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THE INFLUENCE OF SOME SPRAY MATERIALS ON THE INTERNAL STRUCTURE AND CHLOROPHYLL CONTENT OF APPLE LEAVES

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PART I. INTERNAL STRUCTURE OF APPLE LEAVES

INTRODUCTION

The photosynthetic activity of apple leaves and the effect of certain spray residues on the rate of photosynthesis has been the subject of much research in recent years. It has long been known that a great many factors affect the photosynthetic activity of foliage leaves. The factors affecting photosynthesis include the carbon dioxide supply, light, temperature, water supply, chlorophyll content, and the internal factors. In general, it has been found that the residues have caused a reduction in the rate of photosynthesis.

The object of the studies reported in this paper is to attempt to discover some of the reasons for this reduction.

Pickett *(40)*[2] stated that more extensive intercellular space in apple foliage is reflected in greater photosynthetic activity and suggested that the superficial area of the intercellular boundaries thus may be a factor governing at least partly the rate of photosynthesis. He suggested that the more extensive mesophyll of the orchard-grown leaves may be one of the contributing factors in enabling them to be more active in carbon assimilation than the greenhouse-grown leaves. The extent of the internally exposed surface of apple leaves is more important than the chlorophyll content as a factor partially governing photosynthetic activity, according to Pickett and Kenworthy *(45)*.

Heinicke *(22, 23),* Hoffman *(26, 27, 28),* Christopher *(5, 6),* Brody and Childers *(3),* Agnew and Childers *(1),* Southwick and Childers *(51),*and others reported that spray materials reduce photosynthetic activity in foliage leaves and that the stronger the spray material, the greater the reduction in rate of photosynthesis.

Pickett and Birkeland *(43, 44),* reported that the ratio of the internally exposed surface to the externally exposed surface of both greenhouse-grown and field-grown apple leaves is reduced by the repeated application of certain spray materials, and stated that spray materials shock or check normal cell development in apple leaves with each application throughout the growing season, and

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ACKNOWLEDGMENT.—A. L. Kenworthy, former graduate assistant, collected the data in 1938, and E. E. Saunders, former graduate assistant, made the chlorophyll determinations.

1. Contribution No. 186, Department of Horticulture.

2. Italicized figures in parentheses refer to Literature Cited, p. 51.

that the so-called mild sprays do not exert so great a dwarfing effect as do the stronger materials.

This investigation was undertaken in an effort to determine (1) the ratio of the internally exposed surface to the external surface of the leaves of certain varieties of apples, (2) whether the leaves of the varieties used differed in their internal structure, (3) whether spray residues had an effect on the internal structure, (4) whether various spray residues affect the internal structure to different degrees, (5) whether varieties differed in their susceptibility to different sprays, (6) at what stage in the development of the leaf the effect of the spray residues was greatest, and (7) some measurement that may be directly correlated with the ratio of the internally exposed surface to the external surface in order that the volume of necessary data and the time ordinarily required would be reduced.

MATERIALS AND METHODS

The following procedure was used in collecting and sectioning leaves. Portions of the leaves used for microscopic study were located near the midrib and midway between the basal and apical regions. The marginal and midrib portions of each leaf were discarded. These leaf pieces were placed in a 1 percent chromo-acetic acid killing and fixing solution. After 24 hours, they were washed, dehydrated with N-butyl alcohol, and imbedded in paraffin. All sections were eight microns thick, and were stained with safranine O.

Drawings. In order to determine the internally exposed surface of a leaf, drawings must be made from the slide at a high magnification. When these drawings are measured and the magnification is known, the amount of internally exposed surface is readily calculated. The drawings for the preliminary work on this problem were made by using a drawing ocular at a magnification of 440 diameters. Since these measurements were variable, a camera lucida was selected for making drawings which would be higher in magnification and from which more accurate measurements could be made. Each set of drawings included the following: one drawing of a field 50 microns square of each of the first, second, and third layers of palisade cells from a tangential section; one drawing of a field of the spongy mesophyll 50 microns square from a tangential section; and one drawing of a field 50 microns wide, across the spongy mesophyll, in cross section (Fig. 9). The drawings were made by using a camera lucida at a magnification of approximately 1760 diameters.

At first it was rather difficult to determine which layer of palisade cells was in the field when making the tangential drawings. Cross sections of each variety showed that the epidermis dipped where veins were present, and that all leaves of the Wealthy contained three layers of palisade cells, but in a large percentage of the York, the third layer of palisade cells was extremely few in number or missing (Figs. 1 and 2). Above small veins the epidermis was found to consist of a number of layers, sometimes as many as five or six

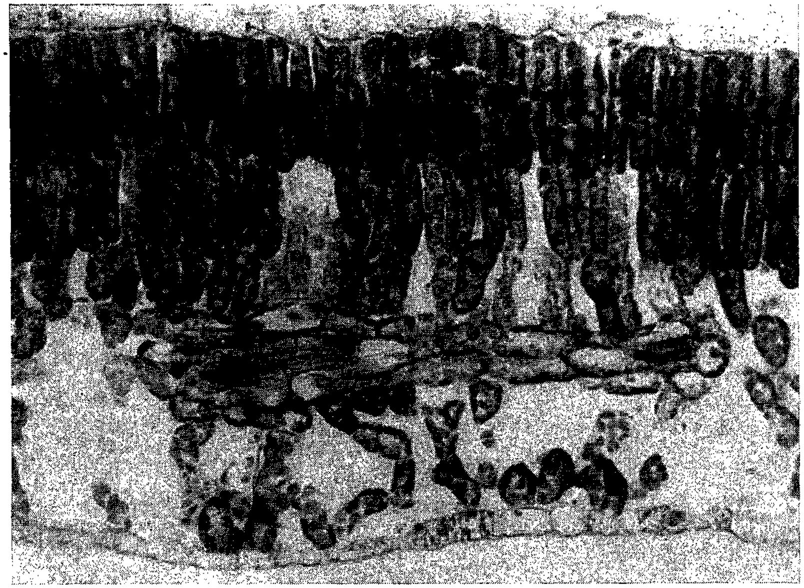

FIG. 1.—Cross-section of Wealthy unsprayed greenhouse leaf (× 315). R value = 14.74; P value = 120.96 microns; N value = 30. 1940.

FIG. 2.—Cross-section of Wealthy sprayed greenhouse leaf (× 315). R value = 8.81; P value = 87.84; N value = 39. 1940.

layers of cells above the parenchyma cells surrounding the bundle, while above large veins the epidermis consisted of a single layer above the parenchyma cells surrounding the bundle. This characteristic of the epidermis was used in determining the layer number of palisade cells, in tangential sections, as outlined below.

Palisade cells were considered to be of the first layer when found in a microscopic field adjacent to cells which were definitely of the

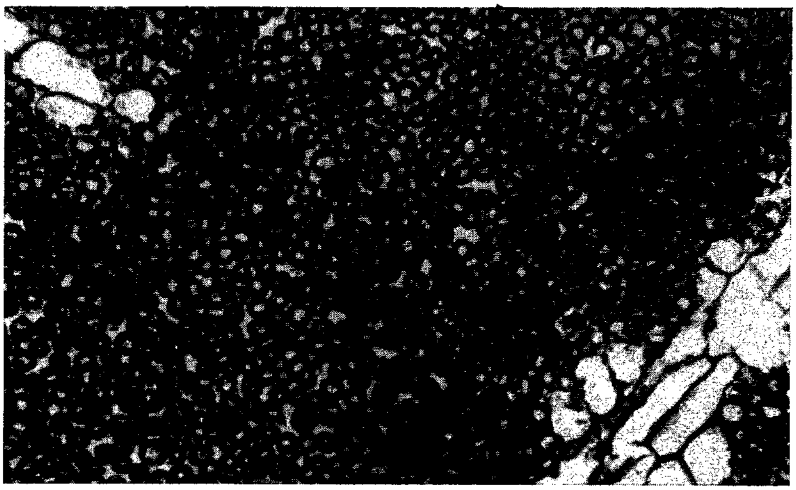

FIG. 3.—Tangential section through the first layer of palisade, York unsprayed greenhouse leaf (× 315). 1940.

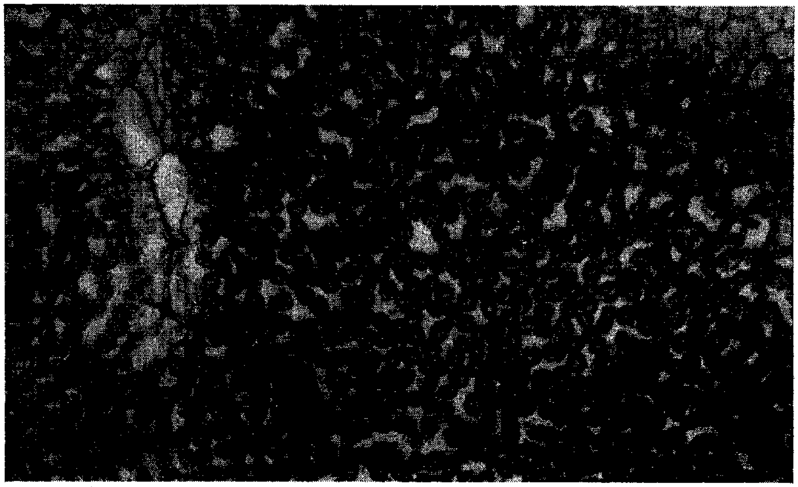

FIG. 4.—Tangential section through the first layer of palisade, York sprayed greenhouse leaf (× 315). 1940.

upper epidermis but not near a vein. The first layer of palisade cells was also considered to be that found in fields which showed vein tracings of epidermal like cells, but including no tracheids (Figs. 3 and 4).

Cross sections of the leaves contained druses or inclusions which were in the second layer of palisade cells only. Such druses or in-

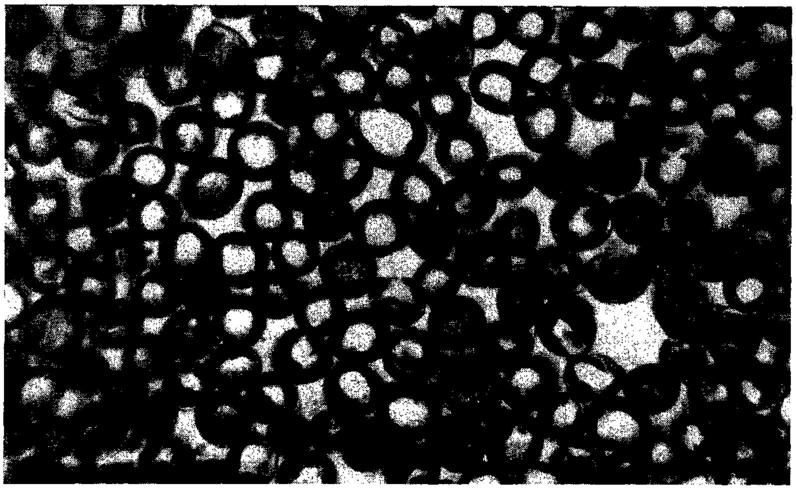

FIG. 5.—Tangential section through the second layer of palisade, Wealthy unsprayed greenhouse leaf (× 700). 1940.

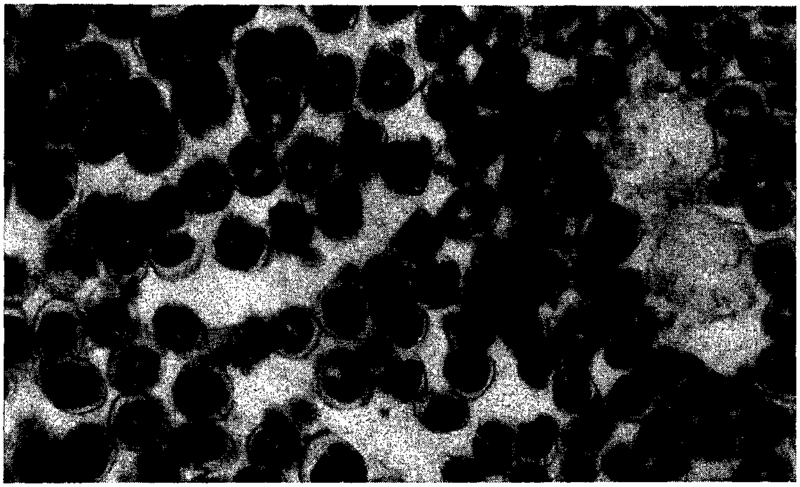

FIG. 6.—Tangential section through the second layer of palisade, Wealthy sprayed greenhouse leaf (× 700). 1940.

clusions were significant in locating fields of the second layer of palisade cells. Also microscopic fields which contained veins with tracheids that disappeared toward the upper palisade cells were considered to be of the second layer (Figs. 5 and 6).

