The Effect of Artificial Climate on the Internal Fruit Color of Redblush Grapefruit[1]

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Abstract. The seasonal decline of lycopene concentration in "Redblush" grapefruit has been attributed to climatic conditions. It has been found that small potted trees and grapefruit grafted onto small potted seedlings can be used in climatic chambers to study the effect of temperature on lycopene concentration in the fruit. The results obtained indicate that high temperatures favor the accumulation and retention of lycopene in the fruit, while lower temperatures favor the decline of lycopene.

Introduction

The red color of "Redblush" grapefruit in Texas reaches a maximum about September 1, and fades to reddish orange by the beginning of the processing season in late November or December. Since this fading bears directly upon the problem of obtaining pleasingly colored juice from red grapefruit, it has been studied previously (3, 5, 6, 7).

It was reported (3, 5) that the maximum concentration of lycopene in "Redblush" grapefruit in Texas occurs between August 15 and September 10. On the basis of studies with fruit which are "off bloom", i.e. set after the normal season of fruit set, Purcell and Schultz (7) concluded that age of fruit can account for part of the seasonal decline of lycopene. It seems evident that an environmental factor is also involved, so an experiment was designed to determine the effect of temperature on lycopene content of "Redblush" grapefruit.

Two-year-old grapefruit trees in pots bloomed abundantly and set 2 to 8 fruit per tree. The development of fruit appeared normal and the trees could be placed in controlled climate chambers. This allowed environmental control over sufficient numbers of fruit for a statistically designed experiment. The first trial indicated the need for more fruit than could be placed in the chambers on potted trees, so a second experiment was designed, using grapefruit grafted onto seedlings (1, 2, 4, 8).

[1]Received for publication July 5, 1967.

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Materials and Methods

Production of fruit and sampling 1963 season: From a group of potted trees, 32 of the more uniform trees with 2 to 8 fruits per tree were selected. The trees were paired into 2 groups according to the number of fruit per tree and each pair was numbered. On July 29, 1963, one group of trees was placed in a chamber with 60°F day temperature and 40° night temperature. The other group was placed in a chamber with 95° day and 85° night. Light in the chambers was provided by banks of G.E.[6] power-groove fluorescent tubes and 100-watt incandescent bulbs. Illumination was 800 ft-c at the top leaves. Day temperatures were held from 8:00 AM until 4:00 PM with night temperatures held from 4:00 PM to 8:00 AM. The lights were on from 6:00 AM until 6:00 PM

At weekly intervals samples of 10 fruits were taken from each chamber. Tree numbers were selected at random and applied to both groups providing more precise comparison than unpaired selection from each chamber. The fruits from each chamber were divided into two lots of 6 and 4. The lots of 6 were taken from the trees with the greatest numbers of fruits so that any effect of the number of fruits per tree would be determined. Samples of 6–8 fruits were randomly picked weekly from around the whole circumference of a single tree in a grove for comparison.

Production and sampling of fruits 1964–1965 season: In 1964, 700 vigorously growing sour orange seedlings were grafted with wood carrying one small "Redblush" grapefruit per tree the first week of June. On July 12, grafted fruits were placed into 4 groups of 100, ranked by fruit size. Group 1 was placed in a chamber with 95°F day temperature and 85° night temperature. Group 2 was placed in a chamber with 70° day temperature and 60° night temperature. Groups 3 and 4 were placed outdoors in the full sunlight. On July 14, a sample of 10 fruits was taken from the remaining trees and analyzed. It was assumed that these fruits were representative of the fruits in each of the 4 groups. On September 15, Group 4 was placed into a chamber and subjected to a temperature of 60° day and 40° night temperature.

Samples of 10 fruits were taken from each chamber every 2 weeks. The fruits from each lot were ranked according to fruit diameter and divided into 3 lots of the 3 largest, 3 medium and 4 smallest.

Samples from the grove were also taken every 2 weeks. Ten trees were given a number and 1 fruit from each tree was taken. The sample was divided into 3 lots as above. The same trees were sampled throughout the experiment. A sample was also taken for fruit quality analysis.

