THE INFLUENCE OF SOME SPRAY MATERIALS ON THE CHLOROPHYLL CONTENT OF APPLE LEAVES

by

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INTRODUCTION

This investigation was undertaken to determine whether the chlorophyll content of apple foliage was altered when combination sprays composed of arsenate of lead and liquid lime-sulphur were used. Arsenate of lead was used because it is the most commonly used insecticide for the control of the codling moth, and liquid lime-sulphur was used because it is the most effective fungicide for the control of apple scab. Commercially, the two spray materials are applied together because of convenience. Liquid lime-sulphur was discontinued in the field toward the end of the season. Verdol summer oil spray was used then with arsenate of lead as a spreader.

When the temperature is high and the relative humidity is low, apple foliage is injured by liquid lime-sulphur and arsenate of lead sprays. According to Hyre (1939) the two spray materials will decrease the rate of photosynthesis even when the leaves appear uninjured. Sachs (1882) stated that the amount of chlorophyll was a limiting factor of the rate of photosynthesis. It seems possible that the conclusions of these two research workers may be related and the lessened rate of photosynthesis be due to the effects of sprays used.

REVIEW OF LITERATURE

Although effects of spray materials upon the size and the photosynthetic activity of apple leaves have been reported by many, Dutton (1932), Hoffman (1934), Young (1934), Schroeder (1935), Christopher (1935), Heinicke (1937), and Hyre (1939), there is little evidence of their influence upon the chlorophyll content. This subject, apparently, has been studied only by Ginsburg (1929) who reported an increase in chlorophyll content of two apple varieties as the result of oil sprays.

According to Willstatter and Stoll (1928) the chemical investigation of chlorophyll began with Berzelius in 1838 when he attempted to isolate the pigment from leaves. Sachs (1882) first recognized that chlorophyll was one of the factors affecting assimilation which was later referred to as photosynthesis, but prior to the researches of Willstatter and his coworkers, knowledge of the chloroplast was uncertain. They succeeded in isolating the pigments in a pure state, and also succeeded in determining their chemical nature. The chloroplasts contain four pigments: chlorophyll component a, chlorophyll component b, and two yellow pigments, carotin and xanthophyll. The method of chlorophyll extraction used by Willstatter and Stoll (1928) was crude and time consuming.

The properties of chlorophyll and methods of extraction and determination have also been clearly defined by Schertz (1928, 1929). Others who have recently studied the properties and methods for determination of the pigments of the chloroplasts are Sprague and Shive (1929), Guthrie (1929), Harriman (1930), Peterson (1930), Hicks and Panisset (1934), Zscheile (1934), Dastur and Buhariwalla (1928), Kuhn (1935), and Loomis and Shull (1937).

According to Schertz (1929) the most important factors affecting the quantity of pigments are rainfall, soil moisture, nutrient elements in the soil, light intensity, temperature, and relative humidity. He reported also that Monteverde in 1892 was the first to attempt to measure the concentration of chlorophyll. His method of determination was by studying the photographed spectra of several different concentrations of alcoholic extracts of leaves. His purpose was not to discover a method for quantitative work, but rather to discover the nature of chlorophyll spectroscopically. This same method was later used by Jacobson and Marchlewski (1912). The disadvantage of the method was that small quantities of chlorophyll could not be studied without great error. The procedure was then modified for a one gram sample by Jacobson (1912). The new method was a comparison of the intensity of the chlorophyll absorption bands of the solution which was unknown with the intensity of the corresponding bands produced by a solution of known concentration.

Lubimenko, according to Miller (1938), observed that shade plants could accomplish the same amount of photosynthesis with a lower illumination than the sun plants, and that the chlorophyll content of the former was higher than that of the latter. Henrici made observations somewhat the same as those of Lubimenko according to Miller (1938). She noted that the chlorophyll content of lowland plants of a species may be 2.3 times more than that of alpine plants of the same species.

Guthrie (1929) used a standard of copper sulphate, ammonium hydroxide and potassium chromate solution for colorimetric determination of unknown solutions of chlorophyll. Schertz (1928) used a crude chlorophyll dissolved in petroleum ether as a standard for his determination of unknown quantity of chlorophyll in a colorimeter. In the method of chlorophyll extraction described by Loomis and Shull (1937) the final chlorophyll is in aqueous solution. Deuber (1928) extracted chlorophyll from fresh leaves of different trees by the method suggested by Schertz (1928), and determined the amount of chlorophyll in the leaves of each kind of tree by comparison with a standard of Guthrie (1929) in a Duboscq colorimeter.

