A STUDY OF THE INTERNALLY EXPOSED SURFACE OF FOLIAGE LEAVES OF FIVE VARIETIES OF APPLES

by

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B. S., Oklahoma Agricultural and Mechanical College, 1937

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1939

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INTRODUCTION

A project was begun at the Kansas State College in 1932 to investigate the relationship of leaf area to tree growth and fruitfulness with the apple. In 1932, Pickett reported on the relationship between leaf area and size of fruit, and in 1934, he reported on the correlation of internal structure of apple leaves to their photosynthetic activity. In 1936, Oberle worked on the photosynthetic activity of Livland and York apple leaves. Kansas Agricultural Experiment Station Technical Bulletin 42 by Pickett on the relation of internal structure and photosynthetic behavior of apple leaves appeared in 1937. Turrell published a formula in 1936 by which one might mathematically express certain internal characteristics of foliage leaves. This formula was used in this study.

This study was designed to discover (1) the differences of the internally exposed cell surface of the leaves of certain varieties of apples and (2) the ratio of the internally exposed surface to the external surface of these leaves.

The varieties of apples used in this study were selected so that comparisons could be made with Pickett's results of similar measurements in previous work. These varieties represent a range of growing seasons.

REVIEW OF LITERATURE

General Anatomy of Dorsiventral Leaves

Eames and MacDaniels (1925) stated that the thin-walled parenchyma between the upper and lower epidermis of leaves is known as mesophyll. Near the upper epidermis there occur elongated, more or less cylindrical, compact cells, which are known as the palisade mesophyll, and are perpendicular to the leaf surface. In leaves in a drooping or more or less vertical position the palisade mesophyll may develop on both sides of the leaf. In the spongy mesophyll the cells lack regularity of shape and are loosely arranged. The radiating arms of the cells in the spongy

mesophyll tend to connect with arms of other cells, and in this manner form a network of cells which is very loosely arranged. The cells of this network connect with the small near-by branches of the veins. These cells also connect with the lower epidermis through which gas enters by way of stomata.

Haberlandt (1928) stated that the palisade mesophyll is recognized as the most important photosynthetic tissue of the leaf. The spongy mesophyll constitutes the physiological link, connecting the photosynthetic tissue with the efferent channels. The products of photosynthesis are diffused through the first layer of palisade mesophyll to the layer beneath it, and on to the spongy mesophyll which carries it to the minute branches of veins. In this manner each palisade cell functions independently of the adjacent cells of the same layer of mesophyll. The spongy mesophyll also contains a small amount of chloroplasts, but photosynthesis is a secondary function of these cells.

Delisle (1938) reported that the size and shape of a leaf within a species are due both to cell number and cell enlargement. The shape of the leaf is not the result of differences in cell shape, but due in part to factors limiting the number of cells and the direction of cell en-

lergement.

Differentiation in Dorsiventral Leaves

Haberlandt (1928) stated that the differentiation of palisade cells is always initiated by the appearance of active anticlinal division in approximately isodiametric mother-cells, and that the palisade cells never arise from the mere elongation of isodiametric meristem elements. The stage of development at which these partitions appear varies in different plants. In Caragana frutescens DC., the palisade tissue appears after the segregation of the principal veins and before the segregation of the smaller veins or vascular bundles. In Ficus elastica Roxbg, the palisade tissue appears contemporaneously with the small veins.

Avery (1933) worked with tobacco plants and found that layers of the palisade and spongy mesophyll were multiplied in a plane parallel to the leaf surface. Cell division ceased first in the epidermis, followed by the spongy mesophyll, and then in the palisade mesophyll. The cells of the palisade mesophyll began to acquire their characteristic shape when the leaf was 1/80 to 1/75 of its final size. Intercellular space did not develop

markedly until the leaf was from 1/4 to 1/3 of its final size.

Mounts (1932) worked with Vitis vulpina L. and Catalpa bignonioides Walt. and concluded that the intercellular space was schizogenous in origin. The greater expansion of the epidermal layers doubtlessly tends to separate the cells of both the palisade and spongy mesophyll, and is an important factor in the development of the intercellular space. The cell layers begin to differentiate into epidermis, palisade and spongy mesophyll when the blade is from five to eight millimeters long.

Intercellular Space and Internally Exposed Surface of Leaves

Eames and MacDaniels (1928) stated that the normal structure of the mesophyll is such that a large cell-wall surface is exposed to the "internal atmosphere" of the leaf. The portion of wall surface of the mesophyll cells exposed to the intercellular spaces varies greatly in different plants.

Turrell (1934) designed formulas for the measurement of the internally exposed surface of mesomorphic, strongly xeromorphic, and succulent leaves. With these formulas,

the ratio (R) of the internally exposed surface to the external surface could be determined. His formula for the measurement of the internally exposed surface and calculation of R for a mesomorphic leaf is:

$$R = \frac{\Sigma(ab...a_nb_n) + l\,(cd + 2\,\frac{ef}{g}) + \frac{hi}{j,}}{2k}$$

In this formula, the part " $(ab...a_nb_n)$" represents the surface exposed in the palisade region; "$l(cd + 2\,\frac{ef}{g})$" represents the exposed surface in the spongy mesophyll; "$\frac{hi}{j,}$" represents the exposed surface of the lower epidermis; and "2k" represents the external surface. The measurements represented by letters were made with a chartometer and planimeter from camera lucida drawings.

Formulas for measurement of the internally exposed surface of leaves of the same types of structure were again published by Turrell (1936) in another form. His formula for the measurement of the internally exposed surface and calculation of R for a mesomorphic leaf follows. For explanation of the formula see page 20.

$$R = \frac{\Sigma lp + L(hc + 2A\,\frac{l_e}{l_t}) + (K^2 - A)\,\frac{l_i}{k}}{2K^2}$$

He checked the accuracy of the instruments used and the formulas were based on calculations made from a theoretical

leaf. The measurements were taken from camera lucida drawings. This formula does not take into consideration the exposed surface of the upper epidermis which, according to Turrell, represents, at most, 0.2 per cent of the internally exposed surface.

Turrell (1937)[1] stated that the two formulas are precisely the same. However, the empirical use of letters in the preliminary paper was changed in the later publication for letters more suggestive of the measurements which they represented. Also the portion of the formula Σlp means $lp + l_1p_1 + l_2p_2 \ldots$. He also stated, "The use of an oil immersion objective is essential. Projection with an 'Edinger' projector was not as satisfactory as the camera lucida."

The palisade region of a leaf has the largest percentage of internally exposed surface, according to Turrell (1936). Working with from three to five drawings, he determined the internally exposed surface of several genera and species, and the ratioof the internally exposed surface to the external surface was computed. In shade leaves of Syringa the ratio was 6.8 while in Eucalyptus it was 31.3; these ratios represented the range of his work.

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[1] Personal correspondence, Nov. 30, 1937.

Factors Affecting the Anatomy of Leaves

Light. Eames and MacDaniels (1925) stated that the number of palisade layers, and the density of cell structure depend largely upon light intensity. Bonnier (1894) compared the same species of plants at various elevations in the Alps and Pyrenees and found that the Alpine leaves had a better developed palisade tissue due to larger cells or an increase in the number of rows.

Lundegardh (1931) found that bright light produced thick, well differentiated leaves, while leaves growing in shady places were thin and poorly differentiated. Certain trees, such as ash and birch, are unable to produce typical shade leaves.

Pfeiffer (1928) stated that the leaves of four-o-clocks, sunflowers, and soybeans decreased in thickness in the order of the following degrees of illumination: full sunlight, full spectrum, visible spectrum, minus violet, blue shade, and red. The outdoor leaves developed two rows of palisade cells, while only one row developed in the shade.

Penfound (1931,1932) observed that leaves of sun-

flower, water pepper, and castor bean had a better development of mesophyll and were thicker when grown in full sunlight than when grown in shade.

According to Hesselman (1904), leaves of forest trees grown in the stronger light had more palisade cells than those in the poorer light. Shade leaves produced more starch than sun leaves of the same species when the light was equal.

Clements and Long (1935) showed that with Helianthus, the greater the per cent of illumination, the greater the thickness of the leaf. The palisade tissue consistently composed more than 50 per cent of the leaf thickness.

Shank (1938), working with leaves of Cornus florida L., found that leaves were thicker and somewhat smaller in the open than in the woods. Also, the leaves were somewhat thicker on the south side than on the north side of the tree.

Leaves from dense shade were found by McDougall and Penfound (1928) to be thinner, had more surface, with less palisade (as to number of layers and depth of individual cells), and a higher per cent of intercellular space and spongy mesophyll than leaves of the same plant in maximum sunlight.

The thickness of sun leaves of evergreen angiosperms was found by Bergen (1904) to be greater than that of the shade leaves. The palisade layer was double in sun leaves and single in shade leaves; the cells next to the epidermis were longer in the sun leaves. The intercellular space was less in upper portions of the mesophyll of sun leaves and a palisade layer occasionally developed next to the lower epidermis of sun leaves.

Turrell (1933) reported that mesomorphic sun leaves, though thin, may have a relatively large internal surface, (R = 11.6 to 18.3), and that xeromorphic leaves of sun species may have an extensive internal surface, (R = 22.2 to 31.3), xeromorphic leaves of shade species may have a limited internal surface, (R = 8.18 to 9.88), while succulent leaves may have a relatively small internal surface, (R = 7.86). In this work, "R" was used to represent the ratio of the internally exposed surface to the external surface of leaves.

Shade leaves have a small internal surface, (R = 6.8 to 9.9), intermediate internal surface for leaves of mesomorphic type, (R = 11.6 to 19.2), and high for xeromorphic sun leaves, (R = 17.2 to 31.3), according to Turrell (1936). The ratio of the internally exposed surface to the external surface is represented by "R".

Clements (1904) stated that decreased light caused a somewhat looser arrangement of mesophyll cells and an increase in thickness of leaf.

Position of Leaf. Working with plants of a marsh, Yapp (1912) found that leaves nearer the ground were larger than those higher in the air or on the stem. Ewart (1906) removed all lateral buds from shoots of Tilia europea L., leaving only the terminal buds. Leaves produced by the terminal buds were much larger than those which grew on normal plants. However, their cells were of the same size as those of ordinary leaves, indicating that leaf enlargement was due to cell division and that this plant is characterized by a constant normal cell size.

Koissejewa (1930) gave a statement of "Zalensky's law", "Zalensky, studying monocotyledonous and dicotyledonous plants on a very extensive and variegated material, showed that the higher up on the tree the leaf grows (or the nearer the end of the branch), the greater the xeromorphic properties it acquired; that is, the epidermis and mesophyll cells are smaller, the conducting strand is thicker, the stomata are greater in number and smaller, and the palisade tissue is more clearly defined." He stated that a similar law was found for Gymnosperms.

Leaves at different nodes of sunflower and water

pepper plants differed with respect to thickness of leaf, and depth of palisade and spongy mesophyll, according to Penfound (1931).

Cowart (1935) reported there was an increase of palisade tissue from the base of apple shoots to the tip, also a parallel increase in per cent of palisade mesophyll and a decrease in intercellular space in the mesophyll. He indicated a negative relationship between these characters and vigor of shoots.

