The Relationship Between the Internal Structure and Photosynthetic Behavior of Apple Leaves

By

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THE RELATIONSHIP BETWEEN THE INTERNAL STRUCTURE AND PHOTOSYNTHETIC BEHAVIOR OF APPLE LEAVES

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Introduction

Several investigators have made studies of the photosynthetic behavior of the foliage leaves of apple (Malus sylvestris) and a few studies have been made of the internal structure of leaves of several species of plants, but the literature does not record any attempts to determine if the extent of the intercellular spaces in the spongy mesophyll of leaves is one of the factors which influence the rate of photosynthesis.

The purpose of the study herein reported is to determine if varieties of apples differ in the structure of the spongy leaf mesophyll, if so, the extent of such differences, and if these possible differences in anatomy have a relationship with variations in photosynthetic behavior of the leaves.

The problem has three distinct phases of investigation: (1) the anatomy of apple leaves, (2) stomatal characteristics and behavior, and (3) photosynthetic behavior. