Technical Bulletin No. T-13

June, 1942

Í

# OKLAHOMA AGRICULTURAL AND MECHANICAL COLLEGE

# AGRICULTURAL EXPERIMENT STATION

W. L. BLIZZARD, Director LIPPERT S. ELLIS, Vice Director

# THE UNEVEN RIPENING OF CONCORD GRAPES: **CHEMICAL AND PHYSIOLOGICAL STUDIES**

By

# JAMES E. WEBSTER and FRANK B. CROSS

Departments of

Agricultural Chemistry Research

and

Horticulture

The Concord grape is the most popular and widely planted variety produced commercially in the United States. Although this grape is generally described as being adapted to a wide range of soil and climatic conditions, there are certain areas in the United States, including most of Oklahoma, where the berries fail to color properly. In Oklahoma, this phenomenon is so pronounced that at harvest time often as many as a quarter of the berries are still green, which practically ruins their market value. Reports from other states indicate that this is not an isolated phenomenon that is peculiar to Oklahoma and parts of the southwest. In other sections of the country, various persons have noted this phenomenon in certain years, although it was not serious enough to damage the market value of the grapes.

The investigations reported in this bulletin were undertaken in an effort to secure information on this problem. The objects of the investigations were (1) to determine the factors responsible for and underlying this condition, (2) what, if anything, can be done to secure more even ripening, and (3) to assess the future possibilities for growing this grape in areas where uneven ripening has been found to occur.

Various treatments of the vines in the field have shown there is little that can be done to materially improve color development if standard good cultural practices are followed. Use of nitrogen fertilizers has slightly improved color formation, while both shading, and irrigation near harvest time, have proven detrimental.

Extensive studies of the grape vine and foliage have failed to show any abnormalities in Concord grapes that would account for the known, low sugar content of the ripening fruit.

These results indicate that in regions where uneven coloring is known to occur it is desirable to replace Concord vines with other varieties, of which there are many, that are adapted to that particular region.

Results secured in this investigation show rather conclusively that the direct cause of uneven coloring in Oklahoma is a partial lack of sugar in the maturing grapes. Berries on a cluster are uneven in their composition and color develops only when the total sugar content exceeds around seven percent. There is a gradual increase in sugar content as maturity approaches; but, by the time all berries are colored, the first to ripen have deteriorated and often have fallen from the cluster. This complete coloring occurs only if the clusters are allowed to remain on the vines long past the usual harvest time. Other differences were found between the green and colored fruit, but the experimental work indicates that these other differences are incidental rather than causative.

# **CONTENTS**

Introduction	5
Review of literature	5
General experimental procedure	8
PART I: Effect of physiological and environmental factors upon	
degree of ripeness at marketing time as indicated by $(1)$	
percentage of colored berries and (2) chemical com-	10
position of juice	14
Environmental factors	14
Application of fertilizers	14
Nitrogen	14
Use of sugar to reduce available nitrates	14
Phosphorus	14
Irrigation	14
Additional light	14
Shading	14
Artificial cooling	16
Weather	16
Summary of environmental studies	10
Physiological factors	17
Leaf pruning	10
Girdling	19
Bagging of fruit	19
DADE II. Chamical changes in the developing fruits of shown	
PART II: Chemical changes in the developing fruits as shown	10
by analysis of perries and juice	19
Changes during ripening	24 94
Solius	24
Ash content	24
Asidity and nU	4 <del>4</del> 94
Sugar	27
Changes during a 24 hour pariod	26
Unanges during a 24-nour period	20
Mineral analysis of herring	26
Mineral analyses of sking	20
Mineral analyses of soods	21
Seeds in relation to color	20
Enzyme studies	20
	40
DADE III, Analyzia of vince and looved comparing Concerd with	
other variation	20
Vine pruninge	29
Vinc prunings	
Leaves	2
Sampling procedure	2
Chloronbyll	20
Carotene and vanthonhyll	20
Detailed leaf analyses	
Total solids	40
Total sugars	<del>1</del>
Ash nercentages	±1
Total nitrogen	41
Detailed ash analyses	41
Summary	43
Literature cited	45

# The Uneven Ripening of Concord Grapes: Chemical and Physiological Studies\*

By JAMES E. WEBSTER and FRANK B. CROSS\*\*

# INTRODUCTION

Ripening of Concord grapes is a cumulative process taking place over an extended period of time. Only a part of the berries color at first, and then as the season progresses more start coloring until finally all have colored. By this time, however, those which colored first usually have become over ripe and have fallen from the cluster. Along with the increase in color as maturity is approached, there is a progressive increase in sugar content. The increased sugar content, however, is found chiefly in the colored berries. The experimental work reported in this paper was designed to study some of the factors known to be associated with color development and photosynthesis in plants, and their relation to grapes, particularly Concord grapes.

### **REVIEW OF LITERATURE**

# General

Much has been written and many experiments have been conducted dealing with the Concord grapes and various phases of its growth, culture, and development. Particularly good bulletins with extensive bibliographies have been written by Colby and Tucker (6), Partridge (30), and Schrader (33), dealing with the growth and fruiting habits of the Concord grape. None of these, however, contain data that bear specificaly on the probem of uneven ripening. Schrader (33) gives detailed analyses for the roots, tops, and shoots at different periods during the year, and discusses these data in relation to pruning and bud formation. Maney and Plagge (22) report chemical analyses on portions of the canes, along with some analyses of roots and trunks. The suggestion is made that pruning vines to extremely long canes and reducing the number of vines per acre may be advantageous under certain conditions. An earlier publication from this station (39) gives some data on the composition of grape leaves, including Concords, but the data are not conclusive.

#### Composition of the Fruit and Juice

Concerning analyses of the fruit, much data is available, many papers having been published dealing with the composition of grapes and grape juices. Probably the best summary of data covering all phases of grape analyses is to be found in the book by Winton and Winton (40), which presents data on such points as changes in composition during ripening, influence of season on composition of the juice, various acids that are present, pectin percentages, colors, and odorous constituents. Hartmann

<sup>\*</sup> In addition to the published articles listed in the bibliography (11, 12, 13, 38, and 39), papers dealing with certain phases of the subject were presented before the American Chemical Society meeting in Kansas City in 1934 and the Midwestern sectional meeting in the same city in 1937. Another paper was presented before the American Society for Horticultural Science at the American Association for the Advancement of Science meeting at Richmond in 1938-39, and an abstract is published in Volume 36 of the proceedings of that association.

<sup>\*\*</sup> The authors wish to express their appreciation to Edward Anderson, Raymond Gentry, and Rodney Black, who assisted with parts of the analytical work.

and Tolman (20) give detailed data on the chemical composition of commercial Concord juices. They report the average sugar content of the juice to be 15.33 g per 100 ml, with a minimum of 11.41 g and a maximum of 17.53 g. No sucrose was found in the pure juice. Tannins present averaged 0.24 g per 100 ml and ranged from 0.07 to 0.37 g. Alwood et al. (1) present detailed data on the development of sugars and acids in grapes during ripening. They state, in general, the sugar should increase and the acid diminish in the berries as long as the leaves function properly. Some decrease may take place if the fruit remains until the leaves have fallen. Ash generally increases during ripening. Detailed ash analyses are given for some samples. Concord fruit samples from Charlottesville, Va., were somewhat lower in sugar and acid than were samples from Sandusky, Ohio. Noyes, King and Martsolf (26) also studied the variations in composition of the fruit as ripening progressed. Like Hartmann and Tolman, they failed to find any sucrose in Concord grapes. However, they also failed to confirm the report of Alwood et al. that sugar increases as long as the leaves function properly, explaining that such an increase near the close of the season is probably due to water losses. From date to date, sugar percentages fluctuated irregularly; and this was explained as probably due to a retardation of sugar formation during cloudy days. Warm, sunny days and cool nights were found to be most favorable to the development of sugar. Tannins varied irregularly during the ripening season. Weights of individual berries remained fairly constant during the ripening season. Bioletti, Cruess, and Davi (4) discuss the changes that take place in vinifera grapes during ripening. Particular attention is called to the care necessary to secure representative samples for analysis. Analyses were made of leaves, but the data are incomplete. An article by Caldwell (5) contains extensive analyses of many varieties of grape juices, with the samples collected over several years. The average sugar content found for Concord juice at Vineland, N. J., was 14.39%, with detectable amounts of sucrose. He states that "There is a very definite and clearly marked effect of climatic conditions during the growing season upon the total sugar content, total astringency and titratable acidity of the fruit produced during that year." Sunshine is claimed as the dominant factor in determining the chemical character of the crop. Attention is also called to the influence of soil and cultural conditions as they affect varietal composition. Dix and Magness (15) list the Concord as the standard all-purpose variety but show it to be relatively low in sugar. Venezia and Gentilini (36) studied the progressive changes that take place in the juice and skin as ripening progresses. Reducing-substances and nitrogen percentages increased in the juice as the season progressed, while the acids decreased. Only traces of tannins were found at later stages of development. In the skin there was a decrease in total and protein nitrogen, while tannins and non-protein nitrogen varied but little.

Peynaud (31) in two articles gives total, ammoniacal and amino nitrogen analyses on clear juice samples taken at different stages of maturity. Various forms of nitrogen are elaborated from the ammonium ion in the grape. Amino nitrogen is chiefly found in ripening grapes and constitutes at maturity 12-25% of the original nitrogen of the grapes. Total nitrogen increases to four or five times its original amount during ripening.

Probably the most extensive studies of changes occurring during ripening of grapes are those reported in a series of chemical bulletins from the Union of South Africa, Department of Agriculture. A bulletin by Villiers (37) discusses at great length the enzyme activity of developing grapes. Catalase activity increased as maturity advanced up until a certain place, and then decreased; oxidases, on the other hand, showed decreased activity as the berries ripened. Respiration of grapes responded to temperature in a way similar to a monomolecular chemical reaction. Temperatures beyond  $40^{\circ}$  C. were found injurious, though respiration was temporarily stimulated. In discussing color of grapes, several observations are made by Villiers, of which the most important seem to be:

- 1. Certain varieties like Red Hanepoot develop much better colours in the Constantia area than they do at such places as Paarl;
- 2. Aeration, i. e., a good supply of oxygen, is essential for colour development, as oxidation by the enzyme oxidase is one of the processes of normal pigmentation;
- 3. Light does not seem essential for all varieties as regards colour development; the effect of light needs further investigation;
- 4. Acidity changes result in corresponding changes in the pigment, and various shades of colour may be obtained by varying the acidity;
- 5. In the writer's estimation, **nutrition**, and hence such factors as soil, fertilizers, etc., are most important. At present it seems that certain mineral constituents are essential for pigment formation, and that the shade of colour varies primarily with acidity.

Frater (16) in his bulletin, which includes an extensive bibliography, gives detailed analyses of the juice, pulp, skins, stalks, and seeds of many varieties. He gives as the average ash content the following figures: pulp 0.23%: skins 1.49%; stalks 1.86%; and seeds 2.01%. A series of three bulletins by Copeman (8, 9) and Copeman and Frater (10) gives detailed analyses regarding the composition of grapes during ripening. Some of their more pertinent conclusions are: Changes in total solids which occur after the berry begins to ripen are mostly due to an increase in sugar content; changes in nitrogen content are not connected with the process of ripening, and the period of maturity cannot be predicted from the nitrogen content; the mineral content of the berry increases during ripening, the absolute amount remaining nearly constant at maturity; and, astringency changes are irregular with a tendency toward decreasing as the fruit matures.