Soil Moisture. Leaves of sunflower, water pepper plants, and castor bean were observed by Penfound (1931, 1932) to be thicker when the plants were grown in soil of a high water content than when grown in soil of a low water content. The palisade and spongy mesophyll were deeper if grown in soil of high moisture. However, the number of rows of palisade and sponge cells was constant under all soil moisture conditions.

Clements and Long (1935) showed that with Helianthus, greater the per cent holard, the greater the thickness of leaf, and that the palisade tissue consistently composed more than 50 per cent of the leaf thickness.

Clements (1904) observed that increased water caused a decrease in thickness and a looser arrangement of the

mesophyll cells, especially of the sponge cells. A decrease in water caused an opposite effect.

Inheritance or Varietal Differences. According to Tenopyr (1918), the cells of plants showed considerable variation in size in the same tissue, but the average cell size for any one tissue of a species or variety is a fairly constant and hereditary character. Cell size depends upon the time or stage of development; the later appearing leaves having smaller cells.

Halma (1929) found that Eureka and Lisbon lemons had three rows of palisade cells, and that the other species possessed only two rows of palisade cells. The percentage of palisade tissue was practically constant for a species, ranging from 30.9 to 31.9 per cent. This percentage varied with age of leaf and illumination.

The rating of Livland, Wealthy, York, Winesap, Geno, Jonathan, and Delicious varieties of apples in ascending order of compactness of mesophyll, and the perimeter of the intercellular space in the spongy mesophyll was reported by Pickett (1923). Field leaves of Livland and Delicious trees had greater intercellular space than greenhouse leaves and orchard grown Livland leaves had more extensive

intercellular space than orchard grown Delicious leaves, according to Pickett (1934). Four varieties of apples were rated by Pickett (1937) on the basis of the extent of intercellular space as judged from tracing projected images of cross sections. In ascending order they rated as follows: Livland, Delicious, Jonathan, and York.

Miscellaneous Factors. According to Lutman (1934), in general, the size of plants was found to be correlated with that of the cell and cell organs. With the potato, the leaves grown with excess nitrogen were crowded with small, short cells with relatively small air spaces; whereas, with an absence of nitrogen the leaves were spongy, and had large intercellular spaces and long slender cells. A study of rape plants showed the length of palisade cells shortened when any variation was made from the complete solution. Buckwheat showed a greater variation than rape.

Hill (1934) found that the leaves of potato plants affected with giant hill were found to be thinner than those of the healthy plants. The cells of the palisade mesophyll of leaves of the diseased plants were smaller than those of healthy plants, with a smaller ratio of width to length, and smaller volume.

Noguchi (1935) and Wolcott (1936) showed that X-ray would change the anatomical structure of leaves. In this regard Noguchi stated that the size and shape of palisade tissue may be disrupted to the extent that it cannot be distinguished from the spongy parenchyma. The longer the irradiation, the greater the disturbance.

Leaves of Livland, Jonathan, and York varieties of apples had a greater extent of intercelluler space in a warm house than in a cool house as reported by Pickett (1937). The reverse was found to be true with the Gano.

PROCEDURE

Field Leaves

Collecting and Imbedding of Leaves. On September 29, 1937 a number of leaves was collected from Livland, Wealthy, York, Winesap, and Jonathan trees in the orchard on the Horticultural Farm. The leaves selected were removed from the south side of the periphery of the tree and near the middle of the twigs.

Portions of the leaf used were located near the midrib and midway between the basal and apical regions. The mar-

ginal and midrib portions of each leaf were discarded. Not more than six pieces, each one centimeter square, were taken from a single leaf. These leaf pieces were placed in a one per cent chromo-acetic acid killing and fixing solution. After leaving them in the killing and fixing solution for 24 hours, they were washed and dehydrated with N-butyl alcohol, (Zirkle 1930), after which the leaf pieces were imbedded in paraffin.

Preparation of Slides. Twelve sets of slides were made for each variety. A set of slides consisted of five cross section and five tangential slides, paried at random. In each set, the cross section slides were made from one leaf piece, and another leaf piece was used in making the tangential slides. The tangential slides were kept in series through the leaf in order to facilitate making drawings. All sections were eight microns thick, fixed on the slides with egg albumin, then stained with one-half to one per cent safranine O in 50 per cent alcohol, and mounted with balsam. Several sections were placed on each slide.

Drawings. Eight pages of drawings were made from each set of slides, except the twelfth, from which twelve pages were made. Four drawings of each leaf region were made

from one slide. One hundred pages of drawings were made for each variety. Each page of drawings included the following: one drawing of a field 50 microns square of each the first, second, and third layers of palisade cells from a tangential slide; one drawing of a field of the spongy mesophyll 50 microns square, from a tangential slide; and one drawing of a field 50 microns wide, across the spongy mesophyll, in cross section (Plate I). These drawings were arranged across the page and from the top to the bottom in the order mentioned. The drawings were made with a camera lucida, using a 1.9 mm. oil immersion objective and a 10x eyepiece with the mirror arm at 120 mm. This produced a magnification of approximately 1760.

In making the tangential drawings, it was difficult to determine which layer of palisade cells was in the field. Cross sections of each variety showed that the epidermis dipped where veins were present, and that all leaves contained three layers of palisade cells (Plates II, III, XXI). Above large veins the epidermis came down as a single layer to the parenchyma cells surrounding the bundle. Above small veins the epidermis was found in a number of layers, there being as many as six layers of cells before reaching the parenchyma cells surrounding the bundle. Con-

sidering this behavior of the epidermis, the layer number of palisade cells, in tangential section, was determined as outlined below.

When a microscopic field was found in which the cells were definitely of the upper epidermis but not near a vein, the adjacent palisade cells were considered to be of the first layer. Also fields which showed vein tracings of epidermal like cells, but including no tracheids, were considered to be of the first layer of palisade cells (Plates VII, VIII, IX, XXII, XXIII).

Microscopic fields which showed veins with tracheids that disappeared toward the upper palisade cells were considered to be of the second layer. Also the leaves of all varieties contained druses or inclusions which were always in the second layer of palisade cells. Such druses or inclusions were significant in locating fields of the second layer of palisade cells (Plates X, XI, XII, XXIV, XXV).

The third layer of palisade cells was determined in a similar manner as the second layer. The cells of microscopic fields, in the palisade region, that contained veins with tracheids which disappeared when the field was moved toward the spongy mesophyll were considered to be of the third layer. The cells of the third layer of palisade tissue were always less compact than the first and second

layers. This fact facilitated differentiation between the second and third layers of palisade cells (Plates XIII, XIV, XV, XXVI, XXVII).

Drawings of the spongy mesophyll were made from microscopic fields that were free of veins. In cross sections, drawings were made from regions that showed the lower epidermis intact. When a cell of the spongy mesophyll was in contact with a palisade cell, the palisade cell was drawn and labeled as such, but was not measured. Due to the warping of leaf pieces in the process of imbedding, absolutely tangential sections were practically impossible. Areas which were uniform in cell size were used in making the drawings in order to eliminate using the tips of palisade cells.

In some preliminary work a drawing ocular was used to make drawings. Drawings made with the drawing ocular were only one-fourth as large as those made with the camera lucida. Twelve pages of drawings were made for each variety and four pages were made from each set of slides. The same sets of slides were used in making camera lucida drawings.

Measurements. Measurements were made from the drawings with a chartometer and planimeter. These measurements

were required for the following formula used by Turrell (1936) for computing the ratio (R) of the internally exposed surface to the external surface of mesomorphic leaves:

$$R = \frac{(lp)+(l_1p_1)+(l_2p_2)+L(hc+2A\frac{l_c}{l_t})+(K^2-A)\frac{l_i}{K}}{2K^2}$$

The measurements represented by these symbols, stated briefly, are as follows:

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;
c - Average length of the exposed cell wall in tangential section of the spongy mesophyll;
A - Average area of cells of spongy mesophyll 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;
h - Average length of vertically exposed cell walls in spongy mesophyll of cross section;
l_c - 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 non-exposed 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 A, l, l_1, and l_2 were recorded in centimeters. The measurement A was recorded in square inches, and measurements l, l_1, and l_2 were recorded in microns. 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.

From the formula the following may be computed: the internally exposed surface of the palisade, $(lp) + (l_1p_1) + (l_2p_2)$; the internally exposed surface of the spongy mesophyll, $L(hc + 2A \frac{l_e}{l_t}) + (K^2 - A) \frac{l_i}{K}$; 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, $(K^2 - A) \frac{l_i}{K}$.

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

The outside diameter of the palisade cells was measured and given symbols of D, D_1, and D_2 respectively. These measurements were taken from the drawings and re-

corded in microns. Twenty cells of each drawing were measured. Should a drawing have less than 20 cells, only 10 were measured. In this manner the average diameter of 2,000 cells of the first and second layers of palisade cells and approximately 1,500 cells of the third layer were obtained for each variety.

The number of cells in the tangential drawings of the spongy mesophyll was needed for the computation of the average exposed surface (c). This determination was designated as N. The number of cells in the cross section drawings of the sponge needed to compute the average vertically exposed cell wall (h) was given the symbol of M.

Table 1. Measurements and calculations of "R" for drawings in Plate I.

Symbol	Centimeters	Microns	Symbol	
p	86.0	517.70	l	45.80 microns
p_1	89.0	535.80	l_1	35.70 microns
p_2	77.0	463.50	l_2	25.90 microns
c	7.2	43.34	A	226.74 sq. microns
h	3.0	18.48	L	7.00 av. no. cells

$$\frac{l_e}{l_t} = \frac{40.0 \text{ cm.}}{44.0 \text{ cm.}} = 0.91 \qquad \frac{l_i}{K} = \frac{10.0 \text{ cm.}}{8.1 \text{ cm.}} = 1.23$$

Calculation of R

Area in palisade $= \Sigma lp = l_1 \times p_1 + l_2 \times p_2 + l_3 \times p_3$

$= 45.8 \times 517.7 + 35.7 \times 535.8 + 25.9 \times 463.5$

$= 54,843.37$ sq. microns

Area in sponge $= L(hc + 2A \frac{l_e}{l_t}) + (K^2 - A)\frac{l_i}{K}$

$= 7(43.34 \times 18.48 + 463.48 \times 0.91) + (2500 - 226.74)\ 1.23$

$= 11,291.24$ sq. microns

$$R = \frac{\Sigma lp + L(hc + 2A \frac{l_e}{l_t} + (K^2 - A) \frac{l_i}{K}}{K^2}$$

$$= \frac{54,843.37 + 11,291.24}{5,000}$$

$= 13.23$

$$\frac{\text{Palisade}}{\text{sponge}} = \frac{54,843.37}{11,291.24}$$

$= 4.88$

Greenhouse Leaves

On January 20, 1938, two-year-old trees of Wealthy, Jonathan, and York varieties of apples were planted in 12-inch clay pots, and plunged into a ground bed in the greenhouse.

On June 14, 1938, leaves from these varieties were collected, killed and fixed, and imbedded, using the same method as with field leaves. From these, one set of slides, 15 microns think, was made from each leaf. From each set of slides, 25 pages of drawings were made and measurements were taken in the same manner as with field leaves.

