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

by

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INTRODUCTION

Photosynthesis, the process by which certain carbohydrates are synthesized from carbon dioxide and water by chlorophyllous cells in the presence of light, occurs in the bodies of green plants. Thus, chlorophyll is essential to photosynthesis, by absorbing light energy, drives the entire process. Carbohydrates obtained in this way could be used, either directly or indirectly, as supplementary sources of human food.

The formation of chlorophyll is not a simple process. Its synthesis has been considered to proceed through a series of intermediates under constant quantitative equilibrium in response to various external and internal factors. Many problems of this synthetic process still await solution, and it is desirable to study by further experiments the formation as well as destruction of this vital compound.

Although we do not know exactly what happens inside the leaf, the progress in the field of biochemistry concerning the chemical and physical properties of chlorophyll has enabled us to acquire some understanding of the process of photosynthesis or assimilation. Until today, humanity lived without having discovered atomic energy, but no man and no animal could survive a day on earth without chlorophyll.

The many advances that have been made in the field of pest control during the past two decades have brought various disorders and changes in the physiological processes of plants due to spray application. About 15 years ago apple growers throughout the

United States feared the codling moth would put them out of business, other insects and mites were of comparatively little concern. Today the situation is reversed, mites and other insects have become problems while the codling moth in most cases is an insect of minor importance. DDT and other organic insecticides are primarily responsible for this reversal. Recently a number of miticides varying in control effectiveness have been introduced for the control of the several species of this pest attacking crop plants. It seemed desirable to know whether these miticides affected any of the physiological processes of plants.

The primary object of this study was to determine whether the chlorophyll content of Jonathan apple leaves was affected quantitatively by the use of miticides, butylphenoxyisoprophyl chloroethyl sulphite (Aramite), bis (P-chlorophenyl) trichloroethanol (Kelthane), O.O-diethyl O-p-nitrophenyl phosphorothioate (Parathion), and dicyclohexylammonium dinitro-O-cyclohexyphenate (DN-111).

REVIEW OF LITERATURE

Englemann, according to Smith (39), found that only the green parts of plants are able to evolve oxygen when placed in an atmosphere containing carbon dioxide. Willstatter and Stoll (49) disclosed upon investigation that the amount of chlorophyll in the leaves had little influence on the rate of photosynthesis, even though carbon dioxide, light, and possibly temperature were not limiting. Emerson (13), however, indicated that their view was not correct. He advocated the possibility of a correlation between the amount of chlorophyll and the rate of photosynthesis. Fleischer

(14) reported essentially the same finding. Photosynthesis is conditioned by the ability of the plant to absorb light by its pigments.

Although the extensive investigations of extracts of chlorophyll in inorganic solvents have advanced our knowledge of their physical and chemical properties, chlorophyll, in living organisms, is always associated with lipoid, protein complex, carotenoids, and minerals in the form of chloroplasts. Actual chemical analysis of the chlorophyll substance have been made by Chibnall (8), Menke (27), Granick (21), and Comar (9). According to Comar (9), the chloroplast substance of spinach leaves has been found to contain about 54 per cent ash. About 11 per cent of total nitrogen is found in the lipoid fraction. Weiber (47) stated that the chloroplasts consist of a colorless stroma or ground substance in which small discs of chlorophyll-impregnated cytoplasm are embedded. The small particles (grana) which have been observed in normal chloroplasts, are considered to be the sole bearers of chlorophyll.

The first chemical investigation of chlorophyll was made by Berzelius, according to Rabinowitch (33). Though he obtained a decomposed product, he succeeded in revealing the long unknown structure of this compound. We owe to Willstatter and Stoll primarily our knowledge of this substance. They isolated chlorophyll in the pure state, and contributed to the knowledge concerning this compound. Willstatter and his associate isolated chlorophyll a and b in pure form in over 200 different species of higher plants, and found them to be identical in chemical composition. Also the molecular structure of these two chlorophylls was determined by

them. Besides chlorophyll a and b, according to Meyer and Anderson (29), chlorophyll c is found in the brown algae and diatoms. Similarly, red algae contain chlorophyll d. In the purple bacteria still another kind of chlorophyll called bacteriochlorophyll is present, whereas the green bacteria contain another apparently similar pigment called bacterioviridin.

There appear to be two possible ways, according to Smith (39), in which the pigments may participate in photosynthesis. One way is by absorption of radiant energy and physical transfer of this energy. The other way is through actual chemical participation of the pigments in the oxidation reduction reaction.

Granick (20) reported that chlorophyll absorbs light energy and this energy is utilized primarily to bring about the decomposition of water. The light energy is thus converted to chemical energy and the chemical energy is only secondarily used to convert carbon dioxide into carbohydrates.

Franck and Loomis (16) stated that in living organisms, in many colloidal suspensions, and in the solid state, chlorophyll exhibits a spectral absorption maximum near 680 millimicrons. However, in solution in organic solvents, chlorophyll exhibits a spectral absorption maximum at shorter wave lengths of about 660 to 673 millimicrons.

Curtis and Clark (11) stated that effective photosynthesis is not necessarily correlated with the absorption band of the spectrum. This was proved by the discovery that yellow light from sodium lamps is fairly effective in photosynthesis although there is no strong absorption band in the yellow. It has been recognized

that the green leaves absorb certain portions of the spectrum selectively with peaks in the band of red rays of 650 to 680 millimicrons and of the blue rays of 420 to 450 millimicrons, with but little absorption in the green band.

Strain (45) studied the absorption spectra and isomerization of the chlorophyll and concluded that the spectral shifts of the absorption band of chlorophyll are due to a change in the physical state of the pigment, not to isomerization.

Chlorophyll possesses another important optical property, fluorescence. Curtis and Clark (11) reported that chlorophyll, either when dissolved in organic solvent or when in the living leaf, exhibits the intensive property of fluorescence. According to their statements, it will absorb light of certain wave lengths and emit light of longer wave lengths. It still remains uncertain whether this peculiar optical characteristic of chlorophyll may be involved in the process of photosynthesis. Wassink, as reported by Franck (15), stated that any influence raising the fluorescence intensity must be an influence which hinders transfer of energy from the excited chlorophyll to the substances which use it for photosynthetic purposes.