#### **Color Formation**

Probably the best discussion of the formation of color in plants and fruits is that in the book by Onslow (27), although since it was published many articles have been written dealing with the classification and composition of anthocyanins. Among Onslow's general statements and conclusions of interest to this problem are the following: "Most general observations bear out the conclusion that increase of anthocyanin is correlated with lowering of temperature: the chromogen for anthocyanin is probably formed in the leaf; and an accumulation of carbohydrates (sugars) and glucosides leads to the formation of anthocyanin." Recently in an article by Rutzler (32) a technique was described which permits a classification of the precursors of anthocyanins, which are claimed to be either flavones or leuco-anthocyanins. As yet the technique has not been appied to fruit, although the paper states such a study is in progress. In a previous article by Bancroft and Rutzler (3), the statement is made that, "Anthocyanin pigments may be obtained in the laboratory or in the plant, by reduction of flavones or by decomposition of leuco-anthocyanins." The statement is also made that the development of anthocyanins is always due to enzymes, and the inference is left that, in tree leaves at least, enzymes are the major factor involved. Moreau and Vinet (24), in studying changes during ripening of the Gamay variety, point out changes in the dextroselevulose ratio, dextrose being the chief sugar in green grapes while at maturity the ratio is approximately one. They also claim that the large increase in sugars over this period comes in part from mobilized reserves in parts of the plant other than the leaves. Overholser (28) confirms earlier work that grapes can color even if the fruit is bagged while green and that there is no appreciable diminution in color from this practice. Annual reports from the Arkansas Station over the past two decades contain many references to uneven ripening of grapes and present statements regarding their results, although in general few data are given. Some of the later results from the Arkansas Station are summarized in the bulletin by Cooper and Vaile (7), in which they discuss uneven ripening along with other topics. Their summary is as follows:

Neither cultural practices nor fertilizer affected uneven ripening materially.

Heavy dormant pruning was accompanied by more even ripening. Summer pruning aggravated uneven ripening.

Thinning Concord grapes to one cluster per shoot materially increased evenness of ripening.

A brief statement by Vaile (35) in the forty-eighth annual report of the Arkansas Station shows a correlation between ripeness and number of seeds per berry. Ripe berries from ten vines averaged 1.75 seeds per berry and the green ones 1.38 seeds each.

Work has been conducted at the Oklahoma Station on the specific problem of uneven coloring of Concord grapes over the past thirteen years. Several articles (11, 12, 13, 38, 39) have been published embodying parts of our data, and these will be discussed in connection with the experimental data.

# GENERAL EXPERIMENTAL PROCEDURE

During the thirteen-year period covered by these investigations, three major lines of study have been followed. In the first of these, the sugar content of the berries and the percentage of colored berries were used as indices of ripeness in an attempt to determine the effect of various physiological and environmental factors upon the evenness of ripening. In the second, samples of juice made from grapes at various stages of ripeness were analyzed to determine the chemical changes which occurred during the ripening process. In the third, chemical analyses of vine prunings and leaves from Concord grapes were compared with analyses of similar portions of the plants of other varieties in an effort to learn whether particular physiological characteristics of the Concord might differ from those of other varieties.

#### Vineyard Location and Management

Nearly all of the analyses were made on samples secured from one of two vineyards maintained by the Oklahoma Agricultural Experiment Station. The one at Stillwater is located on a fine clay loam classified as belonging to the Yahola series; that at Perkins, ten miles south of Stillwater, is on a sandy soil classified as Stidham loamy fine sand. These vineyards have been accorded the best of cultural practices, including cultivation, pruning and spraying. The vines have been trained throughout on the Kniffen trellis system.

The studies dealing with the influence of light, fertilizer, girdling and the like upon the degree of coloring were conducted at the Stillwater vineyard during the years 1932-34. The vineyard plan is shown in Figure 1, which gives the location of the individual plants and also the number and location of plants used for each treatment. Only mature, healthy plants were included in any test. Detailed descriptions of the procedure and equipment used are given later along with the data.

#### **Preparation of Juice Samples**

In preparing samples of juice, the berries were removed from the clusters and allowed to stand over night. In the morning they were crushed and heated to incipient boiling. The crushed mass was then placed in a cheese cloth and the juice expelled by means of a one-gallon fruit press. After one pressing, the cloth was removed and the marc was broken up and returned to the press for another pressing. The juice was then placed in bottles or in enameled cans and pasteurized at approximately  $170^{\circ}$  F., the temperature being secured from an open, water-filled bottle or can respectively.

#### **Analytical Methods**

Carbohydrates. All sugar determinations were run using the Shaffer-Hartmann procedure as outlined in the laboratory manual by Morrow (25). When the samples (whole grapes or ground leaves) were preserved in alcohol, extracts were prepared by decanting the alcohol and extracting residues in continuous extractors until all of the sugars were removed. Alcohol was removed from aliquots of the combined extracts by evaporation on a steam bath, without allowing the samples to reach dryness. From this point the procedure was the same as for the juice. Neutral lead acetate was added in excess and the samples filtered with thorough washing. Neutral potassium oxalate was then added to remove excess lead, and the solutions filtered. Aliquots were used for the determinations. Total sugars were run on aliquots of the previous solution after inversion with "Difco" invertase solution. Dextrose was estimated following the procedure of Lothrop and Holmes (21). Acid hydrolyzable values were secured by hydrolyzing the finely powdered tissue with HC1 according to the Official Methods (2), and then running sugars on the neutralized and filtered but uncleared solutions.

Nitrogen. Total nitrogen was run as directed in the Official Methods (2), using the Gunning modification.

Ash and Ash Analyses. Ash percentages were secured by ashing the samples in platinum at a low red heat. Calcium, phosphorus, and potassium were run as directed in the Official Methods (2) under plant analyses.

Solids. Values for solids, unless otherwise stated, were secured by drying over night in an oven at  $105^{\circ}$ . Juice samples were dried in a vacuum oven at  $75^{\circ}$  C. until constant in weight. In the leaf pigment and leaf composition work, the moisture and ash samples were first dried over electric lights which maintained a temperature of  $60-80^{\circ}$  C. and then later dried at  $105^{\circ}$ .

Density. A Westphal balance was used and the determinations made at  $16\,^\circ$  C.

**Hydrogen Ion Concentration.** Nearly all of the determinations were made using a Welch quinhydrone pH indicator. A few of the later determinations were run using the Coleman glass electrode.

Acidity (Titratable). Five or ten ml of the juice were diluted to 300 ml with distilled water and neutralized using phenolphthalein for an indicator. The results are calculated as percent of tartaric acid.

Tartaric Acid, Cream of Tartar, Free Tartaric Acid, and Alkalinity of the Ash. These were run according to the Official Methods (2) as described under wine analysis.

Astringency. Tannins and related materials were determined essentially as done by Caldwell (5), i. e. by titrating with  $\text{KMn0}_4$  in the presence of indigo-carmine. As indicated by Caldwell, the calculation of values other then tannins is of value chiefly in making comparisons and does not represent absolute values.

**Enzymes.** Iodine reduction and peroxidase determinations were run according to the directions of Miller (23). Oxidase was determined according to Gulhrie's method (19). Catalase was run on 5 ml neutralized samples following the procedure outline by Davis (14).

**Figments.** Pigments were extracted according to the procedure of Shertz (34) and the chlorophyll estimated by comparison with the color standards recommended by Guthrie (18). Carotene and xanthophyll solutions were compared using dichromate standards according to the procedure described by Palmer (29).

# PART I: Effect of Physiological and Environmental Factors upon Degree of Ripeness at Market Time as Indicated by (1) Percentage of Colored Berries and (2) Chemical Composition of Juice.

The general plan in this portion of the investigation was to alter various environment factors and physiological conditions in the grape plant and to determine the effect on the degree of ripeness at picking time by (1) determining the proportion of colored and green berries produced and (2) chemical determination on the juice expressed from the berries.

The environmental factors investigated included various fertilizer treatments, sub-irrigation, temperature and humidity, and light. The physiclogical variations included leaf pruning, girdling the vines, and bagging the fruit. These studies were made in the Stillwater vineyard which was laid out as shown in Figure 1.

Counting of the berry color was done at the time they were removed, by hand, from the clusters. Juice samples were later prepared from these berries as described on page 9. This juice was analyzed during the following winter by the methods described on pages 9 and 10.

Chemical and color count data are assembled in Table I. Detailed figures for each of three years are given in order to show the yearly variations and to illustrate the point that yearly differences are often greater than those shown by many of the treatments. A summary of the three year's results 1932, 1933, and 1934, is given in Table II. Data in the tables are arranged in order of their reducing sugar content, which factor will be shown later (see page 25) to be most intimately connected with coloring of the fruit. The values quoted under "Check" represent the composite value for all of the odd vines of the vineyard whose growth was not affected by the special treatments. Reference to Figure 1 will show that these represent a fair random sampling of the area.

While a part of the data in the summary is for two years and part of three years, this does not appreciably alter the rating of the treatments unless it is to raise relatively the position of the sugar treatments.

A description of each of the treatments, together with a discussion of the results of each follows.



Figure 1. Plan of the experimental vineyard at Stillwater showing location of the special treatments described in Part I. (X marks the location of each vine).

			I	Averages						
Theotheory			ASTRI	IGENCY G.	PER L.		A	BE	RRY COLO	R1
Treatment	% Red Sugars	% Acidity	Total	Non- Tannins	Tannins	Density	Sugar Ratio	Green	Colored	Ripe
				1932						•
Add. light	11.31	.573	1.372	.956	.350	1.0611	1:29.7	9.7	10.2	80.1
Nitrogen	10.83	.398	1.029	.771	.258	1.0605	1:27.5	8.1	3.1	88.8
Superphosphate	10.80	.422	1.090	.842	.239	1.0590	1:25.7	14.8	2.7	82.5
Check	10.43	.360	.993	.786	.208	1.0554	1:29.4	12.3	5.2	82.5
Shade	10.25	.578	1.025	.765	.260	1.0525	1:27.0	53.2	5.5	41.3
Sugar	9.94	.402	.929	.707	.223	1.0521	1:26.2	10.9	15.8	73.3
				1933						
Add. light	10.85	.639	2.081	.975	1.106	1.0542	1:17.3	23.59	4.53	71.88
Nitrogen	9.85	.584	2.035	.994	1.041	1.0569	1:16.9	18.75	3.72	77.53
Superphosphate	9.25	.698	1.968	1.121	.937	1.0531	1:14.7	24.18	3.77	72.05
Irrigation	8.90	.751	2.118	1.022	1.097	1.0495	1:11.9	43.17	7.52	49.31
Water cooled	8.89	.582	1.923	1.106	.817	1.0527	1:15.8	24.31	4.65	71.04
Doplex shade	8.73	.680	2.043	1.218	.825	1.0508	1:12.8	37.23	3.61	59.16
Check	8.62	.647	2.040	1.105	.935	1.0530	1:13.7	27.67	7.31	65.02
Sugar	8.32	.600	1.818	.937	.882	1.0494	1:13.9	36.93	3.98	59.09
Shade	7.74	.623	1.754	1,031	.729	1.0462	1:12.6	24.06	3.49	72.45
Shading cloth	7.37	.571	1.968	1.031	.937	1.0473	1:13.3	28.57	3,60	67.83

 TABLE I.—Analyses of grape juice samples secured from vines grown under different environmental conditions and subjected to special physiological treatments.

<sup>1</sup> Percentage of berries in each classification.

# TABLE I.—Continued)

			ASTRIN	GENCY (G.	PER L.)		، ان ان ا	BE	RRY COLO	R1
Treatment	% Red Sugars	% Acidity	Total	Non- Tannins	Tannins	Density	Sugar Ratio	Green	Colored	Ripe
			Providence of the second s	1934						
Water cooled	9.12	.449	1.623	.869	.753	1.0532	1:21.0	11.69	14.58	73.73
Shading cloth	8.90	.351	1.262	.733	.529	1.0522	1:25.4	5.97	27.74	66.29
Doplex shade	8.88	.377	1.166	.732	.434	1.0511	1:23.6	10.25	23.74	66.01
Nitrogen	8.66	.402	1.187	.759	.428	1.0500	1:22.2	10.57	15.87	73.58
Add. light	8.73	.574	1.218	.828	.390	1.0494	1:15.6	11.56	35.79	52.85
Irrigation	8.44	.480	1.058	.678	.381	1.0468	1:18.0	26.71	44.33	28.96
Checks	8.39	.483	1.210	.765	.445	1.0481	1:17.7	6.17	26.22	67.61
Superphosphate	8.02	.424	1.122	.738	.384	1.0378	1:19.9	10.41	25.48	64.11
Sugar	7.82	.418	1.107	.715	.391	1.0440	1:18.9	1.90	10.66	87.44
Shade	7.56	.420	1.189	.709	.475	1.0460	1:18.0	11.93	42.50	45.57

<sup>1</sup> Percentage of berries in each classification.