Analysis: Fruits from each lot were weighed individually and the diameter of each fruit was measured. The fruits were peeled and again weighed. The peeled fruit of each lot was blended with 3 times

[6]Use of trade names of specific materials does not constitute a recommendation by the U. S. Department of Agriculture to the exclusion of others which may also be available.

its weight of water. With the Waring blendor running at low speed to insure uniformity, samples were drawn into a pipette through the mouth end and weighed into beakers. Duplicate 100 g samples of slurry were analyzed for lycopene and carotene by the procedure previously used (5, 6, 7). Samples were also taken for fruit quality analysis.

The data were examined by regression and analysis of variance procedures to evaluate trends and changes in mg lycopene per fruit, (lycopene yield) and mg lycopene per 100 g carpels (lycopene concentration) and to evaluate differences in trends due to differing environments.

Results

In the 1963 experiment, the visible changes in the trees and fruit in the chambers were striking. The foliage on the trees in the 95°/85°F chamber became greener and the trees started a flush of growth which elongated 12–14 inches within the next 4 weeks. The fruit remained green through most of the test with slight fading and development of some red blush by the end of the 6-week period. The fruit increased in size during the test (Table 1). In the 60°/40° chamber the foliage of the trees became lusterless and no sign of new growth could be found. The fruit were noticeably lighter in color within 2 weeks, becoming lemon yellow by the end of the test. They did not increase appreciably in size during the experiment after the first 2 weeks. Under both temperature regimes the total lycopene yield increased during the first week and decreased during the second week. After the second week the lycopene yield of fruit in the hot and cold chambers diverged sharply and significantly. In the cold chamber, the lycopene concentration and lycopene yield followed the usual decline. In the hot chamber, lycopene concentration and lycopene yield remained close to the original high level until the end of the test on September 3. The concentration of beta carotene increased in both chambers throughout the experiment.

In the 1964 study with grafted seedlings the foliage of seedlings in

Table 1. Changes in lycopene concentration and carpel weight of grapefruit from trees in hot chambers (95°/85°F), cold chambers (60°/40°), and in a grove.

Date	Hot		Cold		Grove		Avg carpel wt in g		
	mg %	mg/fruit	mg %	mg/fruit	mg %	mg/fruit	hot	cold	grove
July 22.....					1.77	2.01			117
July 29.....	2.35	2.42	2.55	2.32			103	91	
Aug. 5.....	2.56	3.48	2.65	2.76			136	104	
Aug. 6.....					3.17	3.71			119
Aug. 12.....	1.68	2.45	1.84	2.02			146	110	
Aug. 13.....					2.27	2.84			125
Aug. 19.....	2.53	3.59	1.54	1.69			142	110	
Aug. 21.....					2.78	2.70			97
Aug. 26.....	3.05	4.27	1.45	1.62			140	112	
Aug. 27.....					2.34	2.53			108
Sept. 3.....	2.73	4.18	1.84	2.06			153	112	
Sept. 4.....					2.98	3.31			111
Sept. 16.....					2.33	3.47			149

Table 2. Growth of grapefruit in a grove and grapefruit grafted onto seedling and held under controlled environment, expressed by fruit diameter and weight of carpels.

Date	Diameter of fruit (cm)					Weight of carpels (g)				
	control	grove	95°/85°	70°/60°	60°/40°	control	grove s	95°/85°	70°/60°	60°/40°
July 14...	5.55	6.35				32.9	55.4			
19...			5.47	5.54				35.6	36.0	
20...	5.50	6.46				34.2	60.3			
Aug. 2...			5.86	5.85				45.0	48.9	
3...	6.23	7.07				44.9	79.7			
16...			5.81	6.01				46.1	57.6	
17...	5.89	7.27				53.0	97.2			
30...			6.14	6.82				62.8	84.8	
31...	6.37	7.51				67.8	121.0			
Sept. 13...			6.05	6.79				67.8	91.0	
14...	6.54	7.62				86.1	141.2			
27...			6.24	7.05				68.0	109.1	
28...	6.41	8.14				78.1	152.7			
29...					6.46					75.5
Oct. 11...			6.40	7.21				86.9	121.4	
12...	6.97	8.56				101.7	186.7			
13...					6.71					89.1
25...			6.03	7.31				77.0	129.2	
26...	6.95	8.86				112.1	216.2			
27...					6.58					90.6
Nov. 8...			6.61	7.26				106.8	128.6	
9...	7.31	8.80				123.1	211.3			
10...					6.97					102.1
22...			6.35	7.59				93.6	151.1	
23...	7.49	8.91				143.5	237.0			
24...					6.98					105.0
Jan. 5(65)		9.14			7.02		255.8			107.7
18...					6.79					97.1

