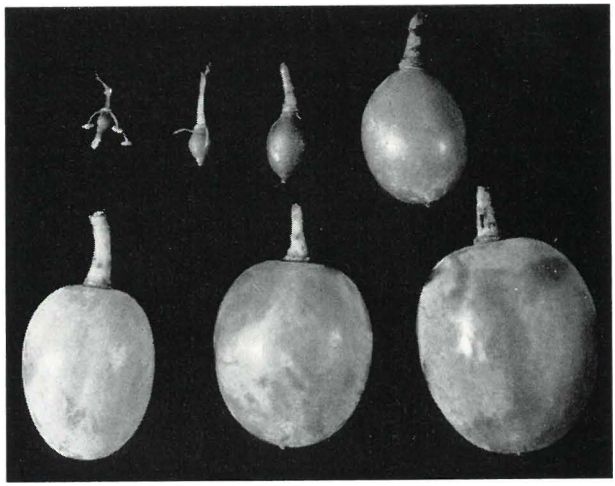

Fig. 1: Morphological changes during the growth of the Sultana berry.
Upper row: Phase 1, from anthesis to the lag period showing berries (left to right) at 0, 2, 9, 23 days from anthesis. Lower row: Phase 2. Post „colour change" development at 44, 58 and 93 days from anthesis (mag. × 2).

The procedures used for maceration [modification of BROWN and BROADBENT (7)] were as follows: For immature fruit a weighed longitudinal wedge of pericarp tissue from five replicate berries was placed in 2 ml of 5% chromic acid for 24 hours The suspension was then agitated for one hour on a wrist action shaker and thoroughly stirred for several minutes using a pasteur pipette fitted with a rubber bulb to complete cell separation. Aliquots were transferred to a haemocytometer slide for cell counts. Mature berries which possess well vacuolated and easily ruptured cells were examined by a modified technique. They were placed in a mixture of 10% nitric acid and 10% chromic acid (1 : 1 volume/volume) for 15—18 hours. Me-

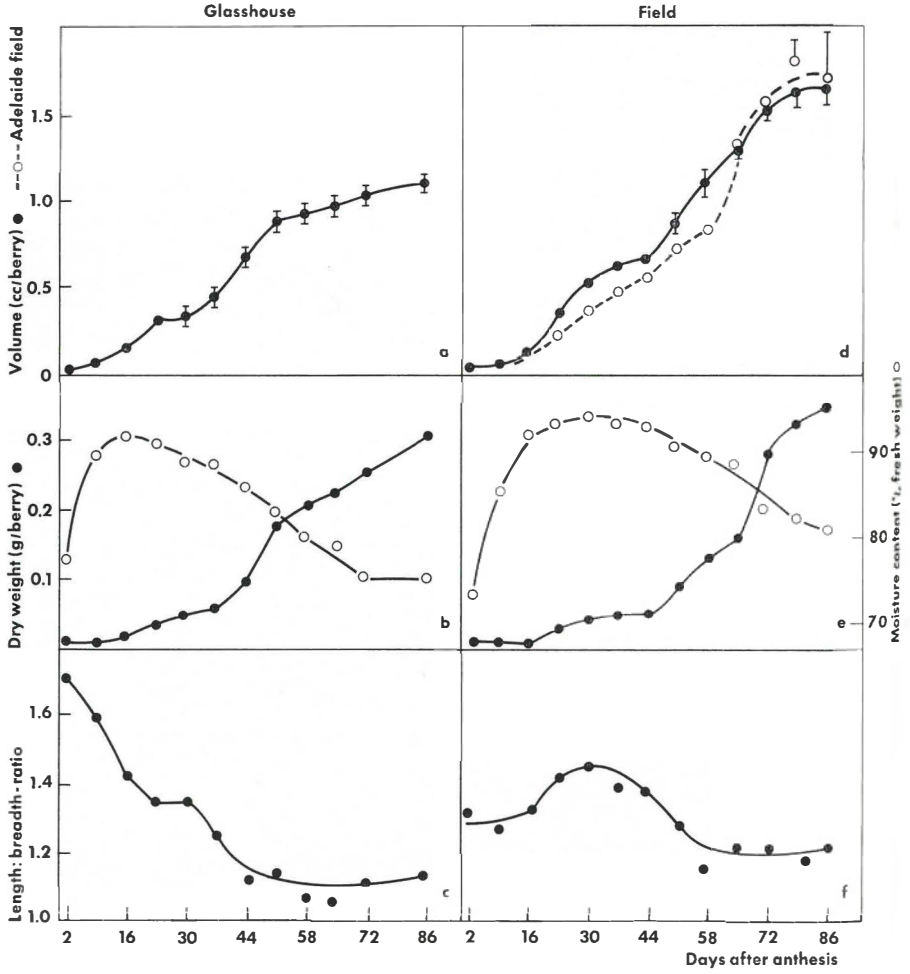

Fig. 2: Sultana berry growth: Changes in volume, dry weight, moisture content and length: breadth ratio of berries grown under glasshouse (a, b, c) and Merbein field (d, e, f) conditions.

I = 2 × std. error indicated in a and f where this figure exceeds the size of the symbol showing mean values.

chanical shaking was omitted but the cell suspension was agitated very cautiously with a wide mouthed pasteur pipette to bring about cell separation.

Cell counts: These were made using a haemocytometer slide which enclosed 3.2 mm^3 of the cell suspension. Replicate counts were made on successive aliquots to give a mean count with a percentage error $\left(\frac{SE}{mean}\ \%\right)$ less than 5%. The number of cells per unit volume of pericarp was derived from these counts and in turn the average volume per pericarp cell and the total number of cells in the pericarp was obtained.

