It has been concluded that peanut and tomato possess an endogenous, time-measuring mechanism, which is slightly temperature dependent, and that for optimal development the external period must be synchronized with the endogenous period of the plant.

ACKNOWLEDGEMENTS

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RELATIONSHIP OF GROWTH AND DEVELOPMENT TO CHANGES IN SUGARS, AUXINS, AND GIBBERELLINS IN FRUIT OF SEEDED AND SEEDLESS VARIETIES OF VITIS VINIFERA [1, 2]

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There are large differences in the degree of seed development in different varieties of *Vitis vinifera* (European grape) ranging from nil in parthenocarpic varieties to complete seed development from several of the four ovules in the berries of certain other varieties. A few varieties are "seedless" because of their stenospermocarpic fruit development, i.e., their seeds begin to develop after fertilization but they abort before the development is complete. These differences in seed development are reflected in growth of the berries. This study was made to relate the development and growth of the fruit of several varieties to changes in their sugar, auxin, and gibberellin contents between anthesis and maturity.

The morphological changes in fruit of a few seeded and seedless varities of grapes have been described (16, 20, 24). Fruit morphogenesis was measured in a semi-quantitative way in the varieties used here to help interpret the other factors measured. Fruit growth studies of grapes are not extensive. Most have the disadvantage that they are based on fruit diameter measurements (33). In this study fresh weight, dry weight and volume of berries, and sugar concentration in the juice were measured.

The most detailed work on auxins in grapes has been done by Nitsch and coworkers. They demonstrated the presence of several types of auxins in nearly mature grapes (14) and made a comparative study of auxin changes during the development of berries of seeded and seedless Concord (16). Gustafson's comparison of auxins in seeded and seedless grapes was made on flowers before anthesis (6). Ferenczy (5) showed the presence of an auxin, possibly IAA[4], in the juice of ripe grapes. The measurement of gibberellin activity was attempted because of

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[4] Abbreviations used are: IAA, 3-indoleacetic acid; IAN, 3-indoleacetonitrile; IAE, ethyl ester of IAA; 4-CPA, 4-chlorophenoxyacetic acid; GA_3, gibberellin A_3 (synonymous with gibberellic acid), similarly for GA_1 and GA_2.

the recently appreciated importance of these compounds as plant growth hormones (22) and the profound effects of exogenous GA_3 on the growth of seedless grapes (28).

Materials and Methods

Five varieties of grapes were chosen to give three types of seed development: A. Perfect (Muscat of Alexandria and Emperor), B. Stenospermocarpic (Seedless Emperor and Sultanina) and C. Parthenocarpic (Black Corinth). The two Emperor varieties mature later than the other varieties. The development of young fruit of Muscat and Sultanina was followed by microscopic examination of longitudinal and transverse sections (prepared in 1934 by Prof. H. P. Olmo) of ovaries taken at intervals from 10 days before to 23 days after anthesis. Measurements were made of dimensions and cell size within different seed and fruit structures to estimate changes in the rate of cell division. For example, counts were made of the number of cells along minimal distances across the pericarp; this was done in five positions on each of six berries at each stage. For Muscat, this number doubled between the third and tenth days after anthesis and during this time many new cross walls were evident. These observations were supplemented by dissection of preserved and lyophilized berries. Few measurements were made of embryos because they were difficult to dissect.

Ten vines of each variety were selected in the university vineyard at Davis during the summer of 1958. In addition, ten vines each of Corinth and Sultanina were trunk-girdled at the end of bloom and 10 days after bloom, respectively. Girdling consisted of removing a complete strip of bark (5 mm wide) down to the cambium after making two parallel knife cuts around the trunk. From these seven lots of vines, flower or fruit samples were taken every 3 to 5 days from the beginning of bloom until maturity. Each sample was a composite of several berries from many clusters on all vines. In all, no more than a tenth of each vine's crop was removed. A sub-sample of 200 berries (gradually decreasing to 50 berries as they enlarged) was weighed, its volume determined by water displacement, then dried at 90° C to measure dry weight. Berries reaching maturity dried very slowly. A second sub-sample was crushed and the percentage of sugars in the free-run juice measured with an Abbé refractometer. This instrument is calibrated against sucrose solutions but it gives a close approximation to the amount of total soluble solids, mainly glucose and fructose, in grape juice. Excluding sampling error, the error in measuring fresh weight was low ($\pm 0.2\%$) but that of volume was high (± 1 to 2%). To help follow the changes in volume, values for density (weight/volume) of each variety were graphed and a smoothed curve drawn (the density remained between 1.00 and 1.01 g/ml until the beginning of sugaring, when it increased gradually to 1.08-1.10 g/ml); volumes were recalculated from the densities read from these curves and from the fresh weights. The size of the sampling error can be gauged by the distance of points from the weight curves shown for Muscat (fig 3). Other varieties behaved similarly so their fresh weight curves are omitted and only smoothed volume curves are shown.

On 16 of the 25 sampling occasions, about one liter of berries was frozen shortly after collecting, held at $-12°$ C and later lyophilized. Samples collected 17 days after anthesis, and later, failed to dry during 2 days lyophilization unless the skins of the berries were cracked; this was done by striking the plastic bag of frozen berries against a bench. A few samples collected between 17 and 25 days after anthesis spoiled because they were not dried completely.