When cross sectional measurements showed no third layer of palisade cells, or too few to be counted, no tangential drawings were made. When present, the third layer was determined in a similar

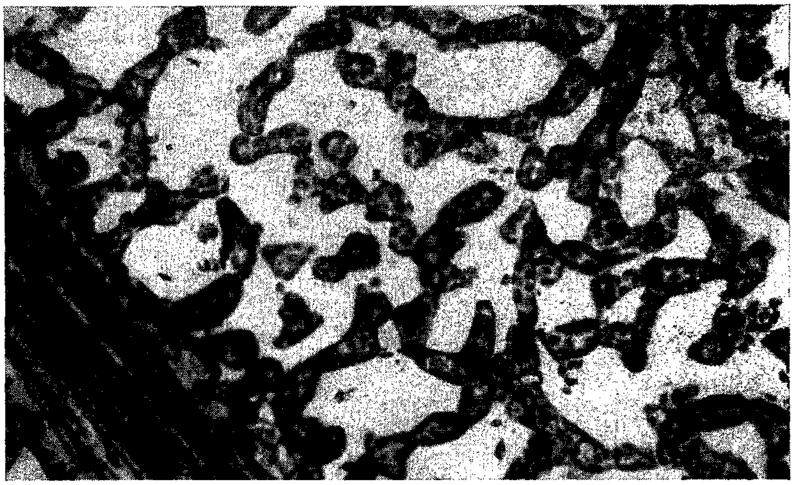

FIG. 7.—Tangential section through spongy mesophyll of Wealthy unsprayed greenhouse leaf (× 315). 1940.

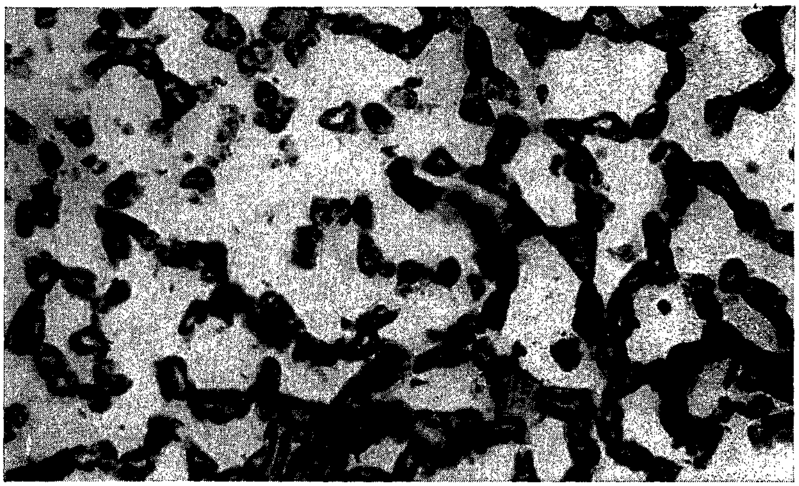

FIG. 8.—Tangential section through spongy mesophyll of Wealthy sprayed greenhouse leaf (× 315). 1940.

manner as the second layer. If the cells of a microscopic field in the palisade region contained veins with tracheids which disappeared when the field was moved toward the spongy mesophyll, they were considered to be the third layer. The third layer of palisade was always less compact than the first and second layers; this facilitated differentiation between the second and third layers of palisade cells.

Microscopic fields that were free of veins were used for drawings of the spongy mesophyll (Figs. 7 and 8). Regions that showed the lower epidermis intact were used for the cross sectional drawings. Palisade cells were drawn and labeled as such when in contact with cells of the spongy mesophyll, but were not measured.

Measurements. Measurements of the drawings were made with a chartometer and a planimeter. The following formula used by Turrell *(54)* for computing the ratio (R) of the internally exposed surface to the external surface of mesomorphic leaves, requires these measurements :

$$R = \frac{(lp) + (l_1 p_1) + (l_2 p_2) + L\left(hc + 2A\frac{l_e}{l_t}\right) + (K^2 - A)\frac{l_i}{K}}{2K^2}$$

The following symbols, stated briefly, represent the foregoing measurements:

p = Exposed perimeter of upper palisade cells in tangential section;
p_1 = Exposed perimeter of second layer of palisade cells in tangential section;
p_2 = Exposed perimeter of third layer of palisade cells in tangential section;
l = Average length of 10 cells, in cross section, of the upper palisade cells; measured directly with eyepiece micrometer;
l_1 = Average length of 10 cells, in cross section, of second layer of palisade cells; measured directly with eyepiece micrometer;
l_2 = Average length of 10 cells, in cross section, of third layer of palisade cells; measured directly with eyepiece micrometer;
L = Average number of tiers of cells in the spongy mesophyll in cross section;
A = Average area of cells of spongy mesophyll in tangential section;
c = Average length of the exposed cell wall in tangential section of the spongy mesophyll;
h = Average length of vertically exposed cell walls in spongy mesophyll of cross section;
l_e = Total length of exposed cell walls making an angle greater than 45 degrees with the vertical in cross section of the spongy mesophyll;
l_t = Total length of exposed and nonexposed cell walls making an angle greater than 45 degrees with the vertical in cross section of spongy mesophyll;
l_i = Average length of inner wall of lower epidermis in cross section;
K = Constant, length of one side of sample area.

All measurements except l, l_1, l_2, and A were recorded in centimeters. The measurements l, l_1, l_2, and were recorded in microns, and measurement A was recorded in square inches. All measurements were transposed to microns or square microns before computing R. The measurements l_e, l_t, l_i, and K were used in ratios of $\frac{l_e}{l_t}$ and $\frac{l_i}{K}$ and these ratios were computed from the centimeter measurements.

The following tissue measurements may be computed from the formula: The internally exposed surface of the palisade, $(lp) + (l_1p_1) + (l_2p_2)$; the internally exposed surface of the spongy mesophyll, $L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_1}{K}$; the horizontally exposed surface of the spongy mesophyll, $L(hc)$; the vertically exposed surface of the spongy mesophyll, $L(2A\frac{l_e}{l_t})$; and the exposed surface of the lower epidermis, $(K^2 - A)\frac{l_1}{K}$.

The foregoing measurements were substituted in the formula and the ratio (R) of the internally exposed surface to the external surface was computed for each individual page of drawings. In Table 1 are presented measurements taken from the drawings in Figure 9.

The number of cells in the cross section drawings of the spongy mesophyll was needed to compute the average vertically exposed cell wall (h), and the number of cells in the tangential drawings of the spongy mesophyll was needed for the computation of the average exposed surface (c). These values were given no symbol in the formula as they were used in determining the value of the above measurements before they were used in the formula.

TABLE 1. *Measurements and calculations of R for drawings in Figure 9.*

SYMBOL.	Centimeters.	Microns.	Symbol.	
p	98.0	589.96	l	62.52 microns.
p_1	97.0	583.94	l_1	42.48 microns.
p_2	78.0	469.56	l_2	33.36 microns.
c	6.8	40.94	A	196.35 microns.
h	5.5	33.11	L	5.00 av. number cells.

$$\frac{l_e}{l_t} = \frac{33.7}{37.5} = 0.90 \qquad \frac{l_1}{K} = \frac{8.9}{8.3} = 1.07$$

CALCULATION OF R

$$\begin{aligned}
\text{Area in palisade} &= \Sigma lp = l \times p + l_1 \times p_1 + l_2 \times p_2 \\
&= 62.52 \times 589.96 + 42.48 \times 583.94 + 33.36 \times 469.56 \\
&= 77{,}354.59 \text{ sq. microns} \\
\text{Area in sponge} &= L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_1}{K} \\
&= 5(33.11 \times 40.94 + 392.7 \times 0.90) + (2{,}500 - 196.35)\ \ 1.07 \\
&= 11{,}009.66 \text{ sq. microns}
\end{aligned}$$

$$\begin{aligned}
R &= \frac{\Sigma lp + L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_1}{K}}{2K^2} \\
&= \frac{77{,}354.59 + 11{,}009.66}{5{,}000} \\
&= 17.67
\end{aligned}$$

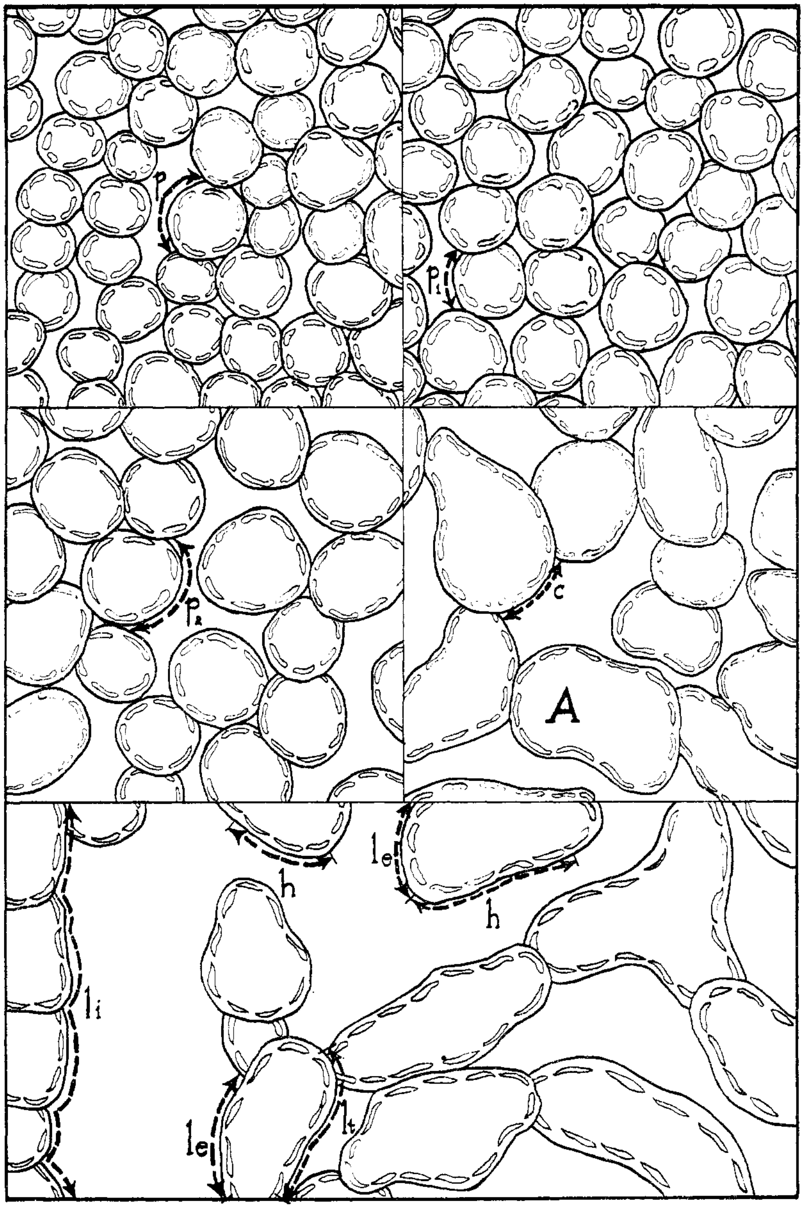

FIG. 9.—Representative set of camera lucida drawings (× 1,760) from a Wealthy apple leaf. Upper left—first layer of palisade cells in tangential section. Upper right—second layer of palisade cells in tangential section. Center left—third layer of palisade cells in tangential section. Center right—spongy mesophyll in tangential section. Bottom—area 50 microns wide across spongy mesophyll in cross section. Refer to page 11 for explanation of symbols.