The formation of chlorophyll is a physiological process that occurs only in the living cells, and the synthesis appears to be complicated. Granick (21) reported that chlorophyll is likely formed in stepwise fashion so that the synthesis will proceed through a series of intermediate compounds with the aid of specific enzymes whose formation is controlled by specific genes.

Protochlorophyll, precursor of chlorophyll, is found in seedling plants grown in the darkness. This yellow pigment, as reported by Frank (17), is converted into chlorophyll upon irradiation. Smith (39) reported that the rate of conversion of protochlorophyll into chlorophyll is very rapid; about 50 per cent being transformed within a minute of illumination of moderate intensity. Frank (17) found that a large amount of carotenoids did not screen the light and prevent the formation of chlorophyll. He also found that the most effective spectrums for the conversion are blue and red. In experiments conducted by him, the major peaks of absorption were found to be 445 millimicrons and 645 millimicrons in protochlorophyll whereas 445 millimicrons and 675 millimicrons were the peaks in chlorophyll. Frank's contribution indicated that there is an excellent correspondence between the absorption properties in both protochlorophyll and chlorophyll. Smith and Koski (42) found that blue light was the most effective part of the spectrum for the conversion of protochlorophyll to chlorophyll. Sayer (35), contrary to Smith and Koski's findings, stated that for equal energy values, the red rays are more effective than the green, and the green more than the blue. He grew plants under colored glass plates and studied the effect of different wave lengths of radiant energy on the formation of chlorophyll in the various seedlings. The wave lengths shorter than 680 millimicrons and longer than 300 millimicrons are effective.

Smith and Young (43) stated that the photochemical transformation of protochlorophyll occurs only when the pigment is in its natural state and not when it is dissolved in organic solvents.

They concluded that the pigment exists in the leaf in some sort of active complex, called the protochlorophyll holechrome which is a pigment-protein complex.

Smith and Benitez (41) found that at -195° C. no photochemical transformation of protochlorophyll into chlorophyll a in etiolated barley occurs. At -70° C. fairly rapid and extensive conversion takes place. The conversion increased in rate and extent with increase in temperature up to 40° C. Leaves heated to 40° begin to lose their capacity for bringing about the phototransformation of protochlorophyll to chlorophyll a. The longer the period of heating, the greater becomes the loss of transformation capacity, and the higher the temperature, the more rapidly the loss takes place. Heating the leaves at 50° C. for five minutes almost completely destroys their transformation capacity.

According to Demerec (12), chlorophyll shows more peculiarities in its inheritance than any other known plant characteristics. In maize there are approximately 100 genetically different characters for chlorophyll. Curtis and Clark (11) stated that studies performed by geneticists have disclosed over 60 distinct generic factors influencing chlorophyll formation. They reported many interesting types of chlorophyll behavior. Certain crosses have been found regularly to produce definite ratios of unusual chlorophyll types. Some of these are almost pure white, never developing chlorophyll or chloroplast and never growing beyond the seedling stage. Still others appear pure white at first but under favorable conditions gradually develop chlorophyll.

Working with the unicellular green algae chlorella, Emerson (13) demonstrated a fairly close relation between the chlorophyll content and the rate of photosynthesis. According to his experiment, the results from individual series led always to the conclusion that photosynthesis reaches its maximum rate at about the same intensity of light, no matter what the chlorophyll content. At very low light intensity of 500 lux and at very high intensity of 7500 lux, the rate of process is relatively independent of change in the concentration of chlorophyll while at the intensities over 100 lux, the rate of process varies rapidly with various concentrations.

Aronoff (3) stated that the increase in the concentration of chlorophyll in solutions does not shift the position of the red absorption maximum in the leaf. Emerson (13) found, in studies on the rate of photosynthesis with various temperatures and varying chlorophyll concentrations, the relationship existed the same as with high intensity.

Smith (40) stated that the transformation of protochlorophyll to chlorophyll took place in an anaerobic atmosphere. It was not accompanied by the evolution of oxygen. Although the etiolated leaves that had been irradiated in air for a short time possessed chlorophyll that was derived from their protochlorophyll, they lacked the power to evolve oxygen. If, after this irradiation, these leaves were stored in air in darkness, they acquired a small capacity for oxygen production.

Frank (17) reported that a number of plants, especially among the unicellular green algae and seedlings of most conifer species,

do not require light for the formation of chlorophyll, and furthermore, he suggested the reduction of two hydrogen atoms from the pyrrole ring IV is presumably brought about in these plants by an enzymatic reaction.

The wave lengths of light effective in bringing about chlorophyll formation seem to be the same as those effective in photosynthesis itself. According to Curtis and Clark (11), several studies have been made of the influence of wave length, intensity, and duration of light on the amount of chlorophyll formed in the leaves when the plants have been grown under varied conditions. These same factors also influence the dry matter content, water content, thickness, and size of the leaves. If the amount of chlorophyll is expressed as percentage of dry or fresh weight, an increase in light intensity may result in a decrease in chlorophyll because of at the higher light intensity, there might have been dry matter as a result of more photosynthesis. If expresson on an area basis, the chlorophyll content might have been higher because the leaves are likely to be thicker at higher light intensity.

Although light is necessary for the formation of chlorophyll, strong light also brings about the disintegration of this compound. In leaves exposed to intense light, synthesis and decomposition of chlorophylls are probably going on simultaneously, according to Meyer and Anderson (29). Shirley (36) found in a number of species of plants that the chlorophyll content per unit leaf weight or per unit leaf area increased with decreasing light intensity until a relatively low intensity was reached. Further decrease in light

intensity below this value caused a decrease in chlorophyll content.

Appleman (2) stated that when etiolated seedlings of barley are illuminated under favorable conditions for the synthesis and accumulation of chlorophyll, catalase activity decreased. He concluded that when rapid chlorophyll synthesis takes place, catalase activity suffers a decrease; while synthesis is blocked, catalase activity rises rapidly if it is at a low level, or it does not decrease if it is at a high level.

Lubimenka and Hubbenet (26) found that the greening of wheat seedlings takes place within definite limits of temperature, beginning between 2° and 4°, attaining its maximum rate between 26° and 30°, and ceasing at/near 48° C. According to them, the increase in the amount of chlorophyll is proportional to the time of exposure to light until it reaches 26° C. and above this, the amount of chlorophyll decreases regardless of the time of exposure to light. Larsen (25) stated that the chlorophyll content in the potato tubers started to accumulate slowly at temperatures of 38° to 39° F. with a very long period of exposure of 600 hours. At the higher temperature of 66.3 F., the chlorophyll content increased rapidly but reached a maximum after 360 hours of exposure and remained relatively constant throughout the rest of the experiment.

