A STUDY OF THE FACTORS INFLUENCING THE RED COLOR ON APPLES.

by

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INTRODUCTION

Inasmuch as the red color of an apple is recognized as one of the principal factors determining the market value of the fruits, it seemed rather important to make a thorough study of the factors which influence its development. Various investigators have mentioned in earlier experiments that color may be affected by pruning, thinning, fertilizer practices and general orchard culture, but later workers have emphasized that the effects of such practices were indirect in that they mainly influenced leaf area per fruit and amount of sunlight reaching the fruit. Only in comparatively recent years have any studies been made on the specific factors influencing red color on apple fruits.

The present investigations were undertaken to determine if possible how a better red color development on apples could be secured under orchard conditions. The studies in this paper may be divided into two main

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* Some work on the project was carried on in 1927 by Mr. W. L. Kerr, while he was a graduate student at the University of Maryland.

groups: first, nutritional effects, and second, light effects.

The effect of the nutritive condition of the tree on development of red color was studied from various angles. One approach to the problem seemed to be through a study of the effect of various mineral nutrients, water, and other materials added to the soil or injected in the trees, or limbs of a tree. Also the nutritional role of leaves in the relation to color development afforded a study in which an attempt was made to correlate leaf area, chemical composition of the fruit and color development.

In studying the effect of light as a factor on red color development the object was to ascertain, if possible, what specific light ray or rays were instrumental in causing red color development.

In connection with the studies on light and nutrition, at attempt was made to correlate any definite effects on color with composition of the fruit.

The chemical nature of the pigment, and the location of the pigment in the fruit tissues were investigated. A study was made to determine if any seasonal chemcial and anatomical changes in certain tissues of the fruit could be correlated with development of color.

REVIEW OF LITERATURE

In view of the fact that this problem necessitated the conducting of many varied experiments, reference to certain literature will be discussed in connection with the specific group of experiments. Other literature dealing especially with studies of anthocyanin will be included in the following discussion.

Although there has been a large amount of work done on anthocyanin pigments of leaves and flowers of various species, there is to date very little work relative to the nature of the red coloring matter in apples. It may be assumed from work presented in another part of this paper that the red coloring matter of an apple is an anthocyanin, or lat least is closely related to this group of compounds.

One of the earliest written accounts of anthocyanin was that of Nehemiah Grew (1). He stated, "And first their colours; where, with respect to several plants and parts, they are more changeable; as Red in flowers; or constant; as Green in Leaves. Which, with respect to several Ages of one Part, are more failing, as Green in Fruits; or durable as Yellow in Flowers." His work was more of a chemical nature. He stated, "It is somewhat surprizing to see how Differingly-colorud Flowers, or Blossoms, how remote so was their colour be from Green, would in a moment pass into a deep degree of that colour upon the touch of an alcalizate liquor."

The work of Buscalioni and Pollaci (6) in 1903 showing the morphological distribution of anthocyanin is outstanding. These workers seemed to find pigment in all plant parts. Various workers have found anthocyanin crystals in different plants. Molisch (26) observed crystals in the red cabbage, and Sorauer (39) observed crystals in *Pyrus communis*.

Undoubtedly the most complete and comprehensive discussion of anthocyanin is that of Onslow (29). While the work of this author checks primarily with anthocyanin development in the leaves and flowers, an occasional mention is made of the anthocyanins in fruits. As to the method of formation of anthocyanins, Onslow presents the following scheme:

$$\text{Glucoside} + \text{water} \rightleftarrows \text{Chromogen (flavone)} + \text{sugar}$$

$$\text{(Chromogen)} + \text{Oxygen} \rightleftarrows \text{Anthocyanin.}$$

It will be observed from this diagram that the author is of the impression that anthocyanins arise from the oxidation of the flavones which are colorless chromogens in the plant sap. From a rather comprehensive review of literature Onslow draws the conclusion that the chromogen for anthocyanins is probably synthesized in the leaves and transported to various parts of the plant, where with an accumulation of carbohydrate anthocyanins are formed.

Everest (9) has given results which tend to show that *in vitro* anthocyanins are formed by a reduction of flavonols. This work has later been substantiated by Willstatter (46).

Since in the present paper no discussion will be made of the chemistry of the anthocyanins, no further review of this subject will be given. Anyone interested in this phase of the subject will find a complete discussion given in the books by Onslow (29) and Everest (9) which are included in the bibliography.

A number of investigators have studies the factors influencing red color in leaves and flowers. Combes (7) found that decortication was a very good means of inducing color formation. He also found that red leaves are higher in carbohydrates. Bonnier (4) observed that plants grown at high altitudes showed an increased color formation. Overton (32) found that leaves of certain plants would redden if placed in a sugar solution. He also observed that high temperatures were detrimental to color formation.

In regard to the effect of light, Sachs (34a) observed that plants may be divided into two groups: (1) flowers which develop color normally in the dark without a previous exposure to light, and (2) flowers which will develop color only if buds have been exposed to light. Senebier (36) found that apples would not redden unless directly exposed to light. Müller- Thurgau (27) found that grapes would develop color without light. Mirande (25) working with onion scales found that anthocyanin would not appear except in diffuse light. Only those rays in the blue end of the spectrum were active in color production. Onslow is of the opinion that any factor which increases the production or hinders the translocation of sugars and chromogens increases anthocyanin development. In the discussion of the conditions influencing the formation of anthocyanin Onslow (29) lists photosynthesis, accumulation of synthetic products, temperature, light, presence of oxygen, drouth, sugar feeding, and organic acids.

PLAN OF EXPERIMENTS

The studies reported in this paper were carried on in a large commercial orchard in western Maryland during the years 1927, 1928, and 1929. The orchard was in sod-mulch culture and had received spring and fall applications of nitrate of soda ranging from five to eight pounds total per year per tree for a period of from five to seven years. The trees were about twenty-five years old and were bearing good crops of fruit annually. They had received a rather careful detailed pruning every year and were making excellent growth. The soil of the orchard is classified as Berk shale.

In spite of the excellent cultural treatments, difficulty has been experienced in obtaining high colored fruits in this orchard.

One reason for poor color development is that the orchard is located on the eastern side of a steep ridge, and thus the trees received comparatively little sunlight in late afternoon. Frequent morning fogs also limit the amount of daily sunlight reaching the trees of such varieties as Williams, Summer Rambo, Wealthy, York Imperial, and Rome. It was these varieties which were used to the greatest extent in the work reported in this paper.

The different studies made include:

(1) Fertilizer Experiments: Spring and fall applications of varying amounts of manure, potassium sulfate, potassium chloride, acid phosphate, lime and sulfur, singly and in combination, were added to trees of the Summer Rambo, York Imperial, Rome, and Wealthy varieties.

(2) Addition of Chemicals to the Soil: Spring applications of varying amounts of citric acid, manganese sulfate, magnesium sulfate, ferric sulfate, and ferric carbonate were added to trees of the York Imperial, Wealthy, and Summer Rambo varieties.

(3) Addition of Sugar to the Soil: Beginning June 10th and ending two weeks prior to picking, varying amounts of sugar were applied to the soil around trees of the Rome, York Imperial, and Williams varieties.

(4) Artificial Feeding: Injection into limbs of solutions of sucrose, glucose, ferric sulfate, ferric carbonate, sodium nitrate, potassium nitrate, hydrochloric acid, malic acid and boric acid. Trees of the Rome, Wealthy, and Williams varieties being used.

(5) Defoliation and Girdling: At monthly intervals limbs were defoliated so as to leave zero, twenty, forty, and one-hundred leaves per fruit on such varieties as Rome and York Imperials.

(6) Half Tree Defoliation: Three weeks prior to harvest leaves were removed from one-half a tree by hand or by spraying the tree with solutions of citric acid,

ferric sulfate, sodium chloride, sulfuric acid, hydrochloric acid, sodium nitrate, calcium nitrate and potassium chloride. Trees of the York Imperial, Jonathan, Summer Rambo were used.

(7) Bagging With Various Colored Cellophane Bags: At monthly intervals beginning June 10th and ending September 10th, fruits of the Rome, Summer Rambo, and Williams varieties were bagged with red, green, purple, transparent, and yellow cellophane bags.

(8) Ultra-Violet Light Studies: Fruits in different stages of maturity were subjected to treatment with ultra-violet light. Fruits of the Rome, York Imperial, Wealthy, and Williams varieties were used.

(9) Use of Ethylene Gas: Fruits of the Rome and Summer Rambo varieties were treated with varying concentrations of ethylene gas.

(10) Irrigation: Beginning July 4th and continuing until harvest time trees of Rome, York Imperial, and Wealthy varieties were irrigated twelve hours a day, twice weekly.

(11) Microchemical and Anatomical Studies: Throughout the growing season fruits of the Grimes Golden, York Imperial, Rome, Summer Rambo, Wealthy, and Williams varieties were used for microchemical and anatomical studies.

(12) Chemical Studies: Chemical analyses were made on fruits from the sugar plots, irrigation plots and bagging work. A detailed discussion of materials and methods will be given under each specific group of experiments. The methods used in macrochemical, michrochemical, and anatomical

studies are described in the "Appendix."

In selecting trees for field studies great care was exercised to secure as uniform trees, with respect to growth, size, crop, anx general location, as possible. As a rule three trees were used per plot in each variety. While this number of trees may seem too few for measuring small differences in color, it must be remembered that any differences which might appear as a result of a given treatment must be large enough to be of practical value.

RESULTS OF FIELD STUDIES

Effect of Fertilizers

Beginning in the spring of 1927 various fertilizers in varying amounts were applied to the soil at different seasons of the year in an endeavor to influence the production of red color. Rome Beauty, York Imperial, Wealthy, and Summer Rambo were used. The materials used, amounts used per tree, number of trees, date of application, date of final observation, and color records are shown for the York Imperial and Summer Rambo varieties in Table I. and Table II.

The method used in arriving at the percentage of fruits in the color groups was to take a random sample of one-hundred fruits around the tree at shoulder height, and to measure the percentage of blush of each fruit. Such a method was used on each tree under a given treatment, and then an average was taken of the percentage of color from all three trees. While such a method does not give the absolute values it does give a comparative measure of the effect of a treatment on color formation when the fruits have an equal and uniform exposure.

TABLE I.

EFFECT OF FERTILIZER TREATMENTS ON RED COLOR ON APPLES OF THE YORK IMPERIAL VARIETY. HANCOCK, MARYLAND SEASON 1928.

Fertilizers.	No. of Trees.	Amount Per Tree.	Applied.	Notes Taken.	Percentage of Fruits in Color Groups.		
					Below 25%	25% to 50%	Above 50%
Check.	3				15	60	25
Manure.	3	1000 lbs.	Sept. 15 1927.	Sept. 28,1928	20	60	15
Manure.	3	500 lb			30	55	15
Check.	3				25	40	35
Acid Phosphate,	3	10 lbs.			15	50	35
	3	15 lbs.			15	50	35
	3	25 lbs.			20	60	15
Muriate of Potash,	3	5 lbs.					
	3	10 lbs.			10	70	20
	3	20 lbs.			10	80	10
Check.	3				10	75	15
Sulphur	3	10 lbs.			15	75	10
Sulphur	3	20 lbs.			20	70	10
Check	3				10	70	20
Lime	3	50 lbs.			15	70	15
	3	100 lbs.			15	70	15
Muriate of Potash and Sulphate of Potash,		10 lbs. 5 lbs.	April 20th, 1928.		10	70	20
	3	20 lbs. 25 lbs.			15	70	15
Lime.	3	25 lbs.	April 20, 1928.	Sept. 28,1928	15	65	20
	3	50 lbs.			15	60	25
	3	100 lbs			20	16	15
Check.	3				25	55	20
Muriate of Potash,	3	5 lbs.			15	70	15
	3	10 lbs.			10	70	20
	3	20 lbs.			15	65	20
Sulfate of Potash,	3	5 lbs.			15	70	15
	3	10 lbs.			15	65	20
	3	20 lbs.			15	70	15
Acid Phosphate.	3	10 lbs.			10	70	20
	3	15 lbs.			15	70	15
	3	25 lbs.			15	65	20

TABLE II.

EFFECT OF FERTILIZER TREATMENTS ON RED COLOR ON APPLES OF THE SUMMER RAMBO VARIETY. HANCOCK, MARYLAND SEASON 1929.

Fertilizers.	No. of Trees.	Amount Per Tree.	Applied.	Notes Taken.	Percentage of Fruits in Color Groups.		
					Below 25%	25% to 50%	Above 50%
					Fruit Picked immature trace blush		
Check	3		Aug. 3 1929		"	"	"
Acid					"	"	"
Phosphate	3	10 lbs.					
					"	"	"
	3	15 lbs.					
					"	"	"
	3	25 lbs.					
					"	"	"
Muriate of	3	5 lbs.					
Potash.					"	"	"
	3	10 lbs.					
					"	"	"
	3	20 lbs.					
					"	"	"
Check	3						
					"	"	"
Sulphur	3	10 lbs.					
					"	"	"
Sulphur	3	20 lbs.					
					"	"	"
Check	3						
					"	"	"
Lime	3	50 lbs.					
					"	"	"
	3	100 lbs.					
Muriate of					"	"	"
Potash and		10 lbs	April 20				
Sulphate of			1928		"	"	"
Potash.		5 lbs.					
					"	"	"
	3	20 lbs.					
		25 lbs.			"	"	"
Lime.	3	25 lbs.			"	"	"
	3	50 lbs.			"	"	"
	3	100 lbs.			"	"	"
Check	3				"	"	"
Muriate of	3	5 lbs.					
Potash.	3	10 lbs.			"	"	"
	3	20 lbs.			"	"	"
Sulphate of							
Potash	3	5 lbs.			"	"	"
	3	10 lbs.			"	"	"
	3	20 lbs.					
Acid phos-	3	10 lbs.			"	"	"
phate	3	15 lbs.			"	"	"
	3	25 lbs					

Table I. gives the effect of various fertilizers on red color development on apples of the York Imperial variety, and Table II. gives the effect of various fertilizers on the red color development on apples of the Summer Rambo variety.

A careful study of the data presented in these Tables shows that addition of fertilizers, either singly or in combination, is without any noticeable effect on red color production on apples on vigorous, well pruned trees.

The foliage on the trees receiving manure was much greener than that of any other treatment, and yet actual color records showed no effect of the treatment on color. This is in direct contradiction of Stewart's (40) findings. He states, "Nitrogen and manure have materially increased the yields, but decreased the color of fruits." Assuming that nitrogen and organic matter are the two most important compounds in manure, so far as orchard practices are concerned, it is possible that a condition may arise where manure would cause a lessening of color, namely; in an orchard which was devitalized through an insufficient amount of nitrogen. The application of manure to such an orchard might cause a rank growth and dense foliage resulting in a shading effect. Shaw (38) was of the opinion that nitrate would interfere with the development of high color in two ways: (1) by delaying maturity, and (2) by causing a more luxuriant growth of the tree which would result in shading the fruit. Attention is again called to the fact that the trees used in the work reported here-in were well pruned, and the stable manure in such amounts as used did not increase the foliage of the tree to such an extent as to cause a shading of the fruit. No evidence was noted as to a delaying of maturity. Schrader and Auchter (35), comparing fall and spring applications of ammonium sulfate and sodium nitrate, were of the opinion that fall applications do not affect color of fruit during the season in which applications were made. Aldrich (2a) presents rather conclusive data that the red color of apples is not decreased the same year that fall application of nitrate of soda are made to bearing apple trees. In his work, application of nitrate of soda three weeks prior to picking in amounts as high as twenty pounds per tree, was without effect on the red color development of apples of the York Imperial varieties. Tukey (42) seemed to find that applications of nitrate of soda to bearing apple trees caused a very heavy set of fruit with a poor color development. The effect of nitrogen may have been indirect in his work since there was a greater number of fruits competing for a given carbohydrate and water supply and as will be discussed in greater detail in another section of this paper, either of these two materials may be a limiting factor in the production of color.

Potash has often been considered as playing an important role in the production of red color on apples. A study of Table I. and Table II. shows no effect however of potash either as a muriate or a sulfate, upon the red color development in these experiments. Even when as much as forty-five pounds per tree of muriate and sulfate of potash was used, there was no effect on color. Stewart (40) seemed to find that potash did give an increase in color, although in a later work he says that sunlight is the important factor in the production of red color. Recent work by Grubb (12) in England seems to show an effect of potash on increasing color in apples, but here again the effects are indirect in that potash was effective in reducing leaf scorch which resulted from a potash deficiency.

Acid phosphate was without effect on red color development under the conditions of the experiment reported here. Earlier workers have reported that this material would occasionally increase color. We find Stewart (40) saying that phosphate gave a slight increase in color formation. However Hedrick (15) basing his conclusions on twelve years results, was unable to show any effect of phosphate on red color development.

Lime in amounts as high as one-hundred pounds per tree was without effect on red color development on apples over a period of three years.

Sulfur in amounts high enough to destroy all sod around the trees was without effect on red color development on apples over a period of three years.

It is evident from the data presented here that red color development on apples was not affected by the use of the various fertilizers used. A condition might be brought about by the usse of nitrogen which would result indirectly in a decrease in color. If nitrogen is applied to an orchard and such applications are not followed by proper pruning, thinning, and soil management, there might result a decrease in the amount of red color due to a very dense growth and a heavy set of fruit. If under such conditions nitrogen was withheld from the orchards and potash, lime, or phosphate added, there would probably result an increase in color, but such an increase would probably occur if these materials had not been added, so the effect on color by use of such materials under the condition mentioned cannot be directly attributable to these substances.

In considering any relation of fertilizers to color development of the fruit, any increase or decrease in color will probably be affected by the thoroughness with which the grower performed his pruning, thinning, spraying, pollination and soil management practices. The marked response of trees to nitrogen necessitates especial attention by the grower, particularly when nitrogen has not been used previously on the orchard.

Effect of Chemicals Other Than Fertilizers Added to the Soil.