Extraction and Measurement of Auxins. Preliminary comparisons of extraction and purification methods led to adopting a procedure similar to that used by Nitsch (13). A weighed sample of lyophilized berries (1-1.5 g dry wt) was ground and extracted with 50 ml methanol at 4° C for 3 to 4 hours. The filtrate was dried under an airstream and the residue extracted four times with acetonitrile and hexane (1:1) in a separatory funnel. After shaking, the clear yellow acetonitrile solution was run off and divided into two or three equal parts (in case repeats were needed), then dried under an airstream and stored at $-12°$ C. Three hours before bioassay, a measured volume of sucrose-buffer solution (15) was added to the residue to give a desired concentration of grape extract. After 1 hour, this was filtered and 0.5 ml aliquots of the filtrate (or dilutions of it) were used for the bioassay.

Auxin was measured by the method of Nitsch and Nitsch (15) using straight growth of sections of Avena first internodes. Their method was modified as follows: seed was grown in vermiculite for 60 hours at 26° C; only two sections were placed in each tube containing 0.5 ml sucrose-buffer-auxin solution and each treatment was replicated four times (26); the tubes were rotated at 0.2 rpm in a roller tube apparatus. A control without IAA and five solutions containing IAA at concentrations between 0.001 and 0.1 μg per 0.5 ml were run with every test.

For each extract, three dilutions were assayed (or two in some repeated runs). Solutions from some extracts with high auxin concentration had to be diluted to as little as 5 mg of berries (dry wt) per 0.5 ml to fall within the test's sensitivity. The strength of solutions from extracts with low auxin concentration was increased to 24 mg per 0.5 ml. Some strong solutions of extract were toxic to the internode sections causing them to become flaccid and reducing their elongation. Bentley (1) has also described this. The trouble from phytotoxicity was confined to extracts of berries sampled later than 14 days after anthesis and ceased when sugaring began. Usually only slight dilutions were needed to remove phytotoxicity and reveal auxin activity. In each run, from 8 to 12 extracts were compared at two or three dilutions of each. Each sample of lyophil-

ized grapes was extracted and assayed two or three times.

The dilutions of grape extracts showed a steeper dosage-response curve than IAA. The average ratio of the slope of the grape extract response curve to the IAA curve was about two. This difference could be due to the presence of inhibitors or of auxins other than IAA. Nitsch and Nitsch (14) have shown that grapes probably contain IAN and IAE as well as IAA. They later (15) showed that the standard curves for IAN and IAE are steeper than that for IAA. Because of this difference in slope the IAA standard curve could not be used to compare results, so the following arbitrary procedure was used: A new standard curve was prepared, double the slope of the IAA curve and intersecting the latter at its midpoint, viz. 0.01 μg IAA per 0.5 ml (this reduced high values and increased low values). From this curve, internode elongation was corrected to "μg—equivalents of auxin". These were adjusted to "μg—equivalents of auxin per gram dry wt. of grapes" and these are called "auxin units".

Extraction and Measurement of Gibberellins. The terms "gibberellin" and "gibberellin activity" are used to include known gibberellins and other gibberellin-like compounds which have the property of causing elongation of seedlings of the dwarf mutants of maize used in the bioassay. Measurements of gibberellin activity were made on all samples which were assayed for auxin content, and, in addition, flowers at anthesis were assayed after separation into ovaries and stamens. The following extraction procedure was used (Phinney, personal communication): a lyophilized sample was weighed (1-8 g dry wt of ovaries, less for stamens), ground with a pestle and mortar, extracted with 30 to 50 ml acetone for 3 hours at room temperature, then filtered; the filtrate was dried under an airstream, extracted with ethyl acetate in the presence of excess anhydrous sodium sulfate to remove water, then filtered; the filtrate was dried under an airstream and stored at −12° C until an hour or two before its bioassay when a measured volume (3-10 ml) of water plus Tween 20 (polyoxyethylene sorbitan monolaurate, 0.05 % v/v) was added. After filtering, 0.1 ml aliquots were applied to each test plant.

The amount of gibberellin in these extracts was measured by Phinney's dwarf maize bioassay (11; Phinney, personal communication). The dwarf mutants d-1 and d-5 were used for these assays (seed kindly supplied by Prof. B. O. Phinney); d-1 was used for the first test of all samples, then, when retesting active samples, comparisons were made with d-5. Unknown extracts, a control solution and standard solutions containing from 0.001 to 0.2 μg GA_3 per 0.1 ml were combined in randomized complete block designs with 4 to 8 replicates. Seven days after treatment the lengths of the first and second leaf sheaths were measured in mm and added (=D). The average of the values for control seedlings (D_o) was subtracted from the individual values (D_x) for control and treated seedlings to give a value for elongation ($E_x = D_x - D_o$). Results were compared by analysis of variance after transformation to log ($E_x + 10$). This transformation gave a straight line relation to concentration of GA_3 on a log scale between 0.001 and 0.2 μg GA_3 per plant and it increased the sensitivity of comparisons at low concentrations.