Results

1. General characteristics of berry growth in glasshouse and field

Sultana berries at various stages of development are shown in Fig. 1. Normally 100 days or thereabouts were required for complete development from full bloom of the flowers to full maturity of the fruit. The upper row of fruits in Fig. 1, commonly referred to as „immature", were at this stage green and firm. Approximately midway between anthesis and maturity the berries changed their appearance (see lower row in Fig. 1). They became translucent, less green in colour, more succulent in texture, and increased their volume approximately threefold. During the later phase of berry growth accumulated organic acids were steadily dissipated and there was a concurrent rise in sugar content (see Fig. 2).

(a) Changes in volume, conformation, moisture content and dry weight

Berry growth, however measured, followed a biphasic pattern under both glasshouse and field conditions. For convenience, development prior to the "colour change" (véraison) is referred to as the first growth period, while subsequent growth up to maturity is referred to as the second growth period. It was found that the lag phase separating these growth periods could be displaced in time and its duration was variable, lasting for at least a week under glasshouse conditions. It was virtually non-existent under South Australian field conditions (Fig. 2a and 2b). Different environmental factors, together with possible differences in cultural conditions between the three situations, are likely to be responsible for this effect. Final berry size, which was clearly different for the three situations, may have been influenced by the same variables.

Berry volume gave a double sigmoid growth curve (Fig. 2a and 2d) similar to the corresponding dry weight curve (Fig. 2b and 2e). Berry volume at maturity was on the average three times greater than the volume during the lag phase, the actual ratios being 3.4 in glasshouse, 3.0 small berried clone, 2.7 Merbein field, 2.5 Adelaide field.

Berry conformation also underwent a series of changes during development. A visual difference to be noted in Fig. 1 is the expansion in the fruit's equatorial plane which occurred during the second period of growth producing the typical berry "fattening". The quantitative result was a decrease in the length to breadth ratio illustrated in Fig. 2c and 2f.

Changes in moisture content (expressed as a percentage of fresh weight) occurred during development (Fig. 2b and 2e). In both the field and the glasshouse a maximum value was reached 3—4 weeks after anthesis, which was followed by a gradual decline as the fruit matured.

(b) Changes in sugar and organic acid content

Data from field grown material at Merbein and Adelaide are presented in Fig. 3. Acid was accumulated by the immature fruit until the lag phase. There was a subsequent rapid fall in acid content which coincided with sugar accumula-