The outside diameter of the upper layer of palisade cells was measured and given symbol D. These measurements were taken from the drawings in microns. The average number of cells of the upper layer of palisade in each drawing was also computed and given the symbol N. Each drawing represented 2,500 square microns of leaf area. The total depth of palisade mesophyll in microns was given the symbol P.

PRESENTATION OF DATA

Greenhouse Leaves, 1938. In January, 1938, twelve two-year-old trees of each of Wealthy, Jonathan, and York varieties of apples were planted in 12-inch clay pots, and plunged into a ground bed in the greenhouse.

In June, 1938, leaves from these trees were collected and twenty-five pages of drawings were made for each variety. Measurements were taken in the same manner as shown in Figure 9. The measurements of the length of palisade cells gave the values recorded in Table 2.

TABLE 2. *Length of palisade cells—microns (average of 250 cells each). Greenhouse-grown leaves, 1938.*

PALISADE LAYER.	Variety.		
	Wealthy.	Jonathan.	York.
First (l)	47.50	38.50	31.20
Second (l_1)	36.30	30.50	24.60
Third (l_2)	25.50	20.20	18.30
Totals	109.30	89.20	74.10

In regard to the length of palisade cells in each layer, as given in Table 2, the varieties ranked: Wealthy, Jonathan, and York. Wealthy had a depth of palisade tissue sufficiently greater than Jonathan to cause it to have a larger total internally exposed surface in the palisade mesophyll than Jonathan, and Jonathan ranked above York.

The average diameter of each layer of palisade mesophyll cells for each variety is recorded in Table 3. This table shows a consistent increase in diameter of palisade cells from the first to the last layer. The diameter was also consistently greatest in Wealthy and least in Jonathan.

TABLE 3. *Diameter of palisade cells—microns (average of 250-500 cells each). Greenhouse-grown leaves, 1938.*

PALISADE LAYER.	Variety.		
	Wealthy.	Jonathan.	York.
First (D)	7.97	7.44	7.83
Second (D_1)	9.01	7.88	8.64
Third (D_2)	10.44	9.44	10.10

The number of cells in cross and tangential sections, from which the compactness of the spongy mesophyll may be computed, and the average area of the cells in tangential section showed that Jonathan had the most compact spongy mesophyll and the smallest cells. Wealthy was approximately of the same compactness but had the largest cells. The York leaves had the least compact spongy mesophyll and the average area of the cells was greater than those of the Jonathan.

The distribution of internally exposed surface in the spongy mesophyll shows a consistent rating of Wealthy, Jonathan, and York varieties in regard to horizontal, vertical, and total exposed surface in the spongy mesophyll. The Jonathan had a greater area of lower epidermis exposed than the Wealthy leaves, with the York leaves having the least.

From the above measurements, the percentage of the total internally exposed surface located in each region or plane of the leaf was calculated. These calculations are shown in Table 4.

TABLE 4. *Percentage distribution of internally exposed surface (calculated from mean of 25 measurements). Greenhouse-grown leaves, 1938.*

PLANE OF EXPOSURE.	Variety.		
	Wealthy.	Jonathan.	York.
First layer	40.84	40.78	40.61
Second layer	28.62	31.36	28.30
Third layer	15.17	14.10	15.96
Total palisade mesophyll	84.63	86.24	84.87
Total spongy mesophyll	15.37	13.76	15.13

The palisade mesophyll contained about 85 percent of the total internally exposed surface (Table 4). The Jonathan variety contained the largest percentage of surface in the palisade mesophyll, and the greatest amount in the second layer of palisade. The Wealthy variety contained the largest percentage of surface in the spongy mesophyll with the greatest amount of exposed inner wall of the lower epidermis of these varieties.

From the preceding measurements, the ratio of the internally exposed surface to the external surface, and the ratio of the surface exposed in the palisade region to the surface exposed in the spongy mesophyll were computed.

The Wealthy leaves had the highest ratio of internally exposed surface to external surface and the York had the lowest. Jonathan leaves had the highest ratio of internally exposed surface in the palisade mesophyll to the exposed surface of the spongy mesophyll while York had the lowest ratio. The ratios were less than for field leaves except for the Wealthy variety. There were significant differences between all varieties. There were no significant differences between P/S values of the varieties.

Greenhouse Leaves, 1940. In January, 1940, 22 two-year-old trees of Wealthy and 21 two-year-old trees of York varieties of apples were planted in 12-inch clay pots, and plunged into a ground bed in a greenhouse. Soon after the buds started to grow, eleven trees of Wealthy and ten trees of York were sprayed with a mixture of 2 1/2 gallons of liquid lime-sulphur and 4 pounds of lead arsenate per 100 gallons of water. This spray was repeated at weekly intervals for 12 weeks. Relative humidity readings were taken at each application and ranged from 42 to 66 and the temperature ranged from 73° F. to 99° F.

In April, 1940, five apparently uninjured leaves were collected from each of the greenhouse trees, the leaves selected being from near the middle of the new shoots and of average size for the tree. Only one piece, about 1 by 3 centimeters, was taken from each single leaf.

Preparation of Slides. Five sets of slides were made for each tree. A set of slides consisted of one slide with cross sections and one slide with tangential sections, each made from the same leaf piece.

Drawings. Two pages of drawings were made from each set of slides resulting in a total of 430 pages of drawings. Measurements of leaves of Wealthy and York trees were made as described in the materials and methods section. The average R, P, N, and D values are recorded in Table 5.

TABLE 5. *Average R, P, N, and D values for greenhouse-grown leaves, 1940.*

VARIETY.	Treatment.	R.	P in microns.	N.	D in microns.
Wealthy	Unsprayed	13.40	104.49	32.48	9.95
Wealthy	Sprayed	10.71	90.86	34.80	8.82
York	Unsprayed	9.44	68.84	35.85	9.46
York	Sprayed	6.88	52.92	39.35	8.36

The Wealthy leaves had a greater R value, longer palisade cells, wider upper palisade cells, and fewer upper palisade cells in an equal area than the York variety (Table 5). Within each variety, the sprayed leaves showed lower R, P, and D values and a greater N value than the unsprayed leaves. These values were derived from the averages of a total of 430 pages of camera lucida drawings from 43 trees.

An analysis of variance was made of each of the following values: R, P, N, and D. Individual tree variation mas compared with variation due to varieties and treatments, to determine whether varieties differed in these values, and whether spray residues had any effect on them. For each of these values the variability or mean square between varieties was highly significantly greater than the variability between trees, and it may be assumed that the mean differences found were due to varietal instead of tree variation. When considering treatments, the variability between treatments was highly significantly greater than the variability between trees for each of the above values also, indicating that the mean differences found were due to treatment variation rather than tree variation. Representative photomicrographs of this series of leaves are shown in Figures 1 to 8, inclusive.

Field Leaves, 1940. In March, 1940, 6 Wealthy, 10 Jonathan, and 10 York two-year-old trees were planted in the field. When the buds on the field trees had started to grow, one-half of the trees of each variety were sprayed at weekly intervals and the rest were left unsprayed. These sprays consisted of 6 applications of 4 pounds of lead arsenate and 2 1/2 gallons of liquid lime-sulphur per 100 gallons of water, 3 applications of 4 pounds of lead arsenate alone per 100 gallons of spray, and 5 applications of 4 pounds of lead arsenate and 2 gallons of oil emulsion per 100 gallons of spray. Temperature readings ranged from 62° F. to 98° F. and relative humidity readings ranged from 23 to 65.

On July 1, 1940, after the last spray in which lead arsenate alone was used, and on August 5, 1940, at the end of the season, five leaves were collected from each of the field trees in the same manner as for the greenhouse trees.

Measurements for the R, P, N, and D values are recorded in Table 6.

The Wealthy had a greater R value, longer and wider palisade cells, with fewer upper palisade cells per given area than the York variety (Table 6). The Jonathan was intermediate in R, P, and N, but the cells were slightly smaller in diameter than in the York. Within each variety and without exception, the sprayed trees showed a lower R value; the palisade cells were less in diameter and length, and greater in number per equal area than the check trees. These values were based on measurements from 260 pages of camera lucida drawings from 26 trees.

TABLE 6. *Average R, P, N, and D values for field-grown leaves, 1940.*

VARIETY.	Treatment.	R.	P in microns.	N.	D in microns.
Wealthy	Unsprayed	15.30	117.08	31.57	10.24
Wealthy	Sprayed	11.96	103.64	35.93	8.20
Jonathan	Unsprayed	13.54	99.60	35.72	9.34
Jonathan	Sprayed	10.26	83.29	37.52	7.81
York	Unsprayed	11.63	83.35	36.78	9.64
York	Sprayed	7.71	60.93	37.06	8.25

An analysis of variance was made of each of the following values: R, P, N, and D. Individual tree variation was compared with variation due to varieties and treatments, to determine whether varieties differed in these values, and whether spray residues had any effect on them.

For R and P, the variability between varieties was so much greater than the variability between trees that one may assume the mean differences found were due to varietal variation rather than tree variation. The variability of N was significant, but there was no significant difference in the variability of the D values. When taking treatment into consideration, the variability between treatments was highly significantly greater than the variability between trees for R, P, and D, indicating the mean differences found were due to treatment variation rather than tree variation. The variability of N was significant.

Covariance, correlation, and regression between the total depth of palisade and the R values for all varieties, treatments, and conditions were calculated to determine whether a short cut could be found for determining the R values from P values. The estimate of the R value or regression coefficient equaled 0.1122P +1.33, and the correlation between P and R was +.88, which is very highly significant. Using this regression coefficient, the ratio of the internally exposed surface to the external surface may be computed from the total depth of palisade mesophyll (Fig. 10).

The midseason group of leaves, taken July 1, 1940, after the last lead arsenate spray, were collected and used as a check on accuracy of sampling and also to determine whether the spray materials had an accumulative effect as the season progressed. For this group of leaves, treated in the same manner as the end-of-season field leaves, only the palisade lengths were measured. The means of their total depth of palisade are compared with the corresponding value of the leaves from the same trees at the end of the season in Table 7.

The differences between unsprayed and sprayed leaves in a variety were approximately the same at midseason as they were at the end of the season (Table 7). The P value for the unsprayed Wealthy leaves was a little higher at the end of the season than at midseason, but the unsprayed Jonathan leaves were somewhat less at the end of the season than at midseason. The York values were virtually identical for both periods.

A comparison of the means of the Wealthy and York varieties in the greenhouse and in the field is presented in Table 8.

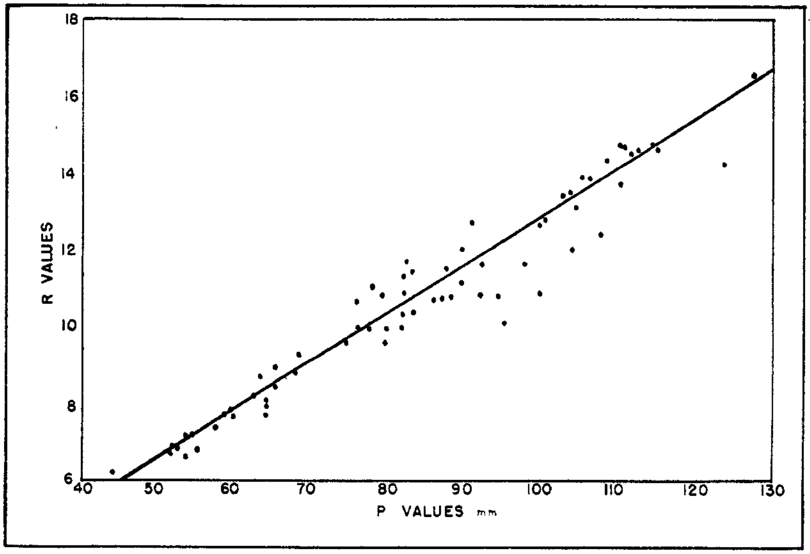

FIG. 10.—Regression line for R and P values for sprayed and unsprayed, field- and greenhouse-grown leaves, 1940. Correlation coefficient $= +.88$ and the regression coefficient $= 0.1122P + 1.33$.