Ruth (1922) found in the case of the common bean, Phaseolus vulgaris, that the chlorophyll content of a square meter of leaf area of the primordial leaves sprayed with a Bordeaux mixture was slightly greater than the chlorophyll content of the same unit of area of the unsprayed primordial leaves. He observed also that the chlorophyll content per unit area of the primordial leaves decreased as the leaves developed after the shedding of the cotyledons. The leaves of the sprayed plants did not equal in size those which were not sprayed.

The chlorophyll content of grasses in Bechuanaland, South Africa, has been reported by Henrici (1926). She found that the low chlorophyll content of grasses was definitely associated with low carbohydrate content. Moisture seemed to be a limiting factor in this experiment. During a severe drought the carbohydrate content of the grasses was one-fourth or one-sixth of the amount that could be obtained from the leaves on a sunny day after a heavy rain. It was observed by this investigator that the chlorophyll and carbohydrate content were low when a low nocturnal temperature prevailed. It was further noticed that the chlorophyll content was variable in the leaves starting at a high initial value when the leaves were young and gradually decreasing with age. The chlorophyll content also was variable during a 24-hour period. She found that the chlorophyll content decreased from early morning to midday and increased again during the ensuing night.

When Deuber (1926) grew soybean seedlings in distilled water and a mineral solution, he found that the seedlings in distilled water became dwarfed and were of a higher concentration of chlorophyll, particularly in the first leaves and to a lesser extent in the cotyledons. The higher chlorophyll content in the dwarfed seedlings was explained by the fact that growth processes are impaired to a greater extent than the processes controlling chlorophyll formation. Deuber (1928) found that the chlorophyll content of the tree leaves was variable, depending upon the kind of tree.

Dastur and Buhariwalla (1928) gave evidence that the chlorophyll content in the leaves of tropical plants decreased with age.

Ginsburg (1929) found that Gravenstein and Wealthy apple leaves sprayed with oil had a greater amount of chlorophyll than the unsprayed leaves of the same varieties. He explained that the greater chlorophyll content in the sprayed leaves of the two varieties was due to the following: (1) Oil sprays may stimulate directly the chloroplast formation in the epidermal cells of the leaf, (2) greater reduction of leaf hoppers on the sprayed leaves compared with the unsprayed leaves, and (3) the spray may reduce the light intensity. Chlorophyll, according to Palladin (1922), accumulates faster in weak mid-light than in strong light which causes a decomposition of chlorophyll.

According to Emerson (1929b), there are two sets of factors that are generally recognized as affecting the rate of photosynthesis in green plants: namely, external and internal. Among the external factors are light, temperature, and the supply of carbon dioxide. Little is known of the internal factors, but chlorophyll is one. Photosynthesis as a function of light intensity with different concentrations of chlorophyll was also investigated by Emerson (1929a). He found that photosynthesis reached its maximum rate at about the same light intensities over the whole range of chlorophyll concentrations used. The same relationship existed with temperature. Emerson's results were interpreted as indicating that photosynthesis may involve an autocatalytic reaction, and that chlorophyll plays some part in the process in addition to its role in light absorption.

Emerson and Arnold (1932) found that the amount of chlorophyll present for each molecule of carbon dioxide reduced at a single flash of light was found to be about 2480 molecules. Lubimenko and Hubbenet (1932) reported that the maximum quantity of chlorophyll was formed in etiolated germinated seedlings at a temperature of 26° to 30° C. The chlorophyll content of kafir leaves was found by Ireland and Yeats (1933) to increase gradually until the grain began to mature. Then, the amount of chlorophyll gradually decreased. They observed also that the chlorophyll content varied in different varieties.

The experimental evidence of Dastur and Desai (1933) indicated that the water content of the leaves of some tropical plants is an important internal factor to which photosynthesis is related. Their results showed that the water content in the process is more important than that of the chlorophyll content. The chlorophyll content, also, is influenced to some extent by the water content of the leaf. The role played by the water content in the process of photosynthesis is not wholly unexpected as the importance of water in other life process of a plant is well known. They were among the first to take into account the water content of a leaf when the relation of other external and internal factors of photosynthesis were being investigated.

Ulvin (1934) found that on the average the leaves of the soybean and radish contained more chlorophyll on the dry weight and the area bases when grown in continuous light than those grown in a shorter photoperiod. He also reported that the chlorophyll content was increased in plants when treated by X ray.

The effects of various methods of storage on the chlorophyll concentration of leaves were studied by Harriman (1930) who found that the leaves of the soybean and nasturtium frozen with dry ice retained all of their chlorophyll. His work with oven drying of leaves at different temperatures showed that the optimum temperature was from 45° to 60° C., but even at these temperatures some chlorophyll was lost. He concluded that low temperatures are ineffective in preventing the loss of chlorophyll unless the leaves are actually frozen. The leaves, in this experiment, were placed on a filter paper for freezing with dry ice.