TABLE II.—Analyses of grape juice samples secured from vines grown under different environmental conditions and subjected to special physiological treatments; summary, 2 and 3 years.

			ASTRIN	GENCY (G.	PER L.)		A	BE	RRY COLC	$\mathbb{R}^{1}$
Treatment	% Red Sugars	% Acidity	Total	Non- Tannins	Tannins	Density	Sugar Ratio	Green	Colored	Ripe
Add. light	10.30	.595	1.557	.919	.638	1.0549	1:20.9	14.95	16.84	68.11
Nitrogen	9.78	.461	1.417	.841	.576	1.0558	1:22.2	12.47	7.56	79.97
Superphosphate	9.36	.515	1.390	.900	.520	1.0500	1:20.1	16.46	10.65	72.89
Check	9.15	.497	1.414	.885	.529	1.0522	1:20.3	15.35	12.91	71.74
Water cooled*	9.01	.516	1.773	.988	.785	1.0529	1:18.4	18.00	9.62	72.38
Doplex shade*	8.81	.529	1.605	.975	.630	1.0510	1:18.2	23.74	13.68	62.58
Sugar	8.69	.472	1.285	.786	.499	1.0485	1:19.7	16.58	10.15	73.27
Irrigation*	8.67	.616	1.588	.850	.738	1.0481	1:14.9	34.94	25.93	39.13
Shade	8.52	.540	1.323	.835	.488	1.0482	1:19.2	26.40	17.16	56.44
Shading cloth*	8.14	.461	1.615	.882	.733	1.0498	1:19.4	17.27	15.67	67.06

\* 2 years only. Percentage of berries in each classification.

# ENVIRONMENTAL FACTORS

# **Application of Fertilizers**

Nitrogen. Nitrogen was supplied to the plant by spreading ammonium sulfate on the soil and cultivating. Two and one-half pounds were used per plant and the application made twice (first of May and the middle of June). This treatment slightly improved coloring, but had a lesser effect upon raising the sugar content. During 1934, a year of unusual heat, nitrogen was an effective coloring agent, as was water cooling.

Use of Sugar to Reduce Available Nitrates. In an effort to determine the effect of reduction of nitrogen, sucrose was applied to the soil around certain plants at the rate of 10 pounds per plant in the spring, two weeks before time for growth to begin. The purpose was to reduce the supply of available nitrates in the soil by rendering them unavailable to the plant through an excess of carbohydrates. Plants thus treated were restricted in growth, the foliage was light green, and the fruit was smaller in size. As might be expected from the slight beneficial effect of nitrogen, the addition of sugar to reduce available nitrogen content of the soil gave an opposite effect, definitely lowering the sugar content and greatly reducing the percentage of ripe berries.

**Phosphorus.** Phosphorus was applied by suspending superphosphate in water and forcing this suspension and solution into the soil to a depth of about 2½ feet, using a Worthley tree-spray gun attached to a power sprayer. This technique was used to insure a better distribution of phosphorus through the soil zone of greatest root distribution in hope of accelerating any plant responses to this element. This treatment was the only one other than the use of nitrogen which raised the sugar content and increased the color, and the increase here was only neglible.

# Irrigation

Irrigation was accomplished by burying perforated pipe at a depth of 18 inches in the soil along each side of a row of vines and three feet distant from the row. Water was secured from the city system and was admitted under pressure through a hand valve as needed to keep the soil moist. Additional soil water during ripening proved definitely harmful, indicating that yearly variation in color may quite often be due to the rainfall distribution. However, this cannot be considered the major factor, for complete drought does not give full coloring, as may be seen by reference to the discussion below of the relation between rainfall figures and ripening (See page 16).

# Additional Light

Additional light was secured by suspending a 100-watt light bulb over each plant at a height of approximately 24 inches. Using an automatic switch, the lights were turned on from 10:00 p. m. until 3:00 a. m. each night during the nine weeks previous to the normal harvesting date. Figure 2 shows the arrangement in use. Additional light increased the sugar content of the juice but did not raise the color count.

#### Shading

Two types of shading were used: one continuous; the other discontinuous but approaching completeness.

The discontinuous shading arrangement is shown in Figure 3. This was a series of telescoping frames arranged to completely cover several plants on two sides and top. The vines were covered from 3:00 p. m.



Figure 2. Installation used to furnish additional light.



Figure 3. Partial shade assembly for reducing the hours of full sunlight.

each day until 7:00 a.m. the following morning. The shades were in use for a period of approximately six weeks previous to average harvesting time. This experiment will be referred to in further discussions and in the tables as the "Shade" treatment.

Figure 4 shows a different system, and one that gave partial but continuous shading. One part of this series was covered with a glass substitute, "Doplex," that was claimed by the manufacturer to allow the passage of a large part of the ultraviolet in sunlight. The name "Doplex" is used when referring to this treatment. Another and similar series was prepared except that the cover in this instance was shading cloth. The cloth permitted considerably less light to pass, although no accurate measurement of intensity was made. This part is referred to in the tables as the "Shading cloth."

Shading of all types proved to be a hindrance to good color development, thus confirming the need of ample sunlight either directly or indirectly for best coloring of grapes.

# **Artificial Cooling**

Another treatment was a type of cooler shown in Figure 5. This consisted of an eave-trough supported at the height of the vine by stakes. Burlap was dropped from this trough and held in place in a lower trough by an iron pipe. The upper trough was kept filled with water by an automatic valve, and this kept the burlap wet except on the most unfavorable days (strong wind and high temperatures). The net result of this treatment was to raise the humidity by a fourth and lower the temperature about  $5^{\circ}$  F. This test will be called "Water cooled" in the tables and discussions.

# Weather

Assuming that in this part of Oklahoma the grapes commence to color about August 1 of each year, the weather records were examined for the months of July and August for three years of this test to see if any correlation might be found betwen the weather and color development of the checks.

The last half of July and the first 20 days of August for 1932 had high maximum temperatures and ample total rain, although the first 17 days of August (normal ripening time) were dry. The percentage of green berries on the check plants was 12.3. In 1933, rain again seemed ample and well-distributed. The temperature ranged somewhat cooler than in 1932, many days having maximums in the eighties or low nineties. The green percentage was 27.6, highest for the three years. The year of 1934 was a drought year with excessively high temperatures, many days having maximums over  $100^{\circ}$  F. The only appreciable rain came after August 20. This year gave the lowest percentage of green berries, 6.2 percent. From these data it would seem that if there is any correlation between Stillwater weather and ripening it is that best coloring results when moisture is low during ripening. This is in conformity with the results from irrigation, which consistently gave the lowest percentage of coloring of any treatment that was tried (See page 14.) This indicates that excessive moisture in the soil during or near ripening increases greatly the percentage of green

# **Summary of Environmental Studies**

From the data in Table II it is apparent that the special treatments have not proven effective in appreciably improving coloring when compared to the check plants. Application of nitrogen fertilizer was the only practical method found to appreciably increase coloring, and even here the results were not outstanding.

# PHYSIOLOGICAL FACTORS

# Leaf Pruning

Data in Table III are typical of the results secured in studying the relation of leaf area to number of clusters. Within limits, color development bears a direct relation to the leaf area per cluster; however, unlimited leaf



Figure 4. A veiw of the vineyard showing Shading cloth and Doplex screens in place.



Figure 5. The arrangement for water cooling listed as "water cooled" in Tables I and II. Water circulates around the top trough and the excess drip is carried away by the bottom trough.

		SUGA	R %		ASTRINO	GENCY (G.	PER L.)		A sid.	BE	RRY COLO	DR1
Treatment	Year	Red.	Total	% Acidity	Total	Non- Tannins	Tannins	Density	Sugar Ratio	Green	Colored	Ripe
Not tipped,	1933	8.05	8.05	.554	1.789	1.060	.729	1.0497	1:14.8	18.94	5.27	75.79
1 Cluster	1934	7.36	7.82	.402	1.370	.853	.517	1.0474	1:19.5	2.87	9.51	87.62
1 Cluster,	1933	8.02	8.02	.564	1.944	1.008	.936	1.0494	1:14.3	14.68	11.32	74.00
10 leaves	1934	7.59	7.74	.464	1.402	.817	.585	1.0452	1:16.8	1082	16.38	72.80
2 Clusters,	1933	8.14	8.14	.535	2.241	1.237	1.004	$1.0590 \\ 1.0427$	1:15.2	25.30	5.19	25.30
10 leaves	1934	7.98	8.16	.534	1.122	.715	.407		1:15.3	20.12	17.77	62.11
1 Cluster, 5 leaves	1933 1934	9.16 6.66	9.16 6.85	.512 .557	1.944 1.057	1.081 .737	.863 .306	$1.0567 \\ 1.0420$	1:18.0 1:12.4	$\begin{array}{c} 17.12\\ 50.17\end{array}$	4.46 27.63	68.42 22.22
2 Clusters,	1933	6.91	7.04	.722	1.632	1.031	.601	1.0513	1:10.5	54.06	6.26	39.68
5 leaves	1934	5.79	5.99	.716	.853	.642	.228	1.0360	1: 8.5	68.82	19.20	11.98
Check	1933	8.62	8.77	.647	2.040	1.105	.935	1.0530	1:13.7	27.67	7.31	65.02
	1934	8.39	8.57	.483	1.210	.765	.445	1.0481	1:17.7	6.17	26.22	67.61

TABLE III.—Analyses of grape juice samples secured from vines having different numbers of leaves per cluster; a summary of two years' results.

<sup>1</sup> Percentage of berries in each classification.

area does not produce complete coloring, nor does it markedly raise the sugar content of the juice. From these data, the optimum leaf number is found to be perhaps slightly more than 10 leaves per cluster. Ten leaves per cluster gave color comparable with the checks and not greatly inferior to an unlimited number of leaves, with a sugar content varying around three quarters of a percent lower than the checks but as high as that of the unlimited number.

#### Girdling

The data in Table IV cover a part of the previous work on leaf area but are primarily concerned with the effect of girdling upon the composition of the fruit, therefore no berry counts were made. As has been suggested by previous workers, girdling slightly raised the percentage of sugars in the juice but the increase shown is small and would not seem to justify the difficulties encountered in the work. Titratable acidity is lowered an appreciable amount on a percentage basis.

#### **Bagging of Fruit**

Although it had already been reported (28) that grapes colored normally when bagged, it was decided to test the results under Oklahoma conditions. Results, given in Table V, show that bagging had little effect on the composition of the juice. Color in the bagged fruit (values not shown) also developed equal to that of the check clusters but at a slower rate, full normal color being attained about ten days to two weeks later.

# PART II: Chemical Changes in the Developing Fruit as Shown by Analyses of Berries and Juice.

When in the course of the experiments reported in Part I it became apparent that no simple edaphic or physiological factor was being found which might account for the uneven ripening of Concord grapes under Oklahoma conditions, attention was turned to an examination of the chemical composition of the berries during the period of ripening. A previous publication (38) presents 1934 data on this subject. The figures given here represent a continuation of these analyses, the juice samples being secured during 1935. The samples were taken at 7 o'clock in the morning except when 24-hour samplings were made. Approximately thirty clusters from six or more vines were used in preparing samples, and whenever possible four or five pounds of berries were used for a pressing. The berries were hand picked from the clusters, sorted, and, as indicated in the data (Table VI, parts A, B and C) the color percentages were secured by weighing the appropriate fraction. Green had no trace of color; colored showed purple in spots or throughout and were most often reddish; ripe berries were those having a full, deep, purplish-black shade. The line of demarcation is much sharper between the green and colored berries than it is between colored and ripe berries, because any berries of questionable color were placed in the "colored" classification.