In this phase of the work various chemicals were applied to the soil in the spring of each year to ascertain if possible whether such chemicals would affect directly or indirectly the red color on fruits. A list of the chemicals used includes: citric acid, magnesium sulfate, manganese sulfate, aluminum sulfate, ferric carbonate, and ferric sulfate. Tables III. and IV. show the chemicals used, the number of trees, the amounts of chemicals, date of application, date of observation on color development, and color observations. The Summer Rambo, Wealthy, and York Imperial varieties were used.

Each chemical was used with a definite purpose in mind. With the compounds containing iron, it was assumed as a working hypothesis that the addition to the soil of iron as an essential element in chlorophyll production, might increase the chlorophyll content of the leaves and as a consequence the phtoynthetic activity of the leaves. Since various workers have found that increased carbohydrates were associated with increased color formation in leaves and flowers it was assumed that any compound which might cause an increase in carbohydrates in the fruit would result in a better color. If iron would thus indirectly increase the photosynthetic activity of the leaves, other conditions being equal, there should result an increase in carbohydrates in those fruits from trees to which iron had been added.

Citric acid was added to the soil to increase the acidity of the soil. Such an increase in acidity might result in a cessation of bacterial activity with a consequent loss of nitrates to the tree. This decrease of available nitrates might result in less growth and allow for an accumulation of carbohydrates within the tree and

TABLE III.

EFFECT OF VARIOUS CHEMICALS ON RED COLOR ON APPLES OF THE SUMMER RAMBO VARIETY AT HANCOCK, MARYLAND. Season 1929.

Material.	No. of Trees.	Amount Per Tree.	Applied.	Records Taken.	Color Range.			
Citric Acid.	3	1 lb.	April, 1929.	July, 1929.	Fruit	Picked	Immature-	Trace Color.
	3	3 lb.			"	"	"	"
	3	5 lbs.			"	"	"	"
Ferric sulfate,	3	3 lbs.			"	"	"	"
	3	5 lbs.			"	"	"	"
	3	10 lbs.			"	"	"	"
Manganese Sulfate,	3	1 lb.			"	"	"	"
	3	3 lbs.			"	"	"	"
	3	5 lbs.			"	"	"	"
Magnesium Sulfate,		3 lbs.			"	"	"	"
	3	6 lbs.			"	"	"	"
	3	10 lbs.			"	"	"	"
Aluminum Sulfate,	3	5 lbs.			"	"	"	"
	3	10 lbs.			"	"	"	"
	3	25 lbs.			"	"	"	"
Check	3							
	3							
	3							
	3							

TABLE IV.

EFFECT OF VARIOUS CHEMICALS ON RED COLOR ON APPLES OF THE WEALTHY VARIETY AT HANCOCK, MARYLAND SEASON, 1929.

Material.	No. of Trees.	Amount Per Tree.	Applied	Record Taken.	Color Range.
Magnesium Sulphate.	3	5 lbs.	April, 1929.	July 1929.	Trace to 90%, Average 30% -- good red.
	3	10 lbs.			
	3	20 lbs.			
Manganese Sulfate.	3	1 lb.			Number of fruits green. Trace to 70%. Average 30%, good red.
	3	3 lbs.			
	3	5 lbs.			
Check.	3				Trace to 90%, Average 30% -- Good red.
Citric Acid	3	1 lb.			
	3	3 lbs.			
	3	5 lbs.			
Aluminum Sulphate.	3	5 lbs.			
	3	10 lbs.			
	3	25 lbs.			
Ferric Sulfate.	3	3 lbs.			
	3	5 lbs.			
	3	10 lbs.			
	3				
	3				

fruits. The acid might also react with various bases in the soil liberating carbon dioxide which might be taken up by the roots, again resulting in an increase in carbohydrates. There is also the possibility that the citric acid could be taken up directly by the tree resulting in metabolic changes within the tree and fruits resulting in increased color of fruits.

The results obtained by the use of chemicals mentioned above on the Summer Rambo and Wealthy varieties are presented in Tables III. and IV.

The data in Table III. shows conclusively that under the conditions of the experiments reported in this work, there was no increase in color development by any of the various chemicals used. It is fairly safe to assume that any differences in color which would have been apparent at picking time, would have been apparent at the date color records were taken, which date was only two or three days prior to picking.

The data in Table IV. also shows that the use of such chemicals as citric acid, magnesium sulfate, manganese sulfate, aluminum sulfate, and ferric sulfate cannot be expected to increase or decrease the red color development on apples of the Wealthy variety, provided such condition exist as were prevalent in the experiments reported here.

In only one case was there any evidence that the application of a chemical to the soil had influenced the metabolism of the tree. Ten pounds of magnesium sulfate, applied to trees of the York Imperial variety, did result in a greener foliage in 1929; however, there was no noticeable result on the red color of fruits from such a treatment.

Effect of Addition of Sugar to the Soil.

The relation between high sugar content of a plant, and the development of anthocyanins has been shown by Overton (32), Combes (7), Willstätter (46), Knudsen (19), and Magness (33),. Knudsen (19), has shown that some plants are able to utilize sugar from nutrient media as a source of energy. He observed that alfalfa grown on agar-agar containing a high amount of sugar developed a very striking red color. Soil workers (34) (44) have shown that the presence of organic compounds in the soil greatly retard the production of nitrate nitrogen by bacteria.

Since plants may directly take up sugar, and since the addition of sugar to the soil should influence the nitrate nitrogen content of the soil, it seems logical to believe that the addition of sugar to the soil would affect the metabolism of an apple tree.

Experiments were laid out to test the effect of addition of sugar to the soil on the red color development on apples. In the work carried on in 1927, commercial glucose was used. During 1928 ordinary brown sugar was used; while in 1929 a commercial brand of corn sugar, "Cerelose" was used.

Thrifty trees of the Williams, Wealthy, Rome, Summer Rambo, and York Imperial varieties were used.

TABLE V. EFFECT OF APPLICATION OF SUGAR TO THE SOIL ON RED COLOR OF WILLIAMS

EARLY RED VARIETY, HANCOCK, MARYLAND, SEASON 1929

Treatment	No. of Trees	No. Fruit	0-25% Blush		25-50% Blush		Above 50% Blush		Rank	Remarks.
			No.	% Tot.	No.	% Tot.	No.	% Tot.		
20# Sugar No Nitrate	3	365	0	0.0	207	57	158	43	4	The blush was in the nature of a solid blush, but rather dull.
10# Sugar No Nitrate	3	616	0	0.0	307	50	312	50	3	The blush was in the nature of streaks. All fruits showing some streaks.
Check No Nitrate	3	179	40	23	78	43	61	34	5	The blush was rather dull.
20# Sugar Plus Nitrate	3	393	30	7.6	100	24.5	263	67	2	Color in the nature of a solid blush. Very clear and bright.
10# Sugar Plus Nitrate	3	203	12	5.9	51	25.1	140	69	1	Same as above
Check plus Nitrate	3	295	34	11	117	39	144	50	3	The blush was rather clear. Some streaks.

Dosages ranged from ten to twenty pounds per tree per application. In some cases there was a monthly application, while in other cases there was only one application about two or three weeks before harvest.

The amount of sugar used, the number of trees, the date of each application, the total number of fruits, and the color results for such sugar treatments are given in Table V. and Table VI.

At harvest time the fruits from each tree were segregated into color classes ranging from zero to twenty-five per cent red color, twenty-five to fifty per cent red color, and above fifty per cent red color. Since all varieties responded in a like manner, actual data are presented only for the York Imperial and Williams variety. Tables V. and VI. present the data for the Williams and York Imperial varieties respectively.

A study of Table V. shows that the application of sugar to the soil has in some way influenced the red color of fruits. It is evident that the addition of sugar has markedly increased the percentage of fruit in the upper class range. There was also a difference in quality of blush where sugar was added. The data also indicate that an application of ten pounds of sugar per tree for this variety was just as efficient, or a little more efficient than twenty pounds. Due to lack of rain, most of the July 10th application probably did not enter the soil until after harvest, so that the effect of this application probably may be counted as negligible.

A study of Table VI. shows that the greatest effect from sugar application on the York Imperial variety can be secured if applications are delayed until about two weeks prior to picking. It was observed in all the sugar work that an increase in color was apparent about two weeks after application, but such an increase was of a transitory nature, in that eventually the differences between the treated trees and the check trees were not apparent. With the York Imperial variety it is apparent that the greatest color was secured when twenty pounds of sugar were added to the soil about two weeks prior to harvest. Results of pressure test determinations indicate that fruits of all varieites were from ten days to two weeks advanced in maturity where sugar was used. This advance in maturity was indicated also by a change of ground color as well as a softness of flesh of the fruit. Such evidence of advance in maturity was apparent about two weeks after the addition of the sugar to the soil. Sugar applications may thus influence color by hastening maturity.

There are at least four ways in which sugar added to the soil might affect the maturity of the fruits, and as a consequence, increase red color development.

First, through the actual intake of sugar as such into the tree resulting in a higher carbohydrate content within the tree and fruit. Magness (23) has shown recently that high color development on apples is associated with high carbohydrate content within the fruit. Although this method of increasing color is a possibility, it is highly improbable in that sugars are very readily broken down by bacterial action, and would probably be transformed into single organic acids before they reached the roots of the tree.

Second, a possibility which suggests itself is the increase in osmotic pressure of the soil solution by the addition of sugar -- such an increase in osmotic pressure hindering the ability of the roots to absorb water, thereby resulting in a physiological drouth condition in the tree. The term "physiological drouth" being used here to designate that condition within a tree resulting from the inability of the tree to absorb sufficient water from the soil due to a high concentration of material about the roots. In no case under observation were trees observed to be suffering from a lack of water.

A third possibility as to how sugar applied to the soil might affect the red color development of apples is by the increase in carbon dioxide in the soil resulting from destruction of the sugar by bacteria; such an increase in carbon dioxide might be taken up by the tree and used in photosynthesis resulting in an increase in carbohydrates in the tree and fruits.

A fourth and most probable manner in which sugar added to the soil could increase the red color development on fruits is the effect of sugar in the nitrogen content of the soil. This effect has been shown by Waksman (44) in experiments in which nitrogen free organic materials are added to the soil increases the amount of total nitrogen in the soil, but decreases the amount of nitrate nitrogen. He was of the opinion that the bacteria could and would use the carbohydrate material as a source of energy using such nitrates as might be present in the soil for their own development. A lessening of nitrate nitrogen in the soil might have an effect on the metabolism of a tree and the maturity of the fruit.

Regardless of the manner in which color may be increased by the addition of sugars to the soil, the fact remains that there is an increase in color development progressing concomitantly with a hastening of maturity where sugar is added to the soil.

Work at the Rothamsted Station (34) has shown that where any form of carbohydrate is used the crop production with wheat is increased the second year. The assumption is that the addition of carbohydrates causes an increase in the number of bacteria influencing nitrogen content in the soil. The second year this increased number of bacteria, some of which die with result in an increased supply of nitrate nitrogen. It has been stated that the color of fruit, where sugar was added to the soil, increased for a time over that of the check, but that such an increase was transitory. It is possible that such a transient difference in color was due to a retarding effect on color, caused by a greater amount of nitrate nitrogen after the supply of sugar had been exhausted by the bacteria. The

The late applications of sugar two weeks before picking would not result in such an increase in nitrate nitrogen reaching the tree prior to picking.

Effect of Irrigation

The general impression among fruit growers in the Eastern fruit sections is that the average annual precipitation in this section of the country is sufficient to produce satisfactory crops of well matured and well colored apples. In view of results secured in other sections of the United States (17), (43), (21) and because of general observations, that the color of apples in the Shenandoah-Cumberland Valley section is occasionally poor in years of low precipitation, experiments were started to determine the value of irrigation as a method of increasing red color development on apples.

The season of 1929 offered an exceptionally good opportunity to study the effects of added water supply on red color development in the Eastern orchards. During the season in question there was a prolonged drouth from mid-June until early September. Throughout the season it was observed that the trees located on rather thin soil did suffer from a lack of water. The size of fruit was materially reduced and on exceptionally light soils there was a loss of fruit, due to premature dropping.

In these experiments, owing to the topography of the land, a "flume-furrow" system of irrigation was practiced. Blocks of trees were selected on sites having a gentle slope and a very light soil. Two such blocks were laid out, one block containing five trees of the Wealthy variety and four trees of the York Imperial variety, and a second block containing six trees of the Rome variety. Six check trees were selected for each block. There was always a guard row left between the treated blocks and the checks.

Irrigation was practiced twice weekly beginning July 4th and ending September 15th. The water was allowed to remain in the furrows for twelve hours at each irrigation. A quantity of water was introduced at the head of the furrow to allow a small amount of run-off at the end of the furrow. The furrows were so spaced that each tree received a supply of water from four furrows, in this manner of spacing at no time after irrigation was started were there any dry areas around the trees. An idea of the porosity of the soil may be gained from the fact that 10,000 gallons of water had been used for the initial irrigation of ten furrows, each twenty-five feet long before the water reached the end of the furrow.

At harvest time all of the fruit of the Rome and Wealthy varieties were picked and segregated into color classes ranging from 0 to 25 per cent color, 25 to 50 per cent color, and above 50 per cent color. With the York Imperial variety, a random sample of 100 fruits per tree was selected and segregated into the above classes. The data are presented for all three varieties in Table VII.

TABLE VII. EFFECT OF IRRIGATION ON RED COLOR ON APPLES. HANCOCK, MARYLAND. SEASON. 1929

Date	Variety	No of Trees	Total No. of Fruits.	Percentage of Fruits in Color Range 0-25%	25-50%	Above 50%
Oct.6	Wealthy. Irrigated	5	4097	52.1	28.1	19.8
	Wealthy. Check	5	1183	74.2	21.3	4.0
	Rome. Irrigated	6	2497	8.9	22.5	68.3
	Rome. Check	6	1313	15.7	24.1	60.2
	York. Irrigated	4	334*	21.4	18.4	60.2
	York. Check	4	319*	18.1	46.8	34.1

* These figures represent a random sample.

A study of Table VII. shows rather conclusively that added water has increased the percentage of fruits in the upper class range. With the Wealthy variety there was an increase by irrigation about 25 per cent in the above 50 per cent class; this increase in the higher color class is reflected by a decided decrease in the 25 to 50 per cent color class in irrigated fruits.

The figures for the Rome variety are not so striking, although there is an increase for irrigated fruits in percentage of fruits in the above 50 per cent class. There is some decrease in the percentage of irrigated fruits in the 0 to 25 per cent class. While not showing a marked increase in favor of the irrigated plots the figures on the Rome variety do show the same trend as those on the York Imperial and Wealthy varieties.

These results are in accord with those of Lewis (21) working in Oregon, of Taylor and Downing (43) working in Idaho, and of Jones and Colver (17) also working in Idaho who state, "In intensity and uniformity of color, the irrigated trees have somewhat the advantage over the non-irrigated trees.

Throughout the growing season the general appearance of the fruits of the York Imperial variety on the irrigated trees was far better than that of the check trees. The percentage of fruit in the above 50 per cent class is very much in favor of irrigation, while the percentage of fruit in the poor color classes are greater for the check trees. At this point it is well to emphasize the fact that amount of red color and not of quality of blush was considered in these determinations.

There was an indication that the form of the fruit had been changed by irrigation. This was especially noticeable with the Wealthy variety. The natural form of this variety under western Maryland conditions is oblate with a rather shallow basin under irrigation the fruits tended to be conical with a deep basin. The lenticel markings were much more pronounced on irrigated fruits. The form of the York Imperial variety was changed from a round-oblate with oblique axis to nearer a round oblate form. The general shape of the fruit of the Rome variety was not changed.

From the data presented it is evident that the color of apples of the York Imperial, Wealthy, and Rome varieties may be improved under Eastern conditions during a dry season by irrigation practices. The size of fruit was also materially increased under irrigation.

Further data under average seasons must be secured before any recommendations relative to irrigation practices as influencing size and color under Eastern conditions can be made. However the importance of conservation of water by proper cultural means and in-coöperation of organic matter in the soil can be emphasized in relation to color development.

Effect of Artificial Feeding

Various chemicals were injected into the limbs of a tree in close proximity to the fruit. In work of this nature there are two methods which may be used. The solid materials may be placed in a hole in the limb, or a solution of the material may be injected into the limb. The latter method was used in this work in that it affords a better means of knowing what amounts of materials are used, and also there is less danger of local injury when the latter method is used.

The materials employed in this work were always made up on a percentage basis, one gram of material to one-hundred cubic centimeters of water being designated as a one per cent solution. Distilled water was not used, but water from the same source was used in every case. A list of materials injected into the tree included, sugar (glucose and sucrose), hydrochloric acid, sodium nitrate, potassium nitrate, ferric sulfate, ferric carbonate, boric acid, malic acid, and magnesium sulfate. Such materials were made up in various concentrations. Due to bacterial action the sugar solutions were changed daily in all cases.

A limb for treatment was chosen which was about one and one-half inches in diameter. Each solution was injected into a separate limb and all solutions were injected into limbs of the same tree. The fruits were thinned to twenty fruits per limb and all leaves removed in excess of those of the same tree. The fruits were thinned to twenty fruits per limb and all leaves removed in excess of those required to give sixty leaves per fruit. Check limbs were treated in a similar manner but only water was injected into the limb. After the removal of leaves a hole one-eighth of an inch in diameter was bored to a depth of three-quarters of an inch in the limb at a distance about two feet from the first fruit. In 1928 a single hole rubber stopper was placed in this hole and connected with a reservoir. During 1929 a piece of rubber tubing was placed in the intake hole on the limb and a piece of glass tubing about three inches forced into the rubber tubing. The 1929 procedure resulted in a very tight connection and allowed no loss of water or solution. The glass tubing was connected to a piece of rubber tubing leading to the reservoir. During 1928 tin pails were used as reservoirs, but due to the fact that these pails rusted badly, one-half gallon glass fruit canning jars were used as reserviours in 1929. The reservoir was always raised a little above the point of intake which resulted in the development of a slight hydrostatic pressure.

After all connections were made the glass tubing was removed from the intake and a syphon started. The intake was flushed with the solutions to be used, and the glass tubing replaced in the intake. The injections usually started about one month before harvest, and the reservoirs were kept filled with the solution to be used until no further

TABLE VIII.

EFFECT OF ARTIFICIAL FEEDING ON RED COLOR ON APPLES OF THE ROME VARIETY. HANCOCK, MARYLAND, SEASON 1929.

Date	Material	Concentration.	Amount.	Date Observed.	Remarks.
Aug. 17th.	Water		3L	Sept. 19th.	Trace to 40% color -- Ave. 25% color -- good color.
Aug. 17th.	Boric Acid.	.125%	3L		Slight defoliation--Slight twig injury--Blush from 25 to 100% Ave. 35% good color.
	Boric Acid	.25%	3L		Complete defoliation--bad twig injury--blush 100%--Solid red color--fruit pithy.
	Hydrochloric Acid.	.125%	3L		Blush trace to 90% Ave. 60% deep red color.
	Cerelose	2%	3L		Some defoliation--Blush 10% to 50% Ave. 25% good color.
	Cerelose	4%	3L		Bad defoliation--Blush 10% to 40% Ave. 20% poor color.
	Cerelose	1%	3L		25% to 75% Ave. 50% good clear red.
	Sucrose	1%	3L		20% to 80% Ave. 60% excellent color.
	Sucrose	.5%	3L		20% to 80% Ave. 60% excellent color.
	Malic Acid.	.5%	3L		Trace to 30% Ave. 15% mostly streaks.
	Potassium Nitrate.	.25%	3L		10 to 40% Ave. 20% fair color.
	Sodium Nitrate.	.25%	3L		Bad leaf injury. Trace to 20% Mostly streaks. Fruit green.

intake was noticed. As a general statement it may be said that there was an intake of material over a period of ten days, the most rapid intake occurring during the first two days. Rome, Williams, and Wealthy varieties were used in this work.

Tables VIII. IX. and X. show the results obtained on color by injection of various materials into the limbs of these varieties. A color record was taken on each fruit at the time of injection of the solution, and daily observations made on foliage or fruit injury. The final color records were taken at harvest time.

A study of Table VIII. on Romes shows some results of interest. The concentrations of boric acid were toxic, but gave a remarkable color development. It was observed that boric acid even in very dilute concentration, would cause a slight leaf fall, but its greatest damage was done in killing of all twig growth. The fruits on limbs treated with this substance did not drop but they did develop a very deep color. The flesh of the fruit was of a "cheesey" consistency and unfit for consumption. Microchemical examination showed that such fruits contained no trace of starch.

Hydrochloric acid (.125 per cent concentration) usually gave a slight defoliation with a consequent better color development. The area of blushed surface and depth of blush were increased. This treatment gave indication of being one of the best means of increasing red color on apples, but it does not seem commercially applicable to American orchards.

The effect of "Cerelose" (glucose) were not as striking as was anticipated in view of the results from the application of this material to the soil. Yet the results substantiate to a small degree the explanation of the effects of sugar when applied to the soil. The higher concentration of "Cerelose" caused a plasmolysis of the leaves with a consequent defoliation. Wilting was usually apparent from twenty-four to forty-eight hours after the initial injection. Wilting and discoloration was first apparent on the types and margins of the leaves and gradually covering the whole leaf. In no case was there noticed a shrivelling or dropping of the leaves due to treatment with "Cerelose." Although the higher concentration of "Cerelose" caused a defoliation, there was no apparent increase in red color on the fruits. The lower concentrations did seem to increase color content of the fruit.

Injection of sucrose of higher concentration caused a plasmolysis and a defoliation in a manner similar to that discussed above, while the lower concentration gave an increase in color development.

Malic and injected into the limbs had an unusual effect in that, although the higher concentration caused a defoliation, no color developed. This is interesting in light of the defoliation work mentioned later where increase in color always results from defoliation.

Potassium nitrate was without effect on color

TABLE IX. EFFECT OF ARTIFICIAL FEEDING ON RED COLOR ON APPLES OF THE WEALTHY VARIETY. HANCOCK, MARYLAND

SEASON 1929

Date	Material	%	Am't	Picked	Remarks
July 24	Hydrochloric Acid.	.125	4L	Aug.6	Slight defoliation-several fruits show better color than check. Bright cast to Blush.
	Malic Acid.	.5	4L		Fruit show greater tendency to streak actual blush less than check. Slight defoliation.
	Boric Acid.	.25	4L		Bad injury-fruit better colored than check. Very poor quality. Falls easily.
	Boric Acid.	.125	4L		Same as above except fruit not so well colored.
	Cerelose	.5	4L		Fruit better colored than check. All fruits show same blush. Average 20% mostly streaks.
	Cerelose	2.5	3L		An occasional fruit shows solid blush. Better than check in amount. Average 20%.
	Water		4L		No increase over check.
	Sucrose	1	3L		No increase over check.
	Sodium Nitrate	.25	4L		No increase over check. Advantitious bud growth.
	Potassium Nitrate	.25 .25	4L 4L		Bad leaf injury--Color not so good as in check.

development or growth. The higher concentration did cause a defoliation which resulted in a better color development.

Sodium nitrate in the concentrations used caused severe defoliation, and adventive bud growth. The color development was hindered and the fruits were greener at maturity than the checks. Schertz (41) has shown that sodium nitrate does increase the chlorophyll content of leaves, and since chlorophyll is found in the apple fruit it is possible that there was an actual increase in chlorophyll content in these fruits. Rumboldt (33) found that the higher metals caused a glossier coat on chestnuts, but no increase in wax on apples was noticed in this work.

The water which in these experiments served as a check was without effect on color development on the fruits or on growth of the limb. The latter statement is not in accord with the findings of Goff (10) in that he noticed an increase in growth where water was injected into the roots of young trees.

Table IX. gives the results obtained by the injection of various solutions into limbs of the Wealthy variety. A study of Table VIII. shows the same general facts as were discussed under Table VIII.

The injection of hydrochloric acid resulted in slight defoliation with its consequent good color development. Boric acid caused severe defoliation, twig injury, poor quality of fruit, and increased color development. Cerelose gave better color development than the check. Sucrose had

TABLE X.

THE EFFECT OF ARTIFICIAL FEEDING ON RED COLOR ON APPLES OF THE WILLIAMS VARIETY. HANCOCK, MARYLAND SEASON, 1929.

Material.	Conc.	Injections.	Total Amount Absorbed	Notes 7/19 (final) (last injection 7/3)
Cerelose.	1%	3	3000 cc	Color a trifle better than check in quality and per centage.
Cerelose	2.5%	3	3000 cc	Color far better than check in quality and per centage.
Sucrose.	1%	3	3000 cc	Not much different in color from check.
Sucrose.	.5%	3	3000 cc	Not much different in color from check.
Malic Acid.	1%	1	1000 cc	Some defoliation -- not as much color as check.
Malic Acid.	.5%	3	3000 cc	A few traces of color averages about 10% streaks.
Boric Acid.	.25%	3	3000 cc	All fruits on limb were streaked one week ago. All fruit has fallen. Fruit has tendency to be mushy.
Boric Acid.	.125%	3	3000 cc	Same as above. No starch. Color better than check.
Hydrochloric Acid.	.5%	1	1000 cc	Same leaf defoliation. Fruit not so large as check. Color far in excess of check.
Hydrochloric Acid.	.25%	3	3000 cc.	No increase in color over check.
Ferric Sulfate.	.25%	1	1000 cc	Bad leaf injury -- more color than check.
Ferric Sulfate.	.125%	3	3000 cc	No leaf injury -- color equals to check.
Sodium Nitrate.	.5%	1	1000 cc	Bad leaf injury -- color less than check. Stimulation of adventition buds.
Sodium Nitrate.	.25%	3	3000 cc	Color equal to check.
Potassium Nitrate.	.5%	3	3000 cc	Color equal to check.

no effect on color development. Sodium nitrate was without effect on red color development, but did increase the green color. Potassium nitrate gave severe defoliation and a color development less than the check. Water was without effect on color development or growth.

Table X. shows the results obtained by injection of various solutions into limbs of the Williams variety. The results found here differ from those found on the Rome and Wealthy variety, differing in degree rather than in kind. The higher concentrations of "Cerelose" gave a very good color development with this variety, while a lower concentration gave a color development only slightly better than the checks. The sucrose solutions were without effect on red color development. Malic acid caused defoliation and retarded color development. Boric acid resulted in defoliation and an early appearance of color. Hydrochloric acid caused defoliation and cessation of growth of the fruit, but an increase in color development. Ferric sulfate caused severe leaf injury with a delayed defoliation and an increase in color formation. Sodium nitrate caused defoliation, adventive bud growth, and an increase in green color with a consequent decrease in red color. Potassium nitrate was without effect on color development.

It should be stated here that whole-tree injection experiments were tried with hydrochloric acid (.25 per cent concentration) on the Rome variety. Four holes were bored about one foot from the ground, equidistant around the trunk and injection made about five weeks before picking time in the manner described above. A continuous supply of solution was kept in the reservoirs and a total of forty-eight liter were used before the experiment was terminated. There was a slight defoliation and a very great increase in color three weeks after injection.

In summation of the artificial feeding studies, it might be said that the effect of injection of materials into the sap stream on the red color development on apples seems to vary with the variety. The Rome variety which does not normally develop a high blush, responded to a low concentration of "Cerelose" and sucrose, while the Williams variety, which develops a striking red blush, responded to a high concentration of "Cerelose," although sucrose was without effect on red color with this variety. All varieties responded alike to boric acid solutions showing an increased color formation, but severe leaf and twig injury. Hydrochloric acid varied in its effect on red color. This material usually caused some defoliation and a noticeable increase in color. Ferric sulfate was without color development and in many cases caused severe injury. Fron (10) secured increased vigor by use of this compound injected into pear trees, but no such effects were noted in the present paper on apple trees.

Several facts not directly pertinent to red color formation were observed during the course of the artificial feeding experiments. The amount of material absorbed by a limb was dependent upon the material used, concentration, time of day of injection, temperature, atmospheric conditions, and variety used. It was observed that the first liter of solution was usually absorbed very rapidly, each succeeding absorption being less rapid. Colloidal solution did not seem to be absorbed at all. Acids, mineral or organic, were absorbed more rapidly than water -- the absorption of hydrochloric acid was the most rapid of any solution used, maximum absorption of this acid being one liter in two hours on a clear day. "Cerelose" was absorbed more slowly than water, but more rapidly than sucrose. Temperatures being constant the Rome variety seemed to possess the greatest ability to absorb the various materials.

Effect of Ethylene

Ethylene gas has been used to ripen tomatoes and to hasten the appearance of red color through such a hastening of maturity. The gas has also been used to blanch celery, and to ripen citrus fruits and bananas. Harvey (14) has shown that the use of the gas on celery results in an increase in reducing sugars. Work (45) found that green tomatoes ripened readily with a production of red color provided the tomatoes were in a certain stage of maturity. Little work has been done showing the effects of this gas on apples.

The assumption that chlorophyll in apples must disappear before anthocyanin will appear, was made when the work was started on ethylene gas. Magness and Diehl (24) state, "As the red pigment develops it apparently replaces the chlorophyll, being developed in the same cells in which chlorophyll was formerly present." This statement is made without showing any data upon which it is based. As will be pointed out in the microchemical studies, anthocyanins do not necessarily replace the chlorophyll, but are usually located in specific cells. If it were true that chlorophyll must didappear before anthocyanins appear, it does not necessarily follow that any treatments which destroys the chlorophyll will increase the red color in apples, in that such a destruction of chlorophyll might so change the composition of the fruit that color cannot develop.

In 1928, several experiments were carried out in which varying amounts of ethylene gas were used on fruits which had been subjected to several different treatments. Fruits of the Summer Rambo variety were placed in air tight steel drums of twenty-five gallons capacity and subjected to concentrations of the gas ranging from one part of gas to 1000 parts of air to one part of gas to 500 parts of air, at a temperature of 80°F. The fruits were treated for one week to such concentration, the gas being renewed daily. At the end of one week it was found that the treated fruit was much softer as measured by the pressure tester than the check, but the chlorophyll content had not been lessened to any great degree. Checks were placed in a similar container, but given no gas treatment.

Working on the assumption that perhaps the waxes of

TABLE XIII

EFFECT OF ETHYLENE GAS ON RED COLOR ON APPLES OF SUMMER RAMBO VARIETY. HANCOCK, MARYLAND. SEASON 1929.

Treatment	Pressure test	Ground Color	Blush	Hours in Ethylene	Pressure Test	Ground Color	Blush
Check	14	2½	none	none	13½	2½	
5% NaOH	14	2½	none	none	12½	2½	
5% NaOH	14	2½	none	120	11½	3½	
Check	13½	2½	none	none	13½	2½	
Injured	13½	2½	none	none	12½	3½	
Uninjured	13½	2½	none	120	12½	2½	
Injured	12½	2½	none	120	12½	3	
1	12½	2½	50%	none	11½	2½	50%
2	12½	2½	50%	120	9½	3	50%
3	13	2½	10%	none	11½	2½	10%
4	13	2½	10%	120	9½	3	10%

the surface of the fruit were hindering the action of the dthylene, fruits of the Summer Rambo variety were washed thoroughly with a warm, five per cent solution of sodium hydroxide and placed in ethylene gas of a concentration of one part gas to 1000 parts of air, for one week. Another lot of fruit which had the skin broken by needle punches was treated for one week using a 1 to 1000 concentration of ethylene. A third lot of fruit was chosen which had varying percentages of blush. This lot was treated with ethylene for one week using a concentration of one part ethylene to 1000 parts of air.

The data for the results obtained by the use of ethylene gas on fruits of the Summer Rambo variety are presented in Table XIII. A study of this table shows that ethylene gas may cause a lessening of chlorophyll content as is shown in the case of fruits having varying percentages of blush. It will be observed that the ground color in this case goes from two and one-half to three. This numbering taken as a basis number, which is a very green ground color, and number four, which is a yellow ground color.

Experiments with fruits of the Rome variety, using one part ethylene gas to 500 parts of air, and one part of ethylene to 200 parts of air under different temperature conditions, were made.

It was observed that at the higher temperature (70°F) and at the higher gas concentration that there was a more rapid disappearance of chlorophyll from the treated fruits than on the check fruits held at a similar temperature. At the lower temperature (37°F) the fruits did not show any lessening of chlorophyll. The keeping quality of the fruit was impaired under the higher gas-temperature conditions. There was no increase in the development of anthocyanin in any treatments which is in accord with the findings of Hibbard (16).

In the gas treatments a green tomato was put in the container with the fruit and it was observed that at 70°F. and all concentrations of gas that the green ground color disappeared in three days, while at the lower temperatures 38°F. the green color disappeared in about six days.

From this work it is apparent that ethylene gas may effect a change in chlorophyll content of apples, with a consequent ripening of the fruit. There exists a possibility that ethylene gas may be used to change a ground color of apples which have a blushed area, from green to yellow, thereby accentuating the red color of the fruit, and making the fruit more attractive.

Effect of Defoliation and Girdling.

The role of leaves in the production of red color on apples has received little attention by horticultural investigators. Onslow (29) is of the opinion that some chromogen is manufactured in the leaves and transported to various parts of a plant, and under favorable conditions gives rise to anthocyanin. It is common physiological knowledge that an increase in leaf area results in increased carbohydrate manufacture. Since carbohydrates and possibly chromogens are manufactured

TABLE XI.

EFFECT OF DEFOLIATION AND GIRDLING ON RED COLOR ON APPLES OF ROME VARIETY. HANCOCK, MARYLAND. SEASON, 1929

FINAL OBSERVATION SEPTEMBER 26, 1929.

Date Defoliated	Treatment	No of flowers per apple	Size (Cm.) Original	Final	Increase	Other Observations
June 16	Defoliated and Girdled	0	9.7	13	3.3	Bronze color. Only few streaks of color
		20	10.2	20.6	10.4	50% good red color. few streaks
		40	10.4	22.4	12.0	80% good red color. Few streaks
		100	10.9	25.7	14.8	80% good red color. No streaks
	Defoliated	0	11.3	22.0	10.7	90% Solid red color. Better than check
		20	9.6	21.8	12.2	50% good red color.
		40	8.9	21.0	12.1	50% good red color
		100	11.5	24.8	13.3	70% good red color
July 16	Defoliated and Girdled	0	16.8			All fruits have fallen
		20	16.8	20.8	4	40% color. mostly streaks
		40	16.4	23.0	5.6	60% color. Mostly streaks
		100	16.6	24.4	7.8	70% good color. Yellow ground color.
	Defoliated	0	15.6	16.5	.9	90% excellent deep red color.
		20	16.6	22.8	6.2	50% good color Mostly streaks.
		40	16.4	24.2	7.8	60% good color
		100	16.8	23.8	7.0	60% deep red color
August 16	Defoliated	0	21.7			All fruits have fallen
		20	19.7	22.3	2.6	30% color Mostly Streaks
		40	21.0	24.9	3.9	40% color Mostly streaks
		100	20.6	25.8	5.2	80% solid red color.
	Defoliated	0	22.2	24.7	2.5	60% color. Bronze.
		20	19.9	23.7	3.8	40% color Mostly streaks
		40	21.5	23.6	2.1	50% color. Mostly streaks.
		100	20.7	24.1	3.4	75% deep red color.

in the leaves that any means which would increase or decrease leaf area should result in changes in red color on apples. Using this assumption as a basis, a number of experiments were carried on in which leaf area per fruit was studied in relation to color development.

Defoliation and Girdling: Starting in early June and continuing at monthly intervals through August, leaves or fruits were removed so as to have a given number of leaves per fruit. At each date, after removal of leaves, the twigs were girdled just back of the last leaf required to give a definite number of leaves for a fruit. Girdling was done by cutting with a knife around the twig, through the bark, but not removing any bark. Assuming that carbohydrates move in the phloem such girdling was used to isolate the fruit from leaves other than the definite number selected. At weekly intervals this cut was again circled with the knife blade so as to sever any re-united phloem starch. Ten fruits showing no color development were selected for each treatment. This method is called "Defoliation and Girdling" in the present paper.

Table XI. and XII. show the results obtained by defoliation and girdling upon red color development on apples of Rome and York Imperial varieties. The color records in Table XI. for the Rome are of interest. If complete defoliation with girdling was practiced three months prior to picking, fruits so treated were checked in growth, remained on the tree, but developed only a bronze color, while if the treatment was applied one month prior to picking, the fruit

TABLE XII.