This study of the changes in the levels of auxins and gibberellins during the development of grapes should be considered as exploratory. Only crude extracts were made and, for gibberellin assays, dilution curves of these extracts were not made. More experiments are needed using berries sampled at more frequent intervals, extracted by several methods, purified by chromatography and tested with more than one bioassay method.

Results and Discussion

Fruit Development. The gross changes in seed and fruit structures during the development of Muscat are shown in figure 1. An estimate of the rate of cell division in different structures of the fruit of Muscat and Sultanina is shown in figure 2.

The development of a Muscat berry is probably typical of all seeded grapes apart from varietal differences in dimensions. It can be considered in two cycles:

Cycle I (0-45 days after anthesis): The growth rates of most parts of the seed and fruit rise to a maximum and then fall. The ratio of seed width and pericarp width remains nearly constant, i.e., both grow at an approximately equal rate. Growth is due to both cell division and cell enlargement. The radial diameters of cells of the outer integument and nucellus increase about three-fold and those of the pericarp and septum about six-fold. These four structures comprise the bulk of the seed and fruit until the endosperm takes the place of the nucellus. Meristematic activity in the outer integument is high; the rate of cell division increases to a maximum at 20 to 25 days then declines to nil at 45 days. The three layers of cells of the inner integument divide anticlinally to accommodate the large size and shape changes of the outer integument. Cell division in the nucellus proceeds at a rate comparable to that in the outer integument but it declines earlier as the endosperm nuclei proliferate. Meristematic activity in the endosperm reaches a peak at 35 days. Only a few divisions occur in the zygote until 25 days after anthesis. Most cell divisions in the pericarp occur between the fifth and tenth days after anthesis (divisions occur also 5-10 days before anthesis). The skin, with four to eight layers of cells, is distinguishable at 16 days and is well defined by 23 days. Meristematic activity in the septum continues throughout cycle I. By 45 days, the seeds are nearly full-sized, but the berry is hard and green and only a third of its mature volume.

Cycle II (45 days - 110 days after anthesis): Berry volume triples and the berry softens and matures. Pericarp width increases two to three times

and all of this increase is due to cell enlargement in the pericarp and septum; there are no cell divisions. Presumably, the embryo continues to grow.

Sultanina berries show similar changes to Muscat except that seed development is imperfect. Pearson (20) has described the excessive growth of the tips of the inner integument which can be seen at anthesis. Olmo's sections show that these abnormalities are evident 11 days before anthesis. The outer integument and nucellus commence growth with rapid cell divisions. The rate of cell division in these parts reaches a maximum 15 days after anthesis and stops by about 30 days. Endosperm nuclei commence divisions but usually, after 20 days, endosperm and nucellus cells start to degenerate. In the mature berry, the stenospermic seed is a slender, soft structure. In one mature seed an undegenerate nucellus was found.

Black Corinth berries grow parthenocarpically.

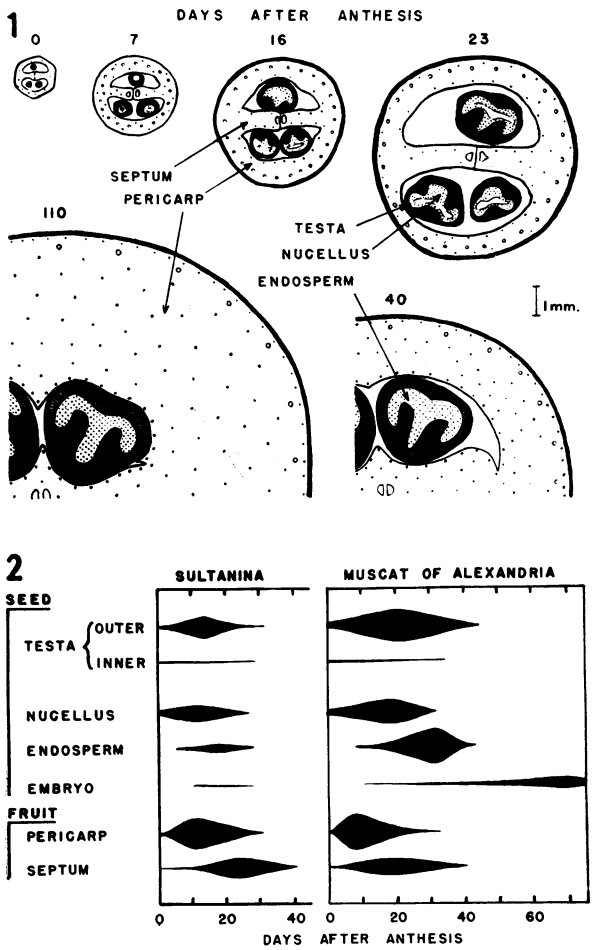


FIG. 1. Diagrams of transverse sections of berries of Muscat of Alexandria at varying times between anthesis and maturity.

FIG. 2. Diagram of changes in rate of cell division in different seed and fruit structures of Sultanina and Muscat. Compiled from sections prepared by Prof. H. P. Olmo. Diagram design after Wright (34).