Date of the earliest sampling was selected to be approximately one month before coloring. The berries were approaching their maximum size by this time, as is shown by the small change in solids content of the green berries during the sampling period. The final sampling date was past the regular harvest time for Concords this year, so that the final sampling period fully covered changes during ordinary ripening.

	SUGA	RS %		ASTRI	NGENCY (G.	PER L)		
Treatment	Red.	Total	- % Acidity	Total	Non- Tannins	Tannins	Density	Sugar Ratio
1 Cluster 5 leaves GIRDLED 1 Cluster 5 leaves	6.81	7.00	.448	1.060	.724	.336	1.0398	1:15.8
NON-GIRDLED	6.66	6.85	.557	1.057	.737	.306	1.0420	1:12.4
2 Clusters 5 leaves GIRDLED 2 Clusters 5 leaves NON-GIRDLED	6.13 5.79	6.13 5.99	.610 .716	.912 .853	.662 .642	.251 .228	1.0358 1.0360	1:10.8 1: 8.5
1 Cluster 10 leaves GIRDLED 1 Cluster 10 leaves NON-GIRDLED	7.93	8.17 7.74	.457 .464	1.305	.832 .817	.474	1.0464	1:17.8 1:16.8
2 Clusters 10 leaves GIRDLED 2 Clusters 10 leaves NON-GIRDLED	8.65	8.79 8.16	.486 .534	1.198	.738 .715	.459 .407	1.0441 1.0427	1:18.2 1:15.3
Checks	8.39	8.57	.483	1.210	.765	.445	1.0481	1:17.7

TABLE IV.—Analyses of grape juices showing the effect of girdling upon the chemical composition of the juice from plants having different numbers of leaves per cluster; 1934.

20

	SUGA	RS %		AS (	TRINGEN G. PER L	CY .)		Acid
	Red.	Total	% Acidity	Total	Non- Tannins	Tannins	Densi <b>ty</b>	Sugar Ratio
Bagged Not Bagged	9.03 9.63	9.12 9.69	.651 .676	1.419 1.353	.867 .827	.552 .526	1.0525 1.0573	1:14.2 1:14.4

TABLE V.—Chemical analyses showing the effect of bagging upon the composition of grape juice; 1935.

# TABLE VI.—Chemical analyses of press juice and whole grapes collected at various stages of growth during the months of July and August, 1935.

Date and	PERC	ENTAGES O	F FRESH WEI	GHT	Alklinity ml. N/10
Color Stage	Color	Water	Solids	Ash	- acid per 100 g.
Green					
July 9	100	90.44	9.56	.416	31.20
July 16	100	89.66	10.34	.467	39.00
July 23					
5 a. m.	100	89.13	10.87	.420	36.90
11 a. m.	100	88.99	11.01	.431	34.90
5 p. m.	100	89.90	11.10	.437	37.10
11 p. m.	100	89.07	10.93	.430	36.00
July 30	100	88.99	11.01	.482	43.78
Aug. 6	<b>6</b> 2. <b>3</b>	87.85	12.15	.482	44.29
Aug. 13	24.7	87.73	12.27	.527	
Aug. 20	14.3	87.98	12.02	.484	48.73
Colored					
Aug. 6	37.4	85.95	14.05	.529	
Aug. 13	37.4	85.52	14.48	.619	53.40
Aug. 20	27.3	84.55	15.45	.607	59.99
Ripe					
<b>Ā</b> ug. 13	37.90				
Aug. 20	<b>58.4</b> 0				
24 hour sampe	. Aug. 14-15				
Green					
Aug. 14					
5 a. m	41.9	87.66	12.34	.578	59 24
11 a.m.	44.0	87.45	12.55	.612	63.80
5 p. m.	40.1	87.50	12.50	.626	62.20
11 p. m.	41.2	87.23	12.77	.653	64.90
Colored					
5 a. m.	32.2	85.61	14.39	.685	65 77
11 a. m.	33.2	85.41	14.59	.666	67.00
5 p. m.	34.3	85.91	14.09	662	68 40
11 p. m.	34.3	85.74	14.26	.666	66.20

Part A: Whole Grapes.

TABLE	VI(	Continu	ed)
-------	-----	---------	-----

· · · · · · · · · · · · · · · · · · ·										
Date and Color Stage	fresh				Acidity		SUGA	R PERCEN	TAGE	
	weight	Sp. Gr.	% Solids	pH	ml. $N/10$	Red.	Total	Sucrose	Dextrose	Levulose
Green										
July 9	100	1.0210	4.28	2.60	38.2	1.16	1.20	.04	.48	.62
July 16	100	1.0251	4.98	2.97	38.4	2.09	2.18	.09	.64	1.43
July 23										
5 a. m.	100	1.0269	5.28	2.91	36.0	2.39	2.52	.12	.70	1.67
11 a. m.	100	1.0280	5.42	2.89	37.0	2.45	2.67	.21	.78	1.62
5 p. m.	100	1.0290	5.55	2.88	37.9	2.46	2.63	.19	.76	1.64
11 p. m.	100	1.0280	5.54	2.83	37.0	2.43	2.76	.31	.76	1.64
July 30	100	1.0316	5.77	3.08	30.4	3.73	3.78	.05	1.00	2.69
Aug. 6	62.6	1.0384	7.21	3.18	25.4	5.89	6.15	.25	2.98	2.67
Aug. 13	24.7	1.0360	7.37	3.21	17.9	5.54	5.71	.17	2.89	2.44
Aug. 20	14.3	1.0390	7.37	3.25	16.6	6.06	6.36	.29	3.29	2.44
Colored										
Aug. 6	37.4	1.0467	8.78	3.29	18.4	7.85	8.16	.30	4.11	3.38
Aug. 13	37.4	1.0452	10.07	3.39	14.0	8.21	8.36	.15	4.15	3.71
Aug. 20	27.3	1.0490	9.61	3.44	12.0	8.80	8.88	.08	4.39	3.97
Ripe										
Aug. 13	37.9	1.0490		3.50	11.1	9.29	9.40	.11	4.63	4.22
Aug. 20	58.4	1.0566		3.57	10.1	9.76	9,99	.22	4.92	4.31
24 hour sample. Aug. 14-15										
Green										
5 a. m.	41.9	1.0378	7.20	3.19	19.8	6.07	6.16	.09	3.12	2.71
11 a.m.	44.0	1.0382	7.10	3.37	21.7	5.64	5.70	.23	2.73	2.68
5 p. m.	40.1	1.0390	7.19	3.40	22.2	5.74	5.98	.23	2.82	2.68
11 p. m.	41.2	1.0390	7.66	3.26	22.2	6.06	6.18	.11	2.86	2,96
Colored										
5 a. m.	32.2	1.0424	8.26	3.40	13.6	7.52	7.55	.03	3.54	3.86
11 a. m.	33.2	1.0429	8.62	3.56	15.7	7.55	8.00	.43	3.65	3.59
5 p. m.	34.3	1.0410	8.02	3.52	14.8	7.24	7.46	.21	3.60	3.32
11 p. m.	34.3	1.0435	8.33	3.47	14.9	7.50	7.66	.15	3.54	3.64
	00	2.0 -00	0.00	01					0.04	

Part B. Juice Analyses.

Part C. Juice Analyses.

•

				UNITS P	ER 100 ML.	JUICE				
	PERCENT. FRESH WE	AGE EIGHT	ALKAL ML. N/I	INITY 10 ACID	Tartaric	Cream of	Free	ASTRI	NGENCY (G	RAMS)
	Color	Ash	Sol.	Insol.	(Grams)	(Grams)	Acid (Grams)	Total	Non- Tannins	Tannins
Green										
July 9	100	.413	35.9	14.1	1.440	.675	.649	.196	.140	.056
July 16	100	.404	36.1	17.0	1.5106	.679	.714	.267	.164	.103
July 23										
5 a. m.	100	.430	39.5	11.8	1.414	.743	.644	.272	.164	.108
11 a. m.	100	.444	40.8	12.6	1.458	.769	.658	.287	.161	.126
5 p. m.	100	.415	39.6	11.4	1.437	.745	.672	.297	.173	.124
11 p. m.	100	.397	39.7	11.6	1.441	.747	.671	.308	.193	.115
July 30	100	.386	43.5		1.335	.819	.490	.243	.151	.092
Aug. 6	62.6	.393	43.6	11.4	1.277	.820	.457	.185	.111	.074
Aug. 13	24.7	.379	41.7	10.4	1.195	.784	.414	.171	.097	.074
Aug. 20	14.3	.375	42.0	11.1	1.170	.789	.373	.164	.104	.060
Colored										
Aug 6	37.4	.365	40.3	10.2	1.063	.759	.304	.129	.095	.034
Aug. 13	37.4	.366	39.6	9.2	1.023	.744	.290	.152	.104	.043
Aug. 20	27.3	.384	44.5	4.9	.947	.836	.206	.160	.108	<b>.05</b> 3
Ripe										
Aug. 13	37.9							.149	.099	.051
Aug. 20	58.4							.176	.114	.043
24 hour sample.	Aug. 14-15									
Green	-									
5 a. m.	41.9	.415	42.1	10.0	1.097	.791	.314	.172	.100	.072
11 a.m.	44.0	.487	50.6	13.8	1.290	.952	.324	.204	.107	.097
5 n.m.	40.1	.497	55.4	12.3	1.357	1.041	.342	.190	.118	.072
11 p. m.	41.2	.430	47.6	9.9	1.142	.896	.286	.185	.105	.080
Colored										
5 g m	32.2	378	36.3	9.8	.641	.682		.155	.092	.063
11 g m	33.2	.413	39.3	9.3	.997	.739	.269	.183	.103	.080
5 n m	34.3	.432	46.6	8.8	.874	.875	.043	.172	.102	.070
11 n m	34.3	.408	40.6	10.1	.856	.764	.095	.168	.102	.067
11 p. m.	01.0									

The Uneven Ripening of Concord Grapes

23

# CHANGES DURING RIPENING

# Solids

The solids content of the green grapes changes surprisingly little during the ripening period, remaining practically constant during the last three weeks. At the same time there is a very definite percentage increase in the solids of colored berries which can be largely accounted for by the concomitant increase in sugars. In the juice, the increase in solids of the colored samples is somewhat less, or about the same as the change in sugar value, thus confirming the general statement that most of the increase in sugars as the berries ripen.

# **Ash Content**

Ash percentages in the green juice in general tend to fall as the season progresses. Colored juice contains approximately the same ash percent as does the green juice at the same time of sampling. In the whole fruit, however, a difference is noticeable in that the colored berries are always somewhat higher in ash content. Unpublished work of later years confirms this fact. Here again a study of the relative sugar and ash contents shows that the increase in ash content is approximately equal to the increase in sugar content, suggesting that the ash is carried along with the sugar to the berries or at least is moved in at the same rate.

# Astringency

Total astrinency values in green grapes reach a maximum and then decrease as the season progresses. Tannins follow much the same course. Total astringency and tannins are somewhat lower in the colored juices, but conversely have a tendency to increase as the season and ripening progresses; for instance, ripe juice on August 20 has a higher astringency content than either the green or colored samples. On the basis of these values and comparing with data for other years (38), it would not seem that astringency values yield any explanation for the uneven coloring of the berries.

# Acidity and pH.

All of the measures of acidity (acidity, pH, tartaric acid) on the green grapes show decreasing values as the season progresses. Later, both colored and ripe juices also show this seasonal decrease. Most noteworthy of the acidity changes is that of titratable acidity in the green juice where at the last periods the values are less than those for the early, coloredjuice samples. Similar results are shown by the data for other years. Therefore, although colored juices are less acid than green samples, it would seem that acidity relationships are more incidental than causative. However, in none of the data taken in the three years have colored juices been found to have a pH value of less than 3.2, therefore it would seem that in most years at least the critical point for color formation is in this pH range. This unquestionably will vary somewhat with weather variations from year to year, and this value should be considered as only suggestive.