Pickett (1937) reported that leaves of the York apple variety had a slightly greater amount of chlorophyll per square meter of leaf area than those of the Wealthy variety. This variation was not sufficiently large to be significant. He concluded that the chlorophyll of the Wealthy leaves could enter into photosynthetic activity more efficiently than the chlorophyll of the York leaves because of the greater amount of internally exposed surface in the mesophyll cells of these leaves.

Pickett and Kenworthy (1940) stated that the amount of chlorophyll is not as significant in the process of photosynthesis as is the internal structure. They found also that the chlorophyll content of the leaves of the Wealthy variety was greater than that of the leaves of the York variety, and that the amount of chlorophyll in the Jonathan leaves was intermediate.

MATERIALS AND METHODS

The apple trees used in this investigation were divided into two series on the basis of the method of culture used. The first series was grown under glass and the second series in the field. This variation in culture system was thought desirable because of the probability that the different environments might have an effect on the content of chlorophyll in both sprayed and unsprayed leaves. The trees in the greenhouse were safe from certain weather injuries which sometimes ruin experimental work with trees growing in the field.

Greenhouse Series

Twenty-two two-year-old trees each of the York and of the Wealthy varieties were planted in twelve-inch clay pots and placed in a ground bed in a greenhouse on January 29, 1940. The trees were spaced thirty-six inches apart in equilateral triangles in which the varieties alternated. Half of the trees were sprayed and the other half were not sprayed. A burlap cover was placed over the trees receiving spray to prevent any of the spray from getting on the foliage of the unsprayed trees.

The first application of spray was made on February 24, and was mixed at the rate of four pounds of arsenate of lead and two and one-half gallons of commercial liquid lime-sulphur, 31.5° Baume, in sufficient water to make one hundred gallons of spray. Twelve applications of spray were made. The dates of the spray applications were: February 24 and 29; March 21 and 26; April 1, 6, 12, 18 and 26; and May 4, 11 and 20.

Sampling and Extraction of Chlorophyll. Leaves were collected between 8:00 a. m. and 10:00 a. m. and chlorophyll determinations made on the following dates: March 25; April 4, 10, 15, 24 and 29; and May 8 and 22.

Duplicate samples of the sprayed and unsprayed leaves were used in this study. When taking the samples the petioles of the leaves were rejected. The sprayed leaves were normal in appearance, and the spray residue was removed by rubbing carefully with a moist cheese cloth. The samples were weighed; the weight ranged from 5 to 10 grams fresh weight. Blueprints were made of the leaves because the quantity of chlorophyll in the leaves was to be expressed as milligrams per square meter of leaf area. The area of the leaves was determined by measuring the blueprints with a planimeter.

The method of chlorophyll extraction used was the one suggested by Loomis and Shull (1937) with modifications. Details of the procedure follow: A sample of leaves was placed into a mortar which contained 60 grams of white quartz sand. A sufficient amount of acetone to moisten the sand was added. The leaves were ground until the sand washed clear upon the addition of a stream of acetone from the wash bottle. Usually, the grinding required 10 minutes. The material was washed with acetone into a Buckner funnel fitted into a suction flask and filtered through number two filter paper. The sand was washed with acetone until the washings were clear. One hundred milliliters of ethyl ether were then poured through the Buckner funnel. The extract in the suction flask was transferred carefully into a liter separatory funnel by pouring down the sides. The flask was washed several times with a small quantity of ethyl ether (total of 50 milliliters) and the washings were transferred into the separatory funnel. One hundred milliliters of distilled water were added carefully.

Careful handling was exercised in all instances to keep the material from emulsifying. Fifteen milliliters of two per cent sodium chloride were added. Distilled water was added slowly and carefully down the side of the separatory funnel until separation occurred. If separation did not occur readily, the addition of a pinch of sodium chloride was necessary. Distilled water was added, and if separation did not occur, the material had formed an emulsion. If the material emulsified, the sample was discarded and new leaves were collected. When separation occurred, the material was allowed to stand twenty minutes to allow complete separation. The bottom layer contained acetone, water, flavones, and anthocyanin pigments. The upper layer contained chlorophyll "a" and "b", carotin and xanthophyll. The lower layer was drawn off and discarded. The remaining material was washed with a one percent sodium carbonate solution until all washings were clear.

The extract was drained into a 350 milliliter Erlenmeyer flask. The separatory funnel was washed several times with small quantities of ethyl ether until clean. Each time the ether washings were added to the Erlenmeyer flask.