EFFECT OF DEFOLIATION AND GIRDLING ON RED COLOR ON APPLES OF THE YORK IMPERIAL VARIETY. HANCOCK, MARYLAND SEASON, 1929.

Date Defoliated	Treatment.	Number of Leaves Per Apple.	(Size Cm) Original	Final	Increase	Color Observations.
June 16th,	Defoliated and girdled.	0	11.5	--	--	All fruits have fallen.
		20	10.0	19.9	9.9	50% good red color.
		40	10.0	21.8	10.9	80% good red color.
		100	10.8	24.0	13.2	90% solid red color.
	Defoliated	0	11.1	22.8	11.7	90% solid red color.
		20	11.5	24.6	13.5	70% good color.
		40	10.2	22.1	11.9	70% good color.
		100	10.7	22.6	11.9	70% good color.
July 16th	Defoliated and girdled.	0	14.8	16.0	1.2	60% color. Broad streaks.
		20	15.3	20.5	5.2	40% color. Broad streaks.
		40	15.4	23.0	6.6	50% fair color.
		100	17.2	25.1	7.9	70% good clear color.
	Defoliated	0	15.8	24.5	8.7	60% fair color.
		20	16.8	23.7	6.9	70% good red color.
		40	16.3	23.1	6.8	70% good red color.
		100	17.2	25.1	7.9	70% good red color.
August 16th.	Defoliated and Girdled.	0	--	--	--	All fruits have fallen.
		20	--	--	--	All fruits have fallen.
		40	--	22.8	--	60% good color.
		100	--	23.4	--	80% excellent color.
	Defoliated	0	--	22.7	--	40% fair color.
		20	--	20.8	--	40% fair color.
		40	--	24.7	--	50% good color.
		100	--	25.6	--	65% good color.

fell from the tree. The reason for such falling probably was the inability of the larger fruits to obtain sufficient moisture without the aid of leaves. The results show that there is a direct relationship existing between the number of leaves and color development; the greater the number of leaves the greater the percentage of color. This fact is in direct accord with the work of Magness (22) on the effect of leaf area on quality.

Table XII. presents the data secured by defoliation and girdling upon red color development of the York Imperial variety. From this Table it is evident that complete defoliation and girdling caused the fruit to drop in every case except one, that of the July treated fruits. An examination of the treated fruit in early September, before it had fallen, showed that no color had developed where defoliation and girdling had been practiced. The lot which wa s treated on July 16th showed a color development in September of 60 per cent, mostly in the nature of streaks. Such a streaking was characteristic of fruits which had been completely defoliated and girdled.

As with the Rome variety, a direct relationship was found to exist between leaf area and color development.

Defoliation Without Girdling: A second method used for isolation of the fruits, after counting the required number of leaves, was to remove all other leaves for a distance of one meter from the fruit. Magness' work (22) indicated that fruits do not draw upon leaves for food at a greater distance than one meter removed from the fruit. Ten fruits showing no color development were selected for each treatment. This method is called "Defoliation without Girdling."

Tables XI. and XII. present the data relative to the effect of defoliation without girdling on red color on the Rome and York Imperial varieties. A study of these tables shows that the completely defoliated fruits did not fall from the tree as occurred when girdling was used with complete defoliation, and the fruit did develop an intense red color.

The fruits treated three months prior to harvest developed a better color than the check. Such an increase in color may be explained either as a result of direct increase in amount of sunlight reaching the fruit, coupled with the ability of the fruit to draw synthesized material from other part of the limb, or by the fact that fruits are more dependent for color development upon leaves during early season than late season. The effect of complete defoliation and girdling seems to be a contradiction of the latter theory, but in such a treatment there must result a great change in the carbohydrate content in the fruit where leaf area is removed and the fruit isolated by girdling. Of the two explanations advanced for the increase in color in the fruit from limbs defoliated early in the season the greater amount of sunlight is the most plausible.

The direct relation between leaf number and color development as shown with defoliation and girdling experiments was not evident probably because the removal of leaves for one meter distance did not isolate the fruit to a given number of leaves.

A study of the growth of the fruit of Rome variety in Table XI. shows that girdling had a greater retarding influence on growth than the removal of leaves for a meters distance. Two explanations of this fact could be made. There may have been a restriction of water by the girdling through a possible injury to xylem tissue and consequent plugging of vessels. If a strip of bark has been removed then undoubtedly the girdled portion would have dried out and inhibited the passage of water through the dried portion. It has been stated that in no case was the bark removed. The second explanation is that fruits do feed on leaves situated at a distance greater than one meter removed from the fruit. The latter explanation seems to be the most plausible of the two advanced in accounting for differences in growth of fruit as secured on girdled and not girdled twigs. The growth figures also show that maximum size is obtained when more than forty leaves are left per fruit.

Complete Defoliation Just Before Harvest: Other experiments on defoliation consisted of the removal of all leaves from one-half a tree by hand two weeks prior to picking, and by use of chemicals sprayed onto the tree with a knapsack sprayer. The former type of experiment is listed in this work as "Half-tree Defoliation," while the latter type is listed as "Chemical Defoliation." The purpose of this work was to ascertain if it were possible to increase the red color development on fruits by the removal of leaves late in the season without incurring any risk due to loss fruit by falling or other injury. It was assumed that the removal of leaves two weeks prior to picking would allow a greater number of fruits to be exposed to the sunlight and consequently there would result a better color development.

Half-Tree Defoliation: Trees of the Summer Rambo, Wealthy, and York Imperial varieties were defoliated, and records made of the number of fruits in the various class ranges. Two weeks after defoliation, color records were again made. The data for the Summer Rambo and Wealthy are presented in Table XIIIa. The same general effects were found to hold for the York Imperial variety.

TABLE XIII (a)

THE EFFECTS OF ONE-HALF TREE DEFOLIATION ON RED COLOR DEVELOPMENT ON APPLES. HANCOCK, MARYLAND SEASON 1928.

Variety	Total No. of Fruits.		Number of Fruits Showing Blush.					
	Check	Treated	Below 25%		25 to 90%		Above 50%	
			Check	Defoliated.	Check	Defoliated.	Check	Defoliated.
Summer Rambo	102	233	90	0	10	121	2	112
Wealthy	86	150	31	0	39	48	16	102

From the figures presented in this Table it is clearly evident that removal of leaves from one-half a tree two weeks prior to picking was certainly beneficial in so far as increasing the red color on apples is concerned. It should be mentioned that this method of treatment did not cause any

TABLE XIV. THE EFFECT OF CHEMICAL DEFOLIATION ON TREES OF THE JONATHAN VARIETY, HANCOCK, MARYLAND. SEASON 1928

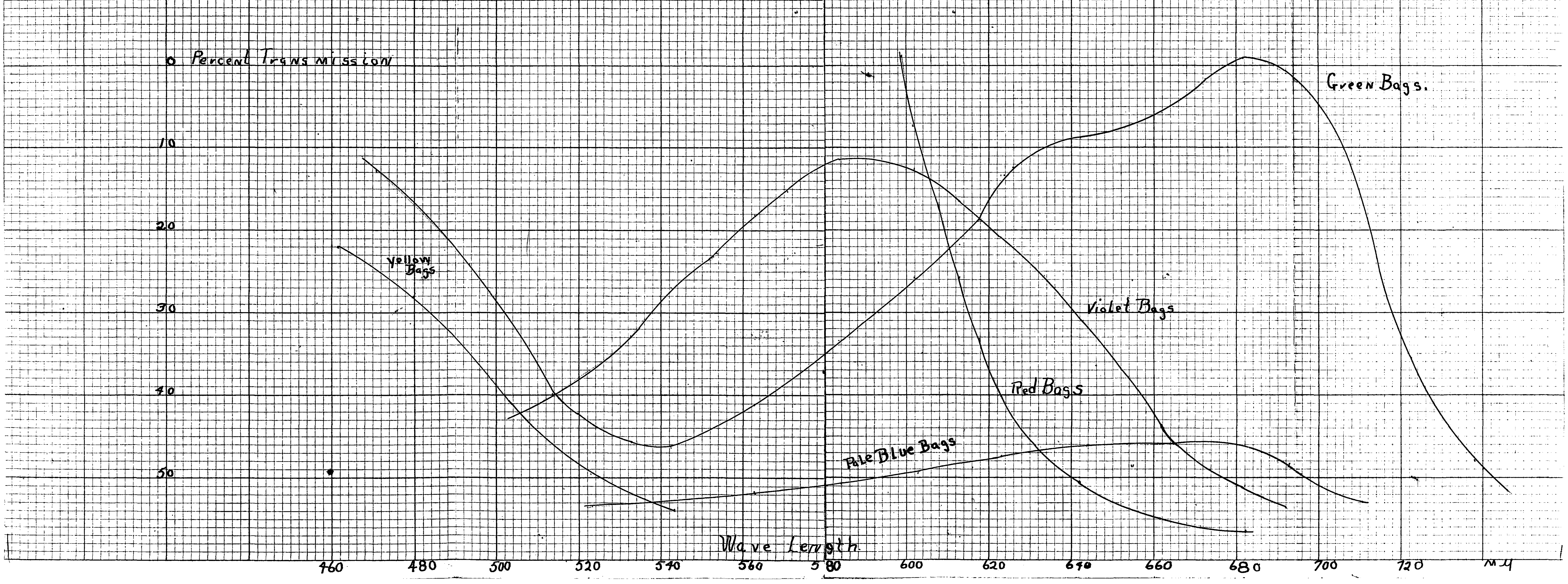
Date Applied	Weather	Material	Concentration	Time of Injury: Leaf	Time of Injury: Fruit	Value	Immediate Action.	Ultimate Action
Aug.27	Cloudy	$NaNO_3$	10%	18 hrs.		Fair	Slight browning of leaves	Leaves drop in 2 das. Twigs injured-no fruit injury.
Aug.28	Clear-hot	NaNO	5%	18 hrs.		High	Slight browning of leaves	Leaves drop in 2 das. No fruit or twig injury.
Aug.27	Cloudy	$CaNO_3$	10%	24 hrs.		Fair	Slight yellowing of leaves	Leaves brown, no fruit or twig injury.
Aug.27	Cloudy	H_2SO_4	1%	12 hrs.	2 hrs.	Nil.	Fruit burned badly	Slight leaf drop
Aug.28	Clear-hot	H_2SO_4	5%	24 hrs.	2 hrs.	Nil	Fruit burned badly	Slight leaf drop
Aug.27	Cloudy	NaCl	10%	4 hrs.		High	Leaves turn yellow	Fruit uninjured Leaves dry-twigs not injured
Aug.28	Clear-hot	NaCl	5%	4 hrs.		Fair	Leaves turn yellow Fruit spotted	Leaves yellow slight injury
Aug.27	Cloudy	NaOH	5%	20 min.	20 min.	Nil.	Leaves burned-twigs burned.	Leaves, twigs and fruit badly injured.
Aug.28	Clear-hot	NaOH	1%	30 min.	30 min.	Nil.	Fruit spotted, leaves burned.	Leaves and twigs injured
Aug.27	Cloudy	K_2SO_4	10%	18 hrs.		Very high	Leaf burned	Leaves drop in 2 das.
Aug.28	Clear-hot	K_2SO_4	5%	18 hrs.		Very high	Leaf burned	Leaves drop in 2 das.

TABLE XV. THE EFFECT OF CHEMICAL DEFOLIATION ON TREES OF THE SUMMER RAMBO VARIETY. SEASON 1928

Date of Application	Weather	Material	Concentration.	Time of Injury Leaf	Time of Injury Fruit	Value	Immediate Action	Ultimate Action
Aug.15	Clear	KNO_3	10%	48 hrs		Slight	Fruit uninjured Leaves show slight browning	Fruit uninjured Leaves brown at edges fruit falls.
							Fruit slightly injured.	Fruit, twigs,& spurs injured
Aug.15	Clear	HCl	5%	20 mins	24 hrs.	Nil	Leaves burned	Leaves burned
							Fruit uninjured	Fruit uninjured but spotted, fruit falls
Aug.15	Clear	Lime-Sulphur	1-10	48 hrs.		High	Leaves slightly browned	Leaves brown, fall easily
Aug.15	Clear	Lime-Sulphur	1-15	69 hrs.		Slight	Fruit uninjured Leaves uninjured	Fruit uninjured Slight browning of leaves
Aug.18	Murky	Na Cl	20%	2 hrs.		High	Fruit uninjured Leaves yellow Flaccide	Fruit slightly spotte Leaves dead
Aug.18	Murky	$FeSO_4$	20%	2 hrs.	24 hrs.	Nil	Fruit slight injury. Leaves blackened	Fruit badly injured fruit falls. Leaves blackened
Aug.17	Rainy	HCl	2%	24 hrs.	24 hrs.	Nil	Fruit burned Leaves slightly injured	Fruit burned-spotted Leaves slightly injured
Aug.18	Murky	HCl	1%	30 hrs.	30 hrs.	Nil	FRuit uninjured Leaves show slight browning	Fruit badly spotted Slight injury on leaves

Figure 1

Light Transmission of Various Colored Cellophane Bags



of the fruit to drop. A precaution which must be observed is not to defoliate the tree during the hottest part of the day as serious sunscald will result on those fruits which are suddenly exposed.

Chemical Defoliation: Due to the fact that many "trial and error" experiments were to be conducted only one limb of a variety was used for each solution. The solutions were made up in the desired concentration and sprayed on the tree by means of a knapsack sprayer about two weeks prior to the picking date. Such chemicals as sulphuric acid, hydrochloric acid, citric acid, ferric sulfate, calcium nitrate, potassium sulfate, lime sulfur spray, and sodium chloride in various concentrations were used. Trees of the Summer Rambo, Jonathan, York Imperial, Rome, and Williams variety were treated. Tables present the data for the Jonathan, Summer Rambo and Rome varieties.

A study of Tables XIV. and XV. shows that the chemicals may be grouped into two classes, based on the effects of the chemicals on the fruit; those which burn the fruit and those which do not. Obviously no spray could be used which would harm the fruit in any manner. Of those chemicals which do not burn the fruit, sodium nitrate offered the greatest possibilities. The action of the material was rapid and there was a minimum of damage to the twigs. Sodium chloride was efficient as a defoliating agent, but it probably could not be used over a period of years due to its ultimate effect on tree growth. Potassium sulfate is another material which was of value in this work. Sulphuric acid, hydrochloric acid, and

TABLE XVI. CHEMICAL DEFOLIATION ON RED COLOR ON APPLES OF ROME VARIETY

HANCOCK, MARYLAND. SEASON 1929

Date Applied	Material	Concentration	Application	Date observed.	Remarks
Sept.10	Sodium nitrate	5%	Heavy	Oct. 6	About 90% defoliation -- no apparent twig injury -- fruit shows blush from 20 to 90% Ave. 60% good red color.
	Sodium nitrate	5%	Light	Oct. 6	About 50% defoliation -- no twig injury -- blush range from trace to 90% Ave. about 40% good color.
	Citric acid	5%	Heavy	Oct. 6	About 50% defoliation -- Leaves Yellowing -- fruit shows acid burn -- blush trace to 90% ave. 50% good color.
	Citric acid	5%	Light	Oct. 6	Same as above except less severe fruit injury.
	Check			Oct. 6	Blush ranges from 10% to 90% Ave. 40% good color.

sodium hydroxide are of no value in this work due to their harmful effects on the fruits and twigs.

Table XVI. shows the effects of varying amounts of two chemicals as sprayed on a tree of the Rome variety in an attempt to defoliate two weeks before picking as a method of increasing red color development. A study of the Table shows that the heavier application of sodium nitrate caused sufficient defoliation to result in good color development, while citric acid caused only a small amount of defoliation and some fruit injury.

The chemical defoliation studies showed that there are chemicals which may be adapted to use as defoliating agents about two weeks prior to harvest as a means of promoting color development on the fruit. Certain other chemicals are absolutely of no value in such work. The application of sodium nitrate as a spray seemed to give best results with all varieties except the Williams. Spraying of any chemicals on trees of this variety two weeks prior to harvest resulted in a loss of fruit from dropping before any leaf area was apparent.

Thinning Experiments: In 1927 Kerr (18) carried on some thinning experiments with the Wealthy variety, which throws additional light on the role of leaves in the production of red color. Table XVII. gives the results of such work.

TABLE XVII.

EFFECT OF THINNING ON RED COLOR DEVELOPMENT ON APPLES OF WEALTHY VARIETY. HANCOCK, MARYLAND SEASON 1927.

Spacing.	Number of Leaves Per Plant	Quality of Color.	Per Cent of Color.
Check	8	Good	5-20
5-6 inches	18	Good	20-30
10 inches	24	Good	30-40
18 inches	40	Good	40-50

From these figures it is evident that while quality of color is not influenced by leaf area in this variety, the percentage of color is increased. These results are in direct agreement with work of Auchter (2) and others on thinning.

In summing up the work dealing with the effect of leaf area on color development on apples it may be safely stated that color development is very closely connected with leaf area. The fact that fruits deprived of their leaves early in the season did not develop color, while they did develop color if they were deprived of their leaves later in the season, seems to substantiate the theory of Onslow (29) as to the manufacture of chromogens in the leaves. A further substantiation of this claim is found in the work of Heinicke (15a) who found that if a variety was grafted onto another variety and all the leaves removed from the stock, the fruit developing on the stock assumed color characteristics of the fruit of the graft.

That varieties differ in the number of leaves required for good color development is shown by the fact that a total of forty leaves per fruit was the minimum required for good color on the York Imperial variety, while a total of twenty leaves per fruit was the minimum required for good color development on the Rome variety. A possible explanation of these differences might lie in the bearing habits of the two varieties. The York Imperial variety being a spur bearer, would require a larger number of leaves in that the leaves on a spur are much smaller than on a terminal growth. The Rome variety being mainly a terminal bearer would require a lower number of leaves in that the size of leaves on terminals are larger than on spurs. Haller and Magness (24a) and Magness (22) have found that varieties differ in regard to leaf area requirements in different sections of the country, being less in sections of high insolation.