PRESENTATION OF DATA

Preliminary Work

The drawings for the preliminary work on this problem were made by using a drawing ocular at a magnification of 440. From each variety 12 pages of drawings were made. Four pages of drawings were made from each set of slides. The average of the measurements from these drawings is

recorded in Table 2. For explanation of the symbols used in the measurements, see page 20.

Considerable variation was found between varieties in regard to all measurements in Table 2. The highest values were obtained with the Livland variety, except for the value of l_e/l_t, and the lengths of the palisade cells. Also, the Livland variety had the highest values of all the varieties for measurements taken from the drawings. In the tangential slides of the Livland variety, the cells of the palisade tissue were more loosely arranged and larger in diameter than the cells of the palisade tissue of other varieties.

A difference of 1.00 between mean R values of varieties was found to be of significance by the use of the "t" table of Snedecor (1938). In a similar manner a difference of 0.89 between mean P/S values was found to be significant. This difference indicates a significant difference between all varieties except the Winesap, Jonathan, and Livland combinations in regard to R values. The differences between P/S values were significant only between the Livland and Jonathan varieties.

Table 2. Measurements of drawings made with drawing ocular (each value is the average of 12 measurements).

Measurement	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
p_t	502.08	403.69	328.36	404.70	417.74
p_1	478.99	374.55	442.84	372.55	426.78
p_2	453.88	385.60	453.88	370.54	426.74
l_t	46.90	51.80	49.50	55.68	55.60
l_1	36.90	39.20	37.40	45.12	44.20
l_2	28.60	28.40	30.00	33.39	31.40
L	6.70	5.10	5.90	5.67	6.00
A	240.91	238.49	187.89	236.34	166.98
c	42.89	45.05	34.14	43.22	34.06
H	18.92	18.69	20.93	20.61	19.42
l_e/l_t	0.86	0.92	0.87	0.84	0.87
l_1/K	1.12	1.07	1.10	1.08	1.08
Palisade	53,380.67	47,526.41	45,639.74	51,350.77	55,461.54
Sponge	10,483.38	8,600.89	8,484.86	9,724.73	8,130.07
R	12.76±0.30	11.19±0.30	10.81±0.34	12.12±0.37	12.70±0.34
P/S	5.15±0.11	5.67±0.30	5.44±0.20	5.59±0.71	6.89±0.25

In order to determine the possibility of duplicating these measurements, 12 more measurements were made of the Winesap variety from other slides. The Winesap variety was selected for this trial because it showed the highest standard error of R values. The results of these drawings are presented in Table 3.

Table 3. Variations of measurements, drawing ocular, Winesap (each value is the average of 12 measurements).

Measurement	First series of drawings	Second series of drawings
p	404.70	413.67
p_1	372.55	391.65
p_2	370.54	418.74
l	55.68	57.82
l_1	45.12	47.85
l_2	33.30	34.20
L	5.67	5.58
A	236.34	208.37
e	43.22	38.15
h	20.61	22.53
l_e/l_t	0.84	0.86
l_1/K	1.08	1.05
Palisade	51,350.77	56,973.30
Sponge	9,724.73	9,209.52
R	12.12±0.37	13.24±0.21
P/S	5.59±0.71	6.43±0.54

As shown by Table 3, the exposed area in the palisade tissue was greater in the second series of drawings; in contrast the exposed area in the spongy mesophyll was less in the second series. The R value for the second series of drawings was significantly greater than in the first series.

With this test showing a significance between R values of two sets of drawings from the same leaves, the accuracy of measurements and size of drawings were questioned. Therefore, a camera lucida was selected for making drawings which would be higher in magnification and from which more accurate measurements could be made. The Winesap variety was used and two series of drawings were made from the same slides used in obtaining the measurements of Table 3. The drawings made with the camera lucida were of an approximate magnification of 1760 and the measurements are presented in Table 4.

Table 4. Variations of measurements, camera lucida, Winesap (each value is the average of 12 measurements).

Measurement	First series of drawings	Second series of drawings
p	685.30	646.70
p_1	612.50	579.40
p_2	540.80	566.90
l	45.10	47.60
l_1	36.20	36.80
l_2	25.10	25.50
L	6.10	6.00
A	174.48	179.29
c	41.83	42.14
h	18.12	16.50
l_e/l_t	0.84	0.85
l_1/K	1.15	1.17
Palisade	67,355.34	66,575.54
Sponge	8,991.61	8,960.24
R	15.26±0.56	14.94±0.41
P/S	7.58±0.39	7.53±0.32

As shown in Table 4, the difference between the R values of the first and the second series of drawings was not significant because the camera lucida drawings were larger and the measurements could be made more accurately. Also, the measurements of the exposed surfaces enclosing small spaces may be made from the larger drawings, whereas, these surfaces appear as cell walls that are in contact with other cell walls in the smaller drawings.

Since the surfaces bordering small spaces can be measured by use of camera lucida drawings, the total internally exposed area in the palisade region was larger for the camera lucida drawings. For some reason, the internally exposed area in the spongy mesophyll was less when calculated from camera lucida drawings.

Because of the greater accuracy of the measurements, a camera lucida was used in the remainder of this work.

Field Leaves

One hundred pages of drawings were made for each variety and measurements were taken in accordance with the method given on page 10. The means of measurements of the tangentially exposed cell walls in the palisade layers are reported in Table 5.

Table 5. Internally exposed palisade cell walls, 2500 square microns of leaf, in tangential section - microns (average of 100 measurements each).

Palisade layer	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
First (p)	562.51	570.69	603.89	641.09	639.10
Second (p_1)	534.94	576.82	629.66	614.76	617.77
Third (p_2)	460.65	513.45	584.49	562.63	554.56
Total	1,558.10	1,670.96	1,818.04	1,818.48	1,811.43

The Winesap variety, according to Table 5, had the greatest total tangentially exposed cell walls. York, Jonathan, Wealthy, and Livland followed in decreasing order. This rating was consistent only in the Wealthy and Livland varieties when the exposed cell walls of each layer of palisade were considered. In the first layer of palisade Jonathan had more than York. In the second layer York had the greatest value with Jonathan having a value exceeding that of Winesap. In the third layer York had more than Winesap (Plates VII, VIII, IX, X, XI, XII, XIII, XIV, XV).

In general, there was a greater amount of exposed cell walls in the first than in the second layer of palisade and a greater amount in the second layer than in the third. The Wealthy and York varieties are exceptions; they had more ex-

posed cell walls in the second layer of palisade tissue than in the first.

The mean lengths of the cells of each layer of palisade of each variety were obtained by direct measurement and are recorded in Table 6.

Table 6. Length of palisade cells - microns (average of 1000 cells each).

Palisade layer	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
First (1)	45.0	47.5	42.1	48.8	43.0
Second (1_1)	35.7	36.8	33.5	38.6	32.7
Third (1_2)	26.1	25.8	22.8	27.7	22.1
Total	106.8	110.1	98.4	115.1	97.8

The greatest total depth of palisade tissue, as shown in Table 6, was found in the Winesap variety, followed in order by Wealthy, Livland, York, and Jonathan. This rating was consistent for all layers, with the following exceptions: Jonathan had longer cells in the first layer than York, and Livland had longer cells in the third layer than Wealthy. Without an exception, there was a decrease in length of cells from the first to the second and third

layers of palisade in all the varieties (Plates II, III, IV, V, VI).

There was a low correlation between the length of palisade cells and the length of the tangentially exposed cell walls. This correlation was not significant according to the table presented by Snedecor (1938), giving the correlation coefficients necessary for significance.

The diameter of the cells for each layer of palisade tissue was determined from the drawings for each variety as outlined earlier. The means of these measurements are presented in Table 7.

Table 7. Diameter of palisade cells - microns (average of 1500-2000 cells).

Palisade layer	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
First (D)	8.20	7.88	7.16	7.46	7.24
Second (D_1)	8.44	8.39	7.07	7.59	7.51
Third (D_2)	10.21	9.75	8.33	8.93	9.21

In Table 7 the varieties rank: Livland, Wealthy, Winesap, Jonathan, and York with an exception in the third layer where the Jonathan value was greater than that of the Winesap (Plates VII-XV). This rating is in a negative

relationship to the tangentially exposed surface presented in Table 5. The correlation between the mean measurement of the diameter of palisade cells and the mean length of the tangentially exposed walls of palisade cells was -0.91. This correlation is highly significant according to Snedecor (1938).

The number of tiers of cells in the spongy mesophyll; the number of cells in a tangential plane of an area of 2500 square microns; the number of cells in a plane across the spongy mesophyll, 50 microns wide; and the average tangential area of the cells in the spongy mesophyll are presented in Table 6.

Table 8. Compactness and size of cells in spongy mesophyll (average of 100 determinations).

Cells		Variety: Livland	Wealthy	York	Winesap	Jonathan
		Number of cells				
Across mesophyll in cross section	(L)	6.8	6.2	5.7	6.4	5.9
In tangential section / 3000 sq. microns leaf surface	(N)	5.3	5.3	5.8	6.8	7.1
In cross section width, 50 microns	(M)	11.5	11.6	11.5	13.5	12.6
		Average area - square microns				
In tangential section	(A)	204.04	216.58	195.63	174.75	164.77

No consistent rating of varieties in regard to compactness of mesophyll is shown in Table 8. There was a direct relationship between the depth of spongy mesophyll and the number of cells present in the microscopic fields. An indirect relationship is indicated between the number of cells per unit area in tangential section and the area of the cells in tangential section. The Livland variety had the greatest depth of spongy mesophyll and the York the least.

The Winesap and Jonathan varieties had the most compact spongy mesophyll in regard to number of cells and cell size; the Livland and Wealthy varieties were the least compact (Plates I-VI, XVI-XVIII).

The distribution of the internally exposed surface of the spongy mesophyll may be calculated as given on page 21. This distribution is presented in Table 9.

Table 9. Distribution of internally exposed surface in spongy mesophyll (square microns per 5000 square microns of leaf surface).

Plane of surface	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
Horizontal	5,325.75	5,261.90	4,631.78	4,615.94	4,161.74
Vertical	2,441.95	2,255.87	1,851.08	1,857.15	1,623.77
Inner surface of lower epidermis	2,640.35	2,534.60	2,693.11	2,697.29	2,732.22
Total	10,408.05	10,051.37	9,178.95	9,170.38	8,517.73

The varieties, as shown in Table 9, rated consistently in regard to the amount of internally exposed surface in each plane and total surface exposed. This rating was in decreasing order: Livland, Wealthy, York, Winesap, and Jonathan. The internally exposed surface of the lower epi-

dermis of all varieties exceeded that exposed in a vertical plane. The greater amount of internally exposed surface in the spongy mesophyll was in the horizontal plane.

From the data presented in the preceding tables, the per cent of the internally exposed surface for each region of the leaves was computed. These calculations are presented in Table 10.

Table 10. Distribution of internally exposed surface - per cent (calculated from mean of 100 measurements).