Saponification was the next step and was accomplished by adding 10 milliliters of a saturated solution of potassium hydroxide in methyl alcohol. The material was then placed in a refrigerator over night.

Distilled water was then added. If saponification of the chlorophyll had occurred, a dark layer would have collected in the bottom of the flask. If saponification had not occurred, this process could be brought about by shaking for ten minutes, or by adding 10 milliliters more of potassium hydroxide in methyl alcohol. The material was transferred from flask into a liter separatory funnel. The flask was washed several times with distilled water and the washings were transferred to the separatory funnel. In the funnel were two distinct layers. The lower one contained the saponified chlorophyll in aqueous solution. The upper layer was yellowish in color and contained ethyl ether, carotin, and xanthophyll. The green layer was drained into another separatory funnel. After being washed several times with distilled water, the yellowish layer was drawn off and discarded. The washings were added to the saponified chlorophyll solution. The saponified chlorophyll solution was washed with small quantities of ether until clear. This was done by draining from one funnel to another. The chlorophyll extract was drained into a liter volumetric flask and was brought to volume by adding distilled water. The ether washings were discarded. The chlorophyll solution was ready for readings on the Duboscq colorimeter.

Colorimetric Readings. The quantity or amount of chlorophyll in the sample was determined in a Duboscq colorimeter by comparison with a sample in which the quantity of chlorophyll was known. The known sample or standard was made by weighing 5x chlorophyll which had been secured from American Chlorophyll, Incorporated, Alexandria, Virginia. Twenty milligrams of

5x chlorophyll were placed in an Erlenmeyer flask; acetone was added to the amount of 60 milliliters, and the material in the flask was shaken until all of the chlorophyll had dissolved. The procedure from this point on was the same as for chlorophyll extraction. The 5x chlorophyll seemed to be free from the yellow pigments as all washings were clear.

The standard was set at 30 millimeters on the colorimeter. Ten readings of the unknown sample were made and the average was used in the calculations.

Field Series

Fifteen two-year-old trees of each of three varieties, Wealthy, Jonathan and York, were planted in the field March 28, 1940. There were three rows of trees. Each row contained one variety and ran north and south. They were divided into five plots east and west, three trees of each variety. The first tree of each variety in a plot from south to north was sprayed, the second tree was not sprayed, and one-half of the third tree was sprayed and the other half was not sprayed. The dates of applying sprays, materials used and their concentrations, and dates of chlorophyll extractions are shown in Table 1.

Table 1. Schedule of spray applications[1] and chlorophyll determinations. Field grown trees, 1940.

Spray materials used	Spray applications dates	Chlorophyll determinations dates
Combination sprays of arsenate of lead and liquid lime-sulphur.	May 4 May 10 May 20 May 25 May 31	June 4
Arsenate of lead only.	June 8 June 15 June 21 June 29 July 6	June 10 June 17 June 24 July 1
Verdol summer oil as a spreader with arsenate of lead.	July 13 July 20 August 2 August 16 September 11	July 15 July 22 August 5 August 28 September 13

Details of sampling and chlorophyll extraction follow: Each sample of leaves weighed at least 5 grams fresh weight. The samples from the sprayed and unsprayed trees were taken in duplicate. When part of the foliage of a tree was sprayed and the other part was not sprayed, only one sample of each part was taken. Only leaves which had received all sprays up to each date of chlorophyll determination were used. Comparable leaves which were not sprayed were used. The procedure of

[1]Lead arsenate was used at the rate of 4 pounds for 100 gallons of spray, 2 1/2 gallons of liquid lime-sulphur, 31.5° Baume for 100 gallons of spray. Verdol summer oil was used at the rate of 1/4 of 1 per cent.

handling the samples from this point on, the method of chlorophyll extraction, and colorimetric determinations were the same as that followed with the greenhouse series.

PRESENTATION OF DATA

These data are presented in two series--(1) greenhouse grown trees and (2) field grown trees. A test of significance was calculated statistically for each series by preparing an analysis of variance. Since the chlorophyll content was so variable, it was desirable to use the statistical method to determine the sources of variation.

Greenhouse Series

The data on chlorophyll content of sprayed and unsprayed York and Wealthy leaves pooled are presented in Table 2. Duplicate determinations were made and presented of unsprayed and sprayed leaves of each variety for a given date with the exception of March 25 and April 4. An analysis of variance is found in Table 3.

Table 2. Chlorophyll content of the York and Wealthy apple leaves pooled. Greenhouse grown trees, 1940.

