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Effect of Temperature on Ontogeny of Berries of *Vitis Vinifera* L. cv. Cabernet Sauvignon[1]

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Abstract. The effect of temperature on development of 'Cabernet Sauvignon' berries was examined in growth cabinets beginning 3 weeks after flowering and terminating 6 weeks after the start of stage III. High temperature, 35/30°C, reduced the amount and duration of berry growth during stage I; it lengthened stage II by inhibiting the onset of stage III. Final berry size was irreversibly reduced by high temperature during stage I. The duration of stage II was also lengthened by lowering the day/night temperature during stage I from 25/20 to 18/13°C. Maximum berry size was attained most rapidly at the intermediate and most slowly at high temperature. Generally, stages I and II were more sensitive to temperature than stage III.

Final berry weight was lowest at 35/30°C. The concentration of total soluble solids was low when both stages II and III were at 35/30 and high when stage I or both stages I and II were at 35/30 and subsequent growth at 18/13. Once stage III had started, temperature had little effect on berry size or total soluble solids. Acidity was highest when all stages of development were at 18/13 and was reduced by high temperature at any stage of development.

Investigations on effects of temp on development of grape berries have been concerned chiefly with effects on size and composition rather than on rate of development. For instance, the temp occurring immediately after bloom (12, 17) and in the subsequent period described as berry enlargement (11, 13) influenced size while that occurring during "berry development" and "berry maturity" (11, 13), as well as during the second half of berry development (10), influenced composition. These studies have taken some account of different growth stages since during its development, the grape berry shows 2 periods of rapid growth (stage I and III) separated by a period of relatively slow growth (stage II).

On the other hand, observations on effects of temperature on rate of development have been mainly concerned with the overall period from bloom until a given degree of ripeness (4, 20). Winkler (20) attributed much of the seasonal variation in this period to differences in temperature and proposed that a constant summation of heat is required. This constancy, however, is not always found (4). Because effects of temperature on duration of the 3 growth stages are inadequately described, we examined effects of temp on the timing of stages I, II, and III in developing 'Cabernet Sauvignon' berries using test vines grown under artificial light in growth cabinets.

Methods and Materials

'Cabernet Sauvignon' vines were established in a glasshouse from cuttings planted into 10 cm plastic pots containing Perlite/John Innes (2:1) mixture. Inflorescence retention was promoted by leaf removal (15). Three weeks after anthesis, vines selected for uniformity of flowering time were repotted into 8-litre plastic containers and transferred to growth cabinets. Light intensity during the 16-hour days was 2500 ft-c (8.7 cal cm^{-2} hr^{-1}), and cabinet temp (°C day/°C night) were 18/13 (low), 25/20 (intermediate) and 35/30 (high). These conditions were chosen because vine leaves reach light saturation at 2500 to 3000 ft-c (14), and dry matter production (2) and photosynthesis (14) by vines at 18°C or 35°C are only marginally less than at the optimum temperature for these processes. Shoots were tipped above the twentieth node and laterals were removed as they appeared. The leaf area was about 2,500 cm^2. Berries were thinned 3 weeks after anthesis to 60 per vine by selectively removing the obviously large or small.

Relative humidity was mainly that of atmosphere around the cabinets and ranged from 40 to 80%, high values occurring immediately after watering.

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The diameters of the same 5 berries on each plant were measured with calipers twice weekly. From these measurements, estimates were made of the time at which growth stages I and II were ending. A group of 4 plants from each temp regime was transferred to each of the other temp when that group was judged to be at the end of stage I. This was repeated with further plants at the end of stage II and these temp regimes were maintained until the end of the experiment. There were 15 treatments (Fig. 1) each with 4 single plant plots. The time at which temperature regimes were changed (vertical arrows in Figs. 3 to 5) corresponded reasonably well with transitions from one growth stage to another, except for the change intended to be at the end of stage I at 35/30 which was about 10 days late.

The date of the first appearance of anthocyanin pigment in each berry was recorded. This date was an additional indicator of the start of stage III. As compositional changes associated with ripening start with the commencement of stage III, berries from a treatment were harvested 6 weeks after the start of stage III. They were weighed and the percent soluble solids (refractometer measurement) and titratable acidity (to phenolphthalein endpoint) determined on expressed juice. The number of seeds per berry, and their wt, was noted for berries from treatment A1, B1 and C1 (Fig. 1). The diameters of all peduncles were recorded at harvest. Differences between means were calculated using Duncan's multiple range test.