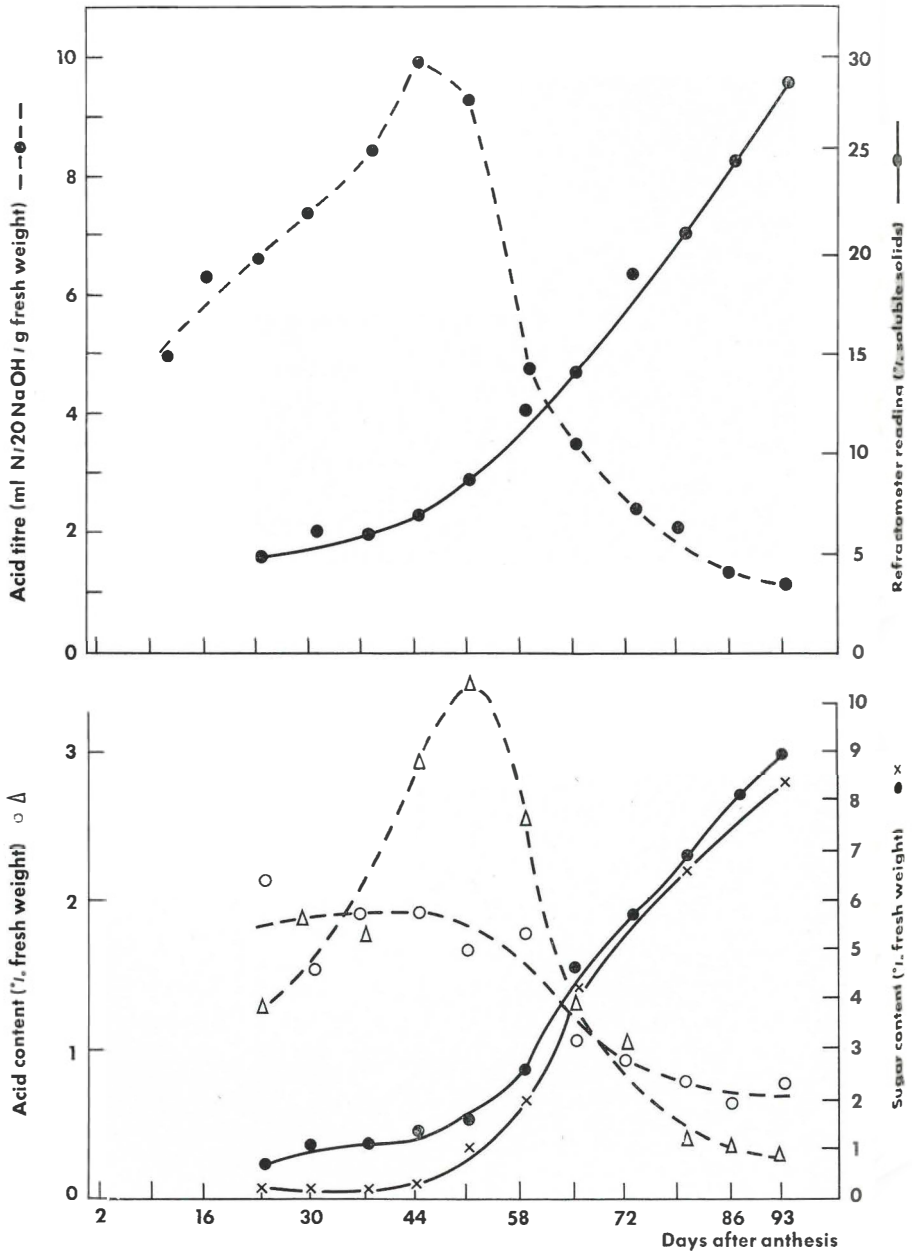

Fig. 3: Changes in acidity and sugar content: Field grown berries at Merbein (above) and Adelaide (below: Δ malic acid, o tartaric acid, • glucose, × fructose).

tion (shown in Fig. 3 above). Fig. 3 below gives detailed data for grapes grown at Adelaide. Here an abrupt decline in total acid followed the lag phase. The maximum acid titre of 10 ml $\frac{N}{20}$ Na OH/g fresh weight reached at Merbein by the seventh week

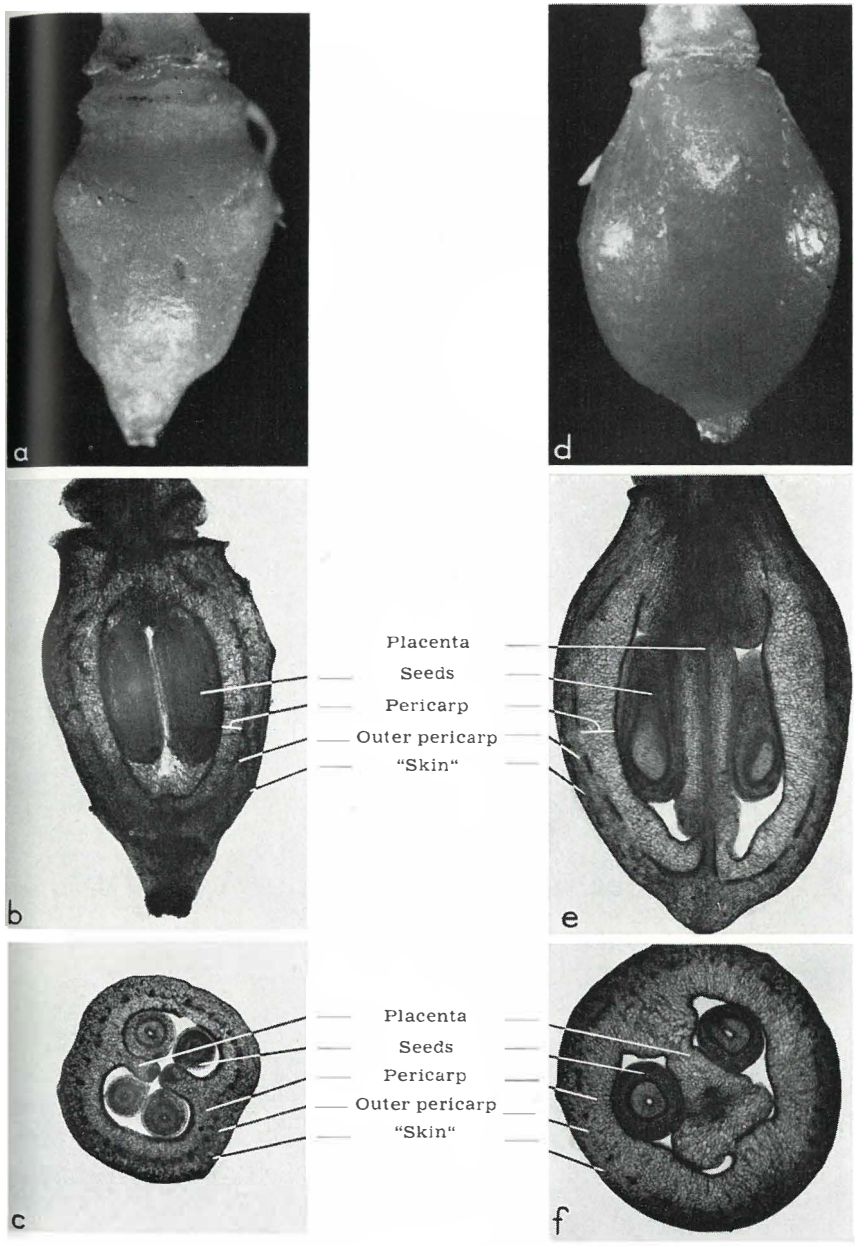

Fig. 4: Gross anatomical features of the developing berry
(unstained hand sections).
a, b, c: 2 days after anthesis (mag. × 16); d, e, f: 9 days after anthesis (mag. × 11).

was equivalent to a total acidity of 3.75% and should be compared with the peak value of 5.10% obtained after 8 weeks at Adelaide.