Plane of exposure	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
Palisade mesophyll	84.6	86.2	86.9	88.6	87.7
First layer	37.8	37.9	36.5	39.4	39.6
Second layer	28.5	29.7	30.3	29.8	29.1
Third layer	18.0	18.5	19.2	19.2	17.7
Spongy mesophyll	15.4	13.8	13.1	11.4	12.3
Horizontal	8.0	7.4	6.7	5.8	6.0
Vertical	3.7	3.2	2.7	2.3	2.3
Inner surface of lower epidermis	4.0	3.6	3.9	3.4	3.9

The most significant fact, presented in Table 10, is the per cent of exposed surface located in the palisade mesophyll. More than 84 per cent of the internally exposed surface was found in the palisade region. The Winesap, although highest in total per cent in the palisade mesophyll, did not have the greatest percentage in any layer. There was no consistent rating of varieties in the per cent of exposed surface located in the different layers of palisade. In the first layer Jonathan had the largest, and in the second and third layers York had the largest percentage. Wealthy was consistently third in all layers. Livland had the largest per cent of exposed surface in the spongy mesophyll and Winesap had the smallest. The lower epidermis consisted of approximately 4.0 per cent of the internally exposed surface in all varieties.

By substituting the measurements recorded in Tables 4, 5, and 8 in the formula given in the procedure, the ratio of the internally exposed surface to the external surface, and the ratio between the area in the palisade mesophyll and the area in the spongy mesophyll were computed. Table 11 presents these ratios.

Table 11. Ratio of internally exposed surface to externally exposed surface (R) and internally exposed surface of palisade to sponge (P/S).

Ratio	Variety				
	Livland	Wealthy	York	Winesap	Jonathan
R	13.36±0.22	14.19±0.20	13.93±0.15	15.85±0.15	13.78±0.17
P/S	5.64±0.12	6.45±0.18	6.78±0.17	8.02±0.16	7.29±0.14

A mean difference of 0.50 between the ratios of internally exposed surface to external surface, presented in Table 11, was significant. This value indicates a significant difference between Winesap and all other varieties, while Livland differed significantly from Wealthy and York. Jonathan was not significantly different from any except Winesap. The R value for Jonathan was doubtlessly low because of being shaded.

By analysis of variance, the variations between varieties were found to be significantly greater than between leaves. However, a number of leaves in each variety had a mean R value which was significantly greater or less than other leaves.

For a significant difference between the values obtained for the ratio of the exposed surface of the palisade

mesophyll to the exposed surface in the spongy mesophyll, a mean difference of 0.38 was found to be necessary. This mean difference was found in varietal comparisons except between Wealthy and York. The analysis of variance showed that greater variation between varieties than between leaves was highly significant.

Greenhouse Leaves

As was stated in the procedure, measurements were taken from leaves of Wealthy, Jonathan, and York trees planted in the greenhouse. The area of exposed cell walls, tangentially, of each layer of palisade tissue is recorded in Table 12.

Table 12. Internally exposed palisade cell walls, per 2500 square microns of leaf, in tangential section - microns (average of 25 measurements each).

Palisade layer	Variety		
	Wealthy	Jonathan	York
First (p)	658.11	667.26	654.74
Second (p_1)	603.44	647.75	578.56
Third (p_2)	455.36	439.70	438.74

As shown in Table 12, the varieties rated consistently: Jonathan, Wealthy, and York except in the third layer of palisade mesophyll where Wealthy had a greater value than Jonathan.

The measurement of the length of palisade cells gave the values recorded in Table 13.

Table 13. Length of palisade cells - microns (average of 250 cells each).

Palisade layer	Variety		
	Wealthy	Jonathan	York
First (1)	47.50	38.50	31.20
Second (l_1)	36.30	30.50	24.60
Third (l_2)	25.50	20.20	18.30
Total	109.30	89.20	64.10

In regard to the length of palisade cells in each layer, as given in Table 13, the varieties consistently rated as: Wealthy, Jonathan, and York. Wealthy had a depth of palisade tissue sufficiently deeper than Jonathan to cause it to have a larger total internally exposed surface in the palisade mesophyll than Jonathan, although

the Jonathan had a greater value for the tangentially exposed cell walls; see Table 12.

The average diameter of each layer of palisade mesophyll cells for each variety is recorded in Table 14.

Table 14. Diameter of palisade cells - microns (average of 250-500 cells each).

Palisade layer	Variety		
	Wealthy	Jonathan	York
First (D)	7.97	7.44	7.83
Second (D_1)	9.01	7.98	8.64
Third (D_2)	10.44	9.44	10.10

Table 14 shows a consistent increase in diameter of palisade cells from the first to the last layer. Also, the diameter was consistently greatest in Wealthy and least in Jonathan.

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 are recorded in Table 15.

Table 15. Compactness and size of cells in spongy mesophyll (average of 25 determinations).

Cells		Variety		
		Number of cells		
		Wealthy	Jonathan	York
Across mesophyll in cross section	(L)	6.4	6.1	5.1
In tangential section / 5000 sq. microns leaf surface	(W)	4.2	5.4	4.3
In cross section width, 50 microns	(M)	9.9	11.3	7.4
		Average area - square microns		
In tangential section	(A)	215.52	168.14	183.17

Table 15 indicates 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 is presented in Table 16.

Table 16. Distribution of internally exposed surface in spongy mesophyll (square microns per 5000 square microns of leaf surface).

Plane of surface	Variety		
	Wealthy	Jonathan	York
Horizontal	6,918.27	4,846.63	3,709.49
Vertical	2,455.17	1,723.13	1,550.71
Inner surface of lower epidermis	2,535.77	2,635.00	2,548.51
Total	11,909.21	9,204.76	7,808.71

Table 16 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 tables, the per cent of the total internally exposed surface located in each region or plane of the leaf was calculated. These calculations are shown in Table 17.

Table 17. Distribution of internally exposed surface - per cent (calculated from mean of 25 measurements).

Plane of exposure	Variety		
	Wealthy	Jonathan	York
Palisade mesophyll	84.64	85.39	84.27
First layer	40.84	40.78	40.61
Second layer	28.62	31.36	26.30
Third layer	15.17	14.10	15.96
Spongy mesophyll	15.36	14.61	15.73
Horizontal	9.04	7.69	7.36
Vertical	3.21	2.74	3.08
Inner surface of lower epidermis	3.31	4.18	5.07

As shown in Table 17, the palisade mesophyll contained 85 per cent of the total internally exposed surface. The Jonathan variety contained the greatest percentage of surface in the palisade mesophyll, and the greatest amount in the second layer of palisade. The York 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. A higher percentage of the exposed surface of the spongy mesophyll is exposed

in the horizontal plane in all varieties.

From the data in the preceding tables, 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. These ratios are presented in Table 18.

Table 18. Ratio of internally exposed surface to external surface (R) and internally exposed surface of palisade to sponge (P/S).

Ratio	Variety		
	Wealthy	Jonathan	York
R	15.31±0.65	12.60±0.14	10.09±0.13
P/S	5.71±0.23	6.00±0.21	5.60±0.28

The Wealthy leaves had, as shown in Table 18, the highest ratio of internally exposed surface to external surface with the York having 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. A mean R difference of 0.68 was found to be significant. This gives significant differences between all

varieties. A mean P/S difference of 0.68 is significant; therefore, there are no significant differences between P/S values of the varieties.

DISCUSSION

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 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.

With field leaves there was no significant correlation between the mean length of the palisade cells and the mean length of the tangentially exposed cell wall of the palisade tissue. For the measurement of these leaves the cross section slides and the tangential slides were not, unless by chance, from the same leaf. With greenhouse grown leaves, the cross section and tangential sections were from the same leaf. The correlation between similar values for these leaves was highly significant. This would indicate a significant correlation between the

length of palisade cells and the length of the tangentially exposed surface should the tangentially exposed surface and the length of the palisade cells be measured from the same leaf.

The data show a general decrease in length of palisade cells from the first to the third layer. Also a general increase of the diameter of palisade cells from the first to the third layer is 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 length of the palisade cells being in a negative relationship with the diameters, and the diameters of the palisade cells being in a negative relationship with 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 which would be in a positive relationship to the tangentially exposed surface.

In the spongy mesophyll there is a positive relationship between the tangential area of the cells and the total area of internally exposed surface 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 calculated 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 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 85 per cent of the total internally exposed surface, the total depth of palisade layers was considered for this relationship. An average for each leaf was determined, thus giving 12 measurements for each

variety.

Analysis by covariance was used and the correlations for each variety and all the measurements together with other relationships are presented in Table 19.

Table 19. Some relationships between the total depth of palisade layers (X) and the ratio of the internally exposed surface to the external surface (Y).

Variety	Degrees of freedom	Sum of squares and products Sx^2	Sxy	Sy^2	Correlation coefficient[1]	Regression coefficient[2]
Livland	11	3,073.73	307.28	31.67	0.985	0.100
Wealthy	11	2,130.24	243.69	31.09	0.947	0.114
York	11	632.25	68.82	9.02	0.912	0.109
Winesap	11	809.23	102.51	15.75	0.908	0.127
Jonathan	11	979.04	127.05	20.50	0.897	0.130
Sums	55	7,624.49	849.35	108.03	0.956	0.111

[1] $Sxy / \sqrt{(Sx^2)(Sy^2)}$

[2] Sxy / Sx^2

The correlations presented in Table 19 are all 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.

In Table 19, Sx^2 equals the variance of the total depth of palisade layers, Sy^2 equals the variance of the ratio of the internally exposed surface to the external surface, and Sxy equals covariance of X and Y. Variance of both measurements 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 greatest variation was found in the Livland variety and the York variety had the least variation.

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 the measurements of field leaves were considered.

The limitations or magnitudes of measurements may be exemplified by the use of ranges of the data collected. The total depth of palisade tissue in field leaves ranged from 63.4 to 145.5 microns. This depth is the sum of the lowest and highest measurement for each layer of palisade mesophyll. The ratio of the internally exposed surface to the external surface ranged from 9.48 to 19.89. The lowest ratio value is within the range of values established by Turrell (1934, 1936) 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 3.07 to 13.65. The above ranges indicate the necessity for taking a large sample and making a large number of determinations.

All of the varieties were found to have three layers of palisade cells. The third layer was frequently, especially in the Livland variety, only partly developed. Two sets of cross section slides of Winesap showed a fourth partial layer. Due to the impossibility of separating the middle two layers of palisade in the tangential section, the fourth layer was termed "palisade-like" spongy mesophyll and was measured with the spongy mesophyll. In two sets of slides, one Winesap and one Livland, were observed a layer of cells that resembled palisade cells just above the lower epidermis. This layer of cells may have developed near the lower epidermis because of high light intensity on the lower surface of the leaf. These cells were also considered as cells of the spongy mesophyll.

Variations between varieties growing in the field may be explained in part by environmental factors. The author is of the opinion, however, that these variations are due to varietal differences. Since a number of factors other than tree and varietal variations were not controlled, the results found herein may be somewhat misleading. In the greenhouse where all factors other than tree and varietal variations were controlled, it was found that the trees had a ratio between the internally exposed surface to the ex-

ternal surface that decreased as the length of the growing season increased. The author believes that similar results could be obtained for all varieties in the field if all factors were the same.