Curtis and Clark (11) stated that when leaves have been thoroughly starved by prolonged growth in the dark so that sugar content has become low, chlorophyll will not develop even on exposure to light under otherwise favorable conditions. Such leaves

will readily develop chlorophyll on exposure to light if floated on various sugar solutions at concentration between 1 and 15 per cent or on glycerol, which is easily changed to sugar in the leaf. Palladin (30), however, found that exposure to excessively high concentrations of sugar (around 35 per cent) reduced greening. Knudson (24) observed that concentration of sugar much lower than this has a marked depressing effect on the chlorophyll content of orchid seedlings. A reasonable explanation is that the higher sugar may decrease the relative availability of some elements, especially nitrogen, which is a constituent of chlorophyll.

Nitrogen, as one of the constituents of chlorophyll, is necessary for the formation of chlorophyll. Granick (21) stated that the chloroplast of tomato and tobacco contains 30 to 40 per cent of the total nitrogen of the leaf blade materials. The protein of the chloroplast constitutes 80 per cent of the chloroplast nitrogen. The chlorophyll a and b account for another 10 per cent of the nitrogen in the chloroplast.

Magnesium also is a constituent of chlorophyll, and a deficiency of this element is likely to result in chlorosis or reduced development of chlorophyll. Kennedy (23) stated that plants grown in full nutrient media increase the photosynthetic rate proportionately with the increase in the length of the dark period. On the contrary, plants grown in magnesium deficient media could not attain the same rate of photosynthesis in the same length of dark period. He concluded that the magnesium content does not bear any relation to the length of the light period, but that deficiency delays the dark reaction in photosynthesis.

Iron is necessary for the formation of chlorophyll, although it has no place in the chlorophyll molecule insofar as has been determined by the analysis of the chlorophyll extracted from the plant. Willstatter and Stoll (49) believed that iron must be a specific catalyst involved in some of the preliminary stages of the production of green pigment. Jacobson and Oertli (22) stated that if iron is supplied at a uniform rate, a good correlation is obtained between iron and chlorophyll content in sunflower leaves. He further suggested that iron is involved in chloroplast formation via protein synthesis because chlorotic leaves may not turn completely green even when a large amount of iron enters the leaf.

Copper, zinc, potassium, sulfur, and phosphorus have some influence on chlorophyll formation even though they do not participate in the formation of the chlorophyll molecule.

Curtis and Clark (11) stated that higher concentration of oxygen is necessary to bring about chlorophyll formation than are ordinarily necessary to keep the tissues alive. That is, tissue which lacks chlorophyll because of growth in the darkness, will fail to become green in light if the oxygen concentration is very low even though they may live for some time.

Willstatter and Stoll (49) reported that there is always a certain surplus of chlorophyll in the leaves. They observed in one of the experiments that the rate of assimilation was the same in the dark as in the pale variety of elm, although in the former the chlorophyll content was 10 times greater.

Sivadjian (37) reported that by use of hygrophotographic methods, the rate of transpiration is correlated with the presence

of chlorophyll. He found the most active transpiration in green parts and negligible in etiolated parts.

Meyer and Anderson (29) believed that the variation of the chlorophyll content is largely influenced by the environmental conditions and the age of the leaf as well as by genetic factors. It appears that the proportion of chlorophyll a to b in leaves may vary considerably, depending upon the light intensity to which the leaves have been exposed. This same finding was reported by Willstatter and Stoll (49). According to them, the proportion of chlorophyll b to a is higher in shade leaves of many species than sun leaves of the same species.

Pickett (31) found the photosynthetic activity of apple leaves to be influenced more by the amount of intercellular space than by chlorophyll content. He determined that leaves of the Wealthy variety made a significantly greater gain in total dry matter per gram of chlorophyll than those of the York variety even though the leaves of the latter variety contained slightly greater amounts of chlorophyll.

Pickett and Kenworthy (32) advanced further evidence that the amount of chlorophyll does not bring into effect the process of photosynthesis as much as does the internal structure of the leaves.

There is little evidence in the literature on the influence of spray materials upon the chlorophyll content of apple leaves. Ginsburg (19) found that Wealthy and Gravenstein apple leaves sprayed with oil had a greater amount of chlorophyll than the

unsprayed leaves of the same varieties. He explained this as follows: (1) oil sprays may stimulate directly the chloroplast formation in the epidermal cells of the leaf, (2) there was a greater reduction of leaf hoppers on the sprayed leaves compared with the unsprayed leaves, and (3) the spray may reduce the light intensity.

Saunders (34) found that the chlorophyll content of York and Wealthy apple leaves grown in a green house was reduced by spray treatments of lead arsenate and liquid lime-sulphur. He concluded that the reduction in chlorophyll content of sprayed leaves was due to the spray residue on the leaves which tended to reduce the light intensity which the leaves received. He found that in field grown trees, the chlorophyll content was not reduced by spraying but that there was a great variation between the unsprayed leaves at the various dates.

Campbell (7) reported that the chlorophyll content of the Jonathan apple leaves, sprayed with DDT, Fermate, wettable sulphur and zinc sulphate, was not reduced significantly by any of the four spray treatments. He observed, however, that the chlorophyll content of Winesap apple leaves was slightly reduced by the same treatment. With these results, he concluded that the chlorophyll content of the Winesap leaves was more sensitive to external factors than that of the Jonathan variety. Furthermore, he stated that the significant variation of the chlorophyll content between dates of both sprayed and unsprayed leaves appeared to be caused by the factors such as soil moisture, light intensity, soil nutrients, relative humidity, and temperature.

According to Wentzler and his associate (48), three applications of nitrogen, Fermate, Crag 341, Phenothiazine, and Parathion sprayed on fully expanded leaves of McIntosh and Stayman Winesap varieties did not result in a significant change in chlorophyll content as compared with leaves of unsprayed leaves.

MATERIALS AND METHODS

Thirty 14-year-old Jonathan trees growing in the orchard at the Horticulture farm near Manhattan were used for the study. The trees were spaced 35 feet apart in rows with 35 feet between the rows. Trees used were assigned spray treatments at random at the time the leaves had fully expanded in late June, 1957. This date coincided with the time that mitecides normally would be applied.