That sunlight is an important factor in the development of red color on apples is shown in the experiments where one-half a tree was defoliated just before picking. In this work the fruits were well advanced toward picking conditions and were apparently in no further need of elaborated food. Under such conditions the admitting of light to the fruit greatly increased the red color.

Effect of Bagging with Various Colored Cellophane Bags.

The effect of the quality of light on red color development on apples has received little attention, while it is common knowledge that the quantity of light is a very important factor. It has long been observed that shaded or partly shaded fruits lack red color as compared with fruits which are fully exposed.

One of the purposes of the study as reported in this paper was to ascertain, if possible, what light ray or rays are effective in the production of red color. To pursue such a study most effectively under field conditions, it was necessary to secure a material which could be easily handled, which would not interfere with the normal growth of the fruit, and which would be inexpensive. Cellophane was the material finally decided upon. Red, green, blue, purple, and transparent cellophane was used.

In 1928 bags of various colored cellophane were placed around fruits which were about two-thirds grown, and upon which no color had yet developed. As a result of these preliminary tests on Williams, Rome, York Imperial, Wealthy, and Summer Rambo varieties, it was observed that only the red colored bags had any influence on color development, and under these bags color was completely inhibited.

The light transmission curves presented in Figure 1 show the percentage of light of the different wave lengths transmitted by the various colored cellophane bags. A study of these graphs show that while there is a variation in percentage of transmission of light rays of the longer wave length, all material with the exception of the red colored cellophane transmitted as high as thirty per cent of visible

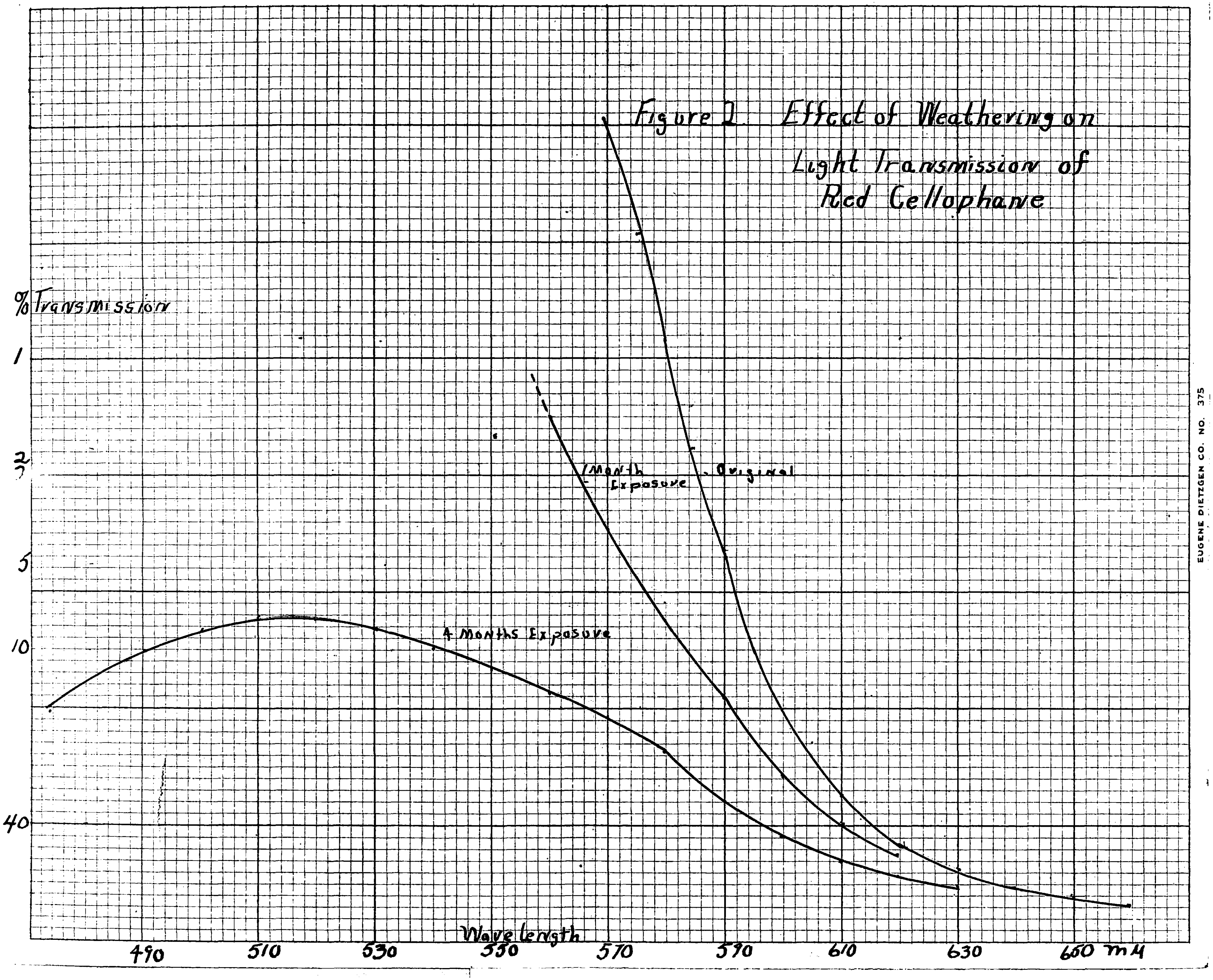


Figure 2 Effect of Weathering on
Light Transmission of
Red Cellophane
% Transmission
1
2
5
10
40
1 Month Exposure
Original
4 Months Exposure
Wave length
490
510
530
550
570
590
610
630
650 mμ
EUGENE DIETZGEN CO. NO. 375

light rays shorter than five-hundred milli-microns. The red cellophane exhibited zero per cent transmission of visible rays shorter than five-hundred and ninety millimicrons. Since red bags do not transmit the blue end of the visible spectrum, and since these bags inhibited red color formation on apples, it is evident that the rays in the blue end of the visible spectrum are the most active in production of red color.

It was also observed in 1928 that fruits which had remained under the red colored bags longer than one month, began to develop color. Spectrophotometric examination of all bags showed that the original red cellophane in double thickness absorbs practically all light rays in the visible spectrum up to 581 milli-microns, which include the blue end of the spectrum, but after more than one months exposure absorption became considerably affected, the red cellophane transmitting ten per cent of the light in the blue end of the spectrum. Figure 2 shows the effect of weathering on light transmission of red cellophane bags.

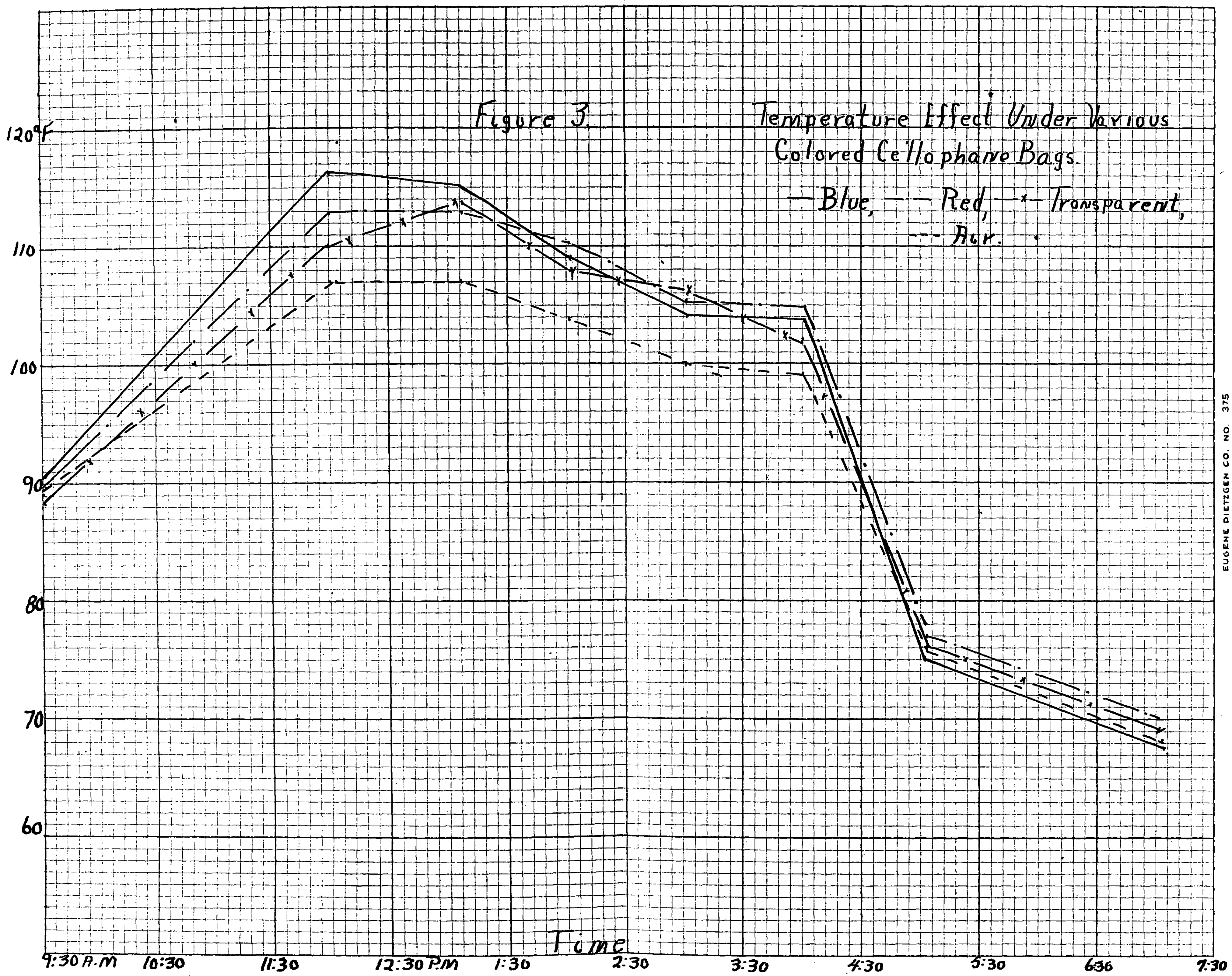
In 1929 only red bags were used. Beginning June 10th, one-hundred fruits each of the Williams and Rome variety were bagged at monthly intervals. Fruits were chosen which had the same exposure and the same leaf area. In all cases fruit was used for the bagging work which had no color development. A different tree was used for each bagging series. At the time of the subsequent bagging from ten to twenty fruits of the previously bagged series were exposed and a daily record made of the rate of color formation. The bags were renewed at monthly intervals.

Williams: With the Williams variety, which ripened approximately July 22nd, only one series was bagged (June 10th). On July 10th bags were removed from twenty fruits. There was no color formation on the bagged fruits while the checks showed a color range from twenty-five to thirty-five per cent good color. Ten of these unbagged fruits, detached from the tree and exposed to the full sunlight for three days, showed only a trace of color. On July 13th, three days after removal of bags, fruits previously under bags, which had not been detached from the tree, showed a color development from twenty-five to thirty-five per cent, and of a better quality than the checks. Six days after bag removal the fruit, previously under bags, left on the tree showed a color development equal to the check in area and had a brighter appearance, while the detached fruit, previously under bags, showed only a slight streaking of color, and bad shrivelling. The remaining fruits under bags were not exposed to full light, but were picked and stored for studies dealing with effect of exposure to ultra violet light on red color development.

Rome: With the Rome variety, four series were bagged, June, July, August, and September. Fruits which were bagged in June and exposed to the full sunlight one month later, but not detached, showed in eight days after bag removal streaks of red color which was equal to the area and percentage of red color on the check.

On August 10th, ten fruits from the June 10th bagging

Figure 3.



Temperature Effect Under Various
Colored Cellophane Bags.
— Blue, — — Red, —x— Transparent,
- - - Air.
120°F
110
100
90
80
70
60
9:30 A.M
10:30
11:30
12:30 P.M
1:30
2:30
3:30
4:30
5:30
6:36
7:30
Time
EUGENE DIETZGEN CO. NO. 375

and July 10th bagging series were exposed to the full sunlight. On August 13th, three days after bag removal the fruits of the June series showed only a streaking of color, while the fruits of the July series showed a color development of from ten to twenty per cent which was equal to that of the check.

On Setptember 10th, ten fruits of the June 10th bagging, July 10th bagging, and August 10th bagging were exposed to the full sunlight. On September 16th it was observed that the fruit of the June series showed only a trace of color development. On the same date fruit of the July series showed a color development of about 55 per cent which was superior to the check. The rate of color formation was more rapid on the August series than on the June series, giving a color development in two days which was equal to that of the check. The rate of color formation on the August series was also more rapid than on the July series. The remainder of the fruit was picked at harvest time and held in cold storage for studies with ultra-violet light.

The use of various colored cellophane bags has shown that the effective range of the spectrum for red color of apples to be the blue end. In all cases where red bags were used there was no sign of red color development. Since the Williams variety was used it is apparent that the results secured were not the same as those found by Overholser (30). The latter writer does not mention the renewal of bags and it is common knowledge that cloth bags do undergo a change during weathering. It is possible that the results observed by Overholser were due to the weathering effect as observed in this work; that bags which have been exposed to weather lose their power of exclusion of certain light rays.

A graph, Figure 3, is presented showing the temperature resulting from the use of the various colored bags. The graph shows that there is a temperature increase from two to ten degrees Fahrenheit where the bags were used. This increase in temperature might explain a partial effect of bags on color formation in that respiration with a consequent utilization of carbohydrates would be increased under high temperatures, as shown by the work of Bushnell (6a) on potatoes. Such an increase must be a negligible factor, however, when it is stated that no effect on color was noted with the transparent bags, yet these bags gave a temperature effect which approximates very closely that of the red cellophane bags.

In summation of the effects of various colored cellophane bags on red color development it might be stated that since the red colored cellophane bags inhibited red color formation, and since spectrophotometric examination showed that these bags eliminated the blue end of the visible spectrum, it would appear that the blue end of the spectrum is necessary for red color development in apples.

Effect of Ultra-Violet Light

While the blue end of the visible spectrum was shown to be excluded by red cellophane bags, under which apple color was inhibited, yet it was not shown whether the rays of the blue end of the visible spectrum or the ultra-violet rays were

TABLE XVIII (a)

EFFECT OF TREATMENT WITH ULTRA-VIOLET LIGHT ON RED COLOR DEVELOPMENT ON APPLES. HANCOCK, MARYLAND. 1929

Exposed August 5, 1929

Variety	Maturity	Treatment before 10 min. Ultra-Violet Exposure	Length of Ultra-Violet Exposure	Treatment after Ultra-Violet Exposure	Effect of Ultra-Violet Exposure on Color.
Williams	Over-ripe (1)	Bagged in Red Cellophane 2 months. Stored in cool cellar 2 weeks.	10 minutes	In manila bags in cool cellar.	No color development in 3 weeks.
Williams	Over-ripe	"	10 minutes	Exposed to sunlight	Streaking of color in 3 weeks
Wealthy	Mature (2)	Bagged in red cellophane 1 month	10 minutes	In manila bags in cool cellar.	no color development in 3 weeks.
Wealthy	Mature	"	10 minutes	Exposed to Sunlight	70% color in seven days.
Wealthy	Mature	Normal	10 minutes	In manila bags in cool cellar.	No color development in 3 weeks
Wealthy	Mature	"	10 minutes	Exposed to sunlight	50% color in 13 days.
Wealthy	Mature	"	0	Exposed to sunlight	50 % color in 24 days.
Rome	Immature (3)	"	10 minutes	In manila bags in cool cellar	No color development in 3 weeks
Rome	Immature	"	10 minutes	Exposed to sunlight	Slight streaking in 13 days.
Rome	Immature	"	0	Exposed to sunlight	Slight bronze color in 25 days
York Imperial	Immature	"	10 minutes	In manila bags in cool cellar.	No color development in 3 weeks
York Imperial	Immature	"	10 minutes	Exposed to sunlight	Slight streaking of color in20 da
York Imperial	Immature	"	0	Exposed to sunlight	Slight bronze color in 1 month

(1) "Overripe" signifies fruit was past prime condition, tending toward mealiness
(2) "Mature" signifies fruit was in "picking ripe" stage of maturity.
(3) "Immature" signifies fruit was picked two months prior to usual harvest date.

effective agents in red color development of apples. Hence, it was important to ascertain if ultra-violet light was important for color formation, and since a source of ultra-violet light is readily obtained, direct exposure of detached apples to ultra-violet light seemed to be a means of study. Some experiments with ultra-violet light were carried out on detached fruit from the Rome, York Imperial, Wealthy, and Williams varieties in different stages of maturity.

The work reported in this paper on ultra-violet light is preliminary. It shows that certain responses may be had by treating fruits of different maturities with ultra-violet light.

Immature fruits having no red color of the York Imperial and Rome varieties, mature fruits of the Wealthy variety, and over-ripe fruits of the Williams variety were exposed to ultra-violet light for ten minutes. After treatment with ultra-violet light the fruits of each variety were divided into two lots, one lot being exposed to the sun, and the second lot held in a darkened, cool cellar. Notes were taken on the color development in the two lots daily.

Table XVIII (a) shows the effect on color development on apples of treatments with ultra-violet light. A study of this Table shows that the stage of maturity of apples is an important factor in the production of red color when fruits are subjected to ultra-violet light and then exposed to the sun. The over ripe fruit of the Williams variety did not develop color characteristic of the variety when it was subjected to ultra violet light. The results with the Wealthy variety are of interest. The fruit which had been under red cellophane bags for one month and then subjected to ultra-violet light developed color much more rapidly than that fruit which had not been bagged. The immature fruits of the Rome and York Imperial variety which were treated, colored more rapidly than fruits which were not treated. Magness (23) observed that fruits subjected to ultra-violet would color more rapidly than fruits which were not exposed. Fruit of all varieties held in the cool cellar did not color.

One lot of twenty fruits of the Rome variety, each from the series which had been bagged at monthly intervals, was subjected to ultra-violet light. The fruit was not colored and had been held in cold storage for three months. Daily exposures to ultra-violet light for fifteen minutes were made for ten days. There was no trace of color development in one month, but there seemed to be a slight loss in chlorophyll content of those fruits which were treated.