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 considerably less than that in the field. Total internally exposed surface of the palisade region was greater in the greenhouse grown Wealthy leaves than in those grown in the field but the reverse was true for the Jonathan and York varieties. Total internally exposed surface of the spongy mesophyll was greater for the greenhouse leaves of Jonathan and Wealthy than for field leaves; the reverse was true for York. The length of the tangentially exposed cell walls of the first layer of palisade tissue was greater in the greenhouse for all varieties than in the field. The length of tangentially exposed cell walls in the second layer of palisade tissue was greater in the field than in the greenhouse for the Wealthy and Jonathan but less for York. The length of tangentially exposed cell walls in the third layer of palisade was less in the greenhouse than in the field for all varieties. The length of the palisade cells in all layers was less in the greenhouse than in the field

for all varieties. 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 to the external surface was greater in the greenhouse than in the field for Wealthy; this ratio was materially reduced for Jonathan and York. This behavior indicates a varietal variation in regard to the effect of light on the anatomy of the leaves, particularly in regard to the ratio of the internally exposed surface to the external surface.

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, more shallow spongy mesophyll of the leaves grown in the greenhouse.

The data presented agree with Turrell (1936) in that the greatest per cent of the internally exposed surface is found in the palisade region. The internally exposed surface found in the palisade mesophyll of apple leaves was very consistently 85 per cent of the total surface exposed internally.

The majority of workers who have studied the photosynthetic behavior of leaves and its relation to leaf anatomy have accepted the structure of the spongy mesophyll as an index to the internal leaf structure. The spongy mesophyll has been studied as the portion of the leaf that has the greatest influence on photosynthesis. Haberlandt (1928) states that photosynthesis is a subsidiary function of the spongy mesophyll. Evidence in regard to this statement may be found in the rate of diffusion of light through the leaf. The palisade region of a leaf would have the greatest intensity of light, with the intensity being materially reduced as it passes through a leaf that is perpendicular to the incident rays of light. The fact that the spongy mesophyll (Haberlandt, 1928) contains only a small percentage of the chloroplasts of a leaf, often as low as 11 per cent, is in agreement with the fact that the spongy mesophyll has a low photosynthetic activity.

If the rate of photosynthesis is related to the extent of the internally exposed surface, the palisade mesophyll of apple leaves would have a greater photosynthetic activity than the spongy mesophyll. The photosynthetic activity of a leaf region would decrease as the region lies more distant from the upper epidermis. This

is supported by the data of Tables 10 and 17 in which the per cent of internally exposed surface consistently decreases from the first layer of palisade to the lower epidermis. The lower epidermis has no photosynthetic value because of the lack of chloroplasts. It contains approximately 4.0 per cent of the total internally exposed surface of apple leaves. When this 4.0 per cent is deducted, only 96 per cent of the internally exposed surface is left to function in photosynthetic activity.

The size of chloroplasts should be considered as an index to photosynthetic activity. Haberlandt (1928) showed that the chloroplasts of the species he studied were larger in the spongy mesophyll than in the palisade mesophyll. Observation of the sections used in this study showed that the same is true for apple leaves. A varietal variation in regard to size of chloroplasts was observed. This variation was associated with the diameter of the palisade cells. The number of chloroplasts was also associated with the diameter of the palisade cells. In all cases a chloroplast was observed to lie against the exposed surface of the cell. The chloroplasts were also observed to be larger in the greenhouse grown leaves than in those from the field. This was attributed to the decrease in

intensity of light. The author believes the relation 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, 1929). 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 farthereat from the source of light or move 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 (1929) 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 above explanation suffices as a reason why a larger percentage of the internally exposed surface of the spongy mesophyll is exposed in a horizontal plane, rather than in a vertical plane. If the hypothesis, that the photosynthetic activity is related to the surface of the chloroplasts exposed to light, is used as a basis to establish a difference in the photosynthetic activity between the palisade and spongy mesophyll, there is need for further supporting evidence.

The size of stomata was observed to vary between varieties. Also, the stomata were larger in the greenhouse leaves than in those from the field. The number of stomata were found to vary between varieties; there were fewer stomata per unit leaf area in the greenhouse than in the field. Pickett (1937) 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 when the diameter of the opening is increased to twice its size and the diffusion rate is proportional to the number of openings available through which the gas may diffuse. 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 has not been established by adequate evidence.

Due to 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. 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 may be used as an index to photosynthetic activity.

SUMMARY AND CONCLUSIONS

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

2. The ratios of the internally exposed surface to the external surface for the field grown leaves of the varieties were: Livland, 13.36; Jonathan, 13.78; York, 13.93; Wealthy, 14.19; and Winesap, 15.85. A mean difference of 0.50 was significant.

3. The ratios of the internally exposed surface to

the external surface of greenhouse grown leaves of the varieties studied were: York, 10.09; Jonathan, 12.60; and Wealthy, 15.31. A mean difference of 0.68 was significant.

4. The ratios of the internally exposed surface in the palisade mesophyll to the internally exposed surface of the spongy mesophyll for field grown apple leaves were: Livland, 5.64; Wealthy, 6.48; York, 6.78; Jonathan, 7.29; and Winesap, 8.02. For greenhouse leaves: York, 5.60; Wealthy, 5.71; and Jonathan, 6.00. A mean difference of 0.38 was significant for field grown leaves, while a difference of 0.68 was necessary for greenhouse leaves.

5. The palisade mesophyll contained 85 per cent or more of the total internally exposed surface of apple leaves.

6. The first layer of palisade mesophyll contained a larger per cent of the total internally exposed surface than the second layer; the second layer more than the third.

7. The spongy mesophyll contained 15 per cent or less of the total internally exposed surface of apple leaves.

8. A larger per cent of the exposed surface in the

spongy mesophyll was in the horizontal plane than in the vertical plane.

9. The inner wall of the lower epidermis composed approximately 4.0 per cent of the total internally exposed surface of apple leaves.

10. The inner wall of the lower epidermis composed a larger per cent of the total internally exposed surface than the cell walls in a vertical plane, but less than those in a horizontal plane of the spongy mesophyll of apple leaves.

11. There was a general decrease in the per cent of the total internally exposed surface as the region considered became more distant from the upper epidermis.

12. From the first to the third layer of the palisade mesophyll the diameter of the palisade cells increased.

13. The length of the palisade cells decreased from the first to the third layers of the palisade mesophyll.

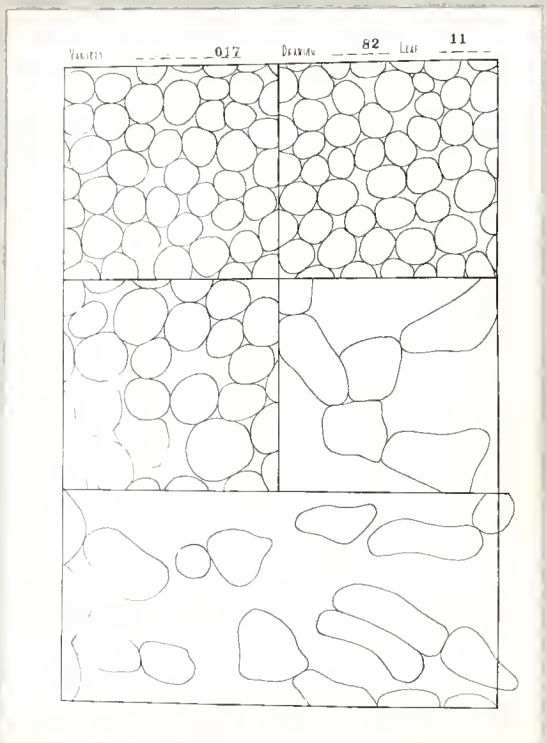
14. There was no consistent rating of the field grown varieties in regard to any of the measurements except those for the diameters of palisade cells.

15. There was a fairly consistent rating of the greenhouse varieties in regard to all measurements, when similar measurements for different tissues were compared.

16. There was a correlation between the total depth of palisade mesophyll and the ratio of the internally exposed surface to the external surface. This correlation was highly significant for field trees of all varieties.

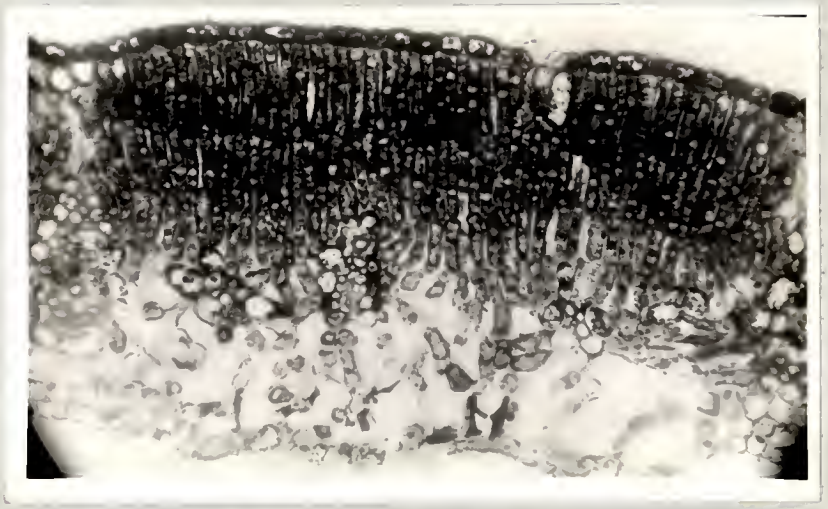
17. The ratio of the internally exposed surface to the external surface was significantly correlated with the ratio of the internally exposed surface of the palisade mesophyll to the internally exposed surface of the spongy mesophyll when all the measurements of field leaves were considered.

Plate I



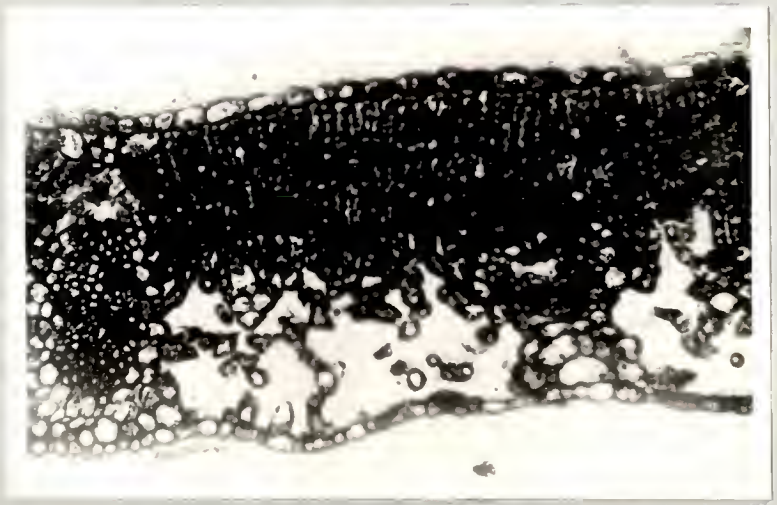
Representative drawings of the Livland variety (x̄). Upper left - first layer of palisade cells in tangential section. Upper right - second layer of palisade cells in tangential section. Center left - third layer of palisade cells in tangential section. Center right - spongy mesophyll in tangential section. Bottom - area 50 microns wide across spongy mesophyll in cross section.