TABLE 7. *Means of total depth of palisade, measured in microns, for field leaves at midseason and at end of season. 1940.*

VARIETY.	Treatment.	Midseason.	End of season.
Wealthy	Unsprayed	111.27	117.08
Wealthy	Sprayed	102.32	103.64
Jonathan	Unsprayed	107.11	99.60
Jonathan	Sprayed	78.37	83.29
York	Unsprayed	82.49	83.35
York	Sprayed	60.08	60.93

The R value and the total depth of palisade layers were greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse (Table 8). The number of cells of the upper palisade per unit area and their diameters showed no great differences as to location.

TABLE 8. *Mean R, P, N, and D values for both varieties and treatments in the greenhouse and in the field. 1940.*

VARIETY.	Treatment.	Location.	R.	P in microns.	N.	D in microns.
Wealthy.....	Unsprayed....	Field...........	15.30	117.08	31.57	10.24
Wealthy.....	Unsprayed....	Greenhouse.....	13.40	104.49	32.48	9.95
Wealthy.....	Sprayed......	Field...........	11.96	103.64	35.98	8.20
Wealthy.....	Sprayed......	Greenhouse.....	10.71	90.86	34.80	8.82
York........	Unsprayed....	Field...........	11.63	83.35	36.78	9.64
York........	Unsprayed....	Greenhouse.....	9.44	68.84	35.85	9.46
York........	Sprayed......	Field...........	7.71	60.93	37.06	8.25
York........	Sprayed......	Greenhouse.....	6.88	52.92	39.35	8.36

Greenhouse Leaves, 1941. In February, 1941, 20 two-year-old trees of each of the Wealthy and York varieties were planted in a ground bed in a greenhouse. Within two or three days after the first new leaves began to appear, one-half of each of ten trees of each variety were sprayed at weekly intervals with a spray composed of 2 1/2 gallons of 33° Beaume liquid lime-sulphur and 4 pounds of arsenate of lead in 100 gallons, and one-half of each of the other ten trees of each variety were sprayed at weekly intervals with a spray composed of 0.25 percent Verdol summer oil spray and 4 pounds of arsenate of lead in 100 gallons. The other half of all 40 trees were left unsprayed.

Three days after the second, fourth, seventh, eighth, and ninth applications, four leaves were collected from each tree, both sprayed and unsprayed portions, for microscopical study. At the tenth spray application, some hitherto unsprayed leaves ten weeks of age were given this application and three days later the final collection of ten leaves was made from each tree.

Only one piece, about 1 by 3 centimeters, was taken from each leaf. One cross-section slide was made from each leaf piece, since the work on the 1940 series indicated that the ratio of the internally exposed surface to the externally exposed surface could be calculated from total palisade depth. A large number of tangential slides were also made, however, for the purpose of comparison with the former work.

The ratio of the internally exposed surface to the externally exposed surface (R) was estimated from the total palisade depth (P) by using $E(R) = 0.1122P + 1.33$. This estimate of R was determined from much previous work substantiated by samples of these data and has a standard error of forecast, *i.e.*, $S(E_R) = 0.249$.

Measurements of leaves of Wealthy and York trees planted in the greenhouse were made as described in the materials and methods section. The average total depth of palisade tissue, P, and the average ratio of the internally exposed surface to the exterior surface R, for all the greenhouse leaves, are recorded in Table 9.

The Wealthy leaves had greater P and R values than leaves of the York variety and each spray material reduced these values of each variety (Table 9).

TABLE 9. *Average P and R values for greenhouse-grown leaves, 1941.*

Value.	York.				Wealthy.			
	Liquid lime-sulphur and lead arsenate.		Oil and lead arsenate.		Liquid lime-sulphur and lead arsenate.		Oil and lead arsenate.	
	Unsprayed.	Sprayed.	Unsprayed.	Sprayed.	Unsprayed.	Sprayed.	Unsprayed.	Sprayed.
P	73.10	60.60	73.74	57.55	115.42	100.68	114.67	99.14
R	9.53	8.13	9.60	7.79	14.28	12.63	14.20	12.45

An analysis of variance of the total depth of palisade tissue was made. The within variation was compared with variation due to varieties and treatments, to determine whether varieties differed in their susceptibility to the spray materials, and whether the spray materials differed in their effect on either variety. This analysis indicated that there was no significant difference between varieties in regard to their susceptibility to different spray materials. There was a highly significant difference between oil and liquid lime-sulphur on York, but no difference on the Wealthy.

Using standard deviation and the "t" value, it was found that the differences between the varieties were highly significant, and the differences between sprayed and unsprayed leaves were highly significant.

A series of leaves was collected after each spray application to determine the stage in the development of the leaves in which the sprays had their greatest effect. These figures are given in Table 10 for each variety, treatment, and collection.

The general trend of the data in Table 10 indicates that the spray applications prevented normal development of the palisade tissue of the apple leaves, having gradually increasing deleterious effects as the season progressed. Leaves which were 10 weeks old before they received a spray application were checked rather severely and held at about the value of the unsprayed leaves at the fifth collection, while the palisade tissue in unsprayed checks continued to grow.

The total depth of palisade tissue data in Table 10 were converted to R values by the method previously described and charted on a graph to illustrate the normal growth of the leaf and the successive retarding effect of the weekly spray applications (Fig. 11).

TABLE 10. *Depth of palisade tissue, microns, of sprayed and unsprayed greenhouse-grown apple leaves, 1941.*

VARIETY.	Treatment.	Leaves collected after spray application.						First spray on leaves 10 weeks old.
		No. 2.	No. 4.	No. 7.	No. 8.	No. 9.	No. 10.	
York.	Unsprayed	39	45	54	62	63	73	
	Liquid lime-sulphur and lead arsenate	36	47	48	49	55	61	65
	Unsprayed	43	42	59	56	63	74	
	Oil and lead arsenate	39	41	49	58	55	58	66
Wealthy.	Unsprayed	55	79	98	104	110	115	
	Liquid lime-sulphur and lead arsenate	58	72	87	86	94	101	111
	Unsprayed	63	75	96	98	104	115	
	Oil and lead arsenate	60	68	83	87	96	99	105

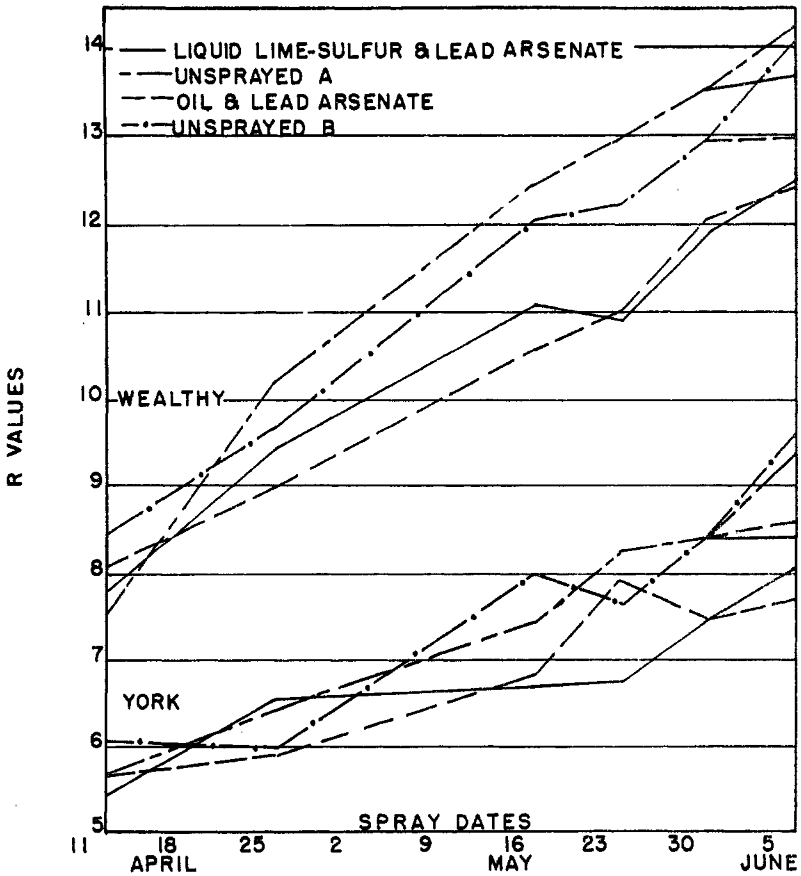

FIG. 11.—Greenhouse-grown leaves, 1941. R values of Wealthy and York varieties at weekly intervals, collected twenty-four hours after spray applications. Liquid lime-sulphur and lead arsenate from same trees as unsprayed A; oil and lead arsenate from same trees as unsprayed B.

Field Leaves, 1941. In April, 1941, 20 two-year-old trees of each of the Wealthy, Jonathan, and York varieties were planted outdoors. Within a few days after the first new leaves began to appear, one-half of each of ten trees of each variety were sprayed at weekly intervals with a spray composed of 2 1/2 gallons of 33° Beaume liquid lime-sulphur and 4 pounds of arsenate of lead in 100 gallons, and one-half of each of the other ten trees of each variety were sprayed at weekly intervals with a spray composed of 2 1/2 pounds of flotation sulphur and 4 pounds of arsenate of lead in 100 gallons of water. The other half of all 60 trees were left unsprayed.

One day after the third, fourth, fifth, sixth, and seventh spray applications, four leaves were collected from each tree, both sprayed and unsprayed portions, for microscopical study. At the eighth spray application, some hitherto unsprayed leaves 10 weeks of age were given this application and the next day the final collection of 10 leaves was made from each tree. At this time also several unsprayed leaves were collected from the median, basal, and apical portions of the shoots on several trees. These field leaves were collected and prepared for microscopic study, using the same method as with the greenhouse-grown leaves.

Measurements of leaves of Wealthy, Jonathan, and York trees planted outdoors were made as previously described. The average total depth of palisade tissue, P, and the ratio of the internally exposed surface to the exterior surface, R, for all field leaves are recorded in Table 11.

The Wealthy leaves had greater R and P values than the leaves of the York variety, and the Jonathan variety was intermediate; each spray material reduced the R and P values of each variety (Table 11).

TABLE 11. *Average P and R values for field-grown leaves, 1941.*

Variety.	Value.	Liquid lime-sulphur and lead arsenate.		Flotation sulphur and lead arsenate.	
		Unsprayed.	Sprayed.	Unsprayed.	Sprayed.
Wealthy	P	114.69	100.67	113.98	105.34
	R	14.20	12.63	14.12	13.14
Jonathan	P	94.70	84.24	94.27	88.92
	R	11.96	10.78	11.91	11.30
York	P	71.06	60.36	64.82	60.84
	R	9.31	8.11	8.60	8.15

An analysis of variance of the total depth of palisade tissue was made. The within variation was compared with variation due to varieties and treatments, to determine whether varieties differed in their susceptibility to the spray materials, and whether the spray materials differed in their effect on the varieties. This analysis of variance indicates that there is no significant difference between varieties in regard to their susceptibility to the different spray materials, and that there is no significant difference between sprays on a given variety. Using the standard deviation and "t" value, the differences between the varieties were highly significant. The differences between York leaves sprayed with flotation sulphur and lead arsenate and the unsprayed were significant, while all other sprays had a highly significant effect.

A series of leaves were collected after each spray application as described in the materials and methods section to determine at what stage in the development of the leaves that the sprays had their greatest effect. These data are given in Table 12, for each variety, treatment, and collection.