the 95°/85°F chamber increased in green color, but did not go through a flush of growth as did the potted trees of the previous years. The seedlings were planted in 1-quart cans and were watered every 48 hours. At these high temperatures moisture was depleted between waterings and the seedlings occasionally appeared in slight water stress. It is believed this periodic stress inhibited new growth and maximum sizing of the fruit. Fruit in the 95°/85° chamber degreened slightly. Most of the fruit had developed a red blush two-thirds through the test. The fruit size increased slowly but continuously (Table 2).

In the 70°/60° F chamber fruit degreening was evident by the fourth week. At the end of the exposure November 22, the fruit were lemon yellow. Fruit size increased continuously. The trees maintained a lush green appearance and went through a flush of growth.

The reaction of fruit grafted on seedlings in the 60°/40° chamber were similar to the reaction of fruit borne on trees in the previous season.

Average fruit diameters and carpel weights at the various harvest dates are shown for each of the 5 treatments in Table 2. Average concentrations (mg% lycopene) and yields of lycopene (mg lycopene per fruit) are given in Table 3. The quadratic curves of best fit for

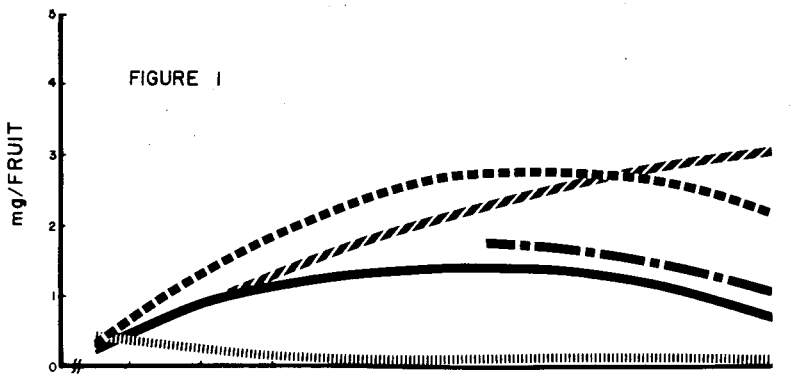
Table 3. Seasonal changes of lycopene concentration and content in grapefruit from a grove compared to grapefruit grafted onto seedlings and held under controlled environments.

Date	Lycopene (mg) %					Lycopene fruit (mg)				
	grove	control	95°/85°	70°/60°	60°/40°	grove	control	95°/85°	70°/60°	60°/40°
July 14...	.80	1.32				.45	.43			
19...			1.39	.89				.46	.32	
20...	1.10	1.47				.66	.51			
Aug. 2...			1.81	.54				.81	.27	
3...	1.29	1.41				1.02	.64			
16...			2.10	.21				.97	.12	
17...	1.60	1.41				1.56	.75			
30...			2.83	.17				1.79	.15	
31...	2.04	2.20				2.44	1.50			
Sept. 13...			2.97	.13				2.08	.12	
14...	1.97	1.81				2.77	1.56			
27...			3.24	.09				2.19	.10	
28...	1.93	1.75				2.94	1.37			
29...					2.37					1.79
Oct. 11...			3.13	.07				2.71	.09	
12...	1.54	1.23				2.89	1.32			
13...					1.79					1.59
25...			3.02	.07				2.32	.09	
26...	1.22	1.16				2.61	1.30			
27...					1.52					1.36
Nov. 8...			3.05	.09				3.17	.12	
9...	1.06	.59				2.24	.72			
10...					1.42					1.44
22...			3.07	.04				2.85	.06	
23...	.96	.53				2.37	.75			
24...					.93					.98
Jan. 5(65)	.50				.60	1.26				.64
18...					.57					.57

the several treatments are shown for lycopene yield in Fig. 1, lycopene concentration in Fig. 2, and fruit growth in Fig. 3. The constants for these curves are given in Table 4.