Plate II



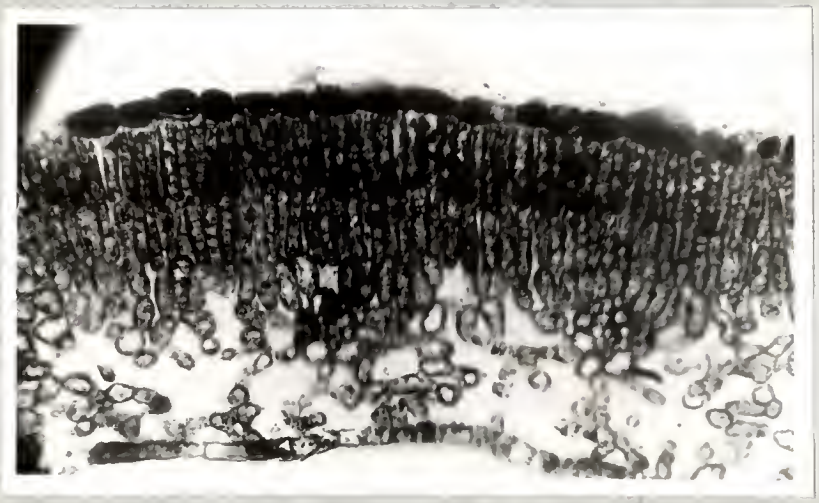
Cross section of Winesap field leaf. The actual mean length of palisade cells, in microns: first layer, 48.8; second layer, 38.6; third layer, 27.7 (x515).

Plate III



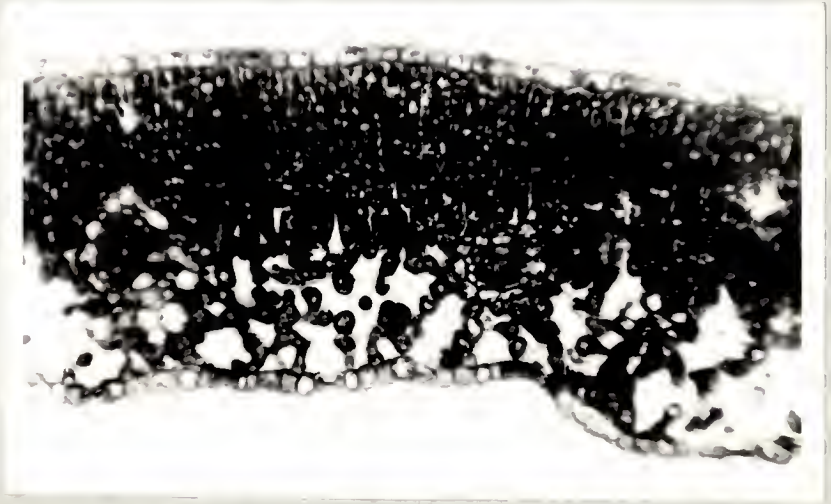
Cross section of Wealthy field leaf. The actual mean length of palisade cells, in microns; first layer, 47.5; second layer 36.8; third layer, 25.8 (x315).

Plate IV



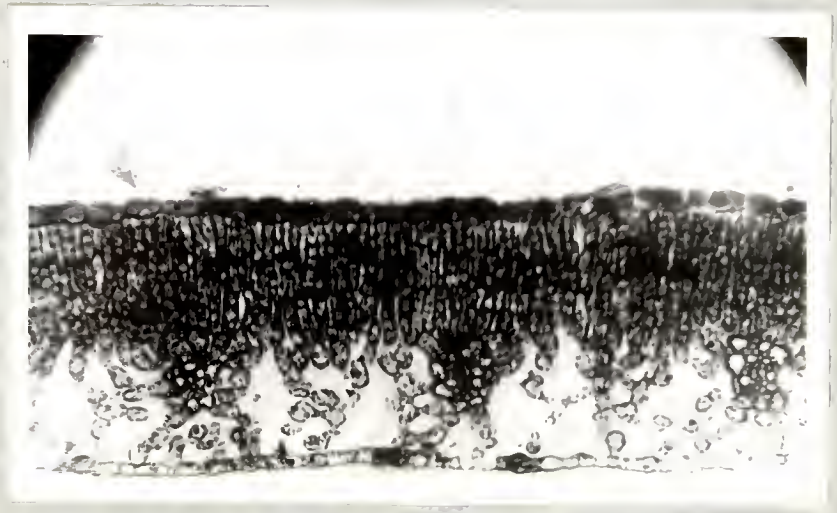
Cross section of Livland field leaf. The actual mean length of palisade cells, in microns; first layer, 45.0; second layer, 35.7; third layer, 26.1 (x315).

Plate V



Cross section of York field leaf. The actual mean length of palisade cells, in microns; first layer, 42.1; second layer, 33.5; third layer, 22.8 (x315).

Plate VI



Cross section of Jonathan field leaf. The actual mean length of palisade cells, in microns; first layer, 43.0; second layer, 32.7; third layer, 22.1 (x315).

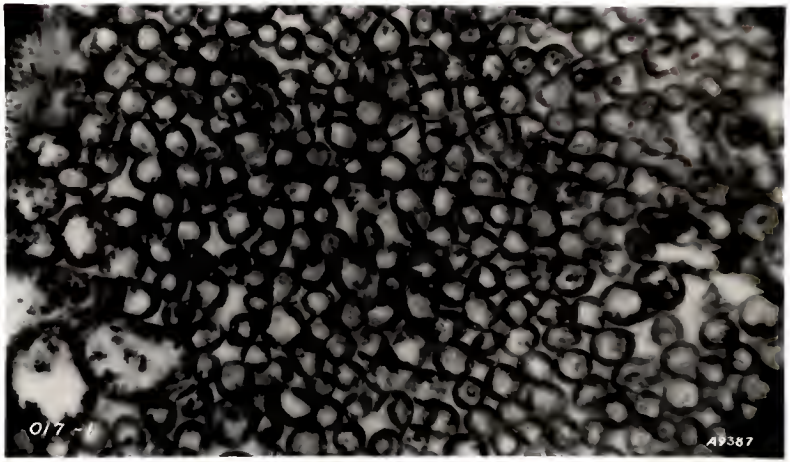
Plate VII

Tangential section through the first layer of palisade cells, Winesap field leaf. Actual length of tangentially exposed cell walls, 641.02 microns per 2,500 square microns; diameter of palisade cells, 7.46 microns (x700).

Plate VIII

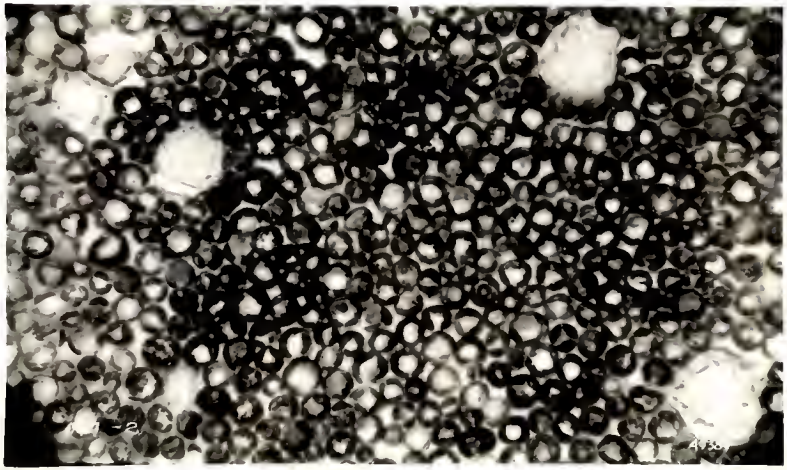
Tangential section of first layer of palisade, York field leaf. Actual length of tangentially exposed cell walls, 603.89 microns per 2,500 square microns; diameter of cells, 7.16 microns (x700).

Plate IX



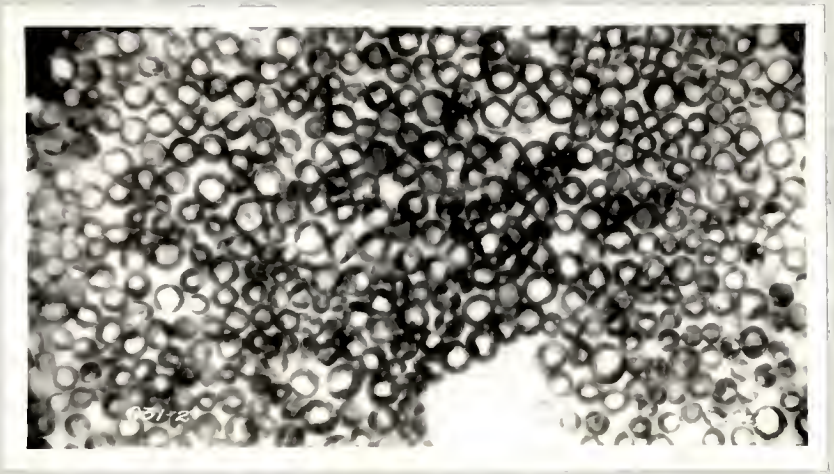
Tangential section through first layer of palisade, Livland field leaf. Actual length of tangentially exposed cell walls, 862.81 microns per 2,500 square microns; diameter of cells, 8.20 microns (x700).

Plate X



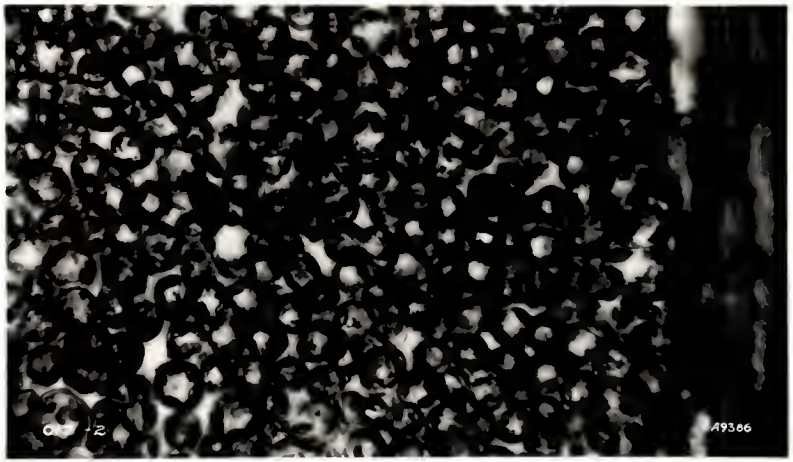
Tangential section through second layer of palisade, Winesap field leaf. Actual length of tangentially exposed cell walls, 614.76 microns per 2,500 square microns; diameter of palisade cells, 7.89 microns (x700).