TABLE 12. *Depth of palisade tissue, microns, of sprayed and unsprayed field-grown apple leaves, 1941.*

VARIETY.	Treatment.	Leaves collected after spray application.						First spray on leaves 10 weeks old.
		No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	
Wealthy.	Unsprayed	44	67	76	85	116	115	
	Liquid lime-sulphur and lead arsenate	41	62	68	73	90	101	110
	Unsprayed	48	72	86	94	107	114	
	Flotation sulphur and lead arsenate	47	73	90	89	102	105	102
Jonathan.	Unsprayed	42	60	74	81	90	95	
	Liquid lime-sulphur and lead arsenate	33	56	68	72	77	84	95
	Unsprayed	47	75	78	86	92	94	
	Flotation sulphur and lead arsenate	43	70	77	81	82	89	95
York.	Unsprayed	30	50	52	56	62	71	
	Liquid lime-sulphur and lead arsenate	29	44	53	49	52	60	65
	Unsprayed	28	39	49	54	55	65	
	Flotation sulphur and lead arsenate	25	36	45	48	62	61	68

The general trend of the data in the above table indicates that the spray applications prevented normal development of the palisade tissue of the apple leaves, gradually accentuating the difference between unsprayed and sprayed leaves as the season progressed. Leaves collected after eight spray applications showed that the liquid lime-sulphur spray reduced the amount of the internally exposed surface more than the flotation sulphur. Leaves which were ten weeks old before they received a spray application were not checked so severely as in the case of the greenhouse-grown leaves. The total depth of palisade tissue data in Table 12 were con-

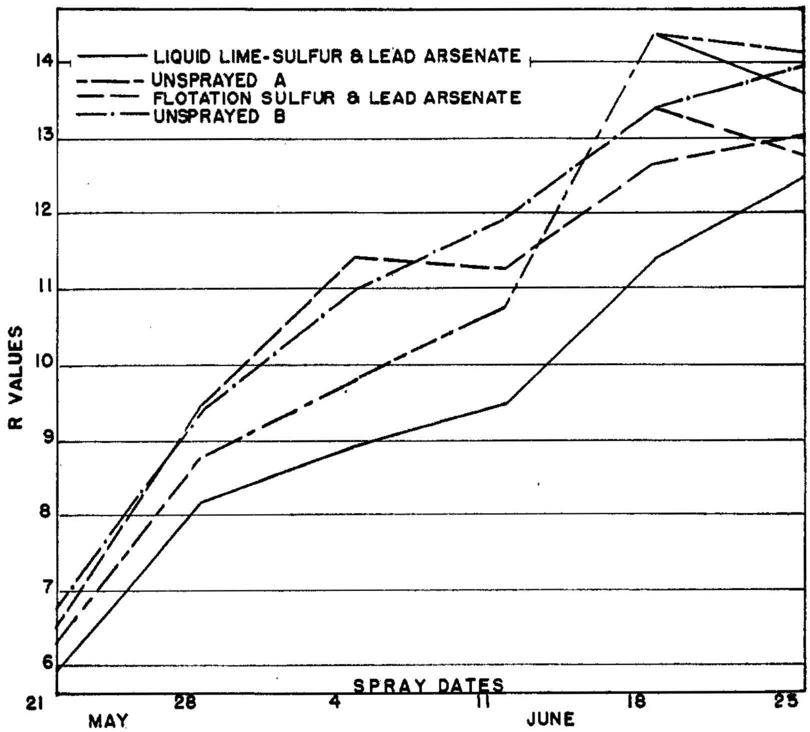

FIG. 12.—Field-grown leaves, 1941. R values of Wealthy variety at weekly intervals, collected twenty-four hours after spray applications. Liquid lime-sulphur and lead arsenate from same trees as unsprayed A; flotation sulphur and lead arsenate from same trees as unsprayed B.

verted to R values by the method already described and charted on graphs to illustrate more clearly the normal growth of the leaf and the successive retarding effect of the weekly spray applications. These data are given in Figures 12, 13, and 14.

Several unsprayed leaves were collected from the median, basal, and apical portions of the shoots on several trees. There was an increase in total palisade depth from the basal towards the apical portion of the shoot in the Wealthy variety, while in the York variety there was an increase from basal to median portions, and then a decrease from the median towards the apical portion (Table 13).

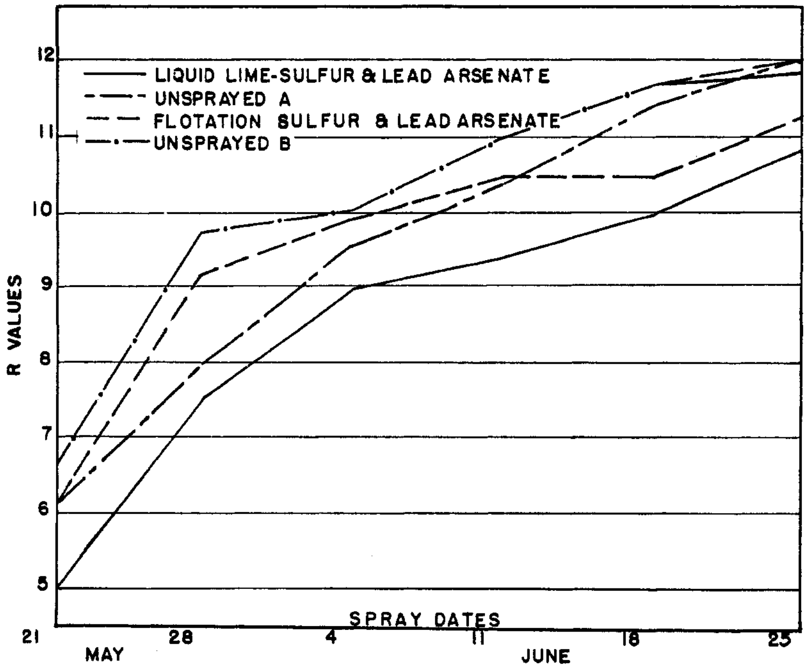

FIG. 13.—Field-grown leaves, 1941. R values of Jonathan variety at weekly intervals, collected twenty-four hours after spray applications. Liquid lime-sulphur and lead arsenate from same trees as unsprayed A; flotation sulphur and lead arsenate from same trees as unsprayed B.

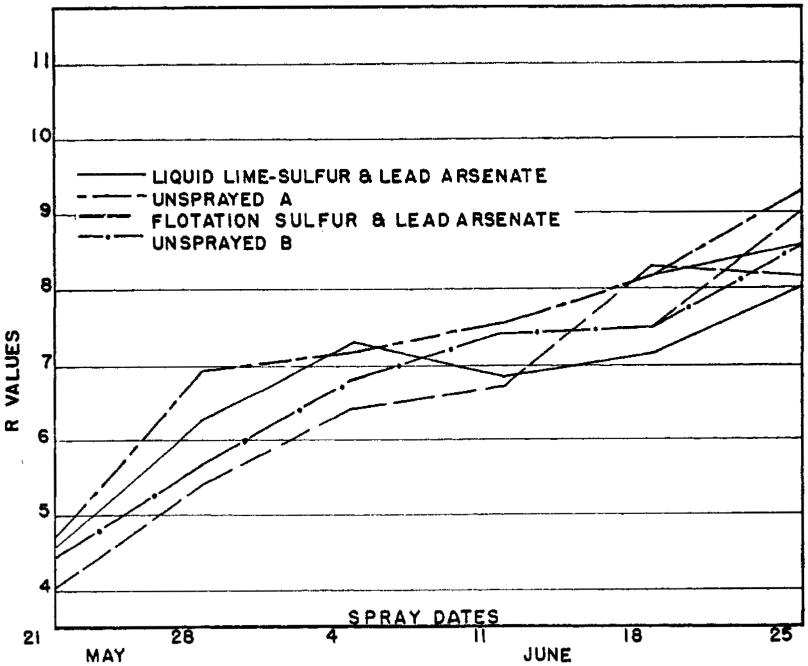

FIG. 14.—Field-grown leaves, 1941. R values of York variety at weekly intervals, collected twenty-four hours after spray applications. Liquid lime-sulphur and lead arsenate from same trees as unsprayed A; flotation sulphur and lead arsenate from same trees as unsprayed B.

TABLE 13. *P values in microns of leaves from different positions on apple shoots.*

Variety.	Position on shoot.		
	Basal.	Median.	Apical.
Wealthy	94.80	109.80	116.70
York	66.00	79.80	69.60

Greenhouse and Field Leaves, 1942. The apple leaf studies in 1942 were carried on in much the same manner as in previous years with the exception that each tree was divided into three parts and each section given a different treatment. This method facilitated a more accurate comparison between spray materials as well as the comparisons between sprayed and unsprayed leaves as it reduced tree variability. It also made possible the study of a larger number of spray materials. The R values for these data are presented in Table 14.

TABLE 14. *R values for 1942 greenhouse- and field-grown leaves.*

VARIETY.	Series A.*		Series B.*	
	Treatment.	R values.	Treatment.	R values.
GREENHOUSE:				
Wealthy	Unsprayed	12.69	Unsprayed	12.81
	Verdol	10.99	Flotation sulphur-lead arsenate	12.00
	Liquid lime-sulphur-lead arsenate	10.94	Bordeaux	11.10
FIELD:				
Wealthy	Unsprayed	14.98	Unsprayed	15.88
	Liquid lime-sulphur	13.04	Flotation sulphur-lead arsenate	14.20
	Liquid lime-sulphur-lead arsenate	12.18	Bordeaux-lead arsenate	12.76
Jonathan	Unsprayed	13.92	Unsprayed	14.06
	Liquid lime-sulphur	11.68	Flotation sulphur-lead arsenate	12.18
	Liquid lime-sulphur-lead arsenate	11.02	Bordeaux-lead arsenate	10.67
York	Unsprayed	10.76	Unsprayed	12.04
	Liquid lime-sulphur	8.74	Flotation sulphur-lead arsenate	9.27
	Liquid lime-sulphur-lead arsenate	8.15	Bordeaux-lead arsenate	8.20

* Each tree in a series received three treatments. There were 12 trees in each series in the greenhouse and 10 trees in each series and each variety in the field.

There was statistical significance between unsprayed leaves and those sprayed with flotation sulphur-lead arsenate in the greenhouse-grown Wealthy leaves, and no difference between those sprayed with Verdol and those sprayed with liquid lime-sulphur and lead arsenate. All other comparisons were highly significant within a series.

There was a significant difference between leaves sprayed with liquid lime-sulphur and those sprayed with liquid lime-sulphur and lead arsenate for all three varieties studied in the field. All sprays in both series reduced the R values to a highly significant degree when compared with unsprayed leaves. There was also a highly significant difference between leaves sprayed with flotation sulphur-lead arsenate and those sprayed with Bordeaux and lead arsenate.

A large number of leaves were collected in which sections were taken from the top, bottom, center, and edge for study to determine whether the R values differed within a leaf if from different portions. The values obtained were: center, 13.86; bottom, 13.46; edge, 12.70; and top, 12.61. The R values from the center and bottom portions of the leaves were significantly larger than those from the edge and top.

DISCUSSION

Greenhouse Leaves, 1938. An analysis of the data collected in this investigation was undertaken in an effort to reduce the volume of data and to shorten the time required in making computations. If some microscopic measurement should be directly correlated with the ratio of the internally exposed surface to the external surface, the volume of necessary data and the time required would be reduced.

When the cross section and tangential sections were from the same leaf, a significant correlation was observed between the length of palisade cells and the length of the tangentially exposed surface.

The data show a general decrease in length of palisade cells from the first to the third layer. A general increase of the diameter of palisade cells from the first to the third layer is also shown. These tendencies would indicate a negative relationship between the length and the diameter of palisade cells. In fact, negative correlation of high significance was found between the diameters and the length of the tangentially exposed cell walls of the palisade cells.

With the lengths of the palisade cells being in a negative relationship to the diameters, and the diameters of the palisade cells being in a negative relationship to the tangentially exposed surface, the total internally exposed surface of the palisade tissue may be calculated from the measurements of the lengths of the palisade cells. This is a positive relationship.