Five treatments, consisting of six trees per treatment, were compared. They included (1) one and one-half pounds of Aramite, (2) one pound of Kelthane, (3) one pound of Parathion, (4) one-fourth pound of DN-111, and (5) the checks to which no spray was applied. The rate of all mitecides listed were for 100 gallons of water. A single application of Kelthane was made on July 9. The other three materials were applied each of July 9 and 24, August 5 and 19, and September 2. Attention was given to obtain thorough coverage of the leaves when spraying.

Sampling and Extraction of Chlorophyll

To minimize differences and to obtain relatively uniform chlorophyll values, leaves were selected from the middle portions of shoots growing in the periphery of the trees. In this way 30 leaves were collected from each tree, and discs one centimeter square were cut from near the midsection of the leaf blade. One disc was removed from each leaf, and plunged immediately into methanol. When taking the samples, the midribs of the leaves were avoided. During the periods of sampling, leaves were collected between 10 and 12 o'clock in the morning to secure as uniform values of chlorophyll as possible.

Chlorophyll determinations were made on each July 23, August 4 and 18, and September 1 and 13.

The method of chlorophyll extraction and determination used was the one suggested by Compton and Boynton (10) with modifications. Thirty discs, one from each of 30 leaves, were placed in about 30 milliliters of methanol. The leaf tissue was left to stand in the solvent for 24 hours in the darkness and then mixed in a Waring blendor for three to four minutes. The solution was then filtered through a Buchner funnel fitted with filter paper and transferred into a 100-milliliter volumetric flask and made up to volume by washing the pulp with several portions of solvent. The extract was read in Klett-summerson photoelectric colorimeter using a light filter which transmitted above 610 millimicrons.

To reduce changes in compositions of the chlorophyll, all samples were kept in the dark except during the operation of

extraction and filtration. Chlorophyll determinations were also carried out by taking samples individually out of the dark so as to minimize the changes of composition of the chlorophyll prior to reading.

Calibration of Colorimeter

Calibration of the instrument by means of a plant extract was accomplished by the method suggested by Benne, et al. (5).

As a calibration standard, wheat leaves were used as a source of chlorophyll. One hundred milliliters of the methanol were prepared as described earlier. A series of dilutions: 10, 25, 35, 50, and 75 milliliters to 100 milliliters solution, respectively, were made from this extract. The percentage of light transmitted by the original and by each of the diluted solutions was measured with the Klett-Summerson photoelectric colorimeter. Twenty-five milliliters of methanol extract were transferred from 100 milliliters of original extract into a separatory funnel containing 50 milliliters of ethyl ether. About 100 milliliters of water was added slowly to avoid the danger of forming an emulsion. The water phase was drained off and discarded. Five or six scrubbings were required for the complete removal of the methanol. When the washing was completed, the ether solution was transferred to a 100-milliliter volumetric flask and made to volume with more ethyl ether. Aliquots of the solution were removed and the color intensity read in the Beckman Spectrophotometer at 6600 Å and 6425 Å. Calculations of the total chlorophyll content expressed as milligrams per liter were made.

Klett-Summerson photoelectric readings corresponding to concentrations of chlorophyll, expressing milligrams per 100 milliliters of solution, are presented in Table 1.

Table 1. The Klett readings of total chlorophyll content of series of diluted solutions expressed in milligrams per 100 millliliters of solution.

Concentration of chlorophyll	Klett reading	Series of solution (Per cent)
0.8896	132	10
2.224	216	25
3.1136	236	35
4.448	253	50
6.672	270	75
8.896	282	100

The readings of the total chlorophyll content of the apple leaves calculated as milligrams per liter were expressed in milligrams per square meter of leaf area.

The dates of spray applications and chlorophyll determinations are presented in Table 2.

Table 2. Schedule of spray application and chlorophyll determination of leaves of 14-year-old Jonathan trees, 1957.

Spray application dates	Chlorophyll determination dates
July 9*	July 23
July 24	August 4
August 5	August 18
August 19	September 1
September 2	September 13

* Kelthane applied only on this date.

RESULTS

The average chlorophyll content of Jonathan apple leaves for each spray treatment at each sampling date is given in Table 3.

Table 3. Average chlorophyll content of Jonathan apple leaves for each spray treatment at each date, expressed in milligrams per square meter of leaf area, 1957.

Date	Aramite	DN-111	Parathion	Kelthane	Check	Average
July 23	244.1	208.0	210.8	213.0	193.7	213.9
August 4	242.7	231.9	255.7	244.1	229.9	240.8
August 18	236.6	237.4	241.9	245.4	265.0	245.2
September 1	334.6	361.3	337.2	410.2	333.0	355.2
September 13	333.5	324.4	287.4	349.4	296.0	318.1
Average	278.3	272.6	266.6	292.4	257.8	

L.S.D. At five per cent = 37.63.

Since the chlorophyll content was highly variable over the entire period of the experiment, the chlorophyll values obtained were analyzed statistically. The results of an analysis of variance are presented in Table 4.

According to the data as shown in Table 4, the variance of between dates and between treatments were greater than the error variance. This indicated that there was some fundamental difference between the series.

The variation in the amount of chlorophyll between dates was significantly great as seen by the F value of 118.56 when a value of but 3.47 is significant at the one per cent level. This

indicated that the chlorophyll content was highly variable at different dates during the experimental period in the Jonathan apple leaves.

Table 4. Analysis of variance of the chlorophyll content of the Jonathan apple leaves, 1957.

Factor	Degree of freedom	Sum of squares	Variance
Total	149	600,349.26	
Between dates	4	435,636.19	108,909.02**
Between spray treatments	4	19,376.48	4,844.12**
Interaction	16	30,513.62	1,907.10*
Error	125	114,822.97	918.57

** Variance which is significant at the one per cent level as shown by the F test.

* Variance which is significant at the five per cent level as shown by the F test.

The variation in the amount of chlorophyll between treatments was also significant. At the one per cent level, an F value of 3.47 is necessary, whereas, an F value of 5.27 was obtained. This denoted that the amount of chlorophyll was highly variable between treatments.

It was found that the variance of interaction was significant. A value of 1.74 at the five per cent level was necessary for significance, whereas a value of 2.06 was obtained. This value, however, much smaller than the variance between dates and treatments, denoted that some other factors affected the results of this experiment.