This is due to defective embryo sac formation and not to defects in any other tissue of the ovule (20). In most berries, the ovule does not develop beyond anthesis; if it does, it develops a hard testa and the berry enlarges. The stimulus of pollination and girdling are needed for good set (17); this is defined by Stout (24) as stimulative parthenocarpy.

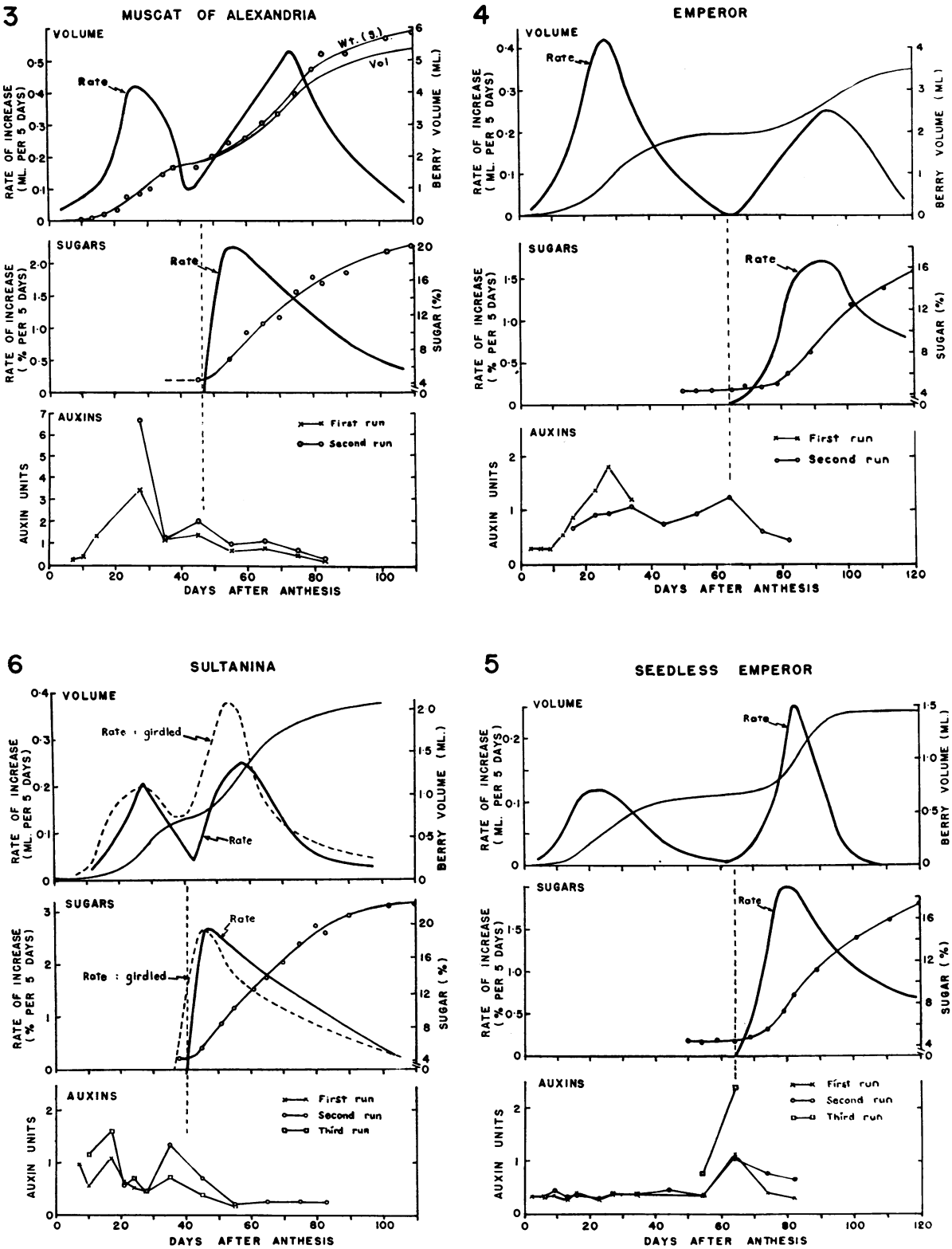
Seedless Emperor berries are stenospermocarpic. Their seed development is very incomplete and variable ranging from none at all to some larger than the stenospermic seeds of Sultanina. No hard testas were found. There is a correspondingly large variation in berry size because the growth of each berry is proportional to the amount of seed growth within. Most berries measure from 9 by 11 to 12 by 15 mm and, in these, seeds rarely lengthen to more than 1.5 mm (c.f. Sultanina seeds which average 3 mm in length and Muscat seeds, 6-7 mm). A few berries enlarge to 16 by 20 mm and some are as small as 5 by 5 mm. Olmo (18) found from one to four small rudimentary seeds in about 70 percent of the berries and, in the rest, no ovule development occurred after anthesis. Considering the slight seed development, it is remarkable that Seedless Emperor berries grow to about 1.5 ml volume compared with 0.25 ml in Corinth.