In the spongy mesophyll there is a positive relationship between the tangential area of the cells and the total area of internally exposed surfect of the spongy mesophyll. There is also a positive relationship between the number of cells per unit area in tangential section and the total internally exposed surface of the spongy mesophyll. With the above two relationships, the internally exposed surface of the spongy mesophyll may be estimated by measuring the area of the cells in tangential section or counting the cells in tangential section.

Using the above relationships, the ratio of the internally exposed surface to the external surface may be computed by means of correlations to the measurements of the length of palisade cells and the tangential area of cells in the spongy mesophyll.

If the tangential area of the cells in the spongy mesophyll is required in order to compute the ratio of the internally exposed surface to the external surface by correlations, the number of microscopic slides required will not be reduced. In order to eliminate the use of tangential slides, a measurement from the cross sectional slides that is correlated with the ratio of the internally exposed surface to the external surface is necessary. Because the palisade mesophyll contained about 85 percent of the total internally exposed surface, the total depth of palisade layers was considered for this relationship.

Analysis by covariance was used and the correlations for each variety were found to be highly significant. Using these correlations, the ratio of the internally exposed surface to the external surface may be computed from the total depth of palisade mesophyll. Due to the variation of cell length in the palisade mesophyll, the measurement of each layer should be made separately and then totaled instead of measuring the total depth of the palisade mesophyll in one measurement. Since the regression coefficients are approximately the same, one regression line may be used to compute the ratio of the internally exposed surface to the external surface for all varieties.

Variance of both the total depth of palisade layers and the ratio of the internally exposed surface to the external surface and covariance between them fluctuated in the same direction between varieties. There was greater variation in the total depth of palisade layers than in the ratio of the internally exposed surface to the external surface because the values of the total depth of palisade layers were numerically greater than the ratio values. The York variety had the least variation.

All of the varieties usually were found to have three layers of palisade cells. The third layer was frequently only partially developed or entirely missing in the York variety and in a few of the Wealthy cross-section slides there was a partial fourth layer. According to Halma *(21),* the Eureka and Lisbon lemons had three rows of palisade cells, while several other species possessed only two rows of palisade cells. Halma *(21)* reported Alexandrov to have found that in grapes, the variety having the greatest number of palisade cells is the most productive and the one with the least number, the least productive. When the third layer was missing or less than half developed, or only a few cells were present, it was omitted in the calculations. The fourth layer was termed "palisade like" spongy mesophyll and was measured with the spongy mesophyll. In a few cross sections a layer of cells that resembled palisade cells was observed just above the lower epidermis. These cells were also measured as spongy mesophyll cells, and were probably developed near the lower epidermis due to high light intensity on the lower surface of the leaf, Bergen *(2).*

Most of the workers who have studied the photosynthetic behavior of leaves and its relation to leaf anatomy have used the structure of the spongy mesophyll as an index to the internal leaf structure, and this spongy mesophyll has been considered as the part of the leaf having the greatest influence on photosynthesis, Delisle *(12),* Eames and MacDaniels *(15).* According to Haberlandt *(20),* photosynthesis is a subsidiary function of the spongy mesophyll, and much evidence supports this view. In a leaf that is perpendicular to the incident rays of light, the first palisade region would have the greatest intensity of light, with the intensity being greatly reduced as it passes through the leaf toward the spongy mesophyll. That the spongy mesophyll has a low photosynthetic activity is again indicated by the fact that it contains but a small percentage of the chloroplasts of a leaf, Haberlandt *(20),* often as low as 11 percent.

If it is assumed that the rate of photosynthesis is related to the degree of the internally exposed surface, the palisade mesophyll of apple leaves would have a greater photosynthetic asticity than the spongy mesophyll, and the photosynthetic activity of a leaf region would decrease as the region lies more distant from the upper epidermis, for the percentage of internally exposed surface decreases from the first layer of palisade to the lower epidermis, as shown by the data of Table 4. The lower epidermis has no photosynthetic value because of the lack of chloroplasts. It contains approximately 4 percent of the total internally exposed surface of apple leaves.

Greenhouse and Field Leaves, 1940. An analysis of the data collected in this investigation was undertaken in an effort to determine (1) whether the leaves of the varieties used differed in their R values, (2) whether spray residues had an effect on the internal structure, and (3) whether some short cut for calculating the R value could be found.

Under both greenhouse and field conditions, with both sprayed and unsprayed leaves, the Wealthy foliage had greater R, P, and D values, and a smaller N value of first layer of palisade cells per unit area than the York variety. The field-grown Jonathan leaves were intermediate. Within each variety and for both locations, the sprayed leaves had lower R, P, and D values with a greater N value than the unsprayed leaves.

An analysis of variance was made of each of the above values studied, R, P, N, and D. Individual tree variation was compared with variation due to varieties and treatments, to determine whether varieties differed in these values, and whether spray residues had any effect on them. The differences in the variability between varieties and that between trees within the varieties in the above values were highly significant, and also, the differences in the variability between treatments and that between trees were highly significant. These facts allow one to assume that there is a significant varietal variation in all of these measurements, and that the spray materials used alter the internal structure of the apple leaves to a significant degree. These results are in agreement with work by Hoffman *(26, 27, 28)*, Christopher *(5)*, Heinicke *(22)*, and Brody and Childers *(3)*, who found that spray residues seriously reduced the rate of photosynthesis in apple leaves as determined by carbon dioxide assimilation towers.

Since the palisade mesophyll contained about 85 percent of the total internally exposed surface, it could be assumed that a measurement having a high correlation with R would probably come from this area. From analysis of variance and other data it appeared that the total depth of palisade layers varied positively and very closely with R. The total depth of palisade layers was considered for this relationship as this would also eliminate the necessity of making tangential slides.

This analysis by covariance for correlation and regression between the total depth of palisade layers and the R values was carried out separately for the greenhouse and field leaves, with each variety, treatment, and location determined separately. The degrees of freedom of number of samples were so few in each case that correlations had to be extremely high to be significant, and regressions could vary much and still not be significantly different. It was therefore decided to pool the lot, using the means for the entire group, including all varieties, treatments, and locations.

Covariance, correlation, and regression between the total depth of palisade and the R values for all varieties, treatments, and locations were calculated to determine whether a short cut could be found for determining the R values from the depth of the palisade. The correlation for the whole group is very highly significant. Using the regression coefficient, the ratio of the internally exposed surface to the external surface may be computed directly from the total depth of palisade mesophyll. Due to the variation of cell length in the palisade mesophyll, the measurement of each layer should be made separately and then totaled instead of measuring the total depth of the palisade mesophyll in one measurement. Since the regression coefficients are fairly close, one regression line may be used to compute the ratio of the internally exposed surface to the external surface for all varieties for rough or preliminary work, but an individual regression for each condition would be more desirable for more accurate results.

A group of leaves was collected from the field trees during midseason and used as a check on accuracy of sampling and to determine whether the spray materials had an accumulative effect as the season progressed. For this group of leaves, treated in the same manner as the end-of-season field leaves, only the palisade lengths were measured. The means of their total depth of palisade were compared with the corresponding values of the leaves from the same trees at the end of the season. The differences between sprayed and check trees were as marked at midseason as at the end of the season, indicating that as soon as the leaves were fully grown the palisade tissue in the sprayed leaves had been reduced as much as it would be from continued applications throughout the remainder of the season. The individual trees within each variety and treatment ranked in the same order as to depth of palisade layers for both periods, which would indicate that the leaf sampling was extensive enough to be highly representative.

A comparison of the means of the Wealthy and York varieties in the greenhouse and in the field showed that the R value and the total depth of palisade layers were greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse. These results are expected and in agreement with literature on the subject, for light has been considered by a number of workers to have the greatest influence on the anatomy of leaves. The intensity of light in the greenhouse is much less than that in the field.

The ratio of the internally exposed surface to the external surface was significantly correlated with the ratio of the internally exposed surface of the palisade mesophyll to the internally exposed surface of the spongy mesophyll when all measurements of field and greenhouse leaves were considered.

The limitations or magnitude of measurements may be exemplified by the use of ranges of the data collected. The total depth of palisade tissue in all leaves ranged from 48.46 to 136.54 microns. This depth is the sum of the lowest and highest measurement for all layers of palisade mesophyll. The ratio of the internally exposed surface to the external surface ranged from 5.89 to 17.97. The lowest ratio value is below the range of values established by Turrell *(53, 64)* for succulent leaves. The highest value is within the range set by Turrell for xeromorphic leaves.

The ratio of the exposed surface in the palisade tissue to the exposed surface in the spongy mesophyll ranged from 4.08 to 12.45. The above ranges indicate the necessity for taking a large sample and making a large number of determinations.

Variations between varieties and treatments in the field may be influenced somewhat by environmental factors, Clements and Long *(8)*, Tenopyr *(52)*, Clements *(7)*, and Penfound *(37, 38)*, but analysis of variance showed that certainly the greater part of the variation was due to varietal differences and differences due to treatment. In the greenhouse where all factors other than tree and varietal variations were controlled, the results were virtually identical with those in the field, except for larger values in the field.

Light has been considered by a number of workers to have the greatest influence on the anatomy of leaves, Eames and MacDaniels *(15)*, Bergen *(2)*, Turrell *(53, 54)*, Hesselman *(25)*, Pfeiffer *(39)*, Lundegardh *(33)*, Clements *(7)*, McDougall and Penfound *(34)*, Penfound *(37, 38)*, Clements and Long *(8)*, Shank *(50)*. Their work indicates that light has an extremely pronounced effect on the anatomy and morphology of foliage leaves. Some leaves are inherently adapted to sun or shade and are thin or poorly differentiated when placed under the opposing condition, but in the majority of cases leaves are thicker when in full sunlight than when in shade, and sometimes an extra layer of palisade mesophyll is formed. The intensity of light in the greenhouse is considerably less than that in the field. Total internally exposed surface of the palisade region was greater in the field-grown leaves than in the greenhouse-grown leaves. Total internally exposed surface of the spongy mesophyll was greater for the field-grown leaves than for the greenhouse-grown leaves. The length of the tangentially exposed cell walls in the second layer of palisade tissue was greater in the field than in the greenhouse-grown leaves. The length of tangentially exposed cell walls in the third layer of palisade in all varieties was less in the greenhouse than in the field and the same was true of the length of the palisade cells in all layers. Compactness of the spongy mesophyll and the tangential area of the cells was less in the greenhouse than in the field.

The ratio of the internally exposed surface in the palisade mesophyll to the internally exposed surface in the spongy mesophyll was materially reduced for all varieties in the greenhouse. This ratio was decreased mainly by the reduction in length of palisade cells, coupled with a less compact, thinner layer of spongy mesophyll of the leaves grown in the greenhouse.

The data presented agree with Turrell *(54)* in that the greatest percentage of the internally exposed surface is found in the palisade region. The internally exposed surface found in the palisade mesophyll of apple leaves was consistently approximately 85 percent of the total surface exposed internally.

The chloroplasts were observed to be larger in the greenhouse-grown leaves than in the field-grown leaves. The decrease in the intensity of light may be considered as the cause of this. The species Haberlandt *(20)* studied had larger chloroplasts in the spongy mesophyll than in the palisade mesophyll. This was also true for the apple leaves studied in this investigation. The size of chloroplasts also varied negatively with the diameter of the palisade cells, and a varietal variation was also in evidence. This would indicate that the size of chloroplasts would vary negatively with the rate of photosynthesis and with ratio of internal exposed surface to external surface, indicating again that internal exposed surface has an effect on rate of photosynthesis. The relation of size of chloroplasts to photosynthetic activity and internal structure offers a field for further research.