Dates of determination	Tree number		variety	Chlorophyll per sq. meter leaf area in mg.				
	un-sprayed	sprayed		un-sprayed	sprayed	Average of all readings	Average of un-sprayed	Average of sprayed
March 25	35	32	York	839.25	890.46	812.35	832.52	792.18
	10	15	Wealthy	825.80	693.90			
April 4	33	34	York	825.24	843.31	807.00	913.10	700.90
	6	5	Wealthy	1000.96	558.49			
April 10	31	36	York	349.05	514.00	594.21	626.08	562.34
	31	36	York	648.31	469.11			
	2	3	Wealthy	744.21	652.77			
	2	3	Wealthy	762.75	613.50			
April 15	37	30	York	1087.39	898.03	911.20	867.20	955.21
	37	30	York	748.83	777.79			
	22	3	Wealthy	627.52	1052.76			
	22	3	Wealthy	1005.08	1092.26			
April 24	41	34	York	1170.23	997.42	1230.88	1470.95	998.30
	41	34	York	974.47	771.95			
	14	15	Wealthy	1673.80	1251.92			
	14	15	Wealthy	2065.80	971.92			

Table 2. (cont.)

April 29	41	28	York	1065.36	897.69	1123.78	1277.37	970.19
	41	28	York	1515.46	813.85			
	4	9	Wealthy	1245.15	1166.29			
	4	9	Wealthy	1283.51	1002.94			
May 8	31	30	York	580.59	536.77	740.11	729.55	750.67
	31	30	York	760.18	443.25			
	8	1	Wealthy	753.34	949.87			
	8	1	Wealthy	824.12	1072.81			
May 29	37	34	York	1703.64	1273.90	1380.32	1509.73	1170.91
	37	34	York	1295.75	1112.84			
	2	3	Wealthy	1664.53	1286.95			
	2	3	Wealthy	1375.00	1009.97			
Average of all unsprayed or sprayed values				1050.53	879.16			
Average of York				968.83	802.88	885.86		
Average of Wealthy				1132.22	955.45	1043.83		

Table 3. Analysis of variance of the chlorophyll content of the York and Wealthy apple leaves pooled. Greenhouse grown trees, 1940.

Source	Degrees of freedom	Sum of squares	Mean square
Between dates	7	3,630,882.42	518,697.48**
" treatments	1	411,106.35	411,106.35**
" varieties	1	349,388.57	349,388.57**
Dates x treatment	7	525,159.07	75,022.72
" x variety	7	502,189.49	71,741.34
Treatment x variety	1	409.10	409.10
Error	31	1,072,181.61	34,586.50
Total	55	6,491,316.61	

**Highly significantly greater than error or within 1 percent level.

In Table 2 the average chlorophyll content of the unsprayed leaves was greater than that of the sprayed leaves. The analysis of variance showed that the variation between the chlorophyll of unsprayed and sprayed leaves was highly significantly greater than the variation due to error. An F value of at least 7.53 was necessary to be highly significant. An F value of 11.88 occurred. This means that the chlorophyll content of unsprayed York and Wealthy apple leaves was highly significantly greater than that of the sprayed leaves.

The variation between the chlorophyll content of the eight dates was highly significantly greater than the variation due to error. An F value of at least 3.27 was necessary to be highly significant, while a value of 14.99 occurred. This indicates that the average chlorophyll content of the two varieties for the eight dates was variable.

The variation in the chlorophyll content between the York and Wealthy apple leaves was highly significantly greater than the variation due to error. To be highly significant or within the 1 percent level, an F value of at least 7.53 was necessary. An F value of 10.10 occurred. This signifies that the chlorophyll content of the Wealthy apple leaves was highly significantly greater than that of the York leaves. An average chlorophyll content for the Wealthy and York apple leaves was 1043.83 and 885.86 milligrams per square meter of leaf area, respectively.

Field Series

The data of the field series are presented in two parts --Part I, the chlorophyll content of sprayed and unsprayed leaves from different trees; Part II, the chlorophyll content of sprayed and unsprayed leaves from the same trees.

Part I. The chlorophyll content of sprayed and unsprayed leaves of the York, Wealthy, and Jonathan varieties pooled is presented in Table 4. Duplicate determinations of sprayed or unsprayed leaves of each of the three varieties are presented. An analysis of variance was calculated for a test of significance. The analysis of variance is presented in Table 5.

Table 4. The chlorophyll content of Jonathan, York and Wealthy apple leaves. Field grown trees, 1940.

Dates of determination	Tree number: unsprayed	Tree number: sprayed	variety	Chlorophyll per sq. meter leaf area in mg.: unsprayed	sprayed	Average of all readings	Average of unsprayed	Average of sprayed
June 4	5	4	York	399.42	983.39	879.11	738.81	1019.41
				342.06	987.25			
	5	4	Jonathan	1188.93	908.36			
				771.76	1036.31			
	5	4	Wealthy	826.47	1000.00			
				904.22	1201.18			
June 10	8	7	York	1011.48	974.97	951.67	900.82	1002.52
				974.97	894.01			
	8	7	Jonathan	905.84	1381.61			
				906.58	928.63			
	8	7	Wealthy	911.36	927.95			
				694.69	907.96			

Table 4. (cont.)

June 24	14	13	York	1130.73	940.28	1188.86	1275.83	1101.90
				1370.89	904.75			
	14	13	Jonathan	1254.42	985.15			
				1282.08	972.92			
	14	13	Wealthy	1316.09	1401.75			
				1300.78	1406.57			
July 1	2	1	York	985.11	684.36	1070.11	1224.45	915.77
				1005.64	808.64			
	2	1	Jonathan	1282.21	1043.04			
				1287.14	1070.64[2]			
	2	1	Wealthy	1513.25	1016.17			
				1273.37	871.80			
July 15	5	4	York	1713.18	1636.94	1817.56	1736.45	1897.19
				1010.31	1867.30			
	5	4	Jonathan	1804.47[2]	2226.14			
				1406.03	1602.96			
	5	4	Wealthy	2292.87	2126.46			
				2191.74	1923.35			

Table 4. (cont.)

July 22	14	13	York	556.22	597.55	557.17	469.76	466.63
				654.92	751.14			
	14	13	Jonathan	479.50	996.98			
				454.27	743.38			
	14	13	Wealthy	336.40	410.12			
				338.26	367.34			
Aug. 28	11	10	York	1120.59	931.18	1096.87	1223.03	970.71
				967.88	895.58			
	11	10	Jonathan	1194.22	952.49			
				1079.13	955.99			
	11	10	Wealthy	1242.33	1057.82			
				1734.03	1031.22			
Sept. 13	2	1	York	1363.30	1271.45	1226.27	1221.60	1230.94
				982.23	1232.03			
	2	1	Jonathan	1048.67	954.74			
				1091.03	1208.58			
	2	1	Wealthy	1411.59	1354.63			
				1432.81	1364.22			

Table 4. (cont.)

Average of all unsprayed or sprayed values	1098.86	1097.85	
Average of York	974.31	1022.53	998.42
Average of Wealthy	1232.52	1089.76	1190.27
Average of Jonathan	1089.76	1122.99	1106.37

[2]Missing value determined according to the formula discussed by Snedecor (1938).

Table 5. Analysis of variance of the chlorophyll content of York, Jonathan and Wealthy apple leaves pooled. Field grown trees, 1940.

Source	Degrees of freedom	Sum of squares	Mean square
Between dates	7	10,847,971.79	1,549,710.11**
" varieties	2	591,960.79	295,908.39**
" treatments	1	24.19	24.19
Treatment x dates	7	1,003,945.98	143,420.85**
Variety x dates	14	1,411,744.41	100,838.39**
Treatment x variety	2	84,525.91	42,262.95
Error	60	1,860,183.81	31,003.06*
Total	93	15,800,356.88	

*Variation significantly greater than treatment or within 5 percent level.

**Variation highly significantly greater than error or within 5 percent level.

According to the analysis of variance, the variation in chlorophyll content between the sprayed and unsprayed leaves of the three varieties was very small compared with the variation due to error. The cause for this small variation can be explained by examining the average chlorophyll content of the sprayed and unsprayed leaves for the eight dates.

Table 6. Average chlorophyll content in milligrams of sprayed and unsprayed York, Jonathan and Wealthy apple leaves for the eight dates. Field grown trees, 1940.

Date	June 4	June 10	June 24	July 1	July 15	July 22	Aug. 28	Sept. 13
Unsprayed	738.81	900.82	1275.83	1224.45	1736.43	469.76	1223.03	1221.60
Sprayed	1019.41	1002.52	1101.90	915.77	1897.19	466.63	970.71	1230.94
Average difference	-270.60	-101.70	173.93	308.68	-160.76	174.48	252.32	-9.34

An average difference of at least ±218.66 milligrams was necessary for significance while at least ±290.81 milligrams was necessary to be highly significant.

In Table 6 the average chlorophyll content of the unsprayed leaves on four of the eight dates was greater than that of the sprayed leaves. On the other four dates the amount of chlorophyll was greater in the sprayed leaves. This interaction was highly significant and was the cause for the small variation in the chlorophyll content due to spraying. The effect of spraying could not be measured because there are other factors which are affecting the chlorophyll content. These factors may be light intensity, relative humidity, moisture supply and temperature.