From Fig. 3 and Table 4, it is obvious that average grapefruit size, as determined by carpel weight, increased linearly (and significantly) as the season progressed for all treatments. There was no evidence of curvature due to reduced growth at later harvests under the harvesting system followed. The rate of carpel weight increase was significantly (P .005) greater for the 70°/60° F than for the 95°/85° treatment. This may have been a function of the observed water stress in 95°/85° plants. Increase of carpel weight on trees placed in the 60°/40° chamber on September 15, was significantly (P .0005) less for the remainder of the season than for the controls, which remained outside.

Lycopene yields increased significantly (P .0005) throughout the experimental period for the 95°/85° F treatment with no indication of slackening off. It decreased significantly (P .0005) under the 70°/60°F treatment coming to an equilibrium at about 0.1 mg lycopene per fruit. Lycopene per fruit in both grove fruit and grafted control plants held outside in full sunlight increased to a maximum about the first half of October then declined. The curvature was sig-



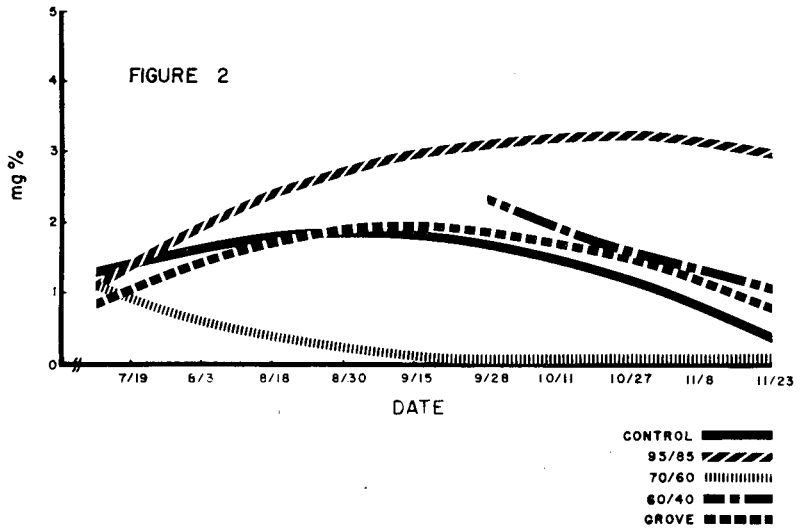


Fig. 1. Lycopene yield of grapefruit under different temperatures plotted against date.

Fig. 2. Lycopene concentration in the carpels of grapefruit under different temperatures plotted against date.

Table 4. Coefficients of the best fitting (least squares) quadratic curves of growth, lycopene concentration, and lycopene yield of grapefruit grafted on seedlings and held under controlled environments.

Environment	Coefficient[b]	Growth (carpel wt g)	Lycopene	
			Concentration (mg %)	Yield (mg/fruit)
95°/85°	a	27.73	0.826	0.024
	b	8.24**	0.628**	0.482**
	c	−0.10	−0.041**	−0.018
70°/60°	a	20.61	1.297	0.438
	b	16.19**	−0.371**	−0.099**
	c	−0.35	0.026**	0.006**
60°/40°	a	−15.63[b]	6.180[b]	1.405[b]
	b	20.23	−0.830**	0.198**
	c	−0.81	0.031	−0.023
Control	a	27.64	1.121	0.068
	b	8.20**	0.334**	0.461*
	c	0.31	−0.041**	−0.040**
Grove	a	40.92	0.638	−0.134
	b	20.23**	0.475	0.849**
	c	−0.04	−0.046**	−0.062**

[a]Yield = $y = a + bX + cX^2$.
[b]Based on only 5 dates.
*Significant at 0.05 level. **Significant at 0.01 level.

nificant, (P .0005) and P .001, respectively). Although both the linear and quadratic trends of lycopene per fruit in grove and control fruit were demonstrated to be significantly different (P .0005 and P .025, respectively). Fig. 1 shows that both treatments followed essentially the same pattern of seasonal production. Lycopene production of fruit from those plants removed from the control environment to the 60°/40° environment declined in the same pattern as from those plants that remained outside.