Ecologists explain the shape of the cells of the palisade and spongy mesophyll by the reaction of the chloroplasts to light, Weaver and Clements *(57)*. A number of workers have observed that the chloroplasts change their position in the cell and the surface they expose when the light intensity varies. When the light intensity is great, the chloroplasts recede to the part of the cell farthest from the source of light or more to a "profile" position. When the light intensity is small, the chloroplasts are well dispersed throughout the cell and the "face" surface is exposed. This behavior, in part, explains why the chloroplasts were larger in a tangential view of the spongy mesophyll than in a cross-section view. Weaver and Clements *(57)* stated that the palisade cells are of an elongated shape because the chloroplasts move to their "profile" position to reduce the amount of chloroplast surface exposed to light; and that the cells of the spongy mesophyll have irradiating arms because the chloroplasts move to a "face" position, thus increasing the surface of the chloroplasts exposed to light.

The size of stomata was observed to vary between varieties. The stomata were also larger in the greenhouse leaves than in those from the field. The number of stomata was found to vary between varieties; there were fewer stomata per unit leaf area in the greenhouse than in the field. Pickett *(42)* found that the size and number of stomata behaved in a similar manner for the varieties of apples used in this study.

The rate of diffusion of gases through an opening is increased proportionally to the diameter of the opening, and the diffusion rate is proportional to the number of openings available through which the gas may diffuse, Brown and Escombe *(4)*. With the above law of diffusion in mind, all varieties may have approximately the same rate of gaseous interchange into the spongy mesophyll. However, this theory has not been established by adequate evidence.

Due to the nature of the internal structure, there is a varietal difference in the facilities for the interchange of gases between the different regions of a leaf. As the diameter of the palisade decreases, there is a proportional decrease in diameter of the space through which gases may diffuse, and more surface exposed for assimilation of gases. The rate of interchange of gases has a direct control over photosynthesis. The above deductions indicate that the diameter of cells of the palisade tissue probably could be used as an index to photosynthetic activity. Also, palisade diameters vary negatively with palisade length, and palisade length varies positively with R value and photosynthetic activity.

The workers who have studied the effect of spray residues on the rate of photosynthesis of apple leaves have shown that these spray materials severely cut down the rate of carbon dioxide assimilation. By the use of carbon dioxide absorption towers they have shown that even where there is no indication of visible injury to the leaves, the rate of photosynthesis has been greatly reduced. The results of this investigation have shown that spray residues greatly reduce the ratio of the internally exposed surface to the exterior surface, largely through shortening the palisade mesophyll cells. It may be deduced from the foregoing evidence that leaf structure has an effect on photosynthesis due to differences in extent of absorption surfaces for gas exchanges, and the conclusion may thus be drawn that spray residues reduce the rate of photosynthesis due in part, to alteration of amount of absorption surface.

Greenhouse and Field Leaves, 1941. An analysis of the data collected in this investigation was undertaken in an effort to determine (1) whether various spray residues affect the internal structure of apple leaves to different degrees, (2) whether varieties differ in their susceptibility to different sprays, and (3) at what stage in the development of the leaf the effect of the spray residues was greatest.

In both the greenhouse- and field-grown apple leaves, the Wealthy had longer palisade cells than the York variety, and the Jonathan was intermediate. Each spray material reduced the length of the palisade cells of each variety for both locations. The "t" test showed that the differences between varieties were highly significant, and the reduction of the palisade by sprays was also highly significant. An analysis of variance indicates that there is no significant difference between varieties in regard to their susceptibility to different spray materials. There is a highly significant difference between oil and liquid lime-sulphur on York in the greenhouse.

Spray applications on both field- and greenhouse-grown leaves prevented normal development of the palisade tissue, gradually increasing the difference between unsprayed and sprayed leaves as the season progressed. Leaves which were ten weeks old before they received a spray application were checked rather severely and held at about the value of the unsprayed leaves at the fifth collection, while those which were still left unsprayed continued to grow normally.

There is a difference in structure of leaves from various positions on the shoot, making it imperative that the investigator in comparing tree, varieties, or treatments, collect his samples from corresponding positions on the shoots.

Turrell *(55)* reported that measurements of alfalfa leaves showed that areas of the late primary, early primary, secondary, tertiary, and quaternary leaves were, on the average, successively smaller. The leaf thickness, upper and lower epidermal thicknesses, palisade and sponge thicknesses, midrib, major-vein, and minor-vein diameters were, in general, greater in the primary leaves and successively less in secondary, tertiary, and quaternary leaves. This is in agreement with Eames and MacDaniels *(15)*, Cowart *(9)*, Moissejewa *(35)*, Ewart *(18)*, Yapp *(58)*, and Penfound *(37)*, whose work indicate that leaves from different parts of the plant differ in mesophyll structure, but the differences are variable and cannot be foretold, since some plants have larger leaves at the apex, and others larger at the base.

That the R value and effect of spray materials on this value are subject to the action of genetic factors is an assumption that is given further strength by the work of Tydeman *(56)* showing that sulphur shyness in apples is controlled by genetic factors and that a number of genes are involved, and by similar results on gooseberries by Crane and Lawrence *(10)*.

The relation of the ratio of the internally exposed surface to the external surface of apple leaves to rate of photosynthesis offers a field for much further research. The spray materials which have been found by other workers to reduce the rate of photosynthesis to the greatest degree were found in this investigation to reduce greatly the R value, and the less caustic spray materials to reduce the R value less. This is in agreement with Heinicke *(23),* Christopher *(6),* Agnew and Childers *(1),* whose work with CO_2 assimilation towers shows that photosynthetic activity is reduced more by caustic sprays than by the so-salled "mild" sprays. The differences in the effect of different spray materials did not prove significant by statistical analysis, but were large nevertheless. This work indicates that milder sprays are preferable if adequate pest control is maintained, and the longer the first spray can be postponed, the better for the foliage. Since the sprays were put on different trees, the tree variability and the small number of trees utilized would require an extremely great difference for statistical significance. The data in this work indicate that the sprays had their greatest effect on the young leaves but also affect leaves in any stage of growth until maturity.

Again assuming that the ratio of the internally exposed surface to the exterior surface is an important factor in photosynthetic activity, the lower R value of the York variety may be offered as one of the factors contributing to its biennial bearing habit in the Missouri Valley. This factor may even be suggested for determining vigor of seedling trees while still in the nursery, and thus save several years in selection.

Greenhouse and Field Leaves, 1942. This experiment was set up so that two different spray materials were applied to different portions of an individual tree. Other portions of the tree were not sprayed, thus making possible a comparison of the materials as well as a comparison of sprayed and unsprayed leaves. Previous data showed that the mild sprays did not reduce the R values nearly as much as did the more harsh sprays, but no two materials were used on the same trees and a great deal of tree variability probably prevented the differences from being significant.

The data from this work show that the differences between Bordeaux and lead arsenate as compared to flotation sulphur and lead arsenate were highly significant, the former the more detrimental to the leaf. There was no difference between Verdol and liquid lime-sulphur and lead arsenate, while the differences between liquid lime-sulphur and liquid lime-sulphur plus lead arsenate were significant, the later the more caustic. As in previous studies, all the sprays reduced the R values when compared with unsprayed leaves, the field leaves had greater R values than the greenhouse leaves, and the varieties ranked in ascending order, York, Jonathan, and Wealthy.

In order to determine whether the R value varied within an individual leaf if sections were taken from different portions, a large number of leaves were collected and portions cut out from the center, bottom, edge, and top portions. The center and bottom sections had larger R values than the edge and top. This indicates that it is necessary to study comparable sections of the leaf when making these studies in order to have a true comparison.

PART II. THE INFLUENCE OF SOME SPRAY MATERIALS ON THE CHLOROPHYLL CONTENT OF APPLE LEAVES

INTRODUCTION

This investigation was undertaken to determine whether the chlorophyll content of apple foliage was altered quantitatively when combination sprays composed of arsenate of lead and liquid lime-sulphur were applied.

When the temperature is high and the relative humidity is low, apple foliage is injured by liquid lime-sulphur and arsenate of lead sprays. Sachs *(47)* stated that the amount of chlorophyll was a limiting factor of the rate of photosynthesis. According to Hyre *(29)* the two spray materials decrease the rate of photosynthesis even when the leaves appear uninjured. It seemed possible that the conclusions of these two workers were related and that the lessened rate of photosynthesis was due in part to the effects of sprays used.

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 protected from certain weather injuries which sometimes ruin experimntal work with trees growing in the field.

Greenhouse Leaves, 1940. Twenty-two two-year-old trees each of the York and of the Wealthy varieties were planted in 12-inch clay pots and plunged in a ground bed in a greenhouse in January, 1940. The trees were spaced 36 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 spray was mixed at the rate of 4 pounds of arsenate of lead and 2 1/2 gallons of commercial liquid lime-sulphur, 31.5° Beaume, to 100 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 cheesecloth. The samples ranged from 5 to 10 grams fresh weight. Blueprints were made of the leaves because the quantity of clorophyll 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 *(31)* with modifications.

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 chlorophyll, grade 5x, which had been secured from American Chlorophyll, Incorporated, Alexandria, Virginia. Twenty milligrams of 5x chlorophyll were placed in an Earlenmeyer 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 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 Leaves, 1940. Fifteen two-year-old trees of each of three varieties, Wealthy, Jonathan, and York, were planted in the field in March, 1940. There were three rows of trees. Each row contained one variety and extended 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 spray schedule included 4 pounds of lead arsenate and 2 1/2 gallons of liquid lime-sulphur for 100 gallons of spray applied May 4, 10, 20, 25, and 31; arsenate of lead alone June 8, 15, 21, and 29; and July 6; and Verdol summer oil at the rate of 1/4 of 1 percent with arsenate of lead on July 13 and 20; August 2 and 16; and September 11.

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 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 statistical significance was made by an analysis of variance. Since the chlorophyll content was highly variable, it was desirable to use the statistical method to determine the sources of variation.

Greenhouse Leaves. The data on chlorophyll content of sprayed and unsprayed York and Wealthy leaves are presented in Figure 15. Duplicate determinations were made of the unsprayed and sprayed leaves of each variety for a given date with the exception of March 25 and April 4.

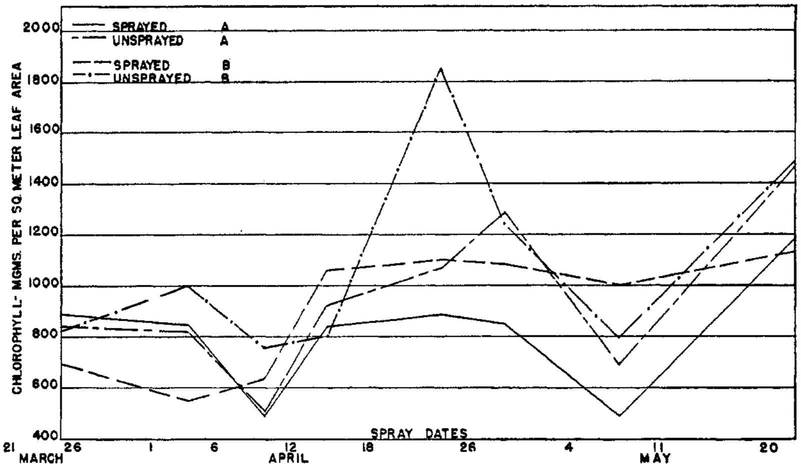

FIG. 15.—Greenhouse-grown leaves, 1940. Mgms. chlorophyll of the York and Wealthy apple leaves per square meter of leaf area. York sprayed and unsprayed, A. Wealthy sprayed and unsprayed, B.

The average chlorophyll content per square meter of leaf area for the York variety was 968.83 milligrams and 802.88 milligrams for the unsprayed and sprayed leaves, respectively, and for the Wealthy were 1132.22 and 955.45.

An analysis of variance showed that the variation between the chlorophyll of unsprayed and sprayed leaves was highly significantly greater than the variation due to sampling. 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 leaves on the eight dates was highly significantly greater than the variation due to sampling. The chlorophyll content of the Wealthy apple leaves was highly significantly greater than that of the York leaves per unit area.

Field Leaves. The data of the field series are presented in two parts—Part A, the chlorophyll content of sprayed and unsprayed leaves from different trees, and Part B, the chlorophyll content of sprayed and unsprayed leaves from the same trees.