FRUIT GROWTH AND CHANGES IN SUGAR. The changes in berry volume and the rate of increase in volume from anthesis to maturity are shown for the five varieties in the top graphs of figures 3 to 7. Similarly, changes in sugar concentration in the juice are shown in the middle graph. The changes in berry volume and sugar concentration brought about by girdling of Sultanina and Corinth are shown by their rate curves.

All varieties show a double-sigmoid growth curve and hence a two-humped rate curve. These humps correspond to cycles I and II mentioned above; similarly shaped growth curves of many fleshy fruits have been commonly described in three periods or stages (see review by Nitsch, 12). The late-maturing varieties, Emperor and Seedless Emperor, show this feature clearly. It is barely perceptible in Corinth because the first growth cycle is small. Winkler and Williams (33) concluded that their measurements of berry diameter of Sultanina did not show a double-sigmoid growth curve (although their graph suggests it); the volume measurements reported here show it clearly.

Sugar concentration in the berries of Muscat, Sultanina, and Corinth rises suddenly, almost on a certain day, and the increase in sugar content reaches its maximum rate within ten days. In the later-maturing Emperors, sugaring begins slowly for 5 to 10 days, then rises to a rapid rate. The maximum rate of sugaring for Corinth is nearly double the maximum rate for the other varieties, probably because of its small size. The increase in sugar concentration of the juice of grapes is remarkable (it changes from 4-20 percent in 30 or 40 days).

A comparison of the sugar and growth rate curves reveals the striking coincidence of the commencement



FIGS. 3, 4, 5, 6. Changes in berry volume, juice sugar concentration, and auxin concentration in berries of Muscat, Emperor, Seedless Emperor and Sultanina from anthesis to maturity. Volume and sugar measurements are expressed as cumulative and rate curves; for girdled Sultanina, only the rate curves are shown. Note that ordinate scales differ for each variety. The term "auxin units" is defined in the text.

of sugaring and the second growth cycle. Corinth shows a slight discrepancy but, having smallest berries, this has the greatest percentage error. Both sugaring and growth rate curves increase sharply and, in each variety, the maximum rate of sugaring is reached a few days before the maximum growth rate. It is possible that the second growth cycle of grape berries could be caused by the influx of sugar into the berry. The changes in auxins and gibberellins (discussed below) and the morphological changes do not suggest other mechanisms to explain why the cells of the pericarp and septum enlarge as they do during this stage. From 80 to 90 percent of the total soluble solids in grape juice are D-glucose and D-fructose in about equal amounts; a 20 % solution of glucose has a theoretical osmotic pressure of about 27 atmospheres. Thomas, Ranson, and Richardson (25, p. 121) quote an osmotic pressure of 40 atmospheres measured in grapes. It is suggested that as sugar moves into the cells of the flesh, water also moves in to adjust diffusion pressure deficits. The berries contain some auxins, probably in sufficient amounts to permit stretching of the cell walls. The literature on changes in sugar and fruit volume in the developing figure (3) and apricot (4) suggests that this hypothesis should be examined in studies on the development of other sugary, fleshy fruits.

Nitsch (12) and others have suggested that the lowered fruit growth rate midway between anthesis and maturity may be due to competition between the fruit and the embryo which is developing during this period. This hypothesis cannot be applied to seedless grapes because there is no embryo development.

Changes in Auxins During Fruit Development. Changes in the level of auxins are shown in the bottom graphs of figures 3 to 7. In all varieties except Seedless Emperor, auxins rise from a low level just after anthesis to a high level during the first few weeks. In the two seeded varieties, Muscat and Emperor, this level is higher and maintained for a longer period than in the seedless varieties Sultanina and Corinth. Nitsch et al (16) have shown a similar difference between seeded and seedless Concord and have related the changes to differences in meristematic activity in the seed. The same could be said for Muscat and Sultanina; meristematic activity in the seed of Muscat is maintained about 10 days longer than Sultanina and, similarly, auxin does not decline to a low level until 35 days after anthesis in Muscat compared with 20 days after in Sultanina. In Emperor, which has big seeds, auxin does not reach a low level until 40 days after anthesis. There is no rise in auxin during cycle I in Seedless Emperor. Corinth provides an exception to this correlation between meristematic activity and auxin production because it has a brief but significant flush of auxin at 11 days after anthesis when its ovule is static; no explanation can be given for this.

In all varieties except Corinth there is a second, smaller rise in auxin concentration reaching a maximum at about the time that the second growth cycle and sugaring begin. It is difficult to explain, on morphological grounds, why auxin should rise as it does at this stage. Wright (34) was able to correlate a second rise in auxin level of Ribes nigrum fruit with meristematic activity in the embryo. This cannot be done in seedless grapes because there is no embryo development. Perhaps the rise in auxin just before sugaring, if real, has some causal relationship with sugaring. Some related facts are that exogenous auxins affect the concentration of sugars in plant tissues (7) and also some workers consider that auxin acts as a trigger in affecting plant metabolism (19). It is interesting that defoliation did not delay the onset of the second growth cycle (33). The mechanism whereby sugars accumulate in grapes and other fruits, apparently against a concentration gradient, remains one of the puzzles of plant physiology. The key to sugaring is probably held by the berries themselves.