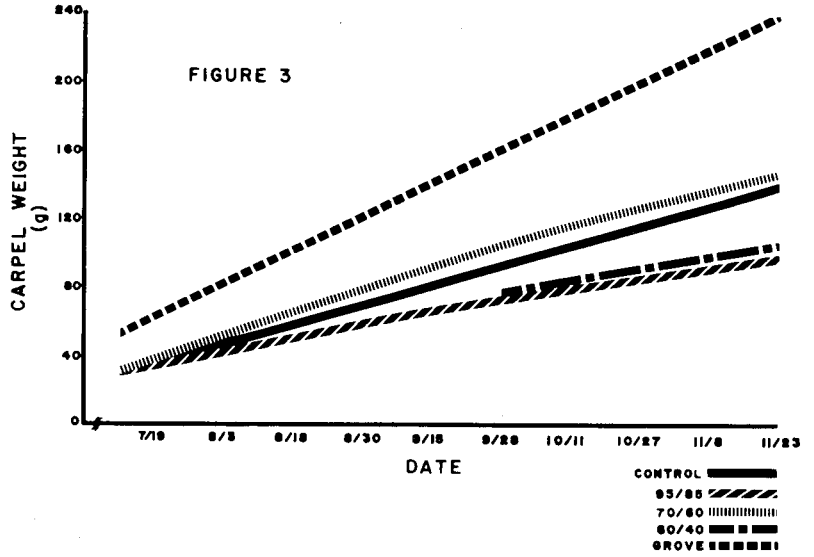


Fig. 3. Growth of grapefruit under different temperatures, indicated by carpel weight plotted against date.

A statistically significant (P .0005) difference was demonstrated in the overall slopes of the lycopene concentration curves of the 2 treatments kept outside, grove fruit and grafted control fruit. Despite this general difference in slope, however, the curvature and seasonal pattern of concentration changes was quite similar, demonstrating that the seasonal changes in concentration of lycopene in grafted fruit kept outside in full sunlight, followed essentially the same pattern of seasonal changes as found in normal fruit.

After placement in the chambers, lycopene concentration of fruit at 95°/85° F rose immediately and significantly (P .0005), and only reached a maximum near the end of the experiment about the beginning of November. Likewise, lycopene concentration of fruit at 70°/60° declined immediately and significantly (P .0005), coming to an equilibrium value close to zero around the middle of September.

Discussion

It appears that fruit borne on small potted trees or fruit-bearing wood grafted onto seedlings mature the fruit essentially the same as normal fruit. It is believed this procedure offers a practical means to study the effects of environment on fruit in climatic chambers. The procedure makes possible the study of a wide variety of environmental effects under closely controlled conditions.

The course of lycopene accumulation in "Redblush" grapefruit can be explained by the postulation of two metabolic systems; lycopene synthesis and lycopene destruction. It is probable that both processes occur simultaneously at least for a while, during fruit maturation. Purcell (unpublished) found that $^{14}CO_2$ was incorporated into lycopene after the lycopene yield had begun to decline. The results reported here and elsewhere (6, 8) can be explained on the basis that the lycopene destroying system is "initiated" as a function of fruit age and gradually increases in activity. The point of maximum lycopene yield occurs when the rate of destruction and synthesis are equal. Periods of high temperature may either increase the rate of lycopene synthesis or inhibit the lycopene destroying system, either of which will cause net synthesis. Periods of low temperature have the opposite effect. The trend of the lycopene concentration is similar to that of lycopene yield but is complicated by continuous sizing of the fruit during this period.

The temperatures chosen for these studies though very dissimilar, are representative of average day-night temperatures in the lower Rio Grande Valley at various seasons. The 95°/85°F chamber is representative of the summer season. This chamber extended "summer" for about 2 months. The 70°/60° chamber is representative of the upper temperatures which occur from November until spring. The 60°/40° chamber is representative of the lower temperatures which occur periodically from the end of November until spring. Usually there is a greater difference between maximum and minimum temperatures than occurred in the chambers. The day-night temperatures averaged over 12-hr periods, give values quite close to those in the chambers.

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