Measurements of berry diameter were adjusted to facilitate the drawing of basic growth curves (no temp change) with secondary curves (following temp change) departing from them. The adjustments were made as follows: on each occasion that vines were transferred to a different temp regime, a mean was calculated for all berries which up to that day had been subjected to the same temp. Means were also calculated for berries which were to remain at that temp and for those which were to be transferred to a different temp. These latter means were adjusted to equal the mean of the group from which they were drawn. For example, when vines were transferred to different temp at the end of stage I, the mean of treatments 1, 4, and 5, the mean of treatment 2, and the mean of treatment 3, were adjusted to equal the mean of treatments 1, 2, 3, 4, and 5. These adjustments were made to each subsequent mean. The average adjustment required was ± 0.16 mm.

Treatments	Temperature Stages			Duration of stages Days		
	I	II	III	I	II	III
A 1				49	28	30
2				49	30	29
3				48	36	23
4				49	30	29
5				49	27	19
LSD (p=0.05)				ns	5.1	5.8
B 1				46	15	28
2				48	16	34
3				45	27	38
4				44	18	32
5				45	16	29
LSD (p=0.05)				ns	7.2	ns
C 1				39	45	28
2				41	22	31
3				41	26	34
4				42	42	26
5				39	41	30
LSD (p=0.05)				ns	8.6	ns
LSD between means for A1, B1 and C1. (p=0.05)				2.5	10.7	ns

Fig. 1. Effect of temperature on the duration of growth stages. The start of stage I was at anthesis and the end of stage III when the berry had attained its maximum diameter. Stage II was that period between stages I and III when diameter increase was less than 0.03 mm per day. Values are means from 4 plants. The temperature treatments were started 21 days after anthesis. The daily temperature regimes were ▨ 18/13; □ 25/20; ■ 35/30; (°C day/night). The treatments were A, B and C initial temp 18/13, 25/20 and 35/30 respectively; 1, temp unchanged; 2 and 3, temp changed at the end of stage I; 4 and 5, temp changed at the end of stage II.

Results

Length of growth stages. The rates of diameter increase during stage II were usually less than 0.03 mm per day. This growth rate has been used to delineate stage II and so fix the end of stage I and the start of stage III. The lengths of the 3 growth stages were then calculated using the time of anthesis as the start of stage I and time of maximum diameter as the end of stage III. The durations of stages I, II, and III for treatment A1 were 49, 28, and 30 days; for B1, 46, 15, and 28 days and for C1, 39, 45, and 30 days respectively (Fig. 1).

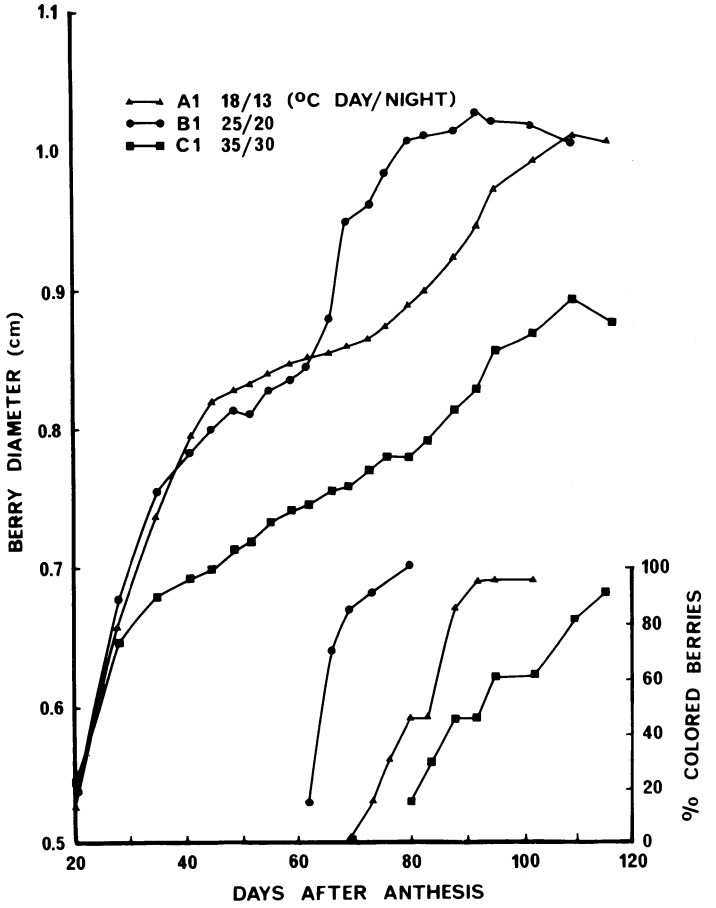

Fig. 2. Effect of unchanging temp regimes 18/13, 25/20 and 35/30 (°C day/night) on berry diameter and on percent colored berries.