These preliminary studies show that immature fruits will respond only slightly to exposure to ultra-violet light. Uncolored, mature fruit which had previously been bagged in red cellophane for one month,responded readily to ultra-violet treatments. Uncolored mature fruits,which had not been bagged, while not responding in color development so rapidly, as the bagged fruit, did increase in color much more rapidly than untreated fruits. The difference in color development being eleven days in favor of the treated fruits. Exposure to ultra-violet light was without effect on uncolored, over-ripe fruits, and on uncolored fruits which had been in cold storage for three months.

It is possible that the amount of carbohydrates in the fruit at the time of exposure to ultra-violet light is a factor in the amount of color which will result from such treatment. The immature fruits and the over-ripe fruits were undoubtedly much lower in carbohydrates than the mature fruits. There are other constituents within the fruit, no doubt, which are likewise important in the production of color, for example, flavonols and chlorophyll. Until more knowledge is gained relative to the amounts of these other substances during the life history of a fruit, it would be enitrely speculative as to how ultra-violet light -- exposures at different stages of maturity -- might affect such substances in the production of red color.

The fact that uncolored fruits when exposed ultra-violet and not placed in the sun, did not color, while those exposed to ultra-violet and placed in the sun did color at a more rapid rate than fruit which was not subjected to ultra-violet light, is interesting, The fact suggests that in the case under discussion, exposure to ultra-violet light acts as a stimulus in the production of anthocyanin development.

RESULTS OF CHEMICAL STUDIES.

In this work an attempt was made to correlate chemical composition with increase in color formation as brought about by the various treatments. Samples for analysis were taken only from those treatments which showed striking results. A list of such treatments includes: (1) applications of sugar to the soil, (2) irrigation, and (3) the effect of red colored

TABLE XIX

THE EFFECT OF ADDITION OF SUGAR TO THE SOIL ON THE COMPOSITION OF THE FLESH FROM THE GREEN AND RED PORTION OF THE WILLIAMS VARIETY OF APPLES.

(Figures Based on Dry Weight)

Date Sampled.	Treatment.	Area	% Dry Matter	Reducing Substances. % Dry Weight	Reducing Substances. % Green Weight.	Total Sugars. % Dry Weight	Total Sugars. % Green Weight.	Acid Hydrolyzables. % Dry Weight	Acid Hydrolyzables. % Green Weight.	Starch % Dry Weight	Total Carbohydrates. % Dry Weight	Total Carbohydrates. % Green Weight	Total Nitrogen. % Dry Matter.
7/23/29	5# Nitrate Spring plus 3# Nitrate Fall.	Red	14.4	38.2	5.5	58.8	8.5	11.4	1.6	8.4	70.2	10.1	.37
		Green	13.9	37.6	5.2	59.4	8.3	12.1	1.6	7.5	71.5	9.9	.40
	3# Nitrate Fall	Red	15.8	30.6	5.1	50.0	8.4	17.7	2.9	10.8	67.7	11.3	.31
		Green	15.6	34.0	5.3	55.6	8.7	19.3	3.0	13.4	74.9	11.7	.30
	5# Nitrate Spring 3# Nitrate Fall plus 20# Cerelose 6/10 and 7/10	Red	16.1	41.5	6.7	63.1	10.2	13.1	2.1	12.9	76.2	12.3	.31
		Green	15.7	39.5	6.3	54.6	9.8	12.0	1.8	11.2	66.6	11.6	.32
	3# Nitrate Fall plus 20# Cerelose 6/10 and 7/20	Red	17.6	37.7	6.6	53.4	9.4	17.0	3.0	10.4	70.4	12.4	.31
		Green	16.0	35.5	5.7	52.4	8.4	16.4	2.6	12.3	68.8	11.0	.31
	5# Nitrate Spring 3# Nitrate Fall 10# Cerelose 6/10 and 7/20	Red	16.2	36.5	5.9	57.7	9.4	15.6	2.4	9.5	73.3	11.8	.27
		Green	15.9	34.0	5.4	59.0	9.3	15.2	2.4	8.1	74.2	11.7	.29
	3# Nitrate Fall plus 10# Cerelose 6/10 and 7/20	Red	15.6	37.6	5.9	58.0	9.1	13.4	2.1	10.2	71.4	15.5	.30
		Green	14.7	40.1	5.9	56.6	8.3	12.4	1.8	9.7	69.0	10.1	.29

cellophane bags. A list of the varieties used for analytical samples includes York Imperial Rome, and Williams. The results of the chemical analyses for such treatments as listed above are presented in the following tables.

Effect of Additional of Sugar to the Soil on Composition: Table XIX. shows the effect of nitrogen application plus "Cerelose" application on the composition of the Williams variety. From this Table it is evident that there is a lower percentage of dry matter in the flesh of the green side of an apple. With the exception of one case there is a higher percentage of dry matter in the flesh of the fruit which had not received sodium nitrate in the spring.

The reducing substances calculated on a dry weight basis are very interesting and significant. The first pertinent fact is that in four cases out of six there is a greater per cent of reducing substances in the flesh from the red side of an apple of the Williams variety. Harley (13) found the same relations to exist. A casual relationship which may exist between high sugar content and anthocyanin development is hard to establish in that both products are to a large extent influenced by the same factors. The application of sugar to the soil resulted in an increase in the reducing substances in the flesh of apples of the Williams variety. Although two applications of sugar were made as has been mentioned, it is questionalbe if the final application was dissolved sufficiently to go in the soil. The application of twenty pounds of "Cerelose" to trees which had received nitrogen in the spring resulted in a noticeable increase in percentage of reducing substances. In a comparison of the figures given in Table XIX. it is essential that the fact that spring applications of nitrogen in itself was instrumental in causing an increase in reducing substances in the flesh of fruits of the Williams variety be kept in mind.

Relative to the effects of treatments on the composition it may be observed that the application of nitrate in the spring has increased the reducing substances content in the flesh of the fruit. These figures are somewhat opposed to those of Archbold (1) who found that reducing substances were usually lower in those fruits grown on fertile soil than those grown on a more sandy soil.

In Maryland experiments the trees had received a fall application of nitrate of soda, then, providing that storage of nitrate was the same in both cases, there should be a like growth response in both cases the following spring. However, the second lot of trees received an application of sodium nitrate the following spring. Its growth potentialities were consequently increased. Then if growth were increased leaf area must have been increased. Also as shown by Schertz (41), application of nitrate of soda increases the amount of chlorophyll per unit weight of leaf. With increased leaf area and increased chlorophyll content in the leaves, it follows logically that there would be an increase in photosynthetic materials. It is assumed here that nitrogen did not cause such an increased growth that the elaborated foods were used up. Under the conditions mentioned by Archbold, there is not necessarily an excess of nitrogen, but there is sufficient nitrogen to cause

TABLE XX

THE EFFECT OF ADDITION OF SUGAR TO THE SOIL ON THE REDUCING SUGAR AND TOTAL CARBOHYDRATE FRACTION FROM THE FLESH OF APPLES OF THE WILLIAMS VARIETY. SEASON 1929.

(Figures Based on Dry Weight)

Sampled	Treatment.	Reducing Sugars		Total Carbohydrates.		Rank in Amount of Blush.
		Red	Green	Red	Green	
7/23/29	5# Nitrate Spring 3# Nitrate Fall	38.2	37.6	70.2	71.5	3
	3# Nitrate Fall	30.6	34.0	67.7	74.9	5
	5# Nitrate Spring 3# Nitrate Fall 20# Cerelose 6/10 and 7/10	41.5	39.5	76.2	66.6	2
	3# Nitrate Fall 20# Cerelose 6/10 and 7/10	37.7	35.5	70.4	68.8	4
	5# Nitrate Spring 3# Nitrate Fall 10# Cerelose 6/10 and 7/10	36.5	34.0	73.3	74.2	1
	3# Nitrate Fall 10# Cerelose 6/10 and 7/10					

a continued growth in either length or thickness, and consequently such growth would utilize carbohydrates and there would be a lessened storage of those materials in the fruit.

The total nitrogen figures are uniform throughout all treatments with the exception of the fruit from the spring nitrated trees. In this case the nitrogen percentage is much higher. The addition of sugar applied on June 10th to such trees resulted in a decrease in total nitrogen in the flesh of the fruits, while addition of sugars to trees which had not received nitrogen in the spring was without effect on the total nitrogen content of the fruit.

The percentage of total carbohydrates are higher in those fruits which had received an addition of sugar and highest for those fruits which had received nitrogen in the spring, together with an application of sugar on June 10th.

Table XX. gives the results obtained by the addition of sugar to the soil on the reducing substances and total carbohydrates in the flesh of apples of the Williams variety. This Table is a condensation of Table XIX. The column headed "Rank" is taken from Table V. and refers to the color rating on fruits from trees which had received sugar on June 10th and July 10th. While there is not a perfect correlation between the rank in color and carbohydrate content in the flesh of fruit, the figures tend to justify the conclusion that there is a relationship between carbohydrate content and red color on apples; these figures are in direct accord with the findings of Overley and Neller (29), and also of the findings of Magness (23).

TABLE XXI. THE EFFECT OF THE ADDITION OF SUGAR AND SODIUM NITRATE TO THE SOIL ON THE COMPOSITION OF THE FLESH OF THE YORK IMPERIAL VARIETY OF APPLES

(Figures based on Dry Weight)

Date Sampled	Treatment	Area	% Dry matter	% Tot. N.	Red Sugar	Tot. Sugar	Acid Hydroly-zables	Tot Carbohy-drates
9/24/29	10# Cerelose monthly Intervals, June, July: August, September	Red	17.7	.22	37.6	49.6	29.1	78.7
		Green	16.6	.21	36.6	49.6	25.3	74.9
	20# Cerelose monthly intervals, June, July: August, September.	Red	17.8	.28	44.2	53.2	31.0	48.0
		Green	17.8	.27	39.4	47.5	27.5	75.0
	Check	Red	16.6	.26	41.5	52.2	26.0	78.2
		Green	15.8	.23	40.0	50.6	26.3	76.9
	10# Cerelose once, August	Red	17.1	.27	42.7	53.8	23.3	77.1
		Green	15.1	.27	42.6	52.9	23.5	76.4
	20# Cerelose once, August	Red	16.6	.19	41.7	53.8	26.1	79.9
		Green	16.4	.18	37.1	48.5	22.7	71.2
	10# Cerelose once, September	Red	16.3	.31	39.9	55.6	23.1	78.7
		Green	15.0	.27	40.8	61.9	23.2	85.1
	20# Cerelose once, September	Red	16.8	.30	42.5	52.7	26.5	79.2
		Green	13.3	.37	45.2	64.8	28.0	92.8
	Check	Red	16.7	.24	38.5	51.0	27.3	78.3
		Green	16.4	.22	38.9	53.6	24.0	77.6
	5# Nitrate September	Red	17.0	.25	39.9	54.3	25.4	79.7
		Green	15.7	.25	40.7	55.0	26.2	81.2
	8# Nitrate September	Red	15.8	.21	43.5	51.8	24.7	76.5
		Green	15.0	.22	41.8	53.7	25.3	79.0

Table XXI. presents the results of application of sugar to the soil on the carbohydrate content in the flesh of apples of the York Imperial variety. The figures in Table XXI. show the same general trend as those given for the Williams variety. The percenta ge of dry matter is lowest in the checks. The trend in dry matter figures is an increase in percentage with the addition of sugar. Eight pounds of nitrate applied two weeks prior to sampling resulted in a decrease in the percentage of dry matter. The highest percentage of dry matter was in the fruits from those trees which had received ten and twenty pounds of sugar at monthly intervals beginning June 10th and ending September 10th.

The reducing substances show an increase when sugar is added to the soil. There is one exception to the statement that of the ten pound application of sugar at monthly intervals. The same exception is evident in the figures for the Williams variety where nitrate was added in the spring. A possible explanation of these results is that ten pounds of sugar added to the soil would increase the number of those organisms which function to produce nitrates in the soil; there would result a temporary decrease in nitrate nitrogen in the soil due to the use of nitrate for bacterial growth; later this increased number of bacteria, once the supply of sugar was exhausted, would result in an increased amount of nitrate nitrogen in the soil. Such changes in the nutrients supplied to the tree may result in a stimulation of growth of

TABLE XXII.

THE EFFECT OF ADDITION OF SUGAR AND SODIUM NITRATE TO THE SOIL ON THE REDUCING SUGAR AND TOTAL CARBOHYDRATE FRACTION FROM THE FLESH OF APPLES OF YORK IMPERIAL VARIETY. SEASON 1929.

(Figures Based on Dry Weight)

Sampled	Treatment.	Reducing Sugars		Total Carbohydrates	
		Red	Green	Red	Green
9/24/29	10# Cerelose Monthly Intervals. June, July, August, September.	37.6	36.6	78.7	74.9
	20# Cerelose monthly Intervals. June, July, August, September.	44.2	39.4	84.0	75.0
	Check.	41.5	40.0	78.2	76.9
	10# Cerelose August only.	42.7	42.6	77.1	74.6
	20# Cerelose August only.	41.7	37.1	79.9	71.2
	10# Cerelose September only	39.9	40.8	78.7	85.1
	20# Cerelose September only	42.5	45.2	79.2	92.8
	Check.	38.5	38.9	78.3	77.6
	5# Nitrate September	39.9	40.7	79.7	81.2
	8# Nitrate September.	43.5	41.8	76.5	79.0

TABLE XXIII. THE EFFECT OF ADDED WATER SUPPLY ON THE COMPOSITION OF THE FLESH OF YORK IMPERIAL APPLES. SEASON 1929.

(Figures Based on dry Weight)

Sampled	Treatment	Area	% Dry matter	% Tot. N.	Red Sugars	Tot. Sugars	Acid Hydrolzables.	Tot. Carbohydrates.
9/25/29	Irrigated twice weekly 24 hrs. each irrigation Beginning July 4.	Red	14.9	.16	46.8	56.1	25.9	81.0
		Blush	14.6	.16	41.7	58.9	27.4	86.3
	Check	Red	16.8	.21	42.4	51.4	26.5	77.9
		Blush	15.0	.25	40.3	50.9	26.8	77.7

the tree and consequent utilization of carbohydrates. The greatest increase in reducing substances is in the fruits from those trees which had received twenty pounds of "Cerelose" (sugar) at monthly intervals.

With two exceptions the percentage of total carbohydrates is higher in those fruits receiving an application of sugar, being highest where the higher amounts are used.

Table XXII. is a condensation of Table XXI. and shows the relationship existing between high carbohydrate content and color development. As with the Williams variety, it may be seen that high sugar content is directly correlated with high color development in the flesh of apples of York Imperial variety.

<u>Effect of Irrigation on Composition</u>: Table XXIII. presents figures showing the effect of irrigation on the composition of the flesh of fruits of the York Imperial variety.

The percentage of dry matter is higher in the non-irrigated fruits. The percentage of reducing substances is higher in the irrigated fruits. Total sugar percentages and total carbohydrate percentages are higher in the irrigated fruits. The total nitrogen percentages is less in the irrigated fruits. A comparison of these figures with the results of color records given in Table VII. will show that high carbohydrate content is correlated with high production of red color on apples. These results are in direct accord with the findings of Taylor and Downing (43).

Table XXIV. presents figures showing the effect of

TABLE XXIV. THE EFFECT OF ADDED WATER SUPPLY ON THE COMPOSITION OF THE FLESH OF ROME APPLES. SEASON 1929

(Figures Based on Dry Weight)

Sampled	Treatment	Area	% Dry matter	% Tot. N.	Reducing Sugars	Tot. Sugars	Acid Hydrol-zables	Tot. Carbohydrates
9/25/29	Irrigated twice weekly beginning July 4, 24 hrs. each irrigation.	Blushed	15.8	.14	41.1	61.7	11.8	73.5
		Green	15.5	.17	41.5	63.1	13.5	76.6
	Check	Blushed	17.0	.16	44.9	63.2	15.4	78.6
		Green	16.0	.16	47.5	64.7	15.6	80.3

TABLE XXV. THE EFFECT OF RED CELLOPHANE BAGS ON THE COMPOSITION OF THE FLESH OF ROME APPLES. SEASON 1929.

(Figures Based on dry Weight)

Sampled	Treatment	Area	% Dry matter	% Tot.N.	Reducing Sugars	Total Sugars	Acid Hydrolyzables	Total Carbohydrate
Sept.25 1929	Bagged 4 months	Green	15.1	.16	43.3	64.8	13.7	78.5
	Bagged 3 months	Green	15.7	.16	47.4	64.7	13.9	78.6
	Bagged 2 months	Green	15.6	.16	48.6	64.0	12.5	76.5
	Bagged 1 month	Green	15.3	.16	49.9	66.3	13.0	79.3
	Green fruit Not bagged	Green	13.4	.17	51.2	71.1	12.7	

irrigation on the composition of fruits of the Rome variety. It is evident that the percentage of dry matter is greater in the non-irrigated fruits. The percentage of total nitrogen is greater in the non-irrigated fruits. The percentage of reducing substances is lower in the irrigated fruits. A comparison of these figures with those of Table VII. showing the color records for this variety under irrigation will show that the differences in amount of color between the two treatments was not very significant, the quality of color, however was much better in the irrigated fruits.

The Effect of Bagging With Red Cellophane Bags on Composition: Table XXV. shows the figures for the effect of bagging with red cellophane bags on the composition of fruits of the Rome variety. It should be stated that only fruits without red color analyzed. The figures for percentage of dry matter shows that there is a decrease in dry matter as duration of bagging increases. The percentage of total nitrogen remains constant. The reducing sugars are interesting. There is a decrease in reducing sugars as duration of bagging increases. When this fact is connected with the statement made previously that color development was most rapid on those fruits which had been bagged for the shortest time, it is seen that there is a relationship between reducing substances and anthocyanin formation. The acid hydrolyzable substances tend to increase as duration of bagging increases.

In summing up the results of macrochemical investigations it is apparent that there is a correlation existing between high sugar content and good color development. Any conditions which favor the accumulation of reducing substances in the fruits favors the production of anthocyanin, and conversely any condition which inhibits the accumulation of reducing substances in the fruit hinders good color development. The application of sugar to the soil resulted in many cases in an increase in reducing substances in the flesh of the fruits. The manner in which such an increase takes place must at the present time be purely hypothetical.