The error variance was subjected to the T test in order to determine a L.S.D. with which to compare the variation in chlorophyll content of the apple leaves on a given date during the growing season. Ordered arrays of average chlorophyll contents of the Jonathan apple leaves for each spray treatment at each date are presented in Table 5.

Table 5. Ordered array of average chlorophyll content of the Jonathan apple leaves expressed in milligrams per square meter of leaf area for each spray treatment at each date, 1957.

July 23	August 4	August 18	September 1	September 13
244.1	255.7	265.0	410.2	349.4
Aramite.....	Parathion	Check	Kelthane *	Kelthane..
213.0 .	244.1	245.4	361.3	333.5 .
Kelthane....	Kelthane	Kelthane	DN-111....	Aramite *
210.8 ..	242.7	241.9	337.2 .	324.4 .
Parathion ..	Aramite	Parathion	Parathion .	DN-111 .
.*			.	ns .
208.0 ns.	231.9	237.4	334.6 .	296.0 .
DN-111 ..	DN-111	DN-111	Aramite .	Check....
..			ns	ns
193.7 ..	229.9	236.6	333.0 .	287.4
Check.....	Check	Aramite	Check....	Parathion
	ns	ns		

L.S.D. At five per cent = 37.63.
ns No significant treatment difference on this date.

As shown in Table 5, there was considerable shifting around of the chlorophyll content of the leaves sprayed with different treatments at the various dates. It is noteworthy that there was no significant increase in chlorophyll content during the first three sampling periods of July 23, August 4, and August 18 whereas a rapid increase in chlorophyll content with greater total amounts

was obtained on September 1 and 13.

On July 23 the chlorophyll content of the leaves sprayed with Aramite was greater although not significantly so than that of the leaves sprayed with the other chemicals. This was the only chemical treatment in which the chlorophyll content was significantly greater than that of unsprayed check leaves. There were no significant differences in the chlorophyll content among all treatments on August 4 and 18 whereas on September 1, the values for leaves sprayed with Kelthane were significantly greater than for leaves receiving the other chemicals as well as the unsprayed check. The leaves sprayed with Kelthane showed a significantly higher chlorophyll content than the unsprayed check leaves, and those sprayed with Parathion. The Aramite-sprayed leaves had a significantly higher chlorophyll content than those sprayed with Parathion, and barely missed being significantly higher in chlorophyll than the unsprayed check leaves.

It was found that the chlorophyll content of unsprayed check leaves, at most dates, was lower than that of the leaves sprayed with chemicals. A noticeable exception was observed on August 18 when the average chlorophyll content of the unsprayed check leaves was higher than that of any of the sprayed leaves.

The daily temperature, radiation, duration of light, and precipitation for the period of July 9 to September 13 are shown in Table 6.

Plate I graphically presents the relationship of the average chlorophyll content of the sprayed to the unsprayed leaves of the Jonathan variety at the different dates.

Table 6. The daily temperature, radiation, duration of light, and precipitation for the period, July 9 to September 13, 1957.

Sampling period	Date	Temperature (° F.) Max.	Min.	Ave.	Duration of light (minutes)	Radiation of light (Langleys)	Precipitation
	July 9	85	67	76	676	644.5	0
	10	86	67	76	155	202.4	0.47
	11	97	70	84	760	666.0	0.06
	12	100	78	89	826	672.9	0
	13	100	73	86	843	671.0	0
	14	98	68	83	742	632.9	0
1	15	101	75	88	803	622.2	0
	16	101	78	90	791	647.6	0
	17	101	75	88	835	651.9	0
	18	100	75	88	830	642.2	0
	19	99	72	86	710	632.2	0
	20	97	69	83	502	516.7	0
	21	82	71	76	104	165.2	1.10
	22	84	71	78	226	304.2	0.99
	23	83	70	76	330	391.3	0
Total					9130	8063.20	2.20
	24	86	58	72	714	597.6	0
	25	89	63	76	582	559.9	0
	26	92	71	82	285	381.5	0
	27	99	76	88	627	662.2	0.02
	28	100	77	88	701	583.1	0
2	29	93	73	83	631	551.1	0
	30	92	70	81	730	585.4	0
	31	94	67	80	798	629.0	0
	Aug.1	97	68	82.5	790	627.4	0
	2	99	71	85.0	786	607.0	0
	3	95	71	83.0	300	436.4	0
	4	86	64	75.0	804	632.7	0
Total					7748	6853.35	0.02
	5	84	57	70.5	812	637.0	0
	6	89	57	73.0	609	638.0	0
	7	93	60	76.5	801	628.8	0
	8	97	65	81.0	793	622.9	0
	9	100	70	85.0	723	596.3	0
3	10	95	68	81.5	741	607.9	0
	11	100	67	83.5	724	594.3	0
	12	100	69	84.5	590	563.6	0

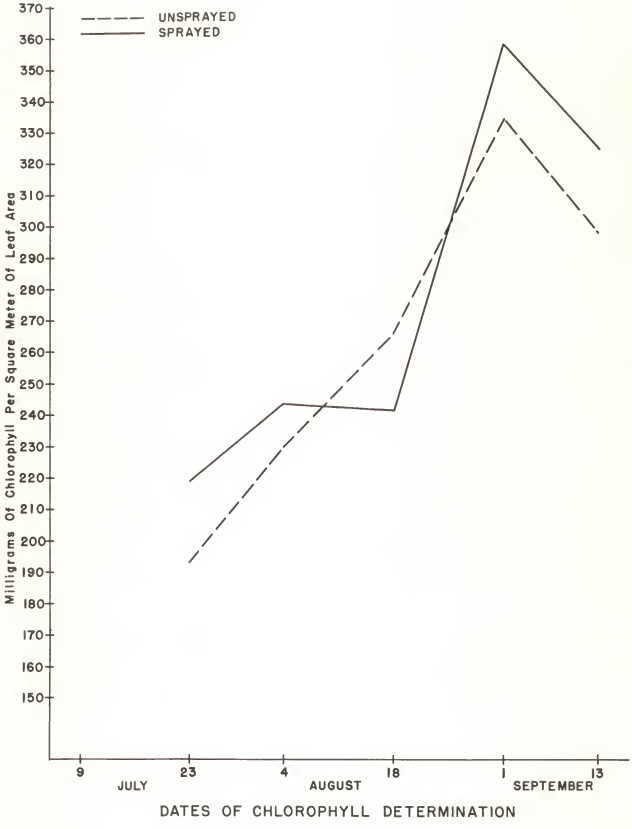
Table 6 (concl.).