The sugar levels are not readily comparable because the Merbein data was based on refractometer measurements (Fig. 3 above) and included was soluble solids

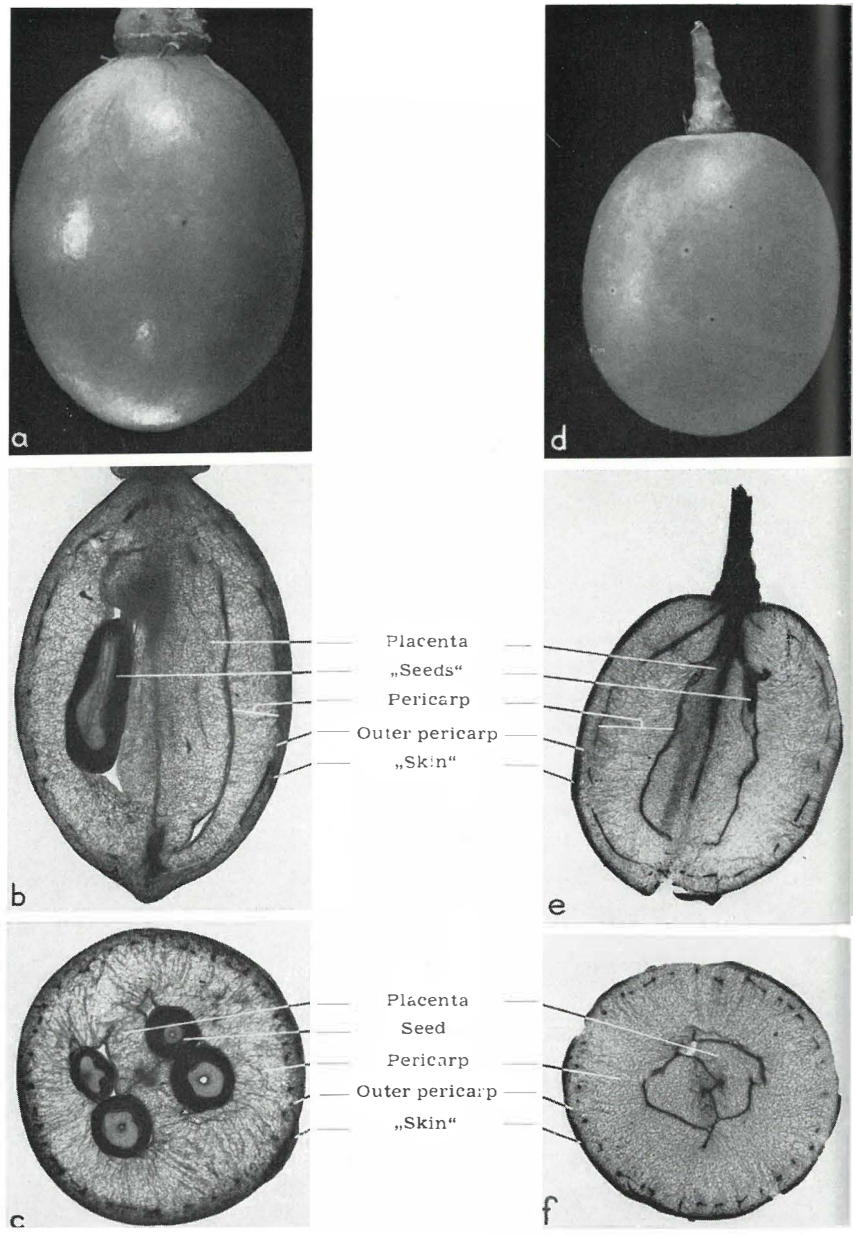

Fig. 5: Gross anatomical features of the developing berry (unstained hand sections).
a, b, c: 23 days after anthesis (mag. × 6); d, e, f: 93 days after anthesis (mag. × 2.6).

other than sugar while the Adelaide data was based on chemical analysis (shown in Fig. 3 below). At both sites sugar accumulation commenced 6 to 7 weeks after anthesis and continued at an almost linear rate for the remainder of the sampling period.

2. Anatomical changes in the developing berry

Fig. 4 and 5 give a macroscopic view of the anatomical change that occurred in sultana berries grown under field conditions at Merbein. Intact berries 2, 9, 23 and 93 days after anthesis were photographed together with hand sections (L.S. and T.S.) of similar material.

The pericarp of the berry refers to the tissue surrounding the locules and extending to the outer ring of vascular bundles beneath the skin. These lines of demarcation were especially clear in the transverse sections.