The effect of irrigation on the composition of the flesh of fruits varies with the variety, likewise does the effect of irrigation on the red color development vary with variety. The York Imperial variety showed a significant increase in color where irrigation was applied to the soil which is correlated with an increase in carbohydrate content under irrigation. The Rome variety did not show such a great increase in color development under irrigation, and likewise there was no increase in carbohydrate content under irrigation. During the season it was observed that the Rome variety was constantly developing new leaves at each growing point. It is highly possible that the development of the new growth would use up the carbohydrates formed, allowing less storage of these materials in the fruits.

Bagging with red colored cellophane bags reduced the amount of reducing substance directly in proportion to the time which the fruits were under the bags. There was likewise an increase in acid hydrolyzable substances in those fruits which were bagged for the longest time.

RESULTS OF MICROCHEMICAL AND ANATOMICAL STUDIES

In 1929 an attempt was made to locate, if possible, any specific region of anthocyanin formation and to determine the general cellular structure of apple fruits in relation to anthoyanin formation. Aside from the work of Kraus (20) and Miss Black (3a)/little work has been published on the anatomical structure of apple fruits.

The present investigation are only preliminary, but some data were obtained which are of general interest. A list of the varieties studied includes, York Imperial, Grimes Golden, Williams, Rome, and Wealthy. Studies were made on the fruits of these varieties throughout the growing season. In making studies the same material was used for anatomical studies as was used for the microchemical studies. In making microchemical studies Miss Eckerson's outline was followed in detail. The results of such studies during the season of 1929 are given below.

Anatomical findings are discussed together with microchemical results.

Starch: The iodine-potassium-iodide test was used throughout the course of study for starch determinations. A thin section was made freehand and placed in water to wash the starch from any ruptured cells. The section was then placed on a slide in a drop of the reagent. The starch grains stained a blue-black, and were usually packed very tightly toward the center of the cell. Starch was more abundant in the outer three or four layers of cells of the flesh in early season, while later in the season it was more abundant near the vascular starch. Starch grains were found only occasionally in the pigment sheath. Starch is more abundant on the green side of an apple throughout the growing season. The microchemical findings on starch are in direct accord with the findings of Bigelow and Gore (3).

Nitrates: A very faint test resulted in young fruits with the di-phenylamine-sulphuric acid reagent. In older fruits no postive test for nitrates was exhibited. The tests for nitrates in the younger fruits was found rather closely related to the chlorophyll area.

Glucose and Fructose: A slight postive test was given with Flückigers regent showing the presence of small amounts of fructose. On warming the slide a very postive test was given showing the presence of large amounts of glucose. These sugars increased throughout the season and were usually more abundant just below the pigment sheath.

Pigment Sheath: All green fruits, young twigs, pedicel of fruit, and petiole of leaf contain a layer of cells, just beneath the epidermis, usually three or four cells deep which give a characteristic flavonol reaction with strong alkalies. This layer of cells has been designated as the pigment sheath. It is in this layer of cells that pigmentation occurs. The depth of pigmentation varies with varieties being from four to six cells deep in the York Imperial variety. The cells of this pigment sheath are rectangular and thick walled during the whole life of the fruit. The cell walls of the fruit flesh are thicker on the blushed side of an apple, thickness diminishing as the core line is approached.

Anthocyanins: The anthocyanins were found in the skins of fruits, the outer layer of cells on young twigs and in the outer layer of cells in pedicel of fruit and petiole of the leaf. In no case were crystals of anthocyanin noticed, furthermore, it was impossible to obtain crystals by the method of Molisch. In this method the sap is pressed from the pigmented area and treated with a drop of acetic acid and the acid allowed to evaporate in a moist atmosphere. A hypertonic solution of potassium nitrate caused slight plasmolysis of the cell sap without a lessening of the color on the cell walls, so evidently while the anthocyanins are in the cell sap, they are also impregnated in the cell wall. The anthocyanins may diffuse back into the flesh of an apple and in a few cases was found quite a distance in the fibro-vascular bundles. The anthocyanin gave a deeper red when treated with acid, a blue-black color when treated with ferric chloride, and a yellow color when treated with strong alkali.

Chlorophyll: Chlorophyll was readily observed in the sub-epiderman cells. It was never observed closer than three cells removed from the epidermis. Where blushed fruits were used only a small amount of chlorophyll could be observed back of the blushed area. In all tests on the blushed fruits a postive reaction was given with the ferrocyanide test for iron in the chloroplastds. This finding certainly does not agree with the general assumption that chlorophyll must disappear before anthocyanins appear.

RESULTS OF PIGMENT STUDIES

Although plant pigments are recognized as being a specific group of substances, yet there is no one qualitative test which, applied to an extract containing a pigment, will show the group of substances to which the pigment belongs. The reason for such a failure of the qualitative test is that in plant tissues there are many substances which may give a postive test with the same reagent. In view of this difficulty experiments were carried out to extract if possible a crude form of the anthocyanin pigment occurring in apples, and make such qualitative tests as necessary to locate the coloring matter in the proper groups.

Ten well colored fruits of the Stayman variety of apples were peeled with a mechanical peeler and the peel ground finely in a food chopper. The resulting mixture was then placed in a 500 cc. Erlenmeyer flask and 200 cc. of ninety-five per cent ethyl alcohol added, and refluxed for one hour. The brightly colored alcoholic extract was decanted into a large beaker, and another portion of alcohol added. After the second extraction there was no coloring matter left in the peels. The two alcoholic extracts were then combined and the alcohol evaporated off on a steam bath. The resulting thick syrup,which had a yellowish brown color, was filtered through a Buchner funnel using paper pulp. The filtrate was placed in a separatory funnel and washed with ether (ethyl) until the ether layer was no longer colored. After the first washing with ether the original extract was of a deep red color. After washing with ether the aqueous layer was drawn off and placed in a refirgerator at 3°C.

The ether washings, which were yellow in color, were combined and the ether driven off. The resulting yellowish amorphous material was tested for its solubility in various substances. The results are given below.

Ether layer evaporated to dryness.

Insoluble in:	Soluble in:
Water	Strong acid
Cold Ethyl alcohol	Strong alkalies
	Ether.

The following qualitative results were obtained from the ether washings:

1. Treated with lead acetate, a yellowish precipitate was formed.

2. Treated with concentrated sulphuric acid, there was an increase in the intensity of the yellow color.

3. Treated with concentrated sodium hydroxide there was an increase in the intensity of the yellow color which when treated with concentrated hydrochloric acid reverted back to its original yellow color.

4. Treated with active hydrogen (mercury added to concentrated hydrochloric acid) the yellow color assumed a pink to red color.

In that the above tests are characteristic of flavones it is evident that flavones occur in apple peels, and that such flavones upon reduction give rise to a red colored substance.

The aquaeous solution mentioned above was allowed to remain in the refrigerator for ten days, but no crystals appeared. It was removed from the refrigerator and dried on a steam bath until a thick paste resulted. This paste was tested for solubility in various reagents, and subjected to a number of qualitative tests. The results appear below.

Soluble in:	Insoluble in:
Cold water,	Ethyl ether
Hot water,	Petroleum ether,
Hot ethyl alcohol,	Amyl alcohol,
Slightly soluble in cold alcohol,	
Methyl alcohol,	
Dilute and Concentrated acids,	
Dilute and concentrated bases.	

An aqueous solution gave the following qualitative tests:

1. Treated with lead acetate a green fluorescent precipitate resulted.

2. Treated with sulphurous acid, a yellowish color resulted.

3. Treated with concentrated sulphuric acid, a deep black color resulted.

4. Treated with dilute sulphuric acid, there was an increase in depth of red color apparent.

5. Treated with concentrated sodium hydroxide, a green color was apparent.

6. Treated with ferric chloride, a dark brown color appeared.

7. A prolonged reduction with active hydrogen resulted in a yellowish liquid which reverted to its original color on standing exposed to the air.

The above qualitative and solubility tests indicate that the red coloring matter in apple peels is an anthocyanin.

A further test was made on this material by use of amyl alcohol to determine the presence of anchocyanidins, which are the non-glucosidal compound resulting from a hydrolysis of tthe anthocyanins. This test is based on the fact that anthocyanins are not soluble in amyl alcohol, while the anthocyainidins are soluble in this reagent. Ten cubic centimeters of the aqueous extract, which had been washed with ether, was pipetted into a test tube containing ten cubic centimeters of amyl alcohol. The tube was stoppered and shaken vigorously for a few minutes. After standing the amyl alcohol layer separated, and did not show a trace of coloring matter. Such a test shows that anthocyanidins are not present in the peel of apples. Another ten cubic centimeter portion of the aqueous extract was hydrolyzed with sulphuric acid and then treated with amyl alcohol. The amyl alcohol layer upon separation was of a brilliant red color, again showing that the red coloring matter of apple peels is an anthocyanin. Treating this red amyl alcohol layer with potassium permanganate, a pinkish red solution resembling that obtained upon reduction of the yellowish ether layer solution with active hydrogen resulted.

The reaction of the red coloring matter of apples to the usual solubility and reagent tests show that an alcoholic extract from red peels of apples contain flavons and anthocyanins.

DISCUSSION

The factors influencing red color development on apples can be grouped under light and nutrition. Light as a factor may be assumed to influence red color development by direct action of light, possibly by aiding in the production of anthocyanins from flavonols; and, indirectly through increasing the photosynthetic activity of leaves resulting in a higher carbohydrate supply for the fruit. Nutritional conditions of the tree and fruit which influence color development are affected largely through an increase or decrease in leaf area, or an increase or decrease in water and other substances essential for color development. It is impossible to say whether the direct effect of light is more important than the nutritional condition of the tree, although color will develop under a wider range of nutritive conditions than light conditions.

That the nutritive factors may be influenced to increase or hinder color development is shown by the experiments on the addition of materials to the soil including water and the injection of materials into the tree as well as leaf area and thinning studies.

The nutritive condition of the tree is subject in a large degree to the application of certain fertilizers to the soil in which the tree is growing. Under general orchard conditions in the United States the use of nitrogen fertilizers is most likely to influence the nutritive conditions of the tree and hence influence color adversely or otherwise. Nitrogen added to the soil often results in an increased set of fruit which would indirectly influence color by a restriction of the elaborated food supply per fruit as a consequence of too few leaves per fruit. Nitrates also cause an increase in growth which may influence color by using the carbohydrates that are essential to color, or by causing such a growth of foliage that the fruit will be shaded and hence poorly colored. On the other hand, there is also a possibility that nitrogen applications may actually increase the photosynthetic activity of the leaves which in turn would be reflected in an increased color development. Successful growers using large amounts of nitrogen in their orchards have reported that with proper pruning, thinning, and soil management, the color of apples has actually increased in years following the application of nitrogen.

The use of phosphorus, potassium, lime, sulfur or other elements essential for plant growth which do not result in a response in growth of the tree or fruit, apparently are without effect on red color development on apples. The use of fertilizers other than nitrogen which increases the growth of cover crops in orchards, may thus indirectly affect red color through the improvement in the water holding capacity of the soil.

While nitrates applied to the soil usually result in an increase growth at the expense of stored carbohydrates, such a growth will result in an increase in leaf area. It is probable that this increase in foliage caused by application of nitrate may be used to advantage through a timely application of certain materials to the soil which in themselves will cause an increase in carbohydrates in the tree and fruit. It will be recalled that defoliation and girdling experiments show that the red color on fruits is closely related to leaf areas. After a large leaf area is once established, if the addition of some form of carbohydrates, such as sugar, is made to the soil, there apparently results a lessening of nitrification in the soil, an increase in carbohydrates in the fruit, and a better color development of the fruit. If this application of sugar is made about two weeks before harvest, the maximum benefits on red color is realized.

A possible modification of the nutrition of the tree seemed to result through artificial injections of materials into the tree. The nature of the changes caused in the tree or fruit is conjectural. In the case of hydrochloric acid injected into the tree there occurred an increase in color which may have been brought about by the resulting defoliation, allowing the light factor to come into play. A second possibility would be through a direct increase in carbohydrates in the fruit resulting from a hydrolysis of starch or some other storage material within the limb into which hydrochloric acid was injected. Sodium nitrate injected into the tree caused a decided change in the metabolism of the limb into which it was injected. As a result of such a metabolic change a greener ground color developed possibly through an actual increase in chlorophyll content of the fruit.

Water seems to be the substance which is most likely to be an important limiting factor in the production of maximum red color. The role of this substance in red color development is probably indirect through its effect on the photosynthetic activity of the leaves or in maintaining the fruits in a normal state of turgor. The response of trees to irrigation in producing highly colored fruit was evidenced in all varieties used. The quantity of color was not always increased, but in every case the quality of color was increased. The increased color was associated with increased content of reducing substances.

Since high carbohydrate content of fruit has been shown to be closely related to color development it is apparent that any factor which increases the carbohydrate content in the fruit, will give an increase in color. Conversely, any factor which causes a decrease in carbohydrates will cause a decrease in color. A heavy set of fruit limiting the number of leaves below a certain minimum per fruit will certainly result in poorly colored fruit. A certain minimum number of leaves which varies with varieties seems to be necessary for the production of good commercial color. Thinning of fruits on trees which are bearing heavy crops is one method by which leaf area per fruit may be increased.

Any study of light as a factor in the production of red color on apples must take into consideration the duration, the quality, and the intensity of the light. It was mentioned before that light may act either directly or indirectly on the production of color on apples. The direct influence of light on color development is probably through a stimulation.in the production of anthocyanins from flavonals. It was shown that exclusion of the blue end of the spectrum which eliminated the rays which are probably responsible for the direct effect of light. However the elimination of the blue end of the spectrum resulted also in a decrease in reducing substances together with an increase in starch in fruits. That such a decrease in reducing substances was instrumental in affecting red color development is shown by the fact that fruits from which the blue end of the spectrum was cut out for the greatest length of time, was the slowest in producing a red color. Here again is direct evidence that red color development and high sugar content are very closely related. A further limitation of the study of the blue end of the spectrum to ultra-violet light shows that ultra-violet light may play some part in relation to red color development possibly acting as a stimulus in production of red color.

The duration and intensity of light no doubt act indirectly on the production of red color on fruits through the increase or decrease in carbohydrate manufacture. In regions where many days of intense and continuous sunlight occur prior to harvest as is the case in the western portion of the United States, apples normally develop a deeper red color than in such regions where the sunlight is less intense and broken by cloudy skies. The production of red color on apples grown at high altitudes is generally assumed to be due to a greater duration of sunlight with the maximum of ultra-violet light.

The production of a heavy foliage by the use of nitrate fertilizers will result in diminuition in the quality and intensity of sunlight reaching the fruit. Obviously the grower has a means of aiding in theproduction of red color through proper pruning. As was shown in those experiments in which all leaves were removed from the tree late in the season with a consequent development of red color, it is possible that a complete or partial defoliation by chemical means, a few days prior to harvest, may prove practicable on winter varieties in assisting in the production of red color even in a region of a lower quantity and intensity of light. A grower in such a region may exact from such light the maximum quantity available for the production of red color.

These studies on the factors influencing red color on apples, while far from being complete, have furnished some information which will aid in analyzing the color problem for the grower and have furnished some leads for future work on this problem. More studies should be made relative to the role of leaf area, water, nitrogen, carbohydrates, and flavonols as well as studies of the chemical nature of the pigment of apples, growth rate of fruit in relation to color, and respiration of developing fruits.

GENERAL CONCLUSIONS

The practical solution of the problem in the production of red color on apples apparently lies in the proper regulation of the nutritive and light factor by specific orchard practices.

Red color on apples apparently will develop under a fairly wide range of nutritive conditions. Maximum color, however is brought about by an abundant supply of soluble carbohydrate and water per fruit. The leaf area per fruit and maintenance of activity ~~of activity~~ of leaves are also important factors to be considered. In attempting to increase the yield of fruit by the use of nitrate fertilizers, attention should also be given to increasing the moisture supply to the fruits as well as increasing the leaf area per fruit. To increase the moisture supply a grower may resort to irrigation, proper tillage practices, thinning, detailed pruning, or use of a mulch. The problem of increasing leaf supply with increased yield may be accomplished by judicious thinning.

The varietal requirements relative to leaf area is an important factor for the grower to consider in his thinning practices. It has been shown in the present work and in the work of Magness (23) that varieties differ with respect to the number of leaves required for best quality and maximum color development. Those varieties which are terminal bearers such as Rome and McIntosh, require a lesser number of leaves per fruit than do spur bearers such as the York Imperial. Proper spraying to prevent loss of leaf area by diseases and proper pruning to admit light to fruit and leaves, are necessary before a grower can expect good color development.

Apparently the exposure of fruit to light is one of the most important factors in so far as color development is concerned. The shading of fruit by too dense foliage as a result of improper pruning, or close planting, is a big reason commercially for failure in the production of red color. Obviously then pruning and thinning are important procedures in orchard practices from the standpoint of admitting light to the fruit. While it is impossible for the grower to increase the amount of sunlight reaching a tree, he can, by judicious pruning and thinning, take advantage of all such light as does reach the tree.

In summation it is probable that the quantity of light and the proper nutritive condition of the tree required for good red color development of apples can be obtained if all orchard operations such as pruning, thinning, fertilization, spraying, and soil management are properly performed.

SUMMARY

1. Experiments to determine the factors influencing the red color on apples were conducted in a large bearing apple orchard near Hancock, Maryland. The orchard was about twenty-five years old, growing in sod andreceiving yearly applications of nitrates, and was in vigorous and fruitful conditions.

2. The use of such fertilizers as potassium sulfate, potassium chloride, and acid phosphate, in combination or alone was without effect on red color development on apples under the conditions of these experiments.

3. Soil applications of such chemicals as citric acid, ferric sulfate, ferric carbonate, manganese oxide, aluminum sulfate, magnesium sulfate, lime and sulfur were without effect on red color development.

4. Addition of sugar to the soil two weeks prior to picking increased the carbohydrate content of the fruit, and also increased red color development.