# Sugar

Considering first the values for dextrose and levulose, it is apparent that during the coloring period the amount of dextrose is equal to or exceds the levulose present, which is in accord with the results of previous workers. During the weeks preceding coloring, however, the levulose exceeds the dextrose, for which no explanation can be given. No particular importance is attached to this point as regarding coloring, for when coloring starts the dextrose-levulose ratios for both green and colored juices fluctuate in the range of 1 to 1.2, and fully colored berries also show the same approximate range. Consequently the dextrose-levulose ratio does not seem to furnish any explanation for the coloring of grapes.

Concurring with reports of most workers in recent years, sucrose is found at all stages of ripeness. It is never, however, present in large amounts, although it may be an important transitional form (See page 26).

In discussing changes in reducing sugars, Figure 6 will prove helpful. This has been prepared from the data in Table VI and is intended to emphasize the most striking chemical change found to be associated with color development. Reducing sugars in general tend to increase in the green grapes as the season progresses, although in no instance has the value for such sugars in green grapes exceeded 6.5 percent or slightly less than 7 percent for total sugars (four year's results). For the one year's



Figure 6. Graph showing changes in sugar concentration of the press juice from grapes as the season progresses. Prepared from the data in Table VI.

results shown in Table VI, this value does not exceed 6.36 percent. Considering next the values found for colored juices, in none of the work has the percentage of reducing sugar been found to be less than 7 percent. As ripening proceeds, this value increases; and in the juice from ripe berries it has reached values as high as 13 percent. In Table VI, the highest value is 9.76 percent for reducing sugars or 9.99 percent for total sugar.

From these data it seems possible to conclude that sugar is at least one of the major factors influencing the development of color in Concord grapes in Oklahoma. Apparently the berries must process at least 7 percent of sugar before color develops, and therefore the low sugar content of Concord grapes in this area is a major factor in explaining their failure to color properly. A study of earlier tables in this paper and a comparison of the sugar content of Concord juices produced in Oklahoma with those from other sections of the country (5, 20, 26, 40) clearly indicates that Oklahoma juices run much below the average for the country as a whole. It may be that this is only an incidental relationship and not causative, but certainly this value of around 7 percent of sugar is definitely associated with color formation.

# **CHANGES DURING A 24-HOUR PERIOD**

Values found in analyses made at intervals of less than 24 hours have been included in Table VI as a matter of convenience in presentation. Only two points stand out as being of possible significance in growth phenomena. There is an appreciable increase in acidity during the day as measured by titration and a decrease in pH; and there is a clearly marked rise in sucrose, most noticeably in the colored samples. This change in sucrose would indicate that it is important as a transport form of sugar, and that when in place in the berries it is inverted to reducing sugars, which in turn suggests the possibility that during daylight hours reducing sugars are used up faster in the ripening berry by respiration than they are supplied to the fruit. This is more evidence showing that reducing sugars are a key factor in the growth and development of color in grape berries.

# **INDIVIDUAL BERRY STUDIES**

Considerable data has been collected dealing with the composition of individual berries on a cluster. Table VII is representative of the results secured and shows quite wide variations in composition of the individual berries. These variations indicate that there are differences in chemical composition long before color differences are apparent.

# **Mineral Analyses of Berries**

Suggestions are found in the literature that various minerals are of major importance in the development of color. This fact, coupled with the known higher mineral content of the colored berries, indicated a need for the mineral analyses reported in Tables VII, IX, and X. Data in Table VIII were secured by removing the seeds from berries, drying the pulp, and then analyzing the dried residue. Interpreting these data, it must be borne in mind that figures for percentages in the colored berries are comparatively low due to the higher total solids content of the colored fruit. Most noticeable fact in the table is the relatively constant ash content of the green berries throughout the sampling period. This indicates, as previously mentioned, that the mineral elements are transported to the berries along with the solids, chiefly sugars. Of the elements tested for, calcium is the only one which showed marked differences and whose percentages varied markedly between the green and colored fruit: and this seems to be a continuation of the trend shown by calcium in the green berries, where the calcium decreases as the season progresses. Iron also decreases progressively, and to a much greater extent. There is no apparent explanation for this striking decrease in iron, and it remains one of the interesting but unsolved problems in this work.

# **Mineral Analyses of Skins**

In an attempt to localize mineral changes at the place where color is developed analyses were made of the skins from green and colored fruit collected over a 24-hour period. These data are shown in Table IX. Little difficulty was experienced in removing the skins of colored berries, but samples from the green berries are subject to considerable sampling error for they needed to be cut away with a knife. The green values, however, represent analyses of the outer layers of skin and flesh, which are roughly comparable to the colored skins. Calcium is again lower in the colored skins, and thus may be considered to be significantly lower. Phosphorus is at times higher in the colored skins and averages slightly higher there, but it is doubtful if the difference is significant. As in Table VIII, iron is lower in the colored samples and consistently so throughout the day.

# TABLE VII.—Individual berry studies: Solids (seeds removed) and ash; 1935.\*

			Solids	Ash
July 19	18 berries	Average	7.51	.474
•	(Green)	High	8.00	.570
		Low	6.87	.390
Julv 26	18 berries	Average	7.78	.440
	(Green)	High	8.41	.493
		Low	7.08	.373
August 2	18 berries	Average	11.77	.540
	(Colored)	High	13.43	.612
		Low	10.46	.437

# (Percentage of green weight.)

\* Random sampling from a cluster.

# TABLE VIII.—Selected mineral analyses of pulp and skins from grapes sampled at weekly intervals during July and August 1935. (Percentage of dried material.)

	G.)			JUL.	Y 23			
	Stage	July 9	July 16	5 a. m.	11 p. m.	July 30	Aug. 6	Aug. 13
Ash	Green Colored	5.00	5.50	5.51	5.45		5.09 4.71	5.39 4.86
K.	Green Colored	2.07	1.84	2.32	2.20	2.23	2.36 2.25	2.04 2.00
Ca.	Green Colored	.59	.56	.59	.57	.50	.48 .32	.44 .32
Ρ.	Green Colored	.15	.12	.10	.11	.09	.09 .11	.10 .10
N.	Green Colored	.98	.94	.92	.90	.91	.90 .77	.95 .87
Fe.*	Green Colored	30.4	18.2	13.4	12.2	11.0	13.8 8.4	11.2 11.0

\* Mg. per 100 g dry weight.

# **Mineral Analyses of Seeds**

Mineral analyses of the seeds, presented in Table X, are incomplete, but show that generally speaking the mineral elements present in grape seeds remain much the same throughout the ripening period.

## Seeds in Relation to Color

Vaile (35) reports that for one year he found a correlation between the number of seeds and color formation. Work at this Station has failed to show any appreciable correlation, as shown by the data in Table XI. These values were secured by weighing the seeds in 200 g of grapes, and of course give no certain indication as to the number of seeds.

 TABLE IX.—Selected mineral analyses of skins only; August 14, 1935.

 (Percentage of dried material.)

•		5 a. m.	11 a.m.	5 p. m.	11 p. m.	Ave.
Ash	Green	7.27	7.90	7.68	7.32	7.54
	Colored	7.56	7.09	7.11	6.97	7.18
K.	Green	3.31	3.45	2.93	3.31	3.25
	Colored	3.16	3.20	3.24	3.28	3.22
Ca.	Green	.41	.29	.30	.34	.34
	Colored	.33	.30	.30	.25	.30
Р.	Green	.109	.101	.120	.104	.109
	Colored	.129	.100	.119	.120	.117
N.	Green	.92	.93	.99	.88	.93
	Colored	.88	.90	.93	.92	.91
Fe.*	Green	18.4	15.2	16.4	14.0	16.0
	Colored	14.0	12.0	.11.6	11.2	12.2

\* Mg. per 100 g dry weight.

# TABLE X.—Analyses of seed from grapes sampled at weekly intervals during July and August, 1935.

(Percentage of dry weight.)

	Galan			JULY	23			
	Stage	July 9	July 16	11 p. m.	5 a. m.	July 30	Aug. 6	Aug. 13
Ash	Green Colored	2.23	2.18	1.87	2.06	1.81	1.93 1.83	1.86 2.04
P.	Green Colored	.275	.265	.248	.282	.237	.268 .243	.261 .261
N.	Green Colored	1.56	.143	1.55	1.40	1.59	1.44 1.55	1.44 1.49
Fe.*	Green Colored	16.8	13.2	15.6	14.8	8.8	13.6 11.2	15.6 21.6

\* Mg. per 100 g. dry weight.

Date	Color	Weight of	Weight of
	Stage	berries (g.)	seeds (g.)
July 16	Green	200	11.6
July 30	Green	200	11.6
August 6	Green	200	11.5
	Colored	200	11 <b>.3</b>
August 13	Green	200	11.6
	Colored	200	12.0

TABLE XI.—Weight of seeds in green and colored grapes;
--

#### **Enzyme Studies**

Data on enzyme analysis presented in Table XII represent a continuation of earlier studies reported in a paper (38) from this Station. Data presented here confirm the fact that catalase activity increases as the season progresses and that ripe juice is much higher in catalase activity than juice from green grapes.

These data also emphasize the importance of oxidase and peroxidase activity during the coloring period, peroxidase activity being particularly pronounced. The large values for oxidase activity in colored juice are not wholly in accord with the results of Villiers (37) who found such activity to decrease as the grapes ripened. There is little in the data to indicate that lack of any of these enzyme activities can account for a failure of color development. Values for green grapes at some time during the ripening period equal or exceed such activity in the colored juice and therefore the variations are judged to be incidental rather than causative.

# PART III: Analysis of Vines and Leaves, Comparing Concord with Other Varieties.

Previous analyses and experiments having indicated that the probable explanation for uneven coloring lay in some transformation within the Concord plant, a series of analyses were made of the prunings and leaves of Concord and other varieties. These tests were run to determine whether Concord vines were abnormal in composition or generally similar to other varieties that colored uniformly.

# VINE PRUNINGS

During 1937 and 1938, Concord, Extra and Worden varieties were pruned at two times during the winter season. At each pruning, all of the canes of each variety were collected separately, immediately ground through a hammer mill, and samples taken for analysis. The samples for each variety was representative of all of the canes from at least a dozen vines, and therefore the results should be quite representative of the vine condition at time of sampling. Both of the early samplings were made while the buds were dormant. The second sampling in 1937 was made when the buds were just starting to break into leaf, and in 1938 it was made when a second set of leaves was just emerging after the first buds had been destroyed by a late freeze.

The most striking point found in examination of Table XIII is a remarkable similarity in composition of the three varieties, both as regards a single sampling time and considered over the two years. The only ap-

Test	Color Stage	July 11	July 18	July 25	Aug. 1	Aug. 8	Aug. 8	Aug. 16	Aug. 22
Iodine.	Green	.15	.22	.25	.15	.22	.15+	.15	.15+
5 ml. juice	Colored					.22	.15	.20	.20
Catalase. 10 ml neut. Samples of	Green		1.05	.55	1.00	1.20	2.05	2.45	3.40
uncent. juice	Colored					3.00	2.00	3.80	5.55
Oxidase. 5 ml samples	Green Colored	0	0	0	0	.50 .26	.47 .47	.16 .75	.18 <b>.51</b>
Peroxidase.	Green	3.64	1.38	1.74	1.26	.61	.49	3.47	4.17
Mg purp. per liter of solution	Colored					.55	.41	2.50	4.23

TABLE XII.—Enzyme activity of press juice from grapes sampled at weekly intervals during July and August, 1935.