With the increase in berry size during cycle II, the auxin concentration, on a dry weight basis, declines to a low level. Recalculated to the amount of auxins per berry, the same general changes are apparent except that, at about 20 days after the beginning of cycle II, auxins show yet another rise: this is probably too late to enter into causal considerations of growth during this cycle. Changes in the dry weights of berries of the five varieties are shown in table I so that auxin (and gibberellin) figures may be converted to amounts per berry.

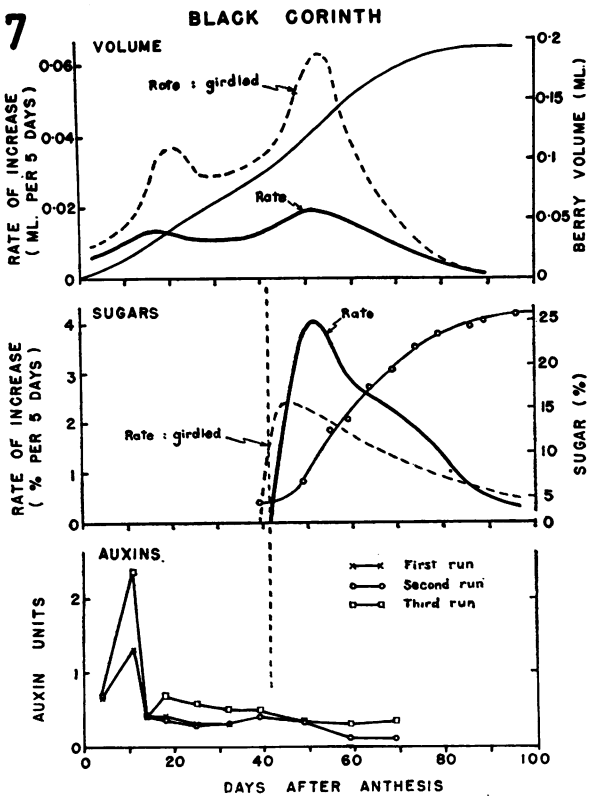
Changes in Gibberellins During Fruit Development. Results of the gibberellin bioassays are shown in figure 8 and table II. Duplicates showed high variability. It must be emphasized that the absence of significant activity in these tests does not mean that gibberellins were absent. Better purification might have revealed smaller amounts or removed masking agents.

The results show that the ovaries of the seeded varieties, Muscat and Emperor, have no significant activity at any stage tested but that their stamens have activity at anthesis. On the other hand the ovaries of the three seedless varieties, especially Seedless Emperor, show activity during the first 14 days after anthesis, but not subsequently. Also, like seeded varieties, their stamens show activity at anthesis. The

Table I
Dry Weights of Berries of 5 Grape Varieties at Certain Intervals After Anthesis

Variety	Dry weight per berry (mg)					
	10	30	50	70	90	Mature*
	Days after anthesis					
Muscat	6	78	170	450	860	1,300
Emperor	3	77	137	150	250	800
Seedless Emperor	3	30	41	45	145	350
Sultanina	4	35	80	270	470	500
Corinth	3	5	11	36	57	60

* Dry weight estimated by slight extrapolation.



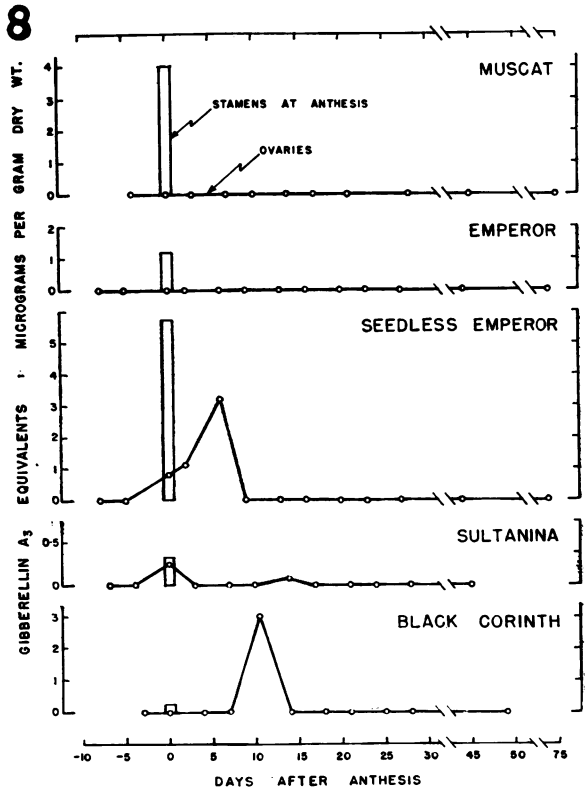


FIG. 7. Changes in berry volume, juice sugar concentration, and auxin concentration in berries of Black Corinth from anthesis to maturity. Volume and sugar measurements are expressed as cumulative and rate curves; for girdled Corinth, only the rate curves are shown. The term "auxin units" is defined in the text.