Sampling period	Date	Temperature (° F.) Max.	Min.	Ave.	Duration of light (minutes)	Radiation of light (Langleys)	Precipitation
	Aug. 13	101	72	86.5	771	593.7	0
	14	100	72	86.5	676	571.0	0
	15	104	75	89.5	742	604.6	0
	16	92	70	81.0	226	195.4	0.01
	17	85	65	75.0	678	584.9	0.01
	18	89	56	72.5	540	467.2	0
Total					9326	7905.6	0.02
	19	90	58	74.0	733	616.2	0
	20	95	65	80.0	358	394.3	0.07
	21	92	66	79.0	377	422.6	0.01
	22	94	72	83.0	317	392.4	0
	23	87	69	78.0	365	280.3	0.06
	24	88	57	72.5	725	622.1	0
4	25	100	57	78.5	764	581.1	0
	26	89	69	79.0	176	194.0	0
	27	88	67	77.5	213	306.9	0.56
	28	91	66	78.5	625	507.6	1.29
	29	64	68	81.0	727	519.3	0
	30	94	74	84.0	726	488.9	0
	31	94	67	80.5	736	540.3	0
	Sept.1	93	69	81.0	660	485.9	0
Total					7502	6321.90	1.99
	2	84	63	73.5	762	597.0	1.70
	3	89	56	72.5	758	576.8	0
	4	82	55	68.5	708	491.9	0
	5	83	61	72.0	692	511.7	0
	6	76	58	67.0	285	242.7	0.78
5	7	76	48	62.0	501	497.7	0
	8	77	46	61.5	725	570.8	0
	9	77	50	63.5	351	408.0	0
	10	71	59	65.0	0	55.2	0.11
	11	70	59	64.5	0	163.7	2.21
	12	82	57	67.5	543	526.5	0.21
	13	81	54	67.5	599	483.9	0
Total					5906	5125.90	4.91

EXPLANATION OF PLATE I

Chlorophyll content of sprayed and unsprayed Jonathan apple leaves.

PLATE I


UNSPRAYED
SPRAYED
Milligrams Of Chlorophyll Per Square Meter Of Leaf Area
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
9
JULY
23
4
AUGUST
18
1
SEPTEMBER
13
DATES OF CHLOROPHYLL DETERMINATION

A graphic comparison of the chlorophyll content of the Jonathan apple leaves receiving each of the four miticides with the unsprayed check leaves is given in Plate II.

The relationship of the chlorophyll content of the Jonathan apple leaves to the average daily mean temperature and precipitation of the period, July 9 to September 13, is shown in Plate III.

DISCUSSION

It was found that the amount of chlorophyll in the Jonathan apple leaves grown in the field varied greatly between dates in which constant changes in the various environmental factors take place.

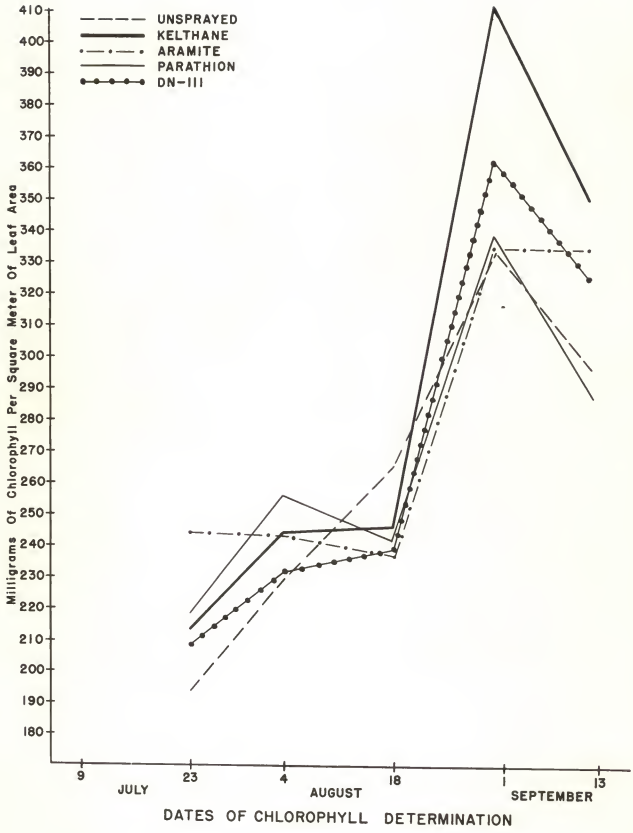
In this experiment, the average chlorophyll content of the leaves increased with the increase in the age of leaves until after September 1 when under the conditions of weaker light intensity and shorter days as well as lower mean temperature, the chlorophyll content in all leaves started to decrease. It was also noteworthy that during the experimental period continuous extreme high temperature, coupled with high light intensity, appeared to depress the formation of chlorophyll.

Terrien, et al. (46) stated that chlorophyll is not a stable substance which, once formed, persists throughout the life of the leaf; its quantity depends on the equilibrium established between its rate of formation and its rate of disappearance. During the growing period the rate of formation is much greater, but the reverse normally occurs in the fall. They stated furthermore that the seasonal change in the chlorophyll content is much influenced

EXPLANATION OF PLATE II

Comparison of chlorophyll content of apple leaves receiving different treatments, 1957.