5. The addition of water to the soil during a dry year, was instrumental in increasing the red color development as well as quality of color on fruits. Shape and quality of fruits were also influenced by such treatments.

6. The injection of sugar solutions, hydrochloric acid, and boric acid into a limb resulted in slight defoliation and an increase in red color of fruits. The injection of ferric sulfate, potassium nitrate, sodium nitrate, and malic acid into a limb resulted in a decrease in color formation.

7. The use of red cellophane bags which do not transmit the blue end of the spectrum inhibited red color development on apples.

8. Ultra-violet light stimulated color production in fruits which were not over-ripe.

9. Leaf area is directly correlated with the production of red color on apples. The removal of leaves in early season inhibits color formation, while the removal of leaves in the late season increases color formation in apples.

10. Ethylene gas, light being excluded, caused a slight reduction in chlorophyll, but no increase in anthocyanin formation.

11. There is a layer of cells just beneath the epidermis, termed "pigment sheath" in this paper, which gives a characteristic flavonol test, and it is in this layer of cells that pigmentation occurs.

12. Chlorophyll does not always disappear before the formation of anthocyanins. The chlorophyll present in apples is not present in the pigment sheath, but in cells just below this sheath.

13. High sugar content is related to high color in apples.

14. Preliminary qualitative tests indicate that there are flavone and anthocyanins, but no anthocyanidins in the peels of red apples.

15. Better colored fruits usually result when all orchard operations such as pollination, thinning, pruning, fertilizer practices, and soil management are correctly done.

LITERATURE CITED

1. Archbold, H. K. Chemical studies in the physiology of apples. Ann. Bot. 39: 132-143. 1925.

2. Auchter, E. C. Five years investigations in apple thinning. W. Va. Agric. Exp. Sta. Bul. 162. 1917.

2a. Aldrich, W. W. Unpublished data. Department of Horticulture, University of Maryland. 1930.

3. Bigelow, W. D. and H. C. Gore. Studies on apples. U.S.D.A. Bur. of Chem. Bul. 94. 1905.

3a. Black, C. A. The nature of inflorescence and fruit of Pyrus Malus. Mem. N. Y. Bot. Gard. 6. 519. 1916.

4. Bonnier, G. De la Variation avec l'altitude des matierer colores des fleurs chez une memeespece vegetale. Bul. Soc. Bot. 27: 1880.

5. Boyle, R. Experiments and considerations touching colours. London. 1664.

6. Buscaloni, L e G. Pollacci. Le antocianine e il loro significato biologico nelle piente. Milan. 1903.

6a. Bushnell, J. The relation of temperature to growth and respiration in the potato plant. Minn. Agric. Exp. Sta. Tech. Bul. 34. 1925.

7. Combes, R. Production d'anthocyane sous l'influenc de la decortication annulare. Bul. Soc. Bot. 56 Paris. 1909.

8. Connors, C. H. Lime studies with hydrangea. Proc. Amer. Soc. Hort. Sci. 18: 78-82. 1921.

9. Everest, A. E. and R. W. Perkins. The natural organic coloring matter. Longmans, London. 1918.

10. Goff, E. S. The application of artificial root pressure on recently transplanted trees. Ann. Rept. Wisc. Agric. Exp. Sta. 14: 23-30. 1903.

11. Grew, N. The anatomy of plants with an idea of a philosophical history of plants, and several other lectures. Read before the Royal Society, London, 1684.

12. Grubb, N. H. The effect of potash on apple trees. Ann. Rept. East Malling Res. Sta. 16: 70-73. 1928.

13. Harley, C. P. Personal correspondence. 1928.

14. Harvey, R. B. and L. O. Regeimbal. Carbohydrate changes in celery under ethylene treatments. Proc. Amer. Soc. Adv. Sci. 80: 73-78. 1926

15. Hedrick, U. P. The effect of wood ashes and acid phosphate on yield and color of apples. N. Y. (Geneva) Agric. Exp. Sta. Bul. 289. 1907.

15a. Heinicke, A. J. Influence of scion leaves on the quality of apples borne by the stock. Proc. Amer. Soc. Hort. Sci. 24: 143-146. 1927.

16. Hibbard, R. P. The physiological effect of ethylene gas upon celery, tomatoes, and certain fruits. Mich. Agric. Exp. Sta. Res. Bul. 104. 1930.

17. Jones, J. S. and C. W. Colver. The composition of irrigated and non-irrigated fruits. Idaho Agric. Exp. Sta. Bul. 75. 1918.

18. Kerr, W. L. Unpublished data, Department of Horticulture, University of Maryland. 1927.

19. Knudsen, Lewis Influence of certain carbohydrates on green plants. Cornell Agric. Exp. Sta. Memoir 154. 1918.

20. Kraus, E. J. Variation of internal structure of apple varieties. Ore. Agric. Exp. Sta. Bul. 154. 1918.

21. Lewis, C. I. et al. Orchard irrigation studies in the Rogue River Valley. Ore. Agric. Exp. Sta. Bul. 113. 1912.

22. Magness, J. R. Relation of leaf area to size and quality in apples. Proc. Amer. Soc. Hort. Sci. 25: 98-102. 1928.

23. Magness, J. R. Observation on color development in apples. Proc. Amer. Soc. Hort. Sci. 25: 106-112. 1928.

24. Magness, J. R. and H. C. Diehl. Physiological studies on apples in storage. J.A.R. 29, 321-346. 1924.

24a. Magness, J. R. and M. H. Haller. Relation of leaf area to growth and composition of apples. Proc. Amer. Soc. Hort. Sci. 22: 1925

25. Mirande, M. Influence de la lumiere sur la formation de l' anthocyane des eçailles des bulbes de Lis. Compt. Rend. Acad. Sci. 175: 189-195. 1922.

26. Molisch, H. Ueber amorphes und Kristallisiertes anthokyan. Bot. Zeit. Leipzig. 63:

27. Müller, Thurgan, H. Uber den Einfluss der Berlaubrung auf das Reifen der Trauben. Wanbau-congress Zu Durkheim. A.D.H. 1882.

28. Neller, J. R. and F. L. Overly. Physical and chemical charactèristics of maturing apples as related to time of harvest. Wash. Agric. Exp. Sta. Bul. 205. 1926.

29. Onslow, Muriel Wheldale The anthocyanin pigments in plants. Cambridge University Press. 1925.

30. Overholser, E. L. Color development and maturity of a few fruits as affected by light exclusion. Proc. Amer. Soc. Hort. Sci. 14: 121-132. 1917.

32. Overton, E. Experiments on the autumn coloring of plants. Nature 59: 21-36. 1899.

33. Rumbold, Caroline, Effects on chestnuts of substances injected into their trunk. Am. Jour. Bot. 7: 21-62. 1920.

34. Russell, E. J. Plant nutrition and crop production. Hitchcock Lecturer for 1924. University of California Press.

34a. Sachs, J. Wirkung des Lichts auf die Blutenbildung unter Vermittlung der Laubblatter. Bot. Ztg. Leipzig 23: 117-139. 1865.

35. Schrader, A. L. and E. C. Auchter. The comparative effects of different fertilizers on bearing apple trees low in vigor. Proc. Amer. Soc. Hort. Sci. 25: 121-127. 1928.

36. Senebier, J. Physiologie Vegetale. Geneva. 1799.

37. Shaw, J. K. Climatic adaptation of apple varieties. Mass. Agric. Exp. Sta. Rept. 23: 1911.

38. Shaw, J. K. Experiments in soil management and fertilization of orchards. Mass. Agric. Exp. Sta. Bul. 209. 1922.

39. Sorauer, P. Handbuch der Pflauzenkrankherten. Band 1 Berlin. 1886.

40. Stewart, J. P. Orchard fertilization. Penn. Agric. Exp. Sta. Bul. 91. 1906.

41. Schertz, F. M. Effect of potassium, nitrogen and phosphorus fertilizers upon chlorophyll pigments. Plant Physiology 4: 1929.

42. Tukey, H. B. and L. C. Anderson. Five years results with fertilizers in three Hudson River Valley apple orchards. N. Y. (Geneva) Agric. Exp. Sta. Bul. 574. 1929.

43. Taylor, E. P. and G. J. Downing. Experiments in the irrigation of apple orchards. Idaho Agric. Exp. Sta. Bul. 99. 1917.

44. Waksman, S. A. The influence of available carbohydrates upon nitrification. Jour. Ind. Eng. Chem 4: 1912.

45. Work, Paul Ethylene ripening of tomatoes in relation to stage of maturity. Proc. Amer. Soc. Hort. Sci. 23:61-64. 1928.

46. Willstatter, R. and H. Mallison. Uber die Verwandtschaft der anthokyane und Flavone. Sitzber. Ak. Wiss., Berlin. 1914.

APPENDIX

MACROCHEMICAL METHODS

Sampling and Preservation. Samples of fruit from the various experimental plots were taken at commercial picking time during the early part of the day, and no greater number of samples were taken at one time than could be preserved in the following six hours. The sample was brought into the laboratory and ten apples of the average size and color were selected for preserving. The fruits were pared with a cannery paring knife so regulated to pare as thin and evenly as possible. The red and green parings were segregated.

After all ten fruits were pared 100 grams of the parings from the red side, and 100 grams of parings from the green side were placed in boiling ninety-five percent alcohol to which had been added .5 gram of calcium carbonate. The alcoholic volume was so adjusted that the final solution would be eighty percent alcohol by volume. The samples were boiled vigorously for fifteen minutes, sealed tightly, and stored in the dark until they could be analyzed.

The pared fruit was halved so as to include in each half a portion of the red and green side of the fruit. Each of these halves then had a slice about one-quarter of an inch thick removed from the cut face. This slice thus had flesh from the red side and the green side of the apple, and could be divided accordingly. The carpellary walls were removed and the remaining tissue was cut into very fine segments. One-hundred grams, constituting a segment from each of the ten apples, were preserved as stated for peels.

In this manner each field sample of ten apples was so divided as to give four samples for chemical analysis; a sample of the red skin, a sample of the green skin, a sample of the flesh from the red side, and a sample of the flesh from the green side.

Determination of Dry Matter: When the samples were to be analyzed they were removed from the dark and in the case of the skins, the alcohol was decanted with as little shaking as possible, into a liter Volumetric flask. After about seven-eiths of the storage alcohol was so decanted the remainder was filtered into the same Volumetric flask. The skins were then transferred to an evaporation dish and placed in an over at 70°C. for twenty-four hours. The preserving flask was washed several times with eighty per cent alcohol and the washings added to the Volumetric flask.

Thepurpose of decanting the major portion of the alcohol fraction of the skin samples was to prevent the filtering of waxes and fats found in peels. It was observed in previous work that when waxes and fats were filtered off and dried it was exceptionally hard to remove them from the filter, and when they were included in the solid material, they greatly hindered grinding by gumming in the mortar.

With the flesh samples the entire alcohol extract was filtered into a liter Volumetric flask, the flesh placed in an evaporating dish and the preserving flask washed several times with eighty per cent alcohol, the washings being added to the volumetric flask.

After the alcohol insoluble fraction had been in the oven for twenty-four hours it was transferred to a tared beaked and placed in the ovenfor another twenty-four hours. The evaporating dish was then washed with alcohol, the washing being added to the original alcohol in the Volumetric flask. The solution was then made up to volume and a suitable aliquot taken for the determination of dry weight of the alcohol fraction.

After the alcohol insoluble residue had been held in an oven at 70°C. for forty-eight hours it was placed in a dessicator to cool, weighed, and ground fine enough to pass through an eighty mesh screen. After grinding, the sample was placed in a sample bottle, returned to the oven at 70°C. for twelve hours, after which time a suitable aliquot was taken for the determination of the various substances.

Determination of Reducing Sugars: Since apple fruit tissue is relatively high in reducing sugars, two courses for the weighing out of an aliquot are open. One may weigh out a small aliquot, and make small dilutions of the extract or one may weigh out a large aliquot and make a large dilution. In the analyses reported in this paper an aliquot was weighed out which with proper and convenient dilutions would allow for the taking of at least a 10 cc. aliquot of the extract for reduction. The aliquot was placed in a paper extraction shell and another aliquot (for starch determinations) from the same sample was weighed out and placed in another shell. One of these shells was superimposed upon the other and the two placed in a Soxhlet extraction tube. An aliquot of the alcohol fraction equal to the sum of the two aliquots from the dried residue was then pipetted into a 250 cc. Erlenmeyer flask. A piece of pumice stone was added to the flask to prevent "bumping." The Soxhlet extraction tube containing the two shells was then fitted to the Erlenmeyer flask and the whole outfit fitted to a Hopkins condenser, placed on a sand bath and refluxed for three hours. The rate of refluxing was regulated so that the Soxhlet tubes would trip from four to six times per hour. Duplicate weighings and determinations were made on each sample.

After extraction was complete, the Soxhlet tube and extraction flask were removed from the sand bath. The Soxhlet tubes were allowed to drip for a few minutes into the extraction flask. The flasks were then placed on a hot sand bath, the temperature of which was maintained by a low flame, and the excess alcohol removed by blowing air into each flask from an air pump. The air was delivered to the flasks by means of copper tubing. This method of removal of alcohol has an advantage over the method of removal on a steam bath in three respects: it is less time consuming, does not require such careful attention, and the temperature during removal is around 47°C. as compared to from 95°C. to 98°C. with the removal by steam bath. It can easily be seen that where air is used to remove the alcohol there is no danger of carmelization of sugars due to high temperatures.

After the alcohol was driven off the samples were transferred with hot water to a 250 cc. Volumetric flask, cleared with lead acetate, made up to volume, and filtered. The excess lead was removed with potassium oxalate. After cleaning and deleading the samples were placed in an electric refrigerator at about 2°C. overnight, it having been observed that samples could be kept at this temperature for seventy-two hours without any change in reducing value. An aliquot then was pipetted off and the reducing value of the sample determined by use of the Munson-Walker modification of the Bertrand method, all values being calculated in terms of dextrose.

The entire procedure was standardized against a sample of glucose as secured from the U. S. Bureau of Standards. This method is to be preferred in that it not only standardizes the potassium permanganate, but it allows for any error which might arise from slight discrepancies in making up the ferric ammonium sulfate, copper sulfate, or sodium-potassium tartrate solutions. In other words the investigator standardizes his whole procedure rather than one solution in the procedure.

Determination of Total Sugars: Fifty cc. of the cleared extract from the reducing sugar sample was pipetted into a 200 cc flask, 5 cc of hydrochloric acid (specific gravity as tested by a pycnometer, of 1.125 to 1.130) added to the flask. The flask was then held at room temperature for twenty-four hours. The acid was neutralized with sodium hydroxide, made up to volume with distilled water and the reducing value of the sample determined. The values were calculated in terms of dextrose.

A point of interest in this determination is that when the aliquot from the cleared portion of the skin extract was treated with hydrochloric acid, a brilliant red color was apparent in the samples from red skins, but not in those from the green skins. This color did not disappear upon neutralization, but when an excess of alkali was added the color changed to a grayish black.

Determination of Acid Hydrolyzable Substances: The weighed aliquot from one of the Soxhlet extraction thimbles, which had been dried in an oven at 70°C after extraction, was placed in a 500 cc. Florence flask. One-hundred cc's of distilled water was added to the flask, together with 10 cc. of hydrochloric acid (specific gravity 1.125) and refluxed for two and one-half hours. The mixture was then cooled, neutralized with sodium hydroxide and transferred to a volumetric flask, made up to volume and filtered. An aliquot was taken and its reducing value in terms of dextrose determined.

Determination of Starch: The larger aliquot was removed from the extraction shell and placed in a 250 cc. beaker. Sufficient water was added to make a thick paste. Fifty cc. of boiling water was then added to the beaker and the contents held at the boiling point for one minute, after which it was transferred to a boiling water bath for one hour. After removal from the bath the material was cooled and 5 cc. of dilute saliva (1 part of saliva to 2 parts of water) was added. The material was then held at 50°C. for one hour and then transferred to a boiling water bath for fifteen minutes. A microscopic examination of the material was then made using a dilute solution of iodine in potassium iodide to test for starch. If any starch were present a second addition of saliva was made. Three digestions were found to be the maximum number of digestions necessary for the complete digestion of starch in apple tissue.

After digestion the sample was transferred to a 250 cc. volumetric flask, using seventy per cent alcohol to wash the beaker. After the transfer was complete, 170 cc. of ninety-five per cent alcohol was added to the flask. The sample was allowed to stand for a few minutes and then made up to volume using seventy per cent alcohol. After making up to volume, the sample was allowed to stand over night. It was apparent that such a procedure greatly aided in filtration. It was observed that if an alcoholic concentration less than seventy per cent was used there was an incomplete precipitation of Pectins, gums, etc. After filtration, an aliquot of the alcoholic portion was pipetted into an Erlenmeyer flask and the alcohol removed by air. Sufficient water was then added to make a volume of 100 cc. Ten cc. of hydrochloric acid (specific gravity 1.125) was then added and the mixture refluxed for two and one-half hours. After refluxing the material was cooled, neutralized, made up to volume and a suitable aliquot chosen for the determination of reducing sugars. All values were calculated in terms of dextrose.

Determination of Total Nitrogen: Total nitrogen was determined by the Kjeldahl-Gunning method modified to include nitrates.

MICROCHEMICAL METHODS

Throughout the growing season microchemical tests were made for such substances as glucose, fructose, starch, cellulose, nitrates, flavones, anthocyanins, and chlorophyll.

A fruit to be studied was brought to the laboratory and a thin section was taken from the area of the fruit upon which determinations were to be made. A very sharp razor was then used to cut free hand sections. Numerous sections were made and placed in distilled water. One of these sections was then placed upon the slide and the desired reagent added, and the section examined under a microscope. In the work carried out in this paper the microchemical outline which Miss Eckerson prepared for use in teaching at the University of Chicago was followed. A study of the changes of the various substances mentioned was carried out during the entire growing season of the fruit.

ANATOMICAL METHODS

The same free hand section as used in microchemical studies were used in the anatomical work. Usually anatomical studies under a microscope were made on the fresh tissue before any reagent was added to insure against a change in cell structure. No measurements were made on cell size and all results are observational.

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APPROVED E. C. Auchter

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