FIG. 8. The gibberellin activity of extracts of grape stamens and ovaries of different age and variety as measured by the d-1 maize bioassay; note the different ordinate scale for Sultanina.

results suggest also that there may be activity in the ovaries of seedless varieties at anthesis. A fourth seedless variety (Olmo's hybrid No. Q25-6) was tested in June, 1959, and found to have significant gibberellin activity in its young berries, although less than Seedless Emperor.

It may be significant that the difference in gibberellin activity between seedless and seeded berries is correlated with their responsiveness to exogenous GA_3. Without exception, the berries of seedless grape varieties have been found to enlarge following treatment with GA_3 at, or soon after, anthesis, e.g., Corinth and Sultanina (28), Black Monukka and Beauty Seedless (Weaver, private communication), Concord Seedless (23), Seedless Emperor and Olmo's hybrid No. Q25-6 (the author, unpublished data). On the other hand, the berries of seeded grape varieties will not enlarge appreciably after treatment with GA_3 (23, 28). These results suggest that gibberellins may be involved in the growth mechanism during cycle I of seedless berries and that the level of naturally-occurring gibberellins may be an important factor limiting their growth. Gibberellins may not be involved in the growth mechanism of seeded grapes or they are destroyed. More information is needed before these questions can be decided.

The interactions between naturally-occurring auxins and gibberellins of seedless grapes are interesting. Seedless Emperor has low auxin content during cycle I yet it grows five times bigger than Corinth. Perhaps this is explained by the high gibberellin activity in its young berries. This idea is supported by the fact that Corinth berries, when dipped in GA_3 solution (500 ppm) at anthesis (28), enlarge to about the same size as, and ellipsoidal shape of, Seedless Emperor berries even though their ovules remain minute. Thus it is possible that natural levels of gibberellins may be the most important factor limiting their size. Girdling and 4-CPA are not as potent as GA_3 in enlarging Corinth berries.

Comparisons of the activity of grape extracts in elongating d–1 and d–5 maize seedlings are shown in table II. It can be seen that, in most cases, both the young berries of seedless varieties and mature stamens of seeded and seedless varieties induce greater elongation in d–5 than in d–1. Obviously, it would have been better to use d–5 to examine changes in developing grapes rather than d–1. A similar differ-

TABLE II

COMPARISON OF ACTIVITY OF GRAPE EXTRACTS IN ELONGATING DWARF-1 AND DWARF-5 MAIZE SEEDLINGS †

Extract Variety	Part	Elongation of 1st and 2nd leaf sheaths mm d-1	d-5	GA_3 Equivs./ 0.1 ml extract g × 10^{-9} d-1	d-5
Gold (seeded)	Stamens	2.0	11.6**	0	1.5
Muscat	Stamens	11.0**	34.9***	2.2	50
Seedless	Stamens	14.3***	22.7***	4	8.3
Emperor	Ovaries††	9.5**	12.9**	1.6	1.8
Sultanina	Stamens	2.8	21.6***	0	9
	Ovaries	3.6	18.0***	0	7
	Pedicels	3.2	4.8	0	1
Corinth	Flowers	1.6	6.9*	0	3

† 0.1 ml of extract (containing from 1-11 mg dry wt of the original grape sample) was applied to each maize seedling. Four or five replicates of pairs of dwarfs were used. Elongations which were significantly greater than control in the analyses of variance are marked by 1, 2, or 3 asterisks when the probabilities that they were significantly greater than controls were better than 20:1, 100:1 and 1000:1, respectively; insignificant values are called nil.

†† Sampled 7 days after anthesis; all other parts in this table were taken at anthesis.

ence in extracts of bean seeds has been found by Phinney and Neely (21) and West and Murashige (30). They purified two crystalline compounds from bean seed. Bean factor I behaved identically with GA_1. Bean factor II was ten times as active on dwarfs -2, -3, -5 and an-1 compared with its activity on d-1. It also differed in its chromatographic behaviour, infra-red spectrum and chemical properties from GA_3, GA_1, and GA_2. Perhaps the activity in grapes is due in part to the presence of bean factor II or other unknown gibberellin-like compounds.

EFFECTS OF GIRDLING ON BERRY GROWTH AND ON CONTENT OF SUGARS, AUXINS, AND GIBBERELLINS. It is common viticultural practice to girdle the trunks or canes of Corinth vines at anthesis to increase fruit set and size and to girdle Sultanina 10 days after anthesis to produce large berries for table use (8). Its effects on berry weight and volume (figs 6 and 7) are apparent within a few days of treatment and these differences continue to increase until maturity. Thus girdling affects both growth cycles. The second growth cycle and sugaring began concurrently a few days earlier in girdled vines of both varieties than in ungirdled. This supports the hypothesis that sugar influx may cause the second growth cycle. The rate of sugar increase in berries on ungirdled vines eventually exceeds that on girdled vines probably because of the bigger crop on the latter.

The effects of girdling on auxin content of the berries are shown in table III. The differences associated with girdling are very small and are far exceeded by variations between tests. The results suggest that the concentration of auxins on a dry weight basis is unchanged. If this is true, the amount per unit volume or fresh weight would also be equal as percentage dry weight and densities are equal in girdled and ungirdled berries at these growth stages. However, the amount per berry would be greater by a factor of 1.5 to 2 in girdled berries because of their greater size. Measurements of gibberellin activity in these samples are too few and too variable to be worth presenting. In four instances, there did not seem to be any large difference on a dry weight basis.