The length of stage I was influenced by temperature such that high < intermediate < low; that of stage II such that intermediate < low < high; whereas the duration of stage III was not affected (Fig. 1).

Berries initially at the low temp (A treatment) were unaffected by raising the temp to the intermediate level. However, an increase to the high temp at the end of stage I lengthened stage II by 8 days and shortened stage III by 7 days. An increase to 35/30 at the end of stage II shortened stage II by 11 days (Fig. 1).

Berries initially at the intermediate temperature (B treatment) were unaffected by a change down to the low temperature at the end of either stage I or stage II, or by a change up to the high temperature at the end of stage II. However, a change to the high temperature early in stage II prolonged stages II and III by 12 and 10 days respectively.

Stage II was shortened as temperature during stage I increased from low to intermediate (Fig. 1, compare treatments A1 with B2, and A2 with B1). The effect was not apparent at the high temp possibly because temp was reduced about 10 days after the start of stage II. When the temp was reduced from the high temp during the first half of stage II, stage II was shortened by about 20 days. The duration of Stage III was unaffected by a reduction to the intermediate or low temp at the end of stage II.

Growth rate. The growth rate during stage III was correlated with the period during which anthocyanin synthesis was initiated, being most rapid when the period of color initiation was short (Fig. 2, B1), and slowest when the period of color initiation was long (Fig. 2, C1). A lengthy period of color initiation and a slower stage III growth rate occurred whenever the transition from stage II to stage III was at the high temp (Fig. 3, A3 and A5; Fig. 4, B3 and B5). This lack of synchrony at the start of stage III could be overcome by lowering the temp (Fig. 5; C2, C3, and C4, and C5).

Characteristics of mature berries. Berry weights ranged from 0.49g to 0.87g (Table 1). These values are low compared with values of 0.83 to 1.28g found for field grown vines. (May, private communication.) Exposure during stage I to the high temp resulted in smaller berries than exposure to either the intermediate or low temp (Table 1). The temp experienced during stage III did not affect final wt.

The mean value for total soluble solids (TSS) was low when stage I was at the low temp (Table 2). As this trend was not consistent for all temp during stages II and III an interaction between stages I and the stage II and III temp was indicated. The means for the different stage III temp show a reduction in TSS with increasing temp during stage III. The effect was most marked when stage I and II were at the high temp which indicates an interaction between stage I and II, and stage III temp. The lowest values for TSS occurred when both stages II and III were at the high temp irrespective of stage I temp and the highest values when stage I or stages I and II were at the high and the remainder at the low temp.

Acidity decreased with an increase in temp during either stage I or stage III (Table 3). Treatment means show that the