The variation in chlorophyll content between varieties was highly significantly greater than the variation due to error. This signifies that the varieties differ in chlorophyll content. The Wealthy variety generally had the greatest amount of chlorophyll per square meter of leaf area while the York variety had the least amount. The Jonathan variety was intermediate in the amount of chlorophyll. The average chlorophyll content of the York, Jonathan and Wealthy leaves was 998.42, 1106.37 and 1190.27 milligrams per square meter of leaf area, respectively.

It was found in this investigation that the variation in amount of chlorophyll between varieties and dates was highly significantly greater than that due to error. An F value of at least 2.37 was necessary for significance, but a value of 3.25 occurred. This denotes that the variation in chlorophyll content of each variety for eight dates was variable. The average chlorophyll content for each variety of the eight dates is presented in Table 7.

Table 7. Average chlorophyll content of York, Wealthy and Jonathan apple leaves for eight dates (milligrams per square meter of leaf area). Field grown trees, 1940.

Date	York	Wealthy	Jonathan
June 4	678.03	932.97	976.34
June 10	965.86	960.49	1030.66
June 24	1086.66	1356.29	1123.64
July 1	870.94	1068.64	1070.75
July 15	1556.93	2133.60	1759.90
July 22	639.95	363.03	668.53
Aug. 28	978.80	1266.35	1045.43
Sept. 13	1212.25	1390.81	1075.75

The variability of the chlorophyll content of each of the three varieties on the eight dates was large. For example, the average chlorophyll content of the York leaves for the eight dates ranged from 639.95 to 1556.93 milligrams per square meter of leaf area. This variation was highly significant.

Part II. In Table 9 is presented the analysis of variance of the data presented in Table 8 which shows the chlorophyll readings for the three dates of the sprayed and unsprayed leaves from the same tree of each of the York, Wealthy and Jonathan varieties.

Table 8. The chlorophyll content of sprayed and unsprayed leaves from the same tree of each of the York, Jonathan and Wealthy apple varieties. Field grown trees, 1940.

Dates of determination	Tree number	variety	Chlorophyll per sq. meter leaf area in mg. un-sprayed	sprayed	Average of all readings	Average of un-sprayed	Average of sprayed
June 17	12	York	986.89	770.42	806.01	837.93	774.08
	12	Jonathan	755.84	680.75			
	12	Wealthy	771.08	871.08			
Aug. 5	3	York	1099.20	1012.17	1229.43	1393.56	1205.35
	6		1106.90	1179.29			
	3	Jonathan	1286.39	1246.15			
	6		1478.90	889.70			
	3	Wealthy	1859.08	1411.80			
	6		1530.92	1493.02			
Aug. 28	12	York	693.60	1007.85	925.88	881.53	970.23
	12	Jonathan	935.98	873.66			
	12	Wealthy	1015.19	1029.19			

Table 8. (cont.)

Average of all unsprayed or sprayed values	1126.65	1038.76	
Average of York	971.64	992.43	982.04
Average of Jonathan	1114.27	992.56	1018.42
Average of Wealthy	1294.02	1201.27	1247.67

Table 9. Analysis of variance of the chlorophyll content of sprayed and unsprayed leaves from the same tree of each of the York, Jonathan and Wealthy apple varieties. Field grown trees, 1940.

Source	Degrees of freedom	Sum of squares	Mean square
Between dates	2	1,170,881.50	585,440.75**
" varieties	2	331,774.97	165,881.48*
" treatments	1	46,350.55	46,350.55
Dates x variety	4	209,106.31	52,276.57
" x treatment	2	77,844.75	38,922.37
Treatment x variety	2	45,225.97	22,612.98
Error	10	223,404.05	22,340.40
Total	23	2,104,588.08	

*Variation significantly greater than error or within 5 percent level.

**Variation highly significantly greater than error or within 1 percent level.

The analysis of variance showed that the variation in chlorophyll content between dates was highly significantly greater than the variation due to error. To be significant an F value of at least 7.56 was necessary, while an F value of 26.20 occurred. This indicated that the amount of chlorophyll among dates was variable. The variation in the chlorophyll content due to spraying was not significantly greater than the variation due to error. An F value of at least 4.96 was necessary for significance, but a value of only 2.07 occurred. This signifies that the amount of chlorophyll in the unsprayed leaves was not significantly greater than that in the sprayed leaves.

According to Table 8 the average chlorophyll content for the sprayed and unsprayed leaves of the three varieties, Jonathan, York and Wealthy, is 1038.75 and 1126.65 milligrams per square meter of leaf area, respectively. The average difference was 50.62 milligrams for the same unit of area while at least 130.03 milligrams was necessary for significance. Even though the unsprayed leaves had more chlorophyll than the sprayed leaves, the difference was not large enough to be significant.