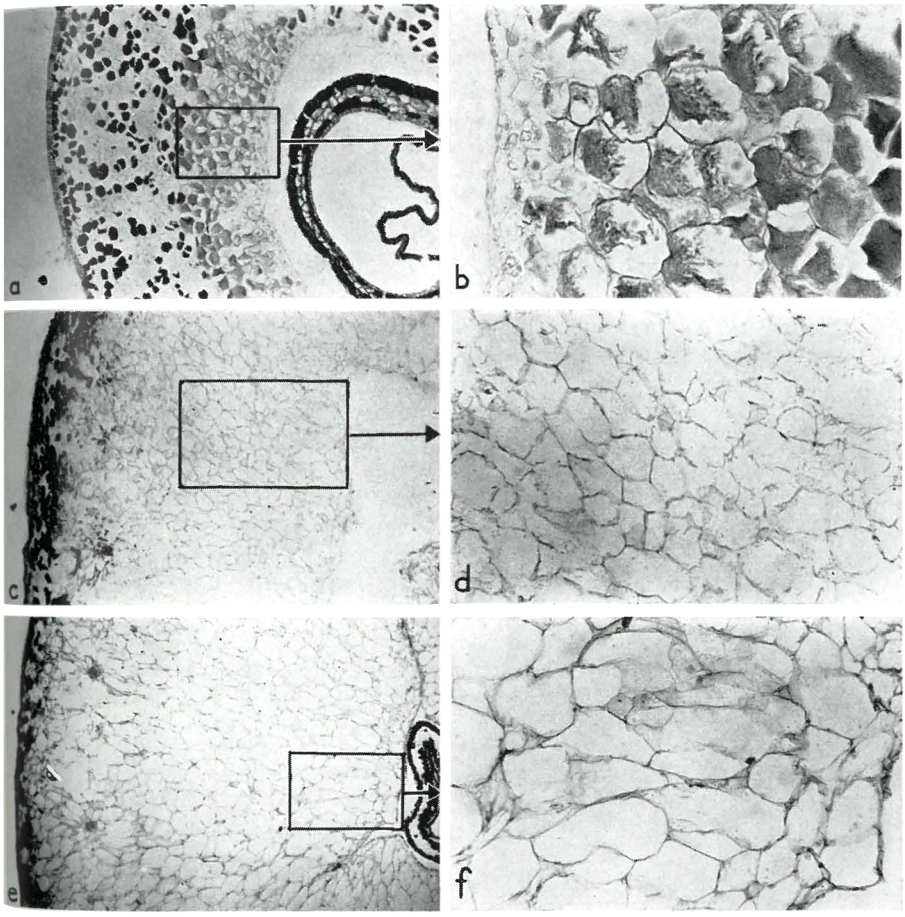

Fig. 6: Cellular changes in the pericarp (stained sections from wax embedded specimens).
a and b: 2 days after anthesis (a: mag. × 68, b: mag. × 308), c and d: 23 days after anthesis (c: mag. × 24, d: mag. × 68), e and f: 93 days after anthesis (e: mag. × 17, f: mag. × 68).

The berry underwent a major change in its morphology between anthesis and maturity. The cuboid parenchymatous pericarp cells of very young fruits became radially elongated as the berry matured. This coincided with berry „fattening" which occurred during the period of sugar accumulation. The degree of radial elongation increased towards the placenta (see Fig. 6). Two days after anthesis the pericarp tissue comprised only 22% of field grown berries (or 9% of glasshouse material), compared with 64% at maturity.

The placenta (or septum) represented by the lobes of tissue between the locules as seen in T.S., enlarged significantly during development despite the incompleteness of seed development (compare Fig. 4c with 5f). The septum and pericarp together formed the major storage sites for sugar accumulation within the berry.

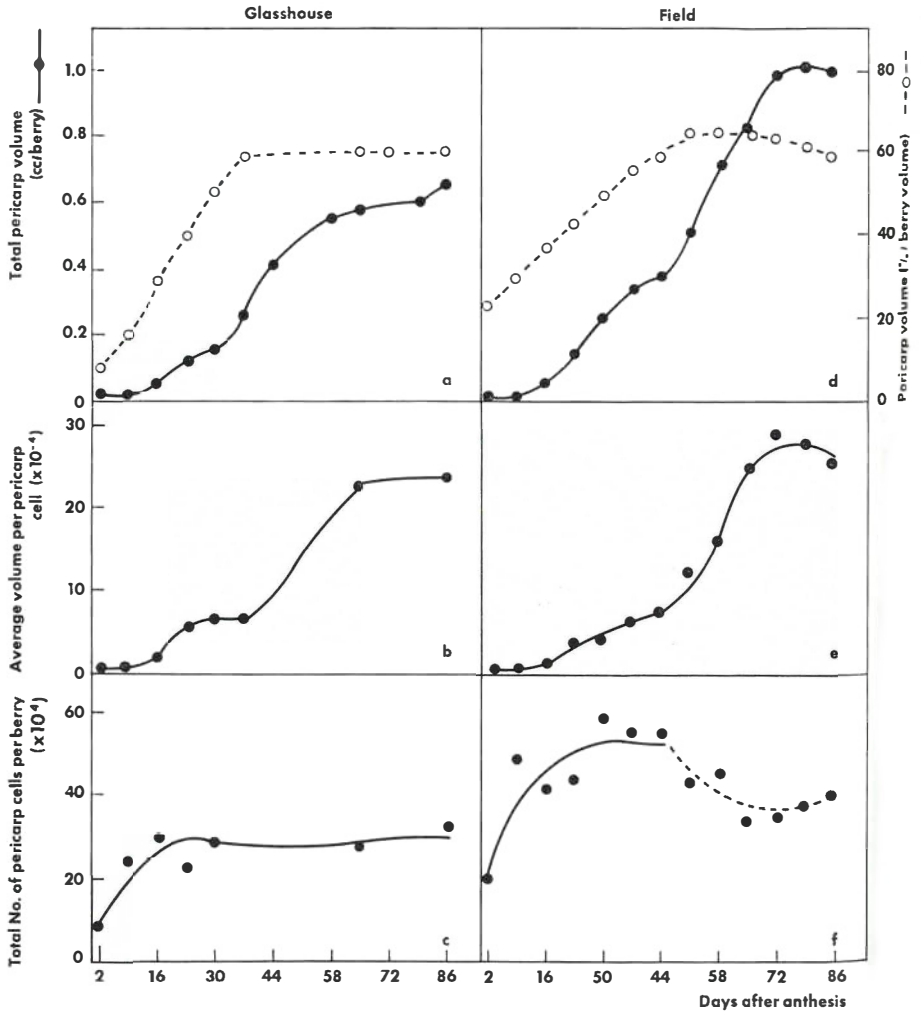

Fig. 7: Growth of the pericarp: Changes in total pericarp volume, average volume per pericarp cell and total number of pericarp cells per berry.