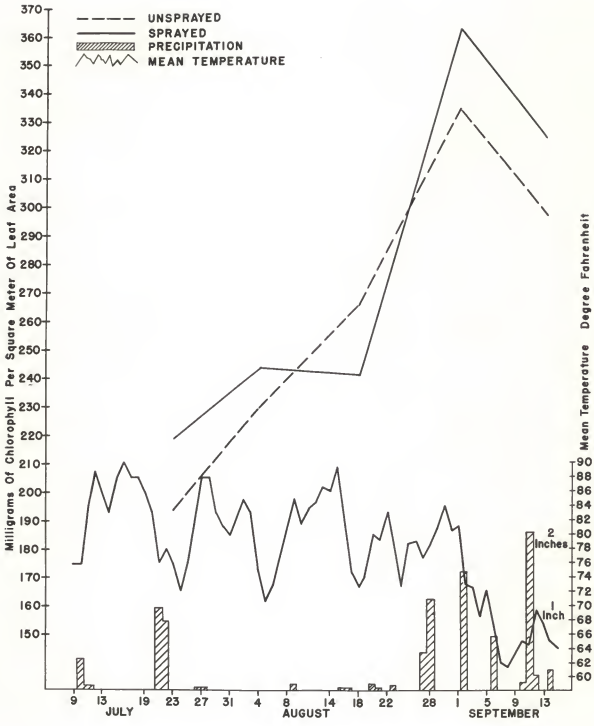
PLATE II


UNSPRAYED
KELTHANE
ARAMITE
PARATHION
DN-111
Milligrams Of Chlorophyll Per Square Meter Of Leaf Area
410
400
390
380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
9
JULY
23
4
AUGUST
18
1
SEPTEMBER
13
DATES OF CHLOROPHYLL DETERMINATION

EXPLANATION OF PLATE III

Relationship of chlorophyll content in Jonathan apple leaves to average daily mean temperature and precipitation, 1957.

PLATE III


UNSPRAYED
SPRAYED
PRECIPITATION
MEAN TEMPERATURE
Milligrams Of Chlorophyll Per Square Meter Of Leaf Area
Mean Temperature Degree Fahrenheit
2 Inches
1 Inch
JULY
AUGUST
SEPTEMBER

by the temperature during the growing season; chlorophyll is more narrowly limited than growth and is not produced when the temperature is too low or too high, although growth may not be suppressed. Smith and Benitex (41) found that the longer the period of heating at 40° C., the greater becomes the loss of transformation capacity of the conversion of protochlorophyll into chlorophyll of barley seedlings.

Shirely (36) found that the chlorophyll content per unit leaf weight or per unit leaf area increased with decreasing light intensity until a relatively low intensity was reached. Further decrease in light intensity caused a decrease in chlorophyll content. Terrien, et al. (46) stated that chlorophyll disappears under the influence of light and of oxygen; the products into which it is transformed are very probably products of oxidation by attacking the neighboring oxidizable material, and it soon attacks the chlorophyll. The average life of chlorophyll in the natural condition seems to be extremely short, since the average life of excited chlorophyll has been estimated at 8×10^{-8} to 8×10^{-11} seconds.

The amount of chlorophyll in the Jonathan apple leaves used in this study was significantly variable between dates. Thus, the results of this test were in agreement with those reported by Campbell (7).

An analysis of variance showed that spraying produced variations in chlorophyll content which exceeded the F value at the one per cent level of significance. A L.S.D. value at the five per cent level was calculated in order to compare the variation in

chlorophyll content of the apple leaves sprayed with different miticides as well as the check leaves. Spraying at some dates appeared to increase the chlorophyll content of the apple leaves.

On July 23 there were no significant differences in chlorophyll content among treated leaves with the exception of the leaves sprayed with Aramite, which were significantly higher than the unsprayed check leaves. The chlorophyll content of Aramite-sprayed leaves on September 13 was significantly higher than that of the Parathion-sprayed leaves and barely missed being significantly higher than that of the unsprayed check leaves. It was noted at other dates that the chlorophyll values of Aramite-treated leaves varied greatly in relation to the unsprayed checks as well as to those leaves receiving the other spray treatments. It is logical to assume that this fluctuation in chlorophyll content was directly related to the mite control obtained (Table 7). Armstrong (1) found the residual action of this material to be effective for an average of seven days. The European red mite Paratetranychus pilosus, Fanzago, is reported by Metcalf and Flint (28) as having an average length of life cycle of about 21 days and from four to eight generations a year. Since the control of mites on Aramite-sprayed leaves was not absolute, it could be expected that the mite population might be variable from date to date in response to varying weather conditions. This is reflected in the chlorophyll readings. Application of Aramite in July and September was apparently made at a critical period in the mite life cycles and reduced the population more effectively than did the spray made at the other dates.

Table 7. Average number of mites per leaf at three dates. Counts are averages of 10 leaves.

Material	Date		
	July 7	August 12	September 10
Kelthane	--	0	0
Aramite	--	1	3
Dn-111	--	2	4
Parathion	--	6	20
Check	10	9	31

Baker (4) stated that the mite feed by piercing the epidermis of the leaf and drawing the liquid content from the cells. The leaf turns pale and stippled around the injured parts. When the infestation is severe, the stippled areas coalesce and cause the leaf to appear sickly, turn rust-red, and then crumple and die. Affected plants are stunted and may be killed.

There were no significant differences in the chlorophyll content among any treatments at the second and third sampling dates although a moderate shifting around of the chlorophyll content of the leaves receiving the different sprays was observed. It can be assumed that prolonged temperature coupled with extreme high light intensity appeared to retard the normal seasonal increase in the chlorophyll content of Jonathan apple leaves at this time. The soil moisture content could possibly be another factor affecting leaf pigment. No appreciable rainfall was received during the second and third treatment periods. It is likely that much of the available soil moisture was utilized by the trees, and that in response to the extreme high temperature, the loss of

water by transpiration was great. Meyer and Anderson (29) stated that desiccation of leaf tissue not only inhibits synthesis of chlorophylls but seems to accelerate disintegration of the chlorophylls already present. Furr and Degman (18) found that Grimes and Delicious grown under soil moisture content six per cent above permanent wilting point showed an appreciable reduction in the rate of photosynthesis made by stomates closing at or near noon. Water, as a carrier and solvent of minerals obtained from the soil, is indispensable for the formation of chlorophyll.

After a rain of about two inches and subsequent moderate climate conditions during the period of August 19 to September 1, the chlorophyll content increased immediately in all treatments though more rapidly in the leaves applied with Kelthane. This increase in chlorophyll made by Kelthane-treated leaves was exceptional and more highly significant than any other treatments. The chlorophyll content in all treated leaves after September 1 started to decrease as if in response to the environmental conditions of the period during which lower light intensity and shorter days with lower temperature prevailed. However, the chlorophyll content of the leaves sprayed with Kelthane was significantly greater than that of the unsprayed check and of Parathion-treated leaves. A significant increase in the chlorophyll content in the leaves sprayed with Kelthane during the last two periods might be attributed to the effectiveness of this chemical as a miticide.