				CAF	CARBOHYDRATES			NITROGEN		
				Sug	ars	Aoid				
	Water	Solids	Ash	Red.	Total	Hydrol.	Sol.	Insol.	Total	
				1937						
3/2/37										
Concord	41.02	58.98	1.60	2.82	5.60	13.71	.073	.486	.559	
Extra	38.57	61.43	1.59	3.32	6.03	14.33	.050	.405	.455	
Norton	39.51	60.49	1.55	3.62	5.01	13.58	.047	.387	.434	
4/15/37										
Concord	46.10	53.90	1.55	.89	1.78	13.37	.046	.407	.453	
Extra	44.60	55.40	1.44	.87	1.86	13.69	.077	.365	.442	
Norton	43.53	56.47	1.44	.90	1.55	13.29	.070	.325	.395	
				1938						
3/8/38										
Concord	44.17	55.83	2.16	2.16	4.41	12.45	.063	.494	.557	
Extra	40.58	59.42	1.56	2.90	4.72	12.34	.052	.399	.441	
Norton	42.82	57.18	1.64	2.80	4.44	11.75	.041	.350	.391	
5/3/38										
Concord	54.63	45.37	1.22	.74	1.00	9.78	.034	.272	.306	
Extra	50.13	49.87	1.23	.71	.94	10.65	.027	.262	.289	
Norton	50.81	49.19	1.28	.68	.89	10.10	.035	.242	.277	

# TABLE XIII.—Analyses of vine prunings from certain varieties during the winter and spring season. (Percentages of fresh weight.)

preciable difference is in the nitrogen content, in which Concord prunings are uniformly higher. This difference is small, however, at the second sampling time each year. From these data it would seem that Concord canes are average in respect to ash, carbohydrate and nitrogen content; and therefore analysis of the canes failed to furnish any explanation for the later failure of the vines to yield the sugar necessary for complete coloring.

#### LEAVES

#### **Leaf Pigment Studies**

The material for a part of this discussion is abstracted from an earlier paper (39), and is included here in order to assemble all of the data in one place. Chemical methods are discussed previously (See page 9) and only the procedure for collecting the samples for pigment analysis will be added here.

Sampling Procedure.—Leaves were collected from the vines in the field by clipping the base of the petioles and placing in paper sacks for transportation to the laboratory. In order to make the analyses as comprehensive as possible, some samples representing old leaves and some representing the newer growth were collected. Age differentiation in the leaves was based upon the location upon the canes, the size, and the shades of green. Some samples were collected in the morning and some during the noon hour. At each sampling, leaves from Concord vines were secured, along with comparable leaves from vines of other varieties. Immediately after cutting, the leaves were taken to the laboratory and a 15 g sample removed, 10 g of which were used for pigment analysis and the remainder for moisture and ash determinations. Samples for analysis were secured by cutting discs from the leaves with a cork borer having an area of 330.06 square millimeters. Care was exercised to avoid cutting any large veins, and 30 to 60 leaves were used for each sample. The total time elapsing between collecting and starting of the chemical analyses did not exceed 1<sup>1</sup>/<sub>2</sub> hours and for the most samples was much less.

The data for 1937 are given in Table XIV. Averages are given in each table so that the composition of Concord leaves may be checked with the average of the other varieties. Although this procedure may be questioned, a check of the individual figures shows that the averages are representative of individual differences at a particular time in a large majority of the comparisons. Weighted averages of data in Table XIV, taking into account the smaller number of Concord samples, do not significantly change the trends.

**Chlorophyll.** In examining the chlorophyll data in Table XIV, it is apparent that, based upon the amounts present, chlorophyll would not seem to be a limiting factor in Concord leaves as regards their photsynthetic activity. In general, chlorophyll is present in slightly greater amounts in Concord leaves than in most other varieties examined, and this is particularly true when the amounts per unit of leaf area are considered.

**Carotene and Xanthophyll.** Results for carotene and xanthophyll were not secured on all samples, but enough are included in Table XV to show that there are but small varietal differences, although here as in chlorophyll the greater average amounts are present in Concord leaves. The amounts present are generally low as compared to many other plants, but the carotene-xanthophyll ratios fall in the usual range of such values.

							CH	LOROPHY	LL		OTITICAN
				~			Mg. per	Mg. per	Mg. per	(In	Mg.)
	Variety	Age	Date	Solids	Water	% Ash	100 g. gr. wt.	dry wt.	sq. cm.	Green	Dry
*1.	Concord	Old	5/24	29.70	70.30	1.61	288.7	972.1	.084	54.5	16.2
2.	Concord	Old	6'/1	32.55	67.45	2.48	279.6	860.0	.050	59.4	19.1
3.	Concord	New	6/1	30.62	69.38	1.92	219.8	717.9	.038	45.7	14.0
4.	Concord	Old	6/15	36.20	63.80	2.71	332.0	917.2	.059	59.1	21.4
5.	Concord	New	6/30	31.98	68.02	1.86	166.5	520.7	.029	56.9	18.2
6.	Concord	New	8/9	38.65	61.35		353.0	526.5	.067	63.9	24.7
7.	Concord	Old	8/10	40.89	59.11	4.36	345.4	844.6	.062	59.0	24.1
8.	Concord	New	8/11	38.07	61.93	3.06	272.4	715.6	.052	62.4	23.8
9.	Concord	New	8/24	41.28	58.85	3.60	244.3	593.6	.048	65.3	26.7
10.	Concord	New	8/25	40.28	59.72	3.00	219.8	545.8	.047	70.4	28.4
	Averages		,	36.01	63.99	2.73	272.2	721.4	.050	59.7	21.7
1.	Extra	Old	5/24	25.08	74.92	1.72	222.5	897.1	.039	57.7	13.4
2.	Extra	Old	6/1	27.35	72.65	2.49	182.7	667.9	.034	61.1	16.7
3.	Extra	New	6/1	29.55	70.45	2.47	148.3	501.7	.027	47.3	14.0
4.	Extra	Old	6/15	29.60	70.40	2.66	177.1	598.3	.033	62.3	18.5
5.	Worden	New	6/30	33.68	66.32	1.72	139.4	414.0	.025	<b>59.6</b>	20.0
	Bailey	New	6⁄30	29.70	70.30	1.55	124.4	419.0	.023	62.3	18.5
6.	Moore Early	New	8′/9	37.82	62.18		440.9	1165.7	.086	64.2	24.3
7.	Ellen Scott	Old	8⁄/10	36.41	63.59	4.05	360.2	989. <b>2</b>	.072	66.2	24.1
8.	Sheridan	New	8/11	34.10	65.90	2.70	225.3	660.7	.041	<b>59.4</b>	20.2
	Campbells	New	8/11	34.33	65.67	3.17	240.6	700.8	.048	65.8	22.6
9.	Fredonia	New	8'/24	43.64	57.36	4.51	188.1	430.9	.032	56.7	24.7
	Bailey	New	8'/24	37.76	62.24	3.07	250.0	662.1	.051	66.9	25.3
10.	Extra	New	8'/25	33.05	66.95	1.94	159.8	483.4	.032	65.1	21.5
	Averages			33.23	66.77	2.67	219.9	683.9	.042	61.1	.20.3

TABLE XIV.—Comparison of solids and chlorophyll content of grape leaves sampled at various times during the growing season; 1937.

\* Numbers indicate corresponding sampling times and conditions. \*\* Area of each cut is 330.06 sq. mm.

		. ,		CARO	TENE	XANTHO	OPHYLL	Ratio (dry wt.)
	Variety	Age	Date	Mg. per 100 g. gr. wt.	Mg. per 100 g. dry wt.	Mg. per 100 g. gr. wt.	Mg. per 100 g. dry wt.	tene: Xanth- ophyll
*								
3.	Concord	New	6/1	17.4	56.8	32.3	105.4	.54
4.	Concord	Old	6/15	17.4	48.1	34.0	93.9	.51
6.	Concord	New	8′/9	16.9	43.8	29.5	76.2	.57
7.	Concord	Old	8/10	20.9	51.0	24.9	60.9	.84
10.	Concord	New	8/25	15. <b>0</b>	37.3	33.5	83.1	.45
	Averages			17.5	47.4	30.8	83.9	.56
3.	Extra	New	6/1	16.0	54.1	24.5	82.8	.65
4.	Extra	Old	6/15	12.7	42.9	18.7	63.2	.68
6.	Moore Early	New	8/9	15.8	41.9	27.7	73.3	.57
7.	Ellen Scott	Old	8/10	16.9	46.4	29.7	81.6	.57
10.	Extra	New	8/25	14.3	43.2	33.2	100.1	.43
	Averages			15. <b>1</b>	45.7	26.8	80.2	.57

TABLE	XV.—C	ompari	son of	carote	ne and	xanthophyll	content	i of	grape
lea	ves sam	pled at	variou	ıs times	during	the growing	season;	1937	1.

\* Numbers refer to same numbers in Table XIV.

# **Detailed Leaf Analyses**

Previous work having indicated that lack of sugars in the berries is the most probable cause of uneven ripening, analyses of the leaves of several varieties were made in seeking an explanation for this fact. The study on pigments had failed to yield any explanation for the low sugar production in Concords, although Table XIV shows some average difference in solids content between Concord and other varieties. This fact, and the knowledge that sugars are elaborted in the leaves, led to a study of seasonal fluctuation in the chemical composition of leaves from 10 grape varieties. These data are given in Table XVI, arranged by sampling dates and stage of maturity.

Previous analyses had shown that Concord and Extra leaves reached their maximum solids content between 3:00 and 5:00 p.m. (Table XVII), therefore the samples for these analyses were collected during the interval 3:30 to 4:30 p.m. Leaves were selected to represent as nearly as possible uniform conditions of age, growth and maturity. The leaves called normal were those fully developed, dark green, and in general located towards the base of the canes. Leaves listed as young were secured from near the ends of the canes and were light green in color as well as smaller in size. Leaves were placed after cutting in  $\frac{1}{2}$  gallon sealed jars and within one hour stored in a freezing room  $(-16^{\circ} C.)$  over night. The following morning, the frozen leaves were ground in a power meat chopper and samples taken for alcohol preservation, and for solids, ash and nitrogen determinations as described on pages 9 and 10. The sampling of August 15 was made when a few of the varieties were fully matured but none had been harvested. By the August 29 sampling, most of the varieties had been harvested. The final sampling time, October 15, found many of the varieties showing some leaf hopper injury, and, due to the preceding hot, dry month, the leaves generally were browned and spotted and some leaves had begun to fall.

				CA	RBOHYDRAT	TES .		N	ITROGEN	
				Sugars				~ 1		
	Solids	Ash	Red.	Total	Sucrose	Acid Hydrol.	Carb.	S01.	Insol.	Total
May 13										
**Normal Leaves	5									
Concord	25.69	1.68	1.04	1.86	.78	3.07	4.93	.055	.762	.817
Sheridan	25.44	2.14	.82	1.73	.86	3.50	5.23	.059	.709	.768
Worden	25.64	1.98	1.02	2.09	1.02	3.29	5.38	.073	.886	.959
Extra	24.63	1.54	1.26	1.81	.52	3.36	5.17	.041	.644	.685
Delaware	28.74	1.94	1.84	2.72	.84	3.92	6.64	.050	.727	.777
Moore Early	25.66	1.77	1.11	1.75	.61	3.82	5.57	.056	.694	.750
Ellen Scott	24.15	1.37	1.89	2.69	.76	3.72	6.41	.051	.664	.715
Bailey	23.49	1.68	1.14	1.68	.51	3.17	4.85	.056	.655	.711
Fredonia	27.55	1.82	1.53	2.64	1.05	4.58	7.22	.080	1.045	1.125
Campbell	27.17	1.94	1.32	1.94	.59	3.29	5.23	.067	.766	.833
June 10										
Normal Leaves										
Concord	30.66	1.93	1.44	2.62	1.12	3.88	6.50	.041	.782	.823
Sheridan	28.52	1.95	1.47	1.94	.45	4.30	6.24	.045	.626	.671
Worden	32.45	2.05	1.61	2.35	.70	4.90	7.25	.036	.718	.754
Extra	27.60	2.02	1.47	1.82	.33	3.08	4.90	.038	.604	.642
Delaware	31.38	2.08	2.02	3.06	.99	4.50	7.56	.038	.878	.916
Moore Early	31.65	1.97	1.73	2.51	.74	4.10	6.61	.041	.740	.781
Ellen Scott	27.72	2.00	1.34	1.94	.57	3.25	5.19	.045	.788	.833
Bailey	27.98	2.13	1.36	1.83	.45	3.43	5.26	.046	.628	.674
Fredonia	31.92	2.12	1.67	2.25	.55	4.01	6.26	.044	.778	.822
Campbell	33.91	2.43	1.64	2.88	1.18	4.64	7.52	.048	.751	.799