The "seeds" of the sultana were prominent features only in the early developmental stages (Fig. 4c and 4f); their arrested development results in a diminutive structure within the mature fruit (Fig. 5e). The complete absence of seeds in Fig. 5f is due to the section being taken through the equatorial plane of the berry whereas the seeds lie closer to the pedicel end.

3. Pericarp growth: The relative extent of cell division and cell enlargement

Cell division and cell enlargement both contributed to pericarp growth in the early post-anthesis stage. Fig. 6 reveals that both the linear number of cells across the pericarp and their average cross sectional area (and so volume) increased during early berry development. Beyond the lag phase there was a change only in cell size. Cell counts made along 6 transects, with a fixed relationship to the plane of the placenta, are shown in Fig. 8. The number of cells increased approximately up to 6 weeks.

A quantitative assessment of growth in the pericarp and the results for glasshouse and field conditions are summarised in Fig. 7. Despite a size difference between glasshouse and field-grown berries, the percentage of final volume occupied by pericarp was similar (see Fig. 7a and 7d). A value of approximately 64% was attained at the onset of the second growth period and this changed very little during ensuing growth. Pericarp volume (cc per fruit) paralleled overall changes in berry volume through the second growth period and contributed substantially to the observable growth pattern of the whole berry. The pericarp tissue was relatively homogeneous, which simplified sampling. The relative importance of cell division and cell expansion in the growth of this tissue was examined in some detail because pericarp is a major site for sugar accumulation.

From haemocytometer counts a value for the number of cells per unit fresh weight of pericarp tissue sampled was obtained, and from this, together with specific gravity, the average volume per cell was calculated. These data are shown in Fig. 7b and 7e. The total number of cells present within the pericarp of the sampled berry was taken as the quotient of pericarp volume and average volume per cell (see Fig. 7c and 7f). The mean volume per cell showed a biphasic increase under both glasshouse and field conditions. The total number of cells present in the pericarp attained its maximum about one week before the onset of the lag, and remained essentially unchanged thereafter. This corresponded to the period from December 19 to December 26, 1966, in the field at Merbein and approximately January 9, 1967, at Adelaide. The apparent decrease in cell number during the second growth period in the field was almost certainly a problem of methodology. An under-estimation of cell number for tissues consisting of well-vacuolated and easily-ruptured cells is a recognized deficiency of the maceration technique.

Average cell volume of pericarp tissue was independently estimated from measurements of length and breadth, assuming the cells to be prolate spheroids. The biphasic growth pattern was again apparent even though the absolute values for cell volume were slightly less when estimated by this method.

4. Berry size as a function of pericarp cell volume and number

Since berry size is ultimately a product of cell size and cell number, genetic and environmental factors could influence berry size by affecting either of these quantities. Some relevant data are given in Fig. 8 which shows berry volume at maturity (histogram) and changes in pericarp cell volume during development.

Genetic effects on berry growth were demonstrated by the two clonal lines grown together in the field at Merbein, the one characterised by small and the other by normal-sized fruit. The smaller berries had, on the average, smaller pericarp cells (see Fig. 8) but the total number of cells in the pericarp was comparable at 55×10^4 for both small and normal-sized berries. Hence cell volume rather than number was the primary determinant of berry size in this comparison. The data in Fig. 8 also suggest that ontogenetic growth patterns were similar under given environmental conditions, despite the genetic differences.