There are several considerations which place importance on the events taking place in the berries of Sultanina during the period from 10 to 15 days after anthesis: the effect of girdling and berry thinning in increasing berry size is maximal (31, 32); treatment with 4-CPA and GA_3 causes greatest enlargement of berries when applied during this period (27, 28); gibberellin activity can be detected in the berries (fig 8) and auxin concentration is high (fig 6). The fact that the rate of cell division in the pericarp is also maximal (fig 2) suggests that girdling and other treatments may increase berry size by increasing the number of cells. The possibility that cell size is also increased should not be excluded.

The effects of girdling on berry size of Sultanina can be duplicated by treatment with low concentrations of 4-CPA and GA_3, so the mechanism of the action of girdling may be due to its effect on naturally-occurring growth regulators. The behavior of seeded varieties supports this idea and emphasizes the

TABLE III

AUXIN CONCENTRATION IN BERRIES OF VARIOUS AGES FROM GIRDLED AND UNGIRDLED CORINTH AND SULTANINA VINES*

Extract Variety	Days after anthesis	Test No.	Auxin units** expressed on dry wt basis Ungirdled	Girdled
Corinth	14	A	0.37	0.36
		B	0.42	0.34
		C	0.43	0.56
	32	A	0.30	0.32
		B	0.33	0.38
		C	0.51	0.37
	49	A	0.36	0.30
Sultanina	17	A	1.08	1.02
		B	1.59	1.92
	24	A	0.51	0.54
		B	0.72	0.61
	28	A	0.45	0.55
		B	0.45	0.51
	55	A	0.20	0.19
		B	0.16	0.15
	75	A	0.25	0.19

* Equal dry weights of girdled and ungirdled berries were compared in each bioassay.

** The term "auxin units" is described in the text.

probably important role of gibberellin; their berries are devoid of (or low in) gibberellin and they do not enlarge much after girdling or spraying with 4-CPA or GA_3. However, girdling affects the concentration of many compounds, e.g., it increases the level of carbohydrates (29) and decreases the level of nitrate-nitrogen in petioles opposite the grape clusters (9). More information is needed before a conclusion can be made on the mechanism of the girdling effect.

General Conclusions

Correlations between growth rate, auxins and meristematic activity have been demonstrated in several fruits and other plant organs. There are examples in fruits, however, where auxin increase can be correlated with increased meristematic activity in the seed (especially the endosperm) but this activity cannot always be correlated with increased growth rate in the fruit, e.g. apple (10) and figure 2. Failure to show a correlation may be due to failure to measure the substances responsible for growth or to a different mechanism of growth.

In the varieties of grapes studied here, only the seeded varieties, Muscat and Emperor, show a correlation between meristematic activity in the seed, level of auxins and the first cycle of berry growth. In Sultanina, the auxin level parallels the rate of cell division in the seed but the rate of increase in berry volume is maintained at a high level 10 days longer. The situation in Seedless Emperor is not as clear because no detailed study of seed development was made but, in any case, auxin content remained low while the berries grew. In Corinth there was a sharp peak in auxin content 11 days after anthesis when there was no seed development at all. It seems more likely that the growth of these seedless grapes is controlled by gibberellin levels rather than auxins or that gibberellins act together with auxins.

The second cycle in berry growth does not coincide with a rise and fall of either auxins or gibberellins. As suggested above, a more acceptable hypothesis to explain this growth cycle is that the movement of sugars into the berries causes an osmotic attraction of water.

Summary

Measurements were made of growth and development of certain parts of the fruit and seed of two seeded and three seedless varieties of *Vitis vinifera* (European grape) from anthesis to maturity. These were compared with changes in the sugar concentration of the juice and the levels of auxins and gibberellins in partially-purified extracts of berries.

The fruit of all varieties showed a double-sigmoid growth curve. In the two seeded varieties, the first growth cycle was paralleled by a rise and fall in meristematic activity in the seeds and in the auxin content of the berries. In the seedless berries, the first growth cycle was greater than would be expected from the berry's auxin content and the seed's meristematic activity. This may be explained by the discovery of gibberellin activity in the young berries of the three seedless varieties. No gibberellin activity was found in seeded berries at any time. These findings, plus the fact that berries of seedless varieties enlarge considerably after treatment with gibberellin, auxin, and by girdling, whereas seeded varieties do not, suggest that gibberellins are important hormones in the fruit of these seedless varieties. Mature stamens of all varieties showed gibberellin activity.

The second growth cycle could not be correlated with morphological, auxin, or gibberellin changes but could be related to the influx of sugars into the berries. It is suggested that sugaring causes this growth cycle by an osmotic attraction of water.

A second rise in auxin level was found to reach a maximum in four varieties at the beginning of sugaring. These events may be related but no explanation can be given for this phenomenon. Trunk girdling of two seedless varieties caused no measurable change in auxin or gibberellin concentration expressed on dry weight, fresh weight, or volume bases, but levels per berry increased because of the large increase in berry volume after girdling.

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