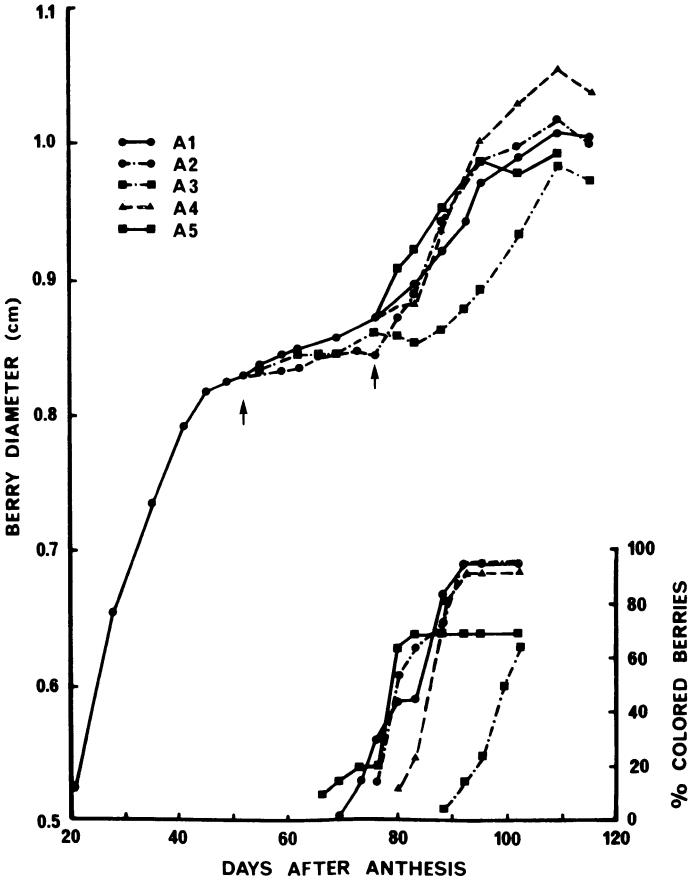

Fig. 3. Effect of changes in temp from an initial 18/13 (°C day/night) on berry diam and on percent colored berries. The temp of treatments A2 and A3 were changed to 25/30 and 35/30 respectively at the end of stage I, and A4 and A5 to 25/20 and 35/30 respectively at the end of stage II. The times of the changes are indicated by vertical arrows. Note that curves cannot be relied on to give actual diameters because values were adjusted to illustrate beginning and end of growth stages.

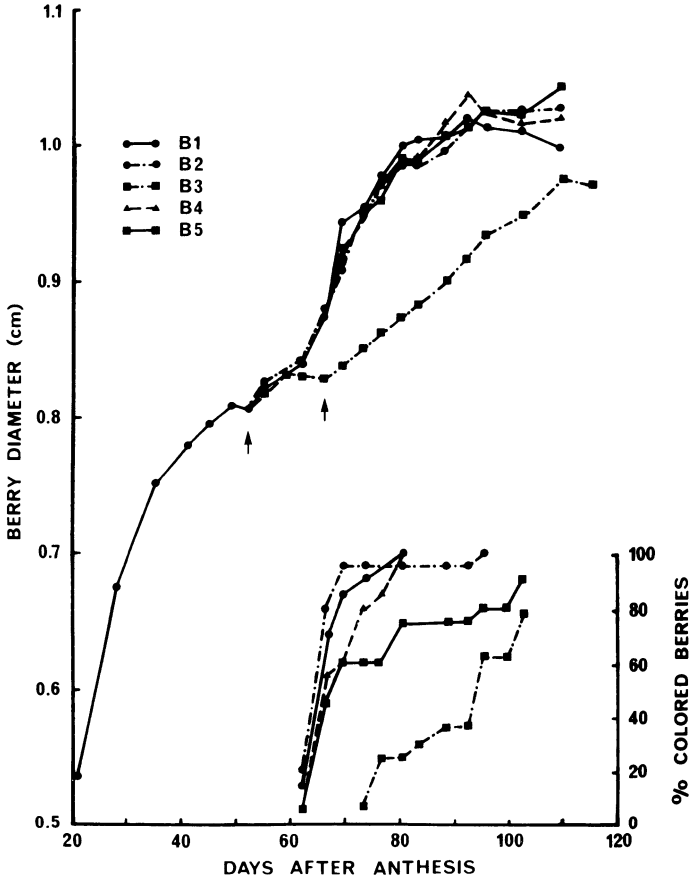

Fig. 4. Effect of changes in temp from an initial 25/20 (°C day/night) on berry diam and percent colored berries. The temp of treatments B2 and B3 were changed to 18/13 and 35/30 respectively at the end of stage I, and B4 and B5 to 18/13 and 35/30 respectively at the end of stage II. The times of the changes are indicated by vertical arrows. Note that curves cannot be relied on to give actual diam because values were adjusted to illustrate beginning and end of growth stages.

Table 1. Effect of temperature on final berry weight (g)[z]. Measurements were made 6 weeks after start of stage III. Each value is the mean of 4 vines.

Stage II & III temp.	Stage I temp[y] 18/13	25/20	35/30	Stage I & II temp.	Stage III temp[y] 18/13	25/20	35/30
18/13	0.73 cde	0.76 cde	0.56 abc	18/13	0.73 cde	0.75 cde	0.67 abcd
25/20	0.77 cde	0.79 de	0.51 ab	25/20	0.76 cde	0.79 de	0.87 e
35/30	0.84 e	0.71 bcde	0.62 abcd	35/30	0.49 a	0.59 abcd	0.62 abcd
Mean	0.78 b	0.75 b	0.56 a	Mean	0.66 a	0.71 a	0.73 a

[z]Values in the body of the table with the same letter are not significantly different ($\alpha = 0.05$).