Plate XI



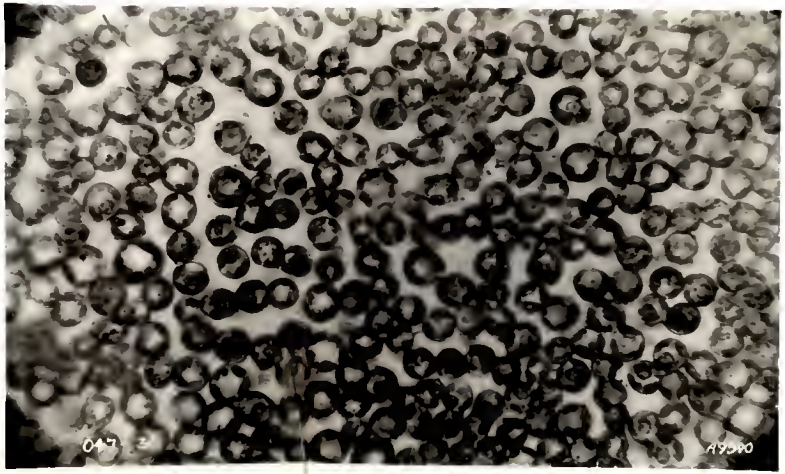
Tangential section through second layer of palisade, York field leaf. Actual length of tangentially exposed cell walls, 629.66 microns per 2,800 square microns; diameter of cells, 7.07 microns (x700).

Plate XII



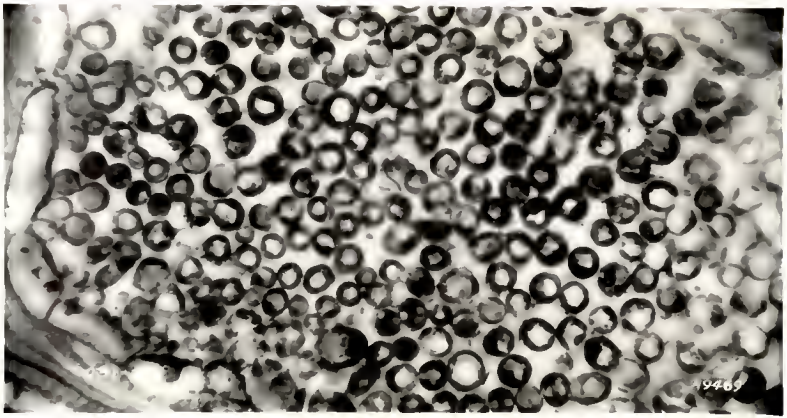
Tangential section through second layer of palisade, Livland field leaf. Actual length of tangentially exposed cell walls, 534.94 microns per 2,500 square microns; diameter of cells, 8.44 microns (x700).

Plate XIII



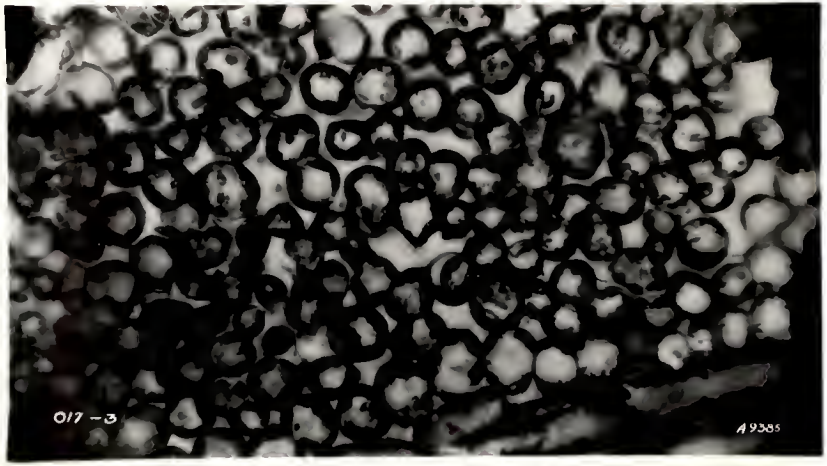
Tangential section through the third layer of palisade, Winesap field leaf. Actual length of tangentially exposed cell walls, 552.63 microns per 2,500 square microns; diameter of cells, 8.93 microns (x700).

Plate XIV



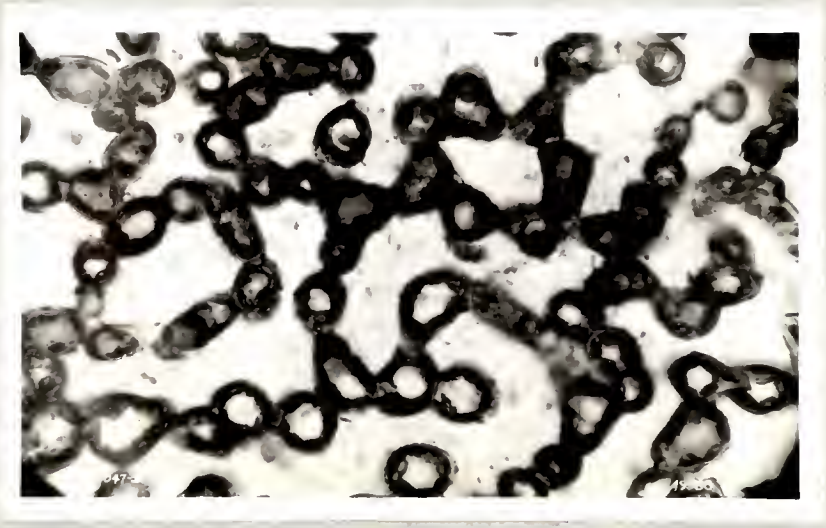
Tangential section through the third layer of palisade, York field leaf. Actual length of tangentially exposed cell walls, 584.49 microns per 2,500 square microns; diameter of cells, 8.33 microns (x700).

Plate XV



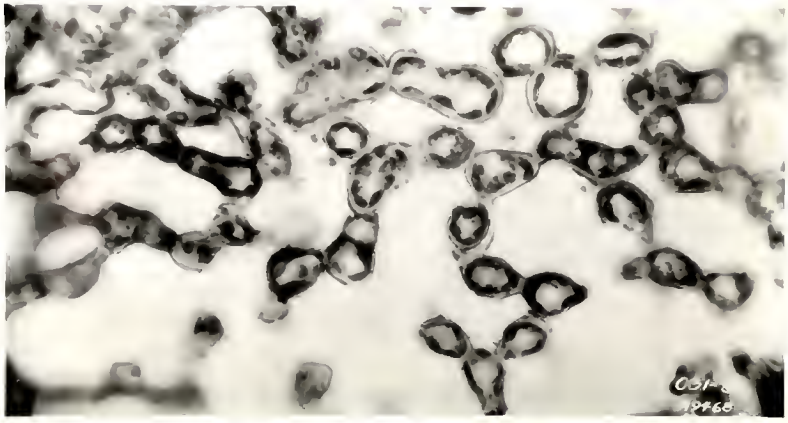
Tangential section through the third layer of palisade, Livland field leaf. Actual length of tangentially exposed cell walls, 460.65 microns per 2,500 square microns; diameter of cells, 10.21 microns (x700).

Plate XVI



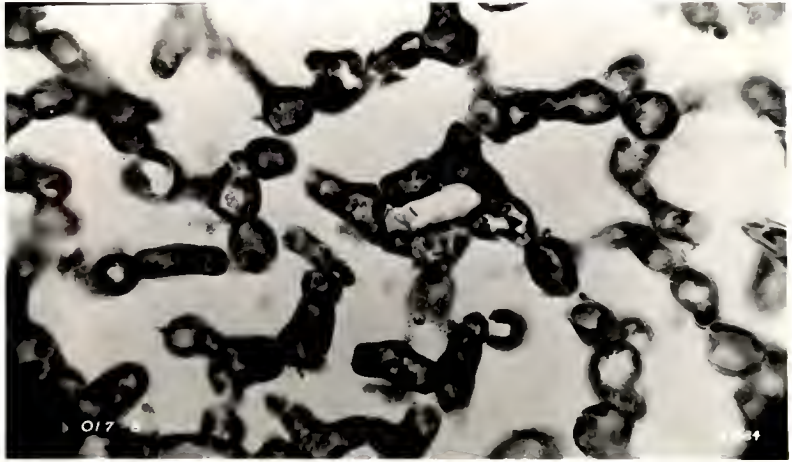
Tangential section of spongy mesophyll of Winesap field leaf. Average actual area of cells, 174.75 square microns. Average of 6.8 cells per 2,500 square microns (x700).

Plate XVII



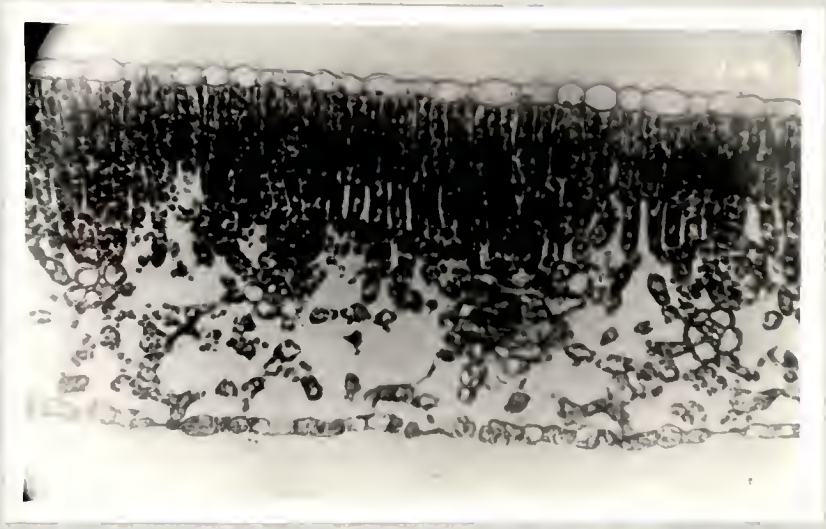
Tangential section of spongy mesophyll of York field leaf.
Average actual area of cells, 198.63 square microns.
Average of 5.8 cells per 2,800 square microns (x700).

Plate XVIII



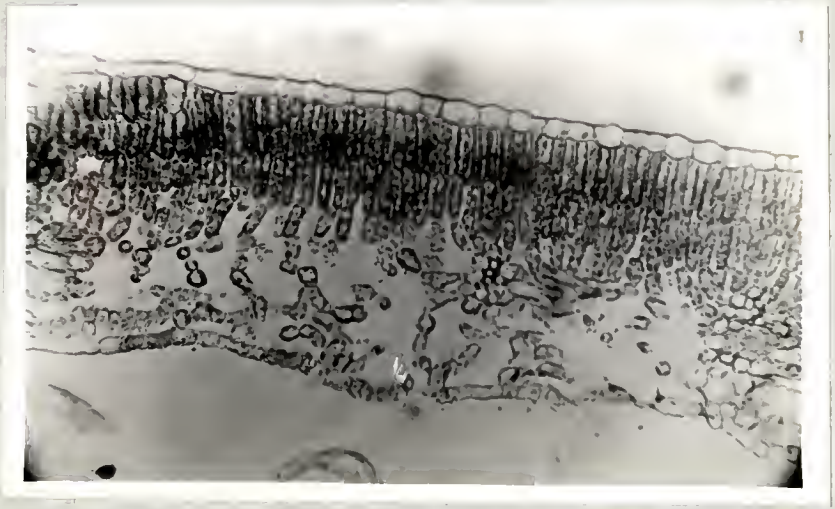
Tangential section of spongy mesophyll of Livland field leaf. Average actual area of cells, 204.04 square microns. Average of 5.3 cells per 2,500 square microns (x700).