Mite counts taken at the three dates show a considerable difference in the degree of control among the different chemical

treatments. Leaves sprayed only once with Kelthane had no mites at any of the dates as compared to a variable population on unsprayed leaves as well as on those receiving the other chemicals. The mite population apparently failed to build up in Kelthane-sprayed leaves as evidenced by the higher chlorophyll reading. According to Spencer and Selhim (44), in late July under California conditions, there were marked increases in citrus red mite infestation regardless of treatment with the exception of the Kelthane-sprayed trees. Mite populations were significantly lower on trees that had been sprayed with Kelthane than on those that received other miticides. They also indicated that the sprays of this chemical had a long-lasting effect, with no evidence of phytotoxicity.

Although the unsprayed check leaves showed an average lower chlorophyll content through most of the test period than all of the sprayed leaves, there was a complete reversal of this situation when the third chlorophyll determination was made on August 18. As stated above, the period August 5 to 18 was characterized by hot weather; on five successive days the maximum temperature equalled or exceeded 100° F. The daily average light intensity was great with no appreciable rainfall. The chlorophyll content of the unsprayed check leaves was higher than that of any sprayed leaves although not significantly so.

In this experiment, changes in the chlorophyll content of the sprayed leaves were apparently not due to the spray residue affecting light intensity as reported by Ginsburg (19) and Saunders (34). None of the chemical used left a heavy residue,

and Kelthane-sprayed leaves had no noticeable residue after a few weeks.

The variance of interaction was significant at the five per cent level. This value presumably was caused either directly or indirectly by various factors which could not be measured in this study.

The importance of chlorophyll is emphasized by the fact that the photosynthetic rate is proportional to cell chlorophyll concentration over a wide range, according to Bonner and Galston (6). They suggested that the amount of chlorophyll content of the cell is perhaps more closely associated with photosynthetic performance than any other internal factors.

Chlorophyll is not a stable substance as Terrien, et al. (46) stated. The amount of chlorophyll in leaves not only varies from species to another but also varies within a variety under different circumstances. Campbell (7) reported that a varietal difference in the chlorophyll content of Winesap leaves was more sensitive to external factors than that of the Jonathan variety when the same spray materials were used.

There was no evidence from this experiment to suggest it but it seems possible that certain chemicals applied as miticides might be absorbed by the leaves and influence chlorophyll formation. No evidence was found in the literature to support or disprove this idea.

SUMMARY AND CONCLUSIONS

The chemicals Aramite, Parathion, and DN-111 were each applied at five dates to 14-year-old apple trees at the rates recommended by the manufacturers. Kelthane was applied only at one date. Quantitative analyses were made at each of five dates to determine the effect of the chemicals on the chlorophyll content of the leaves.

1. The chlorophyll content of the apple leaves was variable between dates; the variation being significant between some dates.

2. Aramite-and Kelthane-sprayed leaves at some sampling dates had significantly greater amounts of chlorophyll than the unsprayed checks and those leaves sprayed with the other chemicals. The increase in chlorophyll was likely due to the effectiveness of the chemicals as miticides.

3. A considerable seasonal variation in leaf chlorophyll levels was observed in all leaves regardless of chemical treatment. There was a general increase in the chlorophyll readings with advancing age of the leaves at the first four sampling dates. Prolonged high temperatures and high light intensities in the middle of the test period appeared to slow temporarily increases in the total chlorophyll content.

4. A significant variance in the interaction factor suggests the influence of external factors on the results of this experiment. These probably include in addition to temperature and light intensity: soil moisture, soil nutrients, and relative humidity.

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To those and others who have aided in this problem, but not especially mentioned, the writer wishes to express his thanks.

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

by

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Korea, 1950

AN ABSTRACT OF A THESIS

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1958

The primary purpose of this study was to find whether or not the chlorophyll content of Jonathan apple leaves was affected quantitatively by applications of certain miticides.

Thirty 14-year-old Jonathan trees growing in the orchard at the Horticulture farm near Manhattan were assigned spray treatments at random at the time the leaves had fully expanded in late June, 1957. Five treatments, consisting of six trees per each treatment, were compared. They included (1) one pound of Kelthane, (2) one and one-half pounds of Aramite, (3) one pound of Parathion, (4) one-fourth pound of DN-111, and (5) the checks to which no spray was applied. The rate of all miticides listed was for 100 gallons of water. A single application of Kelthane was made on July 9. The other three materials were applied July 9 and 24, August 5 and 19, and September 2.

Thirty leaves were collected from each tree, and discs one centimeter square were cut from near the midsection of the leaf blade and plunged into Methanol to keep them for 24 hours for extraction. Chlorophyll determinations were made on each July 23, August 4 and 18, and September 1 and 13. The readings of the total chlorophyll content of leaves calculated as milligrams per liter were expressed in milligrams per square meter of leaf area.

It was found by an analysis of variance that the variation in the amount of chlorophyll between dates and between treatments was significant at the one per cent level. The variance of interaction was significant at the five per cent level, suggesting the influence of factors not measured in this experiment.

Leaves sprayed with Aramite were significantly higher in chlorophyll on July 23 than the unsprayed check leaves. These values at this date were greater also than for the leaves receiving the other sprays although the differences were not significant. Parathion-treated leaves had significantly less chlorophyll on September 13 than those receiving the Aramite sprays. The chlorophyll values for the check leaves barely missed being significantly lower than Aramite-treated leaves on this date.

The Kelthane-treated leaves ranked second in average chlorophyll content at each of the first three sampling dates but were not significantly different from other treatments. However, on September 1, leaves sprayed with this material showed significantly higher chlorophyll values than those for the other treatments. Also, on September 13 significantly greater amounts of chlorophyll were found in Kelthane-sprayed leaves than in the unsprayed check and those treated with Parathion.

The increase in chlorophyll, as noted in the leaves sprayed with Aramite and Kelthane, is probably due to the effectiveness of these materials as miticides compared with the other materials. This is supported by mite counts taken at the beginning, in the middle, and near the end of the test period.

A considerable seasonal variation in leaf chlorophyll levels was observed in all leaves regardless of chemical treatment. There was a general increase in the values of chlorophyll readings with advancing age for leaves at the first four sampling dates. Prolonged high temperature and high light intensities in the middle of the test period appeared to slow temporarily, increases in the total

chlorophyll content.

A significant variance in the interaction factor suggests the influence of external factors on the results of this experiment. These probably include, in addition to temperature and light intensity: soil moisture, soil nutrients, and relative humidity.