The variation in chlorophyll content between varieties was highly significant. The average chlorophyll content of the York, Jonathan and Wealthy was 982.04, 1018.42 and 1247.67 milligrams per square meter of leaf area, respectively.

DISCUSSION OF RESULTS

In the greenhouse where such factors as moisture and temperature were controlled, light intensity was reduced, and the relative humidity was high, the sprayed York and Wealthy apple leaves had lower chlorophyll content. In the field the chlorophyll content was not reduced by spraying, but on four of the eight dates a greater amount of chlorophyll occurred in the unsprayed York, Wealthy and Jonathan apple leaves than in the sprayed leaves. On the other four dates a greater amount of chlorophyll was present in the sprayed leaves. This variation indicates that other factors are altering or affecting the chlorophyll content which were not measured or accounted for in the experiment. Some of these factors may be moisture and temperature (Henrici, 1926), minerals (Deuber, 1928), and light (Palladin, 1922). Any one or all of these factors combined may be the cause of the variation.

The cause for the larger chlorophyll content in the unsprayed greenhouse grown leaves of the York and Wealthy varieties than the sprayed leaves may have been light intensity. The intensity of light coming through the glass in the early spring may be optimum for maximum chlorophyll formation. The spray residue on the leaf reduced the light intensity penetrating through the leaf to the point that chlorophyll formation was inhibited in the sprayed leaves.

In the late spring and summer the light intensity is greater than in the early spring. The light intensity increases gradually from late spring to summer. If spray materials did reduce the chlorophyll content, the high light intensity may have destroyed about the same amount of chlorophyll in the unsprayed field grown leaves. According to Palladin (1922), chlorophyll accumulates faster in weak midlight than in strong light. He states that in weak light the formation of chlorophyll occurs almost exclusively, while in strong light, besides chlorophyll formation, an active decomposition also takes place. This can be interpreted as meaning that high light intensity destroys chlorophyll. The spray residue on the leaf could reduce the intensity of light transmitted through the leaf.

The amount of chlorophyll in greenhouse grown leaves of the Wealthy and York varieties and in field grown leaves of the Wealthy, York and Jonathan varieties varied highly significantly between dates. This is to be expected as plants are subjected to different environmental conditions from time to time. Henrici (1926) found that the chlorophyll content of grasses varied during a 24-hour period and with age of the leaf. Ireland and Yeats (1933) found that the chlorophyll content of kafir increased during the growing season to maturity and then decreased. The variation in chlorophyll content among dates in this study is in agreement with the investigations of other workers.

In this study the chlorophyll content of the Wealthy variety was greater than that of the York variety. The Jonathan variety was intermediate. This is the same as the findings of Pickett and Kenworthy (1940).

This study could be improved by using mature trees as well as the two-year-old trees. Perhaps two mature trees each of York, Jonathan and Wealthy varieties could be used. One of the two mature trees would be sprayed and the other would not be sprayed. Leaf samples should be taken from spurs rather than shoot growth, and records should be kept as to the location of the leaves. Only sun leaves should be used, and all samples of leaves should be taken at the same time of day. Many leaf samples on a given date can be frozen with dry ice for future determination without any loss in chlorophyll, Harriman (1930).

Spraying one part of a tree and not the other part resulted in as large a variation in chlorophyll as occurred when sprayed and unsprayed leaves were taken from different trees. It is doubtful that there is any advantage in continuing this procedure of spraying.

Although the method used in extraction of chlorophyll was as good as any known at this time, studies seeking more accurate methods for this quantitative determination should yield valuable results.

CONCLUSIONS

These data indicate the following:

1. The chlorophyll content of the greenhouse grown York and Wealthy apple leaves was reduced by spraying with arsenate of lead and liquid lime-sulphur.

2. The chlorophyll content of field grown York, Wealthy and Jonathan leaves was not reduced after spraying with arsenate of lead and liquid lime-sulphur.

3. The chlorophyll content of the greenhouse and field grown leaves of the Wealthy variety was significantly greater

than the amount found in leaves of the York variety growing under similar conditions. The field grown leaves of the Jonathan variety were intermediate in the amount of chlorophyll.

4. The chlorophyll content of the York and Wealthy leaves from greenhouse grown trees and of the York, Wealthy and Jonathan leaves from the field grown trees was variable between dates. The variation was highly significant.

5. Such factors as moisture, light intensity and temperature probably caused the large variation in chlorophyll content between dates of both sprayed and unsprayed leaves.

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