[y]Means for stage I temperature or for stage III temperatures with the same letter are not significantly different ($\alpha = 0.05$).

Table 2. Effect of temperature on total soluble solids (°Brix)[z]. Measurements were made 6 weeks after start of Stage III. Each value is the mean of 4 vines.

Stage II & III temp.	Stage I temp[y] 18/13	25/20	35/30	Stage I & II temp.	Stage III temp[y] 18/13	25/20	35/30
18/13	20.1 bcde	22.5 gf	23.6 gh	18/13	20.1 bcde	19.8 bcde	19.2 bc
25/20	20.9 cde	19.8 bcde	21.2 def	25/20	21.55 ef	19.8 bcde	19.9 bcde
35/30	16.3 a	18.7 b	16.9 a	35/30	24.9 h	21.0 de	16.9 a
Mean	19.1 a	20.3 b	20.6 b	Mean	22.2 c	20.2 b	18.7 a

[z]Values in the body of the table with the same letter are not significantly different ($\alpha = 0.05$).

[y]Means for stage I temperatures or for stage III temperatures with the same letter are not significantly different ($\alpha = 0.05$).

Table 3. Effect of temperature on titratable acid (g tartaric acid/100 ml)[z]. Measurements were made 6 weeks after start of stage III. Each value is the mean of 4 vines.

Stage II & III temp.	Stage I temp[y] 18/13	25/20	35/30	Stage I & II temp.	Stage III temp[y] 18/13	25/20	35/30
18/13	0.78 e	0.60 d	0.45 ab	18/13	0.78 e	0.55 bcd	0.51 abcd
25/20	0.58 cd	0.43 ab	0.44 ab	25/20	0.75 e	0.43 ab	0.41 a
35/30	0.49 abcd	0.44 ab	0.42 a	35/30	0.49 abcd	0.49 abcd	0.42 a
Mean	0.62 b	0.49 a	0.44 a	Mean	0.65 b	0.48 a	0.45 a

[z]Values in the body of the table with the same letter are not significantly different ($\alpha = 0.05$).

[y]Means for stage I temperatures or for stage III temperatures with the same letter are not significantly different ($\alpha = 0.05$).

Table 4. Effect of temperature on peduncle diameter (mm)[z,x]. Measurements were made 6 weeks after start of stage III. Each value is the mean of 4 vines.

Stage II & III temp.	Stage I temp[y] 18/13	25/20	35/30	Stage I & II temp.	Stage III temp[y] 18/13	25/20	35/30
18/13	4.07ab	3.83 a	4.03 ab	18/13	4.07 ab	4.44 abc	6.26 de
25/20	4.16 ab	4.66 abc	4.42 ab	25/20	3.90 ab	4.66 abc	5.25 abcd
35/30	4.79 abc	5.38 cde	5.71 cde	35/30	5.27 abcd	6.73 e	5.71 cde
Mean	4.34 a	4.62 a	4.72 a	Mean	4.41 a	5.28 b	5.74 b

[z]Values in the body of the table with the same letter are not significantly different ($\alpha = 0.05$).

[y]Means for stage I temperatures or for stage III temperatures with the same letter are not significantly different ($\alpha = 0.05$).

extent of this effect depended on temp during the other 2 growth stages, suggesting an interaction. Acidity was low in all treatments which had one or more stages at the high temp, and was high where stages I and II were at the low or intermediate temp, and stage III at the low temp.

Peduncles, and to a lesser extent pedicels, of bunches ripened at the high temp were greatly thickened. Increasing peduncle diameter was associated with increasing temp (Table 4).

Temperature did not affect seed number or size. The number of seeds per berry and mean seed wt for treatments with unchanging regimes were respectively (means from 20 berries): Al-1.05 and 30 mg; Bl-1.10 and 3 mg; Cl-1.20 and 27 mg.