Plate XIX



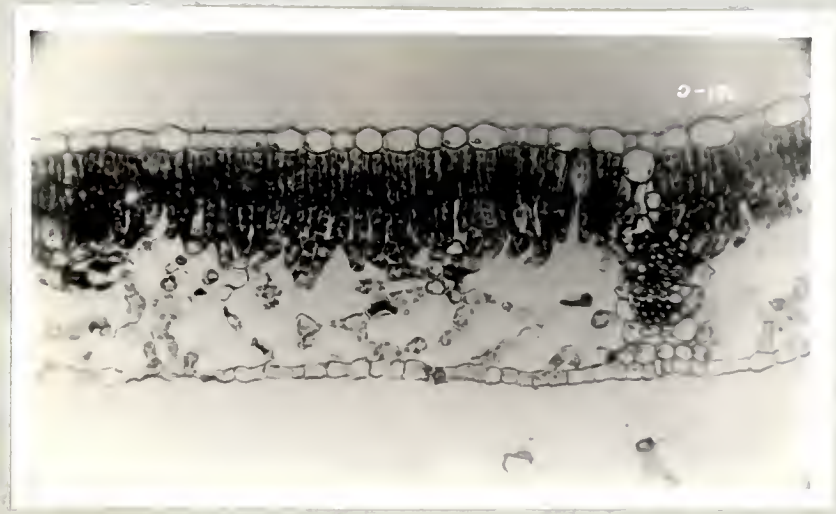
Cross section of healthy greenhouse leaf. Actual mean length of palisade cells in microns: first layer, 47.5; second layer, 36.3; third layer, 25.5 (x315).

Plate XX



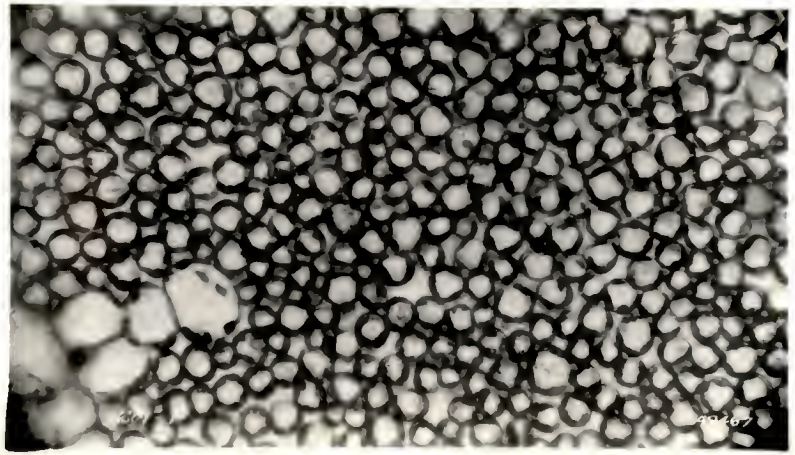
Cross section of Jonathan greenhouse leaf. Actual mean length of palisade cells in microns: first layer, 38.8; second layer, 30.5; third layer, 20.2 (x315).

Plate XXI



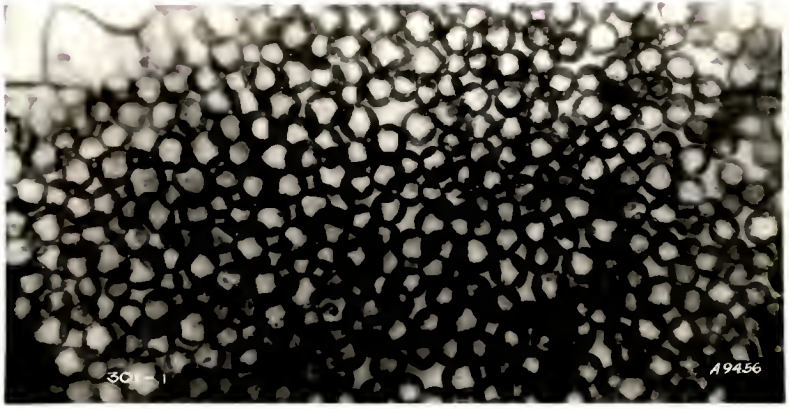
Cross section of York greenhouse leaf. Actual mean length of palisade cells in microns: first layer, 31.2; second layer, 24.6; third layer, 18.3 (x315).

Plate XXII



Tangential section through first layer of palisade cells, Wealthy greenhouse leaf. Actual length of tangentially exposed cell walls, 658.1 microns per 2,500 square microns; diameter of cells, 7.97 microns (x700).

Plate XXIII

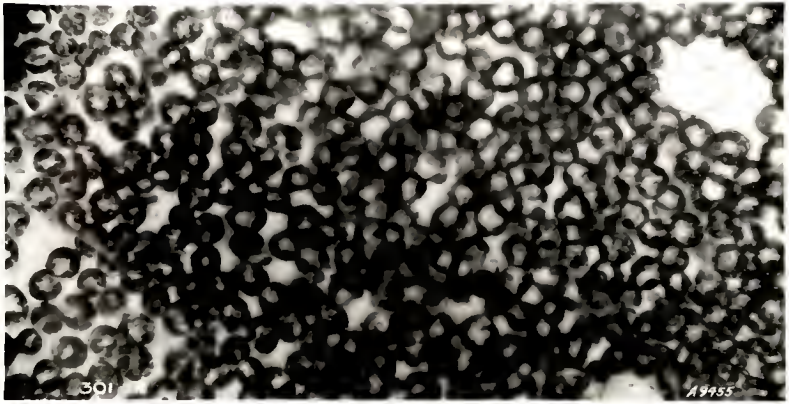


Tangential section through first layer of palisade cells, York greenhouse leaf. Actual length of tangentially exposed cell walls, 654.74 microns per 2,500 square microns; diameter of cells, 7.83 microns (x700).

Plate XXIV

Tangential section through second layer of palisade, Wealthy greenhouse leaf. Actual length of tangentially exposed cell walls, 603.44 microns per 2,500 square microns; diameter of cells, 9.01 microns (x700).

Plate XXV

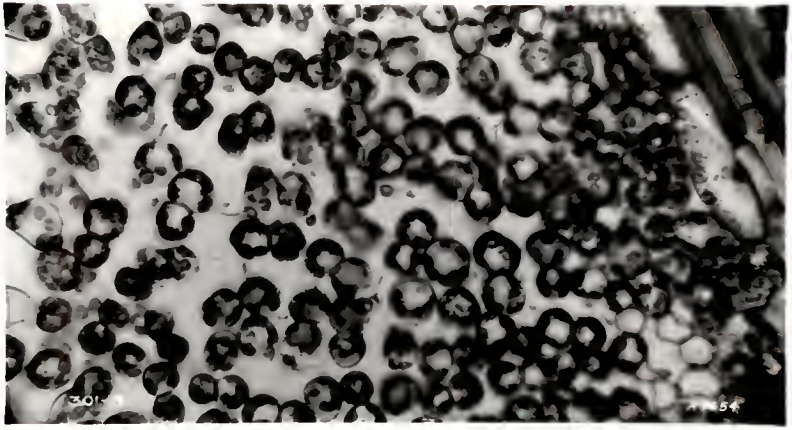


Tangential section through second layer of palisade, York greenhouse leaf. Actual length of tangentially exposed cell walls, 579.56 microns per 2,500 square microns; diameter of cells, 8.64 microns (x700).

Plate XXVI

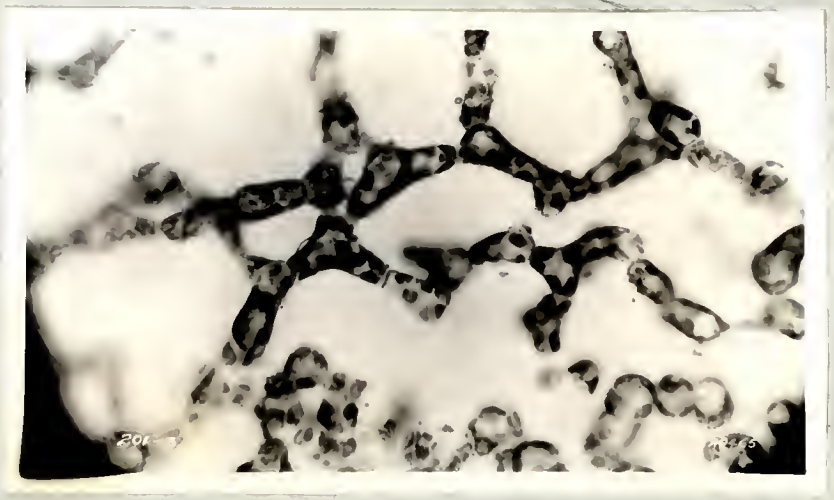
Tangential section through third layer of palisade, Wealthy greenhouse leaf. Actual length of tangentially exposed cell walls, 455.36 microns per 2,500 square microns; diameter of cells, 10.4 microns (x700).

Plate XXVII



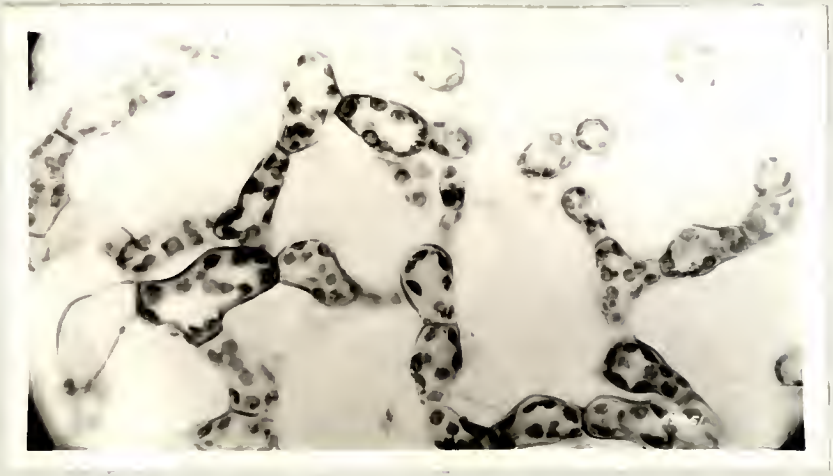
Tangential section through third layer of palisade, York greenhouse leaf. Actual length of tangentially exposed cell walls, 438.74 microns per 2,500 square microns; diameter of cells, 10.1 microns (x700). Note: Cells on the extreme left are of spongy mesophyll and the cells on the extreme right are of the second layer of palisade.

Plate XXVIII



Tangential section of spongy mesophyll of healthy greenhouse leaf. Average actual area of cells, 215.82 square microns. Average of 4.2 cells per 2,800 square microns (x700).

Plate XXIX



Tangential section of spongy mesophyll of York greenhouse leaf. Average actual area of cells, 185.17 square microns. Average of 4.3 cells per 2,500 square microns (x700).

ACKNOWLEDGMENT

The author wishes to express his indebtedness to the following: Dr. Wm. F. Pickett, his major instructor, for suggesting this problem, and for his aid in carrying out the work; Professor H. J. Barnett for his editorial comments upon the manuscript; Dr. H. H. Laude for assistance in statistical analysis of the data; and to his wife, Louise, for assistance throughout the problem.

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