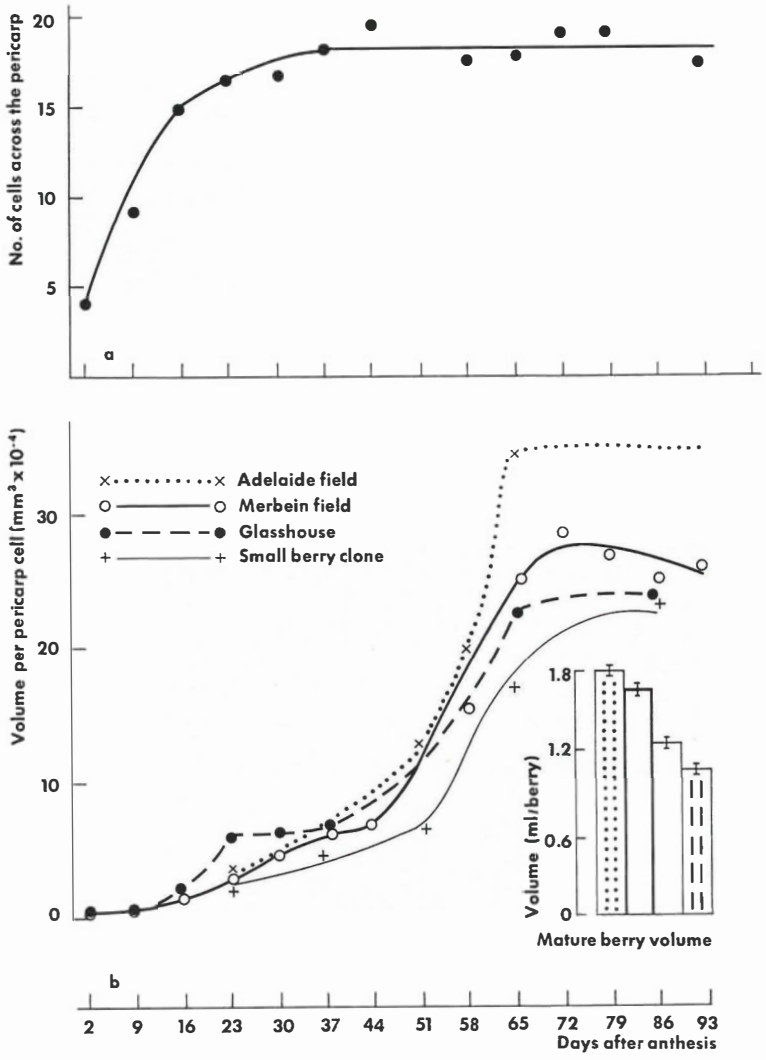

Fig. 8: Pericarp growth at the cell level

a: The number of pericarp cells across a transverse section of embedded berries (Merbein field material); b: Mean volume per pericarp cell and mature berry volume (histogram) Corresponding symbols in curves and histogram. I = 2 × std. error.

Pericarp cell size was again the prime determinant of berry volume in the case of the Adelaide grapes. Grapes from this source were the largest encountered, but they had fewer pericarp cells (50×10^4/berry) than Merbein field-grown material. Their average pericarp cell volume was the largest observed, being 32 % greater than for normal-sized Merbein berries.

In contrast to these situations cell number rather than volume determined the overall size of mature berries grown in the glasshouse. The total number of cells in the pericarp of these berries (30×10^4/berry) was only 55% of the figure for field-grown grapes (Fig. 7c and 7f). Nevertheless the mean volume per pericarp cell was similar to that of field-grown grapes (see Fig. 7).

Discussion

From the present experiments some of the changes that occur during the growth and development of sultana berries have been documented. Many of the changes recorded here such as those of volume, shape (length and breadth), percentage moisture content, dry weight and sugar and acid content were essentially similar to those recorded by other workers (8, 14). However, in the present study information has been assembled on the growth of pericarp tissue in cellular terms. This tissue accounts for over half the volume of mature berries and its growth has a dominant effect on the overall growth of the grape. This is also the situation for apricots (11), peach (6) and apple (5) and may be regarded as a general feature of the growth of many fruits.

In the sultana berry pericarp growth is the product of both cell division and cell expansion up to approximately 25 days after anthesis. From then on pericarp growth is due to cell expansion alone. Cell division in the pericarp begins 5—10 days before anthesis (8). The present results suggest it continues for approximately 25 days after anthesis, a longer period than has been previously reported (8, 14, 15, 16). Wide variations in the duration of division is known to occur in other fruits, for example, mesocarp cells of the apple continue division for 4 to 6 weeks after anthesis (5).

It is evident from Fig. 6 f that differences in cell shape at various positions in the pericarp occur as the berries increase in size. Pericarp cells near the vascular bundles elongate tangentially while those towards the placenta elongate radially. This pattern of development is characteristic of fruits enlarging after division has ceased (9, 11).

Pericarp cell enlargement in sultanas proceeds at a maximum rate during the early post-anthesis period. The pericarp cell volume increases approximately twenty-fold during the first sixteen days of development in berries from field-grown vines at Merbein. The substantial increase in pericarp cell volume is accompanied by an increase in moisture percentage. This period of rapid berry growth corresponds to a stage in the grape's development when it is extremely sensitive to water stress. ALEXANDER (1) found that brief periods of stress for up to 4 weeks after flowering resulted in a substantial berry drop. He also established that the sultana was no longer prone to abscission once the berry had entered the second period of growth. At that stage pericarp cell expansion is the sole determinant of growth while cell division has ceased and the percentage moisture content of the berries is decreasing.

The genetic constitution of the plant can influence fruit growth; for example, selection at Merbein has resulted in a clonal line characterised by small berries (ANTCLIFF, unpublished). These berries are inherently smaller than those borne on neighbouring vines and in the present work it has been shown that this is due to a reduction in the size of the pericarp cells.

Environmental influences can also affect fruit growth and these effects have been studied widely; for example, on apple (20), sour cherry (19), apricot (17), as well as on grapes (12, 13, 14, 15, 16, 21). In general an increased temperature increases fruit growth up to an optimum, beyond which the growth rate drops markedly. In contrast to most fruits, apricot is sensitive to temperature only during the first growth period, final fruit size being independent of temperature during the second growth period (11).

Japanese work suggests that night temperature has a significant influence on grape berry growth (12, 13, 14, 15, 16). In the present study, the glasshouse berries had a mean daily minimum of 20° C and developed more quickly than those in the field at Merbein (min. 18° C) or Adelaide (min. 16° C). A number of studies indicate that the optimum temperature for berry growth is highest during the first two weeks of berry development (14, 15, 16, 21). If the temperature during this period exceeds the optimum temperature, then the fruit's growth can be permanently retarded. The present results suggest that these temperature influences are possibly due to effects on cell division.

Temperature is also known to influence berry shape (14, 15, 16), a low temperature giving a more elongated berry. A possibly similar effect is seen in Fig. 2 c and 2 f, where the glasshouse berries, grown under a higher minimum temperature have a lower length: breadth ratio, i. e. are more nearly spherical than the berries growing under field conditions. Similar observations were made by TUKEY (20) on apples.

There are also indications from the literature that photoperiod affects berry growth. Long days are known to enhance vegetative development (2); however there is a suggestion (loc. cit.) that the reverse holds for berry development where shortening days hasten the ripening process. Such an effect may have caused the glasshouse grapes (13 hour day) to develop more rapidly than the field-grown grapes (14 hour day).