Part A. The chlorophyll content of sprayed and unsprayed leaves of the York, Jonathan, and Wealthy varieties are presented in Figures 16, 17, and 18. Duplicate determinations of sprayed or unsprayed leaves of each of the three varieties were determined. An analysis of variance was calculated for a test of significance.

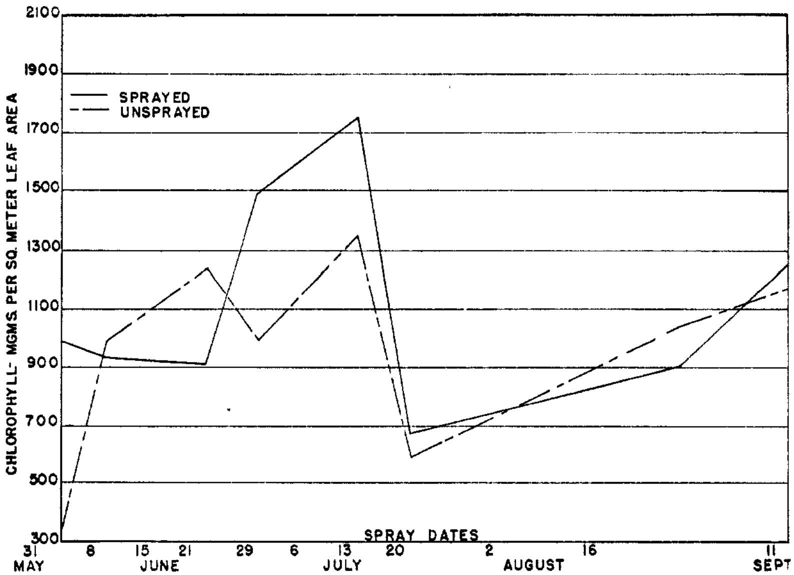

FIG. 16.—Field-grown leaves, 1940. Mgms. chlorophyll of York sprayed and unsprayed apple leaves per square meter of leaf area.

The average chlorophyll content per square meter of leaf area for the York variety was 974.31 milligrams and 1022.53 milligrams for the unsprayed and sprayed leaves, respectively. The average for the Wealthy was 1232.52 and 1089.76; and the average for the Jonathan was 1089.76 and 1122.99.

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 chance.

The variation in chlorophyll content between varieties is shown in Table 15 and was highly significantly greater than the variation due to sampling. This indicates 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

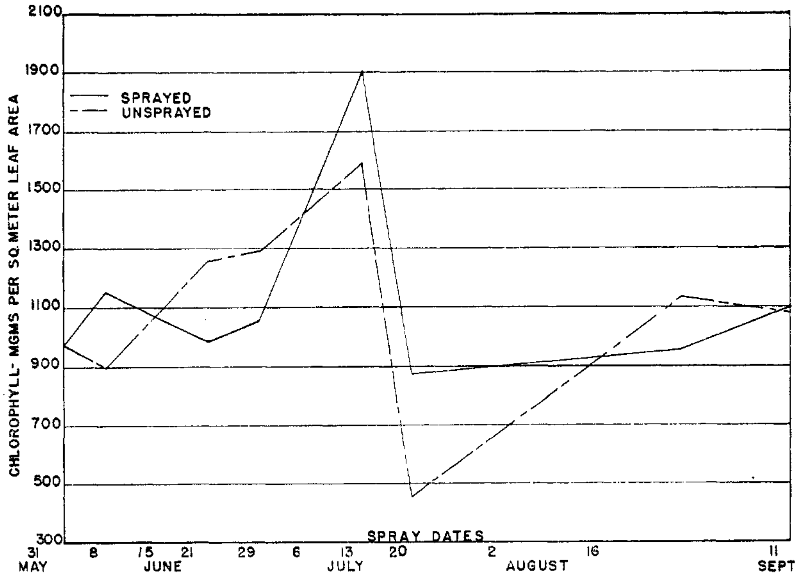

FIG. 17.—Field-grown leaves, 1940. Mgms. chlorophyll content of Jonathan sprayed and unsprayed apple leaves per square meter of leaf area.

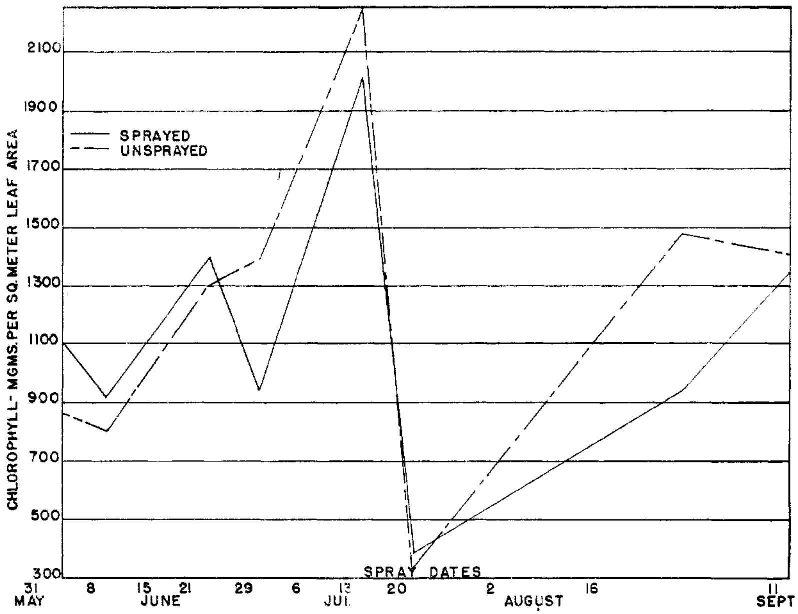

FIG. 18.—Field-grown leaves, 1940. Mgms. chlorophyll of Wealthy sprayed and unsprayed apple leaves per square meter of leaf area.

content of the York, Jonathan, and Wealthy leaf samples was 998.42, 1093.88, and 1190.27 milligrams per square meter of leaf area, respectively. It was found that the variation in amount of chlorophyll between varieties and dates was so great that it was probably not due to chance. 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.

TABLE 15. *Average chlorophyll content of sprayed and unsprayed 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	982.97	976.34
June 10	963.86	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
August 28	978.80	1266.35	1045.43
September 13	1212.25	1390.81	1075.75
Average	998.43	1190.27	1093.88

Part B. 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 were as follows: the average chlorophyll content per square meter of leaf area in milligrams for the York variety was 971.64 and 992.43 for the unsprayed and sprayed leaves, respectively. The average for the Wealthy was 1294.02 and 1201.27; and the average for the Jonathan was 1114.27 and 992.56.

An analysis of variance showed that the variation in chlorophyll content between dates was highly significantly greater than the variation due to sampling. The variation in the chlorophyll content due to spraying was not significantly greater than the variation due to chance. This signifies that the amount of chlorophyll in the sprayed leaves was not significantly less than that in the unsprayed leaves.

The variation in chlorophyll content between varieties was highly significant. Considering both sprayed and unsprayed foliage, 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

Although effects of spray materials upon the size and the photosynthetic activity of apple leaves have been reported by many, Dutton *(14)*, Hoffman *(27)*, Young *(59)*, Schroeder *(49)*, Christopher *(5)*, Heinicke *(22)*, and Hyre *(29)*, there is little evidence of their influence upon the chlorophyll content. Ginsburg *(19)* reported an increase in chlorophyll content of two apple varieties as the result of oil sprays.

According to Schertz *(48)* the most important factors affecting the quantity of pigments are rainfall, soil moisture, nutrient elements in the soil, light intensity, temperature, and relative humidity.

Ruth *(46)* 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.

Ginsburg *(19)* 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 *(36)*, accumulates faster in weak midlight than in strong light which causes a decomposition of chlorophyll.

There are two generally recognized sets of factors that affect the rate of photosynthesis in green plants; namely, external and internal. Comparatively little is known of the internal factors, but chlorophyll is one. Photosynthesis, as a function of light intensity with different concentrations of chlorophyll, was investigated by Emerson *(16)*. 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 *(17)* 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 *(32)* 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 *(30)* 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 (11) indicated that the water content of the leaves of some tropical plants is an important internal factor with which photosynthesis is related. Their results showed that the water content is more important in the process than 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 processes 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.

Pickett *(41)* 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 *(45)* stated that within the limitation of their observations the amount of chlorophyll is not so significant in the process of photosynthesis as is the amount of internally exposed surface. 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.

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 than the unsprayed leaves. 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 which were not measured or accounted for in the experiment altered or affected the chlorophyll content. Some of these factors may be moisture and temperature, Henrici *(24)*, minerals, Deuber *(13)*, and light, Palladin *(36)*. Any one or all of these factors combined may have been 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 reduced 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 *(36),* chlorophyll accumulates faster in weak midlight than in strong light. He states that the formation of chlorophyll occurs almost exclusively in weak light, 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 the 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 since the plants were subjected to different environmental conditions from time to time. Henrici *(24)* found that the chlorophyll content of grasses varied during a 24-hour period and with age of the leaf. Ireland and Yeats *(30)* 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 agrees with the findings of Pickett and Kenworthy *(45).*

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.

SUMMARY AND CONCLUSIONS

1. The formula used in this study is adequate to determine the ratio of the internally exposed surface to the external surface of apple leaves.

2. The ratios of the internally exposed surface in the palisade mesophyll to the internally exposed surface of the spongy mesophyll for greenhouse leaves ranked: York, Wealth, and Jonathan in ascending order.

3. The palisade mesophyll contained 85 percent or more of the total internally exposed surface of apple leaves; the spongy mesophyll contained 15 percent or less of the total internally exposed surface.

4. The first layer of palisade mesophyll contained a larger percentage of the total internally exposed surface than the second layer; the second layer more than the third. From the first to the third layer of the palisade mesophyll the diameter of the cells increased. The length of the palisade cells decreased from the first to the third layer.

5. The correlation coefficient between the total depth of the palisade layers and R values, by pooled covariance, including all varieties, treatments, and locations as one, was highly significant.

6. In the field-grown leaves, the differences between leaves from unsprayed and leaves from sprayed trees within a variety were approximately the same at midseason as they were at the end of the season.

7. The R values and total depth of palisade layers of leaves were much greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse.

8. The R values and total depth of palisade layers of unsprayed leaves were much greater for any variety and location than for the corresponding sprayed leaves.

9. The R values and total depth of palisade layers of leaves ranked in ascending order: York, Jonathan, and Wealthy for any treatment, location, or year.

10. The average number of upper palisade cells per unit area for the greenhouse-grown leaves was greater in the York than in the Wealthy leaves and greater in the sprayed than in the unsprayed. They ranked in the same order in the field with the Jonathan variety intermediate.

11. The average diameter of the first layer of palisade cells was reduced by sprays and was greater in the field than in the greenhouse, and varieties ranked in the following ascending order: Jonathan, York, and Wealthy.

12. The spray materials used shocked or checked normal cell development with each application consistently throughout the growing season in both greenhouse- and field-grown leaves for all varieties. The development of the palisade tissue in leaves which were ten weeks old before they received a spray application was checked in growth to a level between the unsprayed and regularly sprayed leaves.

13. The so-called mild sprays did not have so great a dwarfing effect on the palisade tissue as did the stronger materials.

14. There was an increase in total palisade depth in leaves from the basal towards the apical portion of the shoot in Wealthy, while in York there was an increase from the basal to the median portion, and then a decrease from the median towards the apical portion.

15. The lower and central portions of the leaf blade had greater R values than the top and edge portions. The differences were significant.

16. The reduction in photosynthetic activity brought about by some spray residues on apple foliage may be explained in part by the reduction of the amount of internally exposed surface caused by the residues.

17. The chlorophyll content of the greenhouse-grown York and Wealthy apple leaves was reduced by spraying while that of the field-grown York, Wealthy, and Jonathan was not reduced.

18. The chlorophyll content of the Wealthy leaves was much greater than that of the York leaves growing under similar conditions. Jonathan was intermediate in this respect. Such factors as moisture, light intensity, and temperature probably caused the large variation in chlorophyll content between collection dates of both sprayed and unsprayed leaves.

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