Discussion

The use of temp or heat summation to describe the time required for berries to develop from anthesis to a given sugar concentration assumes that temp has equal value at all stages of berry development. Our results indicate that this assumption, while useful in the field, is not valid for 'Cabernet Sauvignon' grapes for the range of temperatures investigated. The response to temp in terms of rate of development, depended on the stage of development. Stages I and II were more sensitive to temp than stage III. The absence of any significant effect of stage III temp on berry size or sugar accumulation when stages I and II had been at the lower temp (Tables 2 and 3) confirms other results with this cultivar (3, 10). However, sugar accumulation in some cultivars is sensitive to stage III temp (9, 10, 13). When stages I and II were at the higher temp, soluble solids were reduced with increasing stage III temperature. Inhibition of the start of stage III by the high temp and consequent uneven

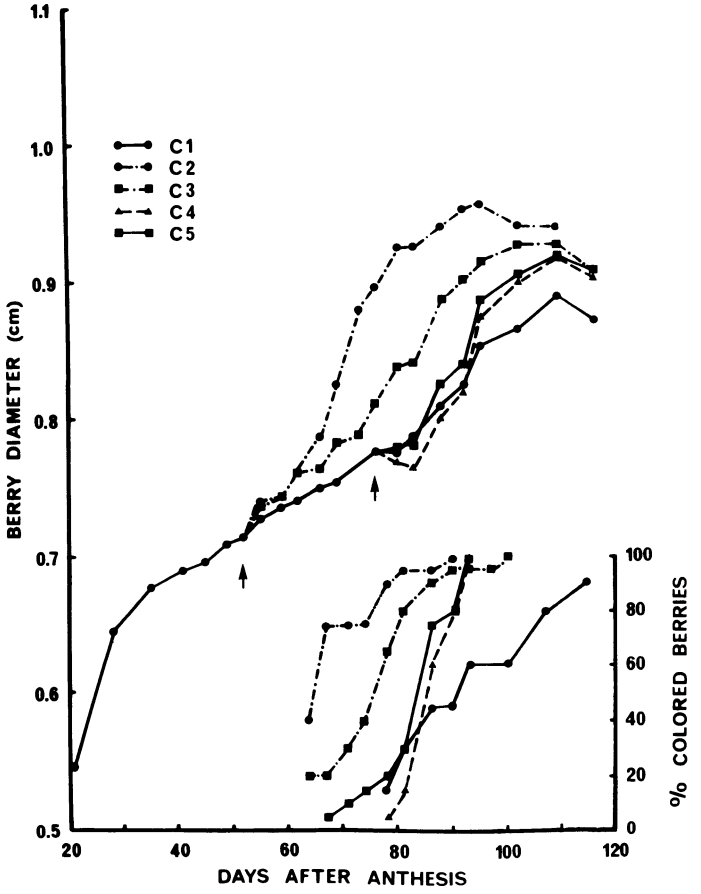

Fig. 5. Effect of changes in temp from an initial 35/30 (°C day/night) on berry diam and changes in percent colored berries. The temp of treatments C2 and C3 were changed to 18/13 and 25/20 respectively after the start of stage II and C4 and C5 to 18/13 and 25/20 at the end of stage II. The times of the changes are indicated by vertical arrows. Note that curves cannot be relied on to give actual diameters because values were adjusted to illustrate beginning and end of growth stages.

ripening probably accounts for this effect.

The differential response by grape berries to temp during their development may help explain the lack of constancy in heat summation values between anthesis and ripeness found in some situations (4).

The duration of stage II appears to be influenced by 2 temp sensitive systems. This is indicated by the shortening of stage II as temp during stage I was increased and the lengthening of stage II by high temp during stage II.

Exogenously applied growth regulators can also shorten or lengthen stage II of berry development (5, 7, 8); they also influence the morphology of pedicel and rachis (18, 19). The similarities in response to growth regulators and temp suggest that temp may influence berry development by its effect on endogenous growth regulators.

The failure of fruits to ripen normally at high temp has been attributed to reduced ethylene synthesis (1) and, in view of the status of ethylene as a fruit ripening hormone, it seems reasonable to speculate that the delay in the onset of stage III induced by high temp is an ethylene effect, either on ethylene concentration or on the sensitivity threshold to ethylene. It has been shown that, in grapes, ethylene hastens the onset of stage III (8), as does abscisic acid (7), but there is no increase in its concentration associated with the onset of stage III as there is with abscisic acid (5). However, an ethylene requirement for the initiation of stage III has not yet been established.

The temp treatments were begun 3 weeks after anthesis by which time cell division would have ceased (6). Therefore, treatment differences in berry size were probably due to effects of temp on cell enlargement, in agreement with conclusions of others (12, 16). The reduction in berry size caused by the high temp during stage I could not be reversed by lowering the temp for stages II and III. It is possible that berry size at the end of stage I determines the potential for size increase during stage III. It is unlikely that berry size was influenced by seed development because the temperature treatment had no effect on seed size or number of seeds per berry.

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