Summary

The anatomical development of the sultana-grape berry has been followed from anthesis to maturity on material grown under glasshouse and field conditions including field-grown clonal lines differing in final fruit size. Fresh weight, volume, berry dimensions, moisture content and dry weight were measured on whole berries. Pericarp growth was studied at the cell level. Pericarp growth is basically responsible for the overall growth of the berry and this tissue represents 64% of the mature fruit's total volume.

The period required for complete berry development (approximately 100 days) falls into two major growth periods separated by a lag phase. Before the lag phase pericarp growth results partly from cell division but mainly from cell enlargement. After the lag phase pericarp growth results entirely from cell enlargement. Cell division in the pericarp ceases about one week before the lag phase.

Berry size differences between clonal lines were primarily due to differences in the size of pericarp cells. Berry size differences between fruits grown in the glasshouse and in the field at Merbein were due to differences in both pericarp cell number and cell size.

Acknowledgements

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Literature Cited

1. ALEXANDER, D. McE.: Effect of high temperature regimes or short periods of water stress on development of small fruiting sultana vines. Austr. J. Agric. Res. **16**, 817—823 (1965).
2. ALLEWELDT, G.: Tageslänge und Temperatur — Regulatoren für Wachstum und Ruhe der Pflanzen. Umschau **64**, 661—665 (1964).
3. BAIN, J.: Morphological, anatomical and physiological changes in the developing fruit of the Valencia orange *Citrus sinensis* (L) Osbeck. Austr. J. Bot. **6**, 1—24 (1958).
4. — — : Some morphological, anatomical and physiological changes in the pear fruit *(Pyrus communis* var. Williams Bon Chrétienne) during development and following harvest. Austr. J. Bot. **9**, 99—123 (1961).
5. — — and ROBERTSON, R. N.: The physiology of growth in apple fruits. 1. Cell size, cell number and fruit development. Austr. J. Sci. Res. Ser. B **4**, 75—91 (1951).
6. BRADLEY, M. V.: Mean cell size in the mescarp of mature peaches of different sizes. Proc. Amer. Soc. Hort. Sci. **73**, 120—124 (1959).
7. BROWN, R. and BROADBENT, D.: The development of cells in the growing zones of the root. J. Exp. Bot. **1**, 249—263 (1951).
8. COOMBE, B. G.: Relationship of growth development to changes in sugars, auxins and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. **35**, 241—250 (1960).
9. CRANE, J. C.: Growth substances in fruit setting and development. Ann. Rev. Plant. Physiol. **15**, 303—326 (1964).
10. HARDY, P. J.: Sucrose breakdown and synthesis in the ripening grape berry. Austr. J. Biol. Sci. **20**, 465—470 (1967).
11. JACKSON, D. T. and COOMBE, B. G.: The growth of apricot fruit. 1. Morphological changes during development and the effects of various tree factors. Austr. J. Agric. Res. **17**, 465—477 (1966).
12. KOBAYASHI, A., OKAMOTO, S., YUKINAGA, H., and NAKANISHI, S.: Studies on the thermal conditions of grapes. I. Effects of night temperatures on the growth and resipiration of several fruit trees including grapes. Bull. Kyoto Univ. Inst. Food Sci. **24**, 20—28 (1960 a).
13. — — YUKINAGA, H., FUKUSHIMA, T. and WADA, H.: Studies on the thermal conditions of grapes. II. Effects of night temperatures on the growth, yield and quality of Delaware grapes. Bull. Kyoto Univ. Res. Inst. Food Sci. **24**, 29—42 (1960 b).
14. — — , — — and ITANO, T.: Studies on the thermal conditions of grapes. III. Effects of night temperatures at the ripening stage on the fruit maturity and quality of Delaware grapes. J. Jap. Soc. Hort. Sci. **34**, 25—32 (1965 a).
15. — — , — — and NII, N.: Studies on the thermal conditions of grapes. IV. Effect of day and night temperatures on the growth of Delaware grapes. J. Jap. Soc. Hort. Sci. **34**, 77—84 (1965 b).
16. — — , — — and MUTSUNAGA, E.: Studies on the thermal conditions of grapes. V. Berry growth, yield and quality of Muscat of Alexandria as affected by night temperature. J. Jap. Soc. Hort. Sci. **34**, 151—158 (1965 c).
17. LILLELAND, O.: Growth study of the apricot fruit. Proc. Amer. Soc. Hort. Sci. **27**, 237—245 (1930).
18. MARTIN, D., LEWIS, T. L. and CERNY, J.: The physiology of growth in apple fruits. VII. Between-tree variation of cell physiology in relation to disorder incidence. Austr. J. Biol. Sci. **7**, 211—220 (1954).
19. TUKEY, H. B.: Growth of the embryo, seed and pericarp of the sour cherry *(Prunus cerasus)* in relation to season of fruit ripening. Proc. Amer. Soc. Hort. Sci. **31**, 125—144 (1934).
20. — — : Some effects of night temperatures on the growth of McIntosh apples. I. Proc. Amer. Soc. Hort. Sci. **68**, 32—43 (1956).
21. — — : Effects of controlled temperatures following bloom on berry development of the Concord grape *(Vitis labrusca)*. Proc. Amer. Soc. Hort. Sci. **71**, 157—166 (1958).
22. VALMAYOR, R. V.: Cellular development of the Avocado from blossom to maturity. Univ. of Florida Ph. D. thesis (1964).
23. WILEY, H. W.: Principles and practice of agricultural analysis. 2nd Ed. Easton, Pa. Chem. Publ. Co. (1914).

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