# TABLE XVI.—Chemical analyses of leaves from 10 varieties of grapes; 1938.\* (Percentage of green weight.)

# TABLE XVI.—(Continued.)

			(.	. crocinage	or green					
				CA	RBOHYDRAT	TES		N	IITROGEN	
				Sugars		Acid	motol.	Sol	Insol.	Total
	Solids	Ash	Red.	Total	Sucrose	Hydrol.	Carb.		211001	100002
June 14										
Young Leaves										
Concord	30.60	1.64	1.46	2.00	.51	4.35	6.35	.048	.760	.808.
Sheridan	30.49	1.81	1.78	2.12	.32	4.99	7.11	.041	.729	.770
Worden	31.34	1.66	1.84	2.23	.35	5.12	7.35	.051	.722	.772
Extra	29.54	1.59	1.67	1.96	.28	4.30	6.26	.038	.740	.778
Delaware	29.24	1.72	1.96	2.40	.42	4.29	6.69	.055	.727	.782
Moore Early	32.08	1.69	1.56	2.06	.48	4.55	6.61	.038	.717	.755
Ellen Scott	25.81	1.59	1.64	2.03	.37	3.79	5.82	.056	.781	.837
Bailey	28.99	1.66	1.46	1.79	.31	4.15	5.94	.038	.673	.711
Fredonia	29.94	1.57	1.91	2.05	.13	4.55	6.60	.046	.731	.777
Campbell	29.94	1.98	1.57	2.03	.44	4.29	6.32	.057	.720	.777
July 11										
Normal Leaves										
Concord	34.99	2.58	1.40	2.14	.70	4.58	6.72	.055	.788	.843
Sheridan	34.05	2.56	1.67	2.34	.64	4.35	6.69	.034	.620	.654
Worden	38.19	2.66	1.57	2.34	.74	4.48	6.82	.046	.812	.858
Extra	29.50	2.41	1.43	1.74	.29	3.36	5.10	.043	.590	.633
Delaware	34.88	2.75	1.88	2.50	.59	4.63	7.13	.044	.721	.765
Moore Early	35.35	2.45	1.59	2.41	.78	4.25	6.66	.043	.735	.778
Ellen Scott	30.52	2.53	1.43	2.01	.55	3.41	5.42	.056	.797	.853
Bailey	31.02	2.36	1.68	2.16	.46	4.01	6.17	.044	.632	.676
Fredonia	36.20	2.69	1.54	2.49	.90	5.59	7.08	.041	.717	.758
Campbell	35.28	3.18	1.60	2.33	.69	3.93	6.26	.044	.732	.776

(Percentage of green weight.)

# TABLE XVI.—(Continued)

				CAF	RBOHYDRAT	ES		NITROGEN		
		-		Sugars		Acid Hydrol.	Total Carb.	Sol.	Ins <b>ol.</b>	Total
	Solids	Ash	Red.	Total	Sucrose					
August 15		the second								
Normal Leaves										
Concord	36.34	3.13	1.38	2.08	.67	3.42	5.50	.040	.748	.788
Sheridan	35.36	3.35	1.43	2.23	.76	2.81	5.04	.024	.534	.558
Worden	39.64	3.41	1.48	2.56	1.03	3.87	6.43	.036	.744	.780
Extra	32.97	3.19	1.27	1.79	.49	3.04	4.83	.035	.623	.658
Delaware	36.04	2.97	1.75	3.03	1.22	3.62	6.65	.35	.782	.817
Moore Early	37.32	3.15	1.45	2.37	.87	3.54	5.91	.035	.705	.740
Ellen Scott	32.51	3.39	1.16	1.69	.50	1.92	3.61	.061	.778	.839
Bailey	31.35	2.88	1.39	1.87	.46	2.97	4.84	.031	.619	.650
Fredonia	37.87	3.49	1.52	2.41	.84	4.47	6.88	.028	.628	.656
Campbell	36.46	4.07	1.48	2.48	.95	3.22	5.70	.036	.672	.708
August 29										
Normal Leaves										
Concord	36.62	3.16	1.56	2.41	.81	3.24	5.65	.055	.781	.836
Sheridan	37.11	3.70	1.56	2.45	.85	3.70	6.15	.040	.560	.600
Worden	43.08	4.01	1.95	2.98	.92	3.94	6.92	.052	.762	.814
Extra	32.94	3.25	1.43	1.73	.28	2.40	4.13	.053	.627	.680
Delaware	36.00	3.86	1.87	2.60	.69	3.09	5.69	.064	.740	.804
Moore Early	39.20	3.32	1.63	2.93	1.24	3.40	6.33	.046	.708	.754
Ellen Scott	33.76	3.78	1.20	2.08	.84		-	.064	.814	.878
Bailey	32.53	3.10	1.35	1.93	.55	3.06	4.99	.045	.629	.674
Fredonia			.00							
Campbell	37.41	3.85	1.48	2.49	.96	2.92	.5.41	.050	.742	.792

(Percentage of green weight.)

# TABLE XVI.—(Continued.)

	(Percentage of green weight.)											
	Solids			CARBOHYDRATES						NITROGEN		
				Sugars		د. د.	mak-1	G.,1	Tunal	motol.		
		Solids	Solids	Solids	Ash	Red.	Total	Sucrose	Hydrol.	Carb.	501.	Insol.
October 1												
Young Leaves												
Concord	40.86	3.14	2.18	3.48	1.24	3.48	6.96	.046	.954	1.000		
Sheridan	40.70	3.60	2.32	3.57	1.19	4.21	7.78	.054	.777	.831		
Worden	43.76	3.36	2.49	3.71	1.16	5.67	9.38	.044	.906	.950		
Extra	37.91	3.81	2.15	3.11	.91	2.77	5.88	.054	.902	.956		
Delaware	40.56	3.97	2.47	3.46	.94	4.70	8.16	.043	<b>.97</b> 2	1.015		
Moore Early	42.05	3.03	2.78	4.00	1.16	5.73	9.73	.044	.919	.963		
Bailey	39.63	3.81	1.89	3.00	1.05	3.15	6.15	.044	.824	.868		
Fredonia	42.69	3.93	2.62	3.89	1.20	5.63	9.52	.033	.731	.764		
Campbell	40.60	3.44	3.00	4.64	1.56	3.44	8.08	.055	.991	1.046		
October 15												
Normal Leaves												
Concord	42.75	3.76	2.20	3.67	1.40	4.17	7.84	.044	.768	.812		
Sheridan	38.98	4.47	2.18	2.90	.68	4.27	7.17	.033	.359	.592		
Worden	43.92	3.92	1.99	3.55	.53	5.07	8.62	.048	.672	.720		
Extra	38.19	3.94	2.03	2.97	.91	3.45	6.42	.033	.663	.696		
Delaware	41.19	4.52	2.61	3.40	.75	4.02	7.42	.041	.767	.808		
Moore Early	43.82	3.79	2.16	3.87	1.62	4.90	8.77	.044	.704	.748		
Ellen Scott	38.94	4.68	1.79	2.63	.84	2.84	5.47	.044	.764	.808		
Bailey	37.20	4.07	2.16	3.23	1.07	3.48	6.71	.033	.597	.630		
Fredonia	41.39	4.25	2.70	3.38	.65	4.49	7.87	.030	.702	.732		
Campbell	40.33	4.31	2.45	3.40	.90	3.54	6.94	.054	.734	.788		

\*Samples cover the entire season and are of normal and of young leaves. \*\* Normal leaves are the older, dark-colored leaves and exclude the young, newly formed leaves toward the ends of the canes.

	Time	Solids	Ash
Concord	7:00 a.m.	20.63	1.25
	10:00 a.m.	30.07	1.67
	3:00 p. m.	<b>3</b> 3.78	1.84
	5:00 p. m.	31.66	1.77
Extra	8:00 a.m.	22.08	1.45
	11:00 a.m.	26.69	1.77
	3:30 p. m.	28.55	1.86
	5:30 p. m.	27.99	1.87

TAELE XVII.—Daily fluctuations of solids and ash in leaves; May, 1937. (Percentage of fresh weight.)

Unfortunately perhaps for this test, the year was one in which ripening vois more uniform than is generally found. The average percentage of green berries was only 8.72% at Perkins, where these leaf samples were collected. The percentage of green berries ranged from 1.8% to 16.8% for the 17 Concord vines used in these tests.

In Tables XVI and XVII, the two varieties most subject to uneven ripening, Concord and Sheridan, are placed at the top. The Sheridan is of particular interest because it shows even more than Concord the peculiarity of uneven ripening; and these two are to be compared with the other varieties that normally are less subject to this fault. In 1938, the berries of all varieties listed, except Concord and Sheridan, colored uniformly. For a clearer picture of the data, certain fractions are shown in Figures 7, 8 and 9.



Figure 7. Seasonal variations in the percentages of solids and total sugar content of Worden, Concord, Sheridan, and Extra grape leaves. 1938.

Total Solids. Figure 7 shows the total solids and total sugars percentages throughout the season for selected varieties, comparing two varieties that in 1938 ripened unevenly with two that colored fully. Data do not include young leaves except in the first sampling, which of necessity must be young because it is the early growth. Curves for total solids are interesting in that the two uneven-coloring varieties are located generally midway between Worden, the highest variety, and Extra, the lowest. Noteworthy also is the fact that all of the curves show steadily increasing amounts of solids throughout the season, with a slight flattening of the curve around the time the grapes are ripening. There is little indication here that Concord and Sheridan leaves differ in general composition from those of the varieties which ripened normally. Table XVIII compares the percentage increase in dry matter of the 10 varieties. Here again the poor-coloring varieties are intermediate in percentage increase; and, for Concords at least, there is certainly no indication that there is a lack of



Figure 8. Seasonal changes in the ash percentages of Concord, Worden, Sheridan, and Extra grape leaves; 1938.



Figure 9. Seasonal changes in the percentages of total nitrogen in the leaves from Concord, Worden, Sheridan, and Extra vines; 1938.



Figure 12. Seasonal changes in the phosphorus percentages of leaves from four selected varieties; 1938.

#### SUMMARY

1. Coloring became apparent only after the berries had increased in sugar content beyond approximately 7 percent.

2. The use of nitrogen fertilizers slightly improved coloring but not enough to prove of any great practical value.

3. Certain of the special treatments—additional light, use of superphosphate and of sugar as a fertilizer—failed to improve coloring as compared to the checks.

4. Various shading treatments and sub-irrigation markedly lowered the percentage of colored berries.

5. Sugar percentages were increased somewhat by the use of additional light. Nitrogen fertilization also gave increased sugar percentages but to a lesser degree than did additional light.

6. Leaf pruning tests indicated that at least 10 leaves should be present for each cluster.

7. Unlimited leaf area gave slightly better coloring than did 10 leaves per cluster.

8. Girdling, while slightly increasing the amount of sugar in the juice, did not give an increase commensurate with the labor and difficulties involved.

9. Bagging did not appreciably alter the composition of the juice, nor did it noticeably affect ultimate color development.

10. Solids and ash percentages in the berries increased continuously until after normal harvest time.

11. Acidity and pH of the press juice decreased steadily as the grapes matured. Cream of tartar generally increased.

12. Reducing sugars increased as maturity approached. Sucrose was always present, but never in large amounts.

13. The importance of water in relation to coloring is emphasized by the

results for sub-irrigation, where the addition of extra water to the soil during and preceding coloring gave the smallest percentage of colored berries found in any of the treatments.

14. Green berries were never found, even during ripening, to contain more than 6.5 to 7 percent sugar.

15. During coloring, the dextrose-levulose ratio varied from 1.0 to 1.2 and in one instance 1.3.

16. Tannins increased slightly in colored fruit as ripening progressed, but first increased and then decreased steadily in the uncolored fruit.

17. During the day, pH decreased slightly and sucrose increased appreciably until late afternoon or evening and then decreased.

18. Marked differences are found in the solids and ash content of individual berries on a cluster while all are still green.

19. Ash in the green berries remained quite constant during the sampling period (one month). On a dry weight basis, there was slightly less in the colored fruit.

20. In comparing green and colored berries and skins, calcium was the only mineral element found to vary appreciably. It was consistently lower in the colored samples. Iron was also slightly lower.

21. Minerals in the seeds of colored and green fruit did not vary significantly.

 $22.\ \mathrm{No}\ \mathrm{correlation}\ \mathrm{was}\ \mathrm{found}\ \mathrm{between}\ \mathrm{seed}\ \mathrm{content}\ \mathrm{and}\ \mathrm{color}\ \mathrm{develop-ment}.$ 

23. Catalase activity was found to increase as the season progressed and it was markedly higher in colored than in green fruit.

24. Oxidase and peroxidase activity were pronounced during coloring.

25. Prunings from Concord Norton and Extra vines were not significantly different in chemical composition.

26. Concord leaves were average as regards their chlorophyll, carotene and xanthophyll content when compared to other varieties.

27. Leaves reached their maximum solids content around late afternoon.

28. Total solids in leaves (Concords and others) increased as the season progressed. Ash percentages increased similarly.

29. Total sugar percentages in the leaves increased slightly for a time and then decreased somewhat during and immediately preceding coloring. Later, after the fruit had been harvested, the percentages again rose until sampling ceased.

30. Nitrogen percentages fluctuated somewhat but in general covered a relatively narrow range of values.

31. Potassium and phosphorus, particularly the latter, remained relatively constant in the leaf throughout the season. Calcium increased constantly as the season progressed.

32. None of the leaf analyses indicated any unusual fluctuations in the composition of Sheridan and Concord leaves that might be of value in explaining uneven coloring of the fruit.

Worden	71.29	
Moore Early	70.84	
Concord	66.41	
Ellen Scott	61.24	
Campbell	60.23	
Bailey	58.37	
Extra	55.05	
Sheridan	53.22	
Fredonia	50.23	
Delaware	43.32	

TABLE XVIII.—Percentage incre	ease of	dry d	matter	in	leaves	during	the	
season of 1938.*								

\* Data compiled from Table XVI.

solids synthesized by the leaves. Neither would there seem to be an unreasonable piling up of the products of photosynthesis, for there are two varieties with higher solids content that ripen uniformly.

**Total Sugars.** Figure 7 also shows values for total sugars, and here again can be noticed the parallel trend of the curves, in general an increasing percentage up until the berries begin to mature and then a drop until after harvest with a later increase continuing until frost. Sucrose is always present in variable amounts, but the variations seem to be without special significance.

Ash Percentages. Ash percentages (Figure 8) show gradually increasing percentages for all of the varieties and seem without special significance. Some detailed analyses are shown in Figures 10, 11 and 12.

Total Nitrogen. Nitrogen values for these same four varieties are shown in Figure 9. Trends here are not so consistent, with first one variety and then another being high and low. In general, Concords are near the top, and Sheridans are near the bottom if not actually last. From this it would not seem that total nitrogen in the leaves is a key factor in determining color development. Soluble nitrogen values are low; and, except for a small decrease just before the berries mature, they do not vary greatly.

Detailed Ash Analyses. Samples of leaves from Concord, Worden, Sheridan and Extra vines were analyzed at four times during the year for potassium, calcium and phosphorus, and the results are shown in Figures 10, 11 and 12. Potassium results vary most, but even here the changes are small and quite similar except that in the Sheridan there is a marked decrease after picking of the fruit. Calcium values rise continually throughout the year in much the same manner as do the solids, and without any significant variation. Phosphorus percentages remain almost constant throughout the year, with perhaps a small dip at picking time. These curves are noteworthy chiefly for the constant values they show compared to the general ash percentages which rise as shown in Figure 8. Specifically, no significant ash variations are apparent that might explain uneven coloring.



Figure 10. Seasonal changes in the potassium percentages of leaves from four selected varieties; 1938.



Figure 11. Seasonal changes in the calcium percentages of leaves from four selected varieties; 1938.

# LITERATURE CITED

(1) Alwood, W. B., Hartmann, B. J., Eoff, J. R., Ingle, M. J., and Sherwood, S. F.

1916. Development of Sugar and Acid in Grapes During Ripening. U. S. D. A. Bull. 335. 28 pp.

- (2) Association of Official Agricultural Chemists
   1930. Official and Tentative Methods of Analysis, Third Edition.
- (3) Bancroft, Wilder D., and Rutzler, John E., Jr.
  - 1938. The Colloid Chemistry of Leaf and Flower Pigments. I. The Precursors of the Anthocyanins. J. Am. Chem. Soc., 60: 2738-2743.
- (4) Bioletti, F. T., Cruess, W. V., and Davi, H.
  - 1918. Changes in the Chemical Composition of Grapes During Ripening. Publication in Agricultural Sciences, Univ. of Calif. Press. Vol. 3: No. 6, pp. 103-130.
- (5) Caldwell, J. S.
   1925. Some Effects of Seasonal Conditions Upon the Chemical Composition of American Grape Juices. J. Agr. Res. 30: 1133-1176.
- (6) Colby, A. S., and Tucker, L. R.
  - 1933. Some Effects of Severity of Pruning on Growth and Production in the Concord Grape. Ill. Agr. Exp. Sta. Bull. 393. 179-206.
- (7) Cooper, J. R., and Vaile, J. E.
   1939. Response of American Grapes to Various Treatments and Vineyard Practices. Ark, Agr. Exp. Sta. Bull. 378. 74 pp.
- (8) Copeman, P. R. v. d. R.
  - 1924. An Investigation Into Some Physical and Chemical Changes Occuring in Grapes During Ripening. Union of South Africa Dept. of Agr. Sci. Bull. 30. 38 pp.
- (9) ——
   1927. Some Changes Occurring During the Ripening of Grapes. Union of South Africa, Dept. of Agr. Sci. Bull. 60: 19 pp.
- (10) —, and Frater, G.
   1926. Some Physical and Chemical Changes Occurring During the Ripening of Grapes. Union of South Africa, Dept. of Agr. Sci. Bull. No. 50. 54 pp.
- (11) Cross, F. B., and Webster, J. E.
  - 1934. Physiological Studies of the Uneven Ripening of Concord Grapes. Proc. Am. Soc. for Hort. Sci. Vol. 32: pp.361-364.
- (12) \_\_\_\_\_, and Webster, J. E.
  - 1935. Physiological and Chemical Studies of Grapes. Assoc. of Southern Agric. Workers. Proc. of the 34, 35, and 36 Annual Convention, pp. 325-326. (A Summary).

- (13) \_\_\_\_\_, and Webster, J. E.
  - 1935. Color Development of Concord Grapes Associated with the Number of Leaves Per Cane. Proc. Am. Soc. for Hort. Sci. Vol. 33: pp. 440-441.
- (14) Davis, Wilmer E.
   1926. The Use of Catalase as a Means of Determining the Viability of Seeds. Boyce Thomp. Inst. for Plant Res. Prof. Paper. No. 2: pp. 6-12.
- (15) Dix, I. W., and Magness, J. R.
   1937. American Grape Varieties. U. S. D. A. Circular 437. 33 pp.
- (16) Frater, G.
   1924. The Composition of Ripe Wine Grapes from the Government Viticultural Station, Paarl. Union of South Africa, Dept. of Agr. Sci. Bull. No. 32. 30 pp.
- (17) Griffin, R. C.
   1927. Technical Methods of Analysis. McGraw-Hill Book Co., Inc., New York. 936 pp. (Second edition.)
- (18) Guthrie, John D.
   1929. A Stable Colorimteric Standard for Chlorophyll Determinations. Amer. Jour. Bot. 15: pp. 86-87.
- (19) ——\_\_\_\_\_\_
   1930. An Iodimetric Method for Determining Oxidase Activity. J. Am. Chem. Soc., 52: pp. 3614-3618.
- (20) Hartmann, B. G., and Tolman, L. M.
   1918. Concord Grape Juice: Manufacture and Chemical Composition. U. S. D. A. Bull. 656. 26 pp.
- (21) Lothrop, R. E., and Holmes, R. L.
  - 1931. Determination of Dextrose and Levulose in Honey by Use of Iodine-Oxidation Method. Ind. and Engr. Chem. Anal. Ed. 3: p. 334-339.
- (22) Maney, T. J., and Plagge, H. H.
  - 1934. A Study of Production and Physiology of Concord Grape Vines as Affected by Variations in the Severity of Pruning. Proc. Amer. Soc. for Hort. Sci. Vol. 32: pp. 392-396.
- (23) Miller, Lawrence P.
  - 1933. Effect of Sulfur Compounds in Breaking the Dormancy of Potato Tubers and in Inducing Changes in the Enzyme Activities of the Treated Tubers. Cont. Boyce Thompson Inst. 5: pp. 29-81.
- (24) Moreau, L., and Vinet, E.
  - 1932. Study of the Phenomenon of the Color Change During the Ripening of Grapes. Compt. Rend. Acad. Agr. France 18: pp. 198-202. Chem. Abs. 26: p. 3278.
- (25) Morrow, Clarence Austin.
  - 1927. Biochemical Laboratory Methods. John Wiley and Sons, Inc. New York. 350 pp.

46

- Noyes, H. A., King, H. T., and Martsolf, J. H.
  1922. Varieties in the Concord Grape During Ripening. J. Assoc. Offic. Agr. Chem. 6: pp. 197-205.
- (27) Onslow, Muriel Wheldale
   1925. The Anthocyanin Pigments of Plants. Cambridge Univ. Press. 2nd Ed. 314 p.
- (23) Overholser, E. L.
   1917. Colcr Development and Maturity of a Few Fruits as Affected by Light Exclusion. Proc. Am. Soc. for Hort. Sci. p. 73-85.
- (23) Talmer, L. S.
   1922. Carotinoids and Related Pigments. Chemical Catalog Co. 316 pp.
- (30) Partridge, N. L.
   1925. The Fruiting Habits and Pruning of the Concord Grape. Mich. Agr. Exp. Sta. Tech. Bull. 69. 39 pp.
- (31) Feynaud, E.
   1939. Sur les variations de l'azote du raisin au cours de la maturation. Rev. Vit. 90: pp. 189-195 and 213-225.
- (32) Rutzler, John E., Jr.
   1939. The Precursors of the Anthocyainins of Autumn Foliage. J. Am. Chem. Soc. 61: pp. 1160-1163.
- (33) Schrader, A. Lee
   1926. The Concord Grape—Pruning and Chemical Studies in Relation to the Fruiting Habits of the Vine. Md. Agr. Exp. Sta. Bull. No. 286. 118 pp.
- (34) Schertz, F. M.
  - 1928. The Extraction and Separation of Chlorophyll (A+B), Carotin and Xanthophyll in Fresh Green Leaves, Preliminary to Their Quantitative Determination. Plant Physiol. 3: pp. 211-216.
- (35) Vaile, J. E.
   1936. Factors Influencing The Uneven Ripening of Grapes. Forty-eighth Annual Report. Ark. Agr. Exp. Sta. Bull. No. 337. p. 57.
- (36) Venezia, M., and Gentilini, L.
  1934. La maturazione dell' uva. Ricerche sperimentali sul succo e sulle bucce. Ann. Staz. Sper. Viticolt. Enol. Congeliano. 4: pp. 207-218.
- (37) Villiers, F. J. de.
   1926. Physiological Studies of the Grape. Union of South Africa, Dept. of Agr. Sci. Bull. No. 45. 97 pp.
- (38) Webster, J. E., Anderson, Edward, and Cross, F. B.
  - 1934. Chemical and Enzymatic Studies of the Uneven Ripening of Concord Grapes. Proc. Am. Soc. for Hort. Sci. Vol. 32: pp. 365-369.

- (39) ——, Black, Rodney, and Cross, F. B.
   1937. Relationship of Leaf Pigments and Total Solids Content to Uneven Coloring of Concord Grapes. Proc. of Am. Soc. Hort. Sci. 35: pp. 475-479.
- (40) Winton, Andrew L., and Winton, Kate Barber
   1935. Structure and Composition of Foods. Vol. II. Vegetables, Legumes, Fruits. John Wiley and Sons, Inc. New York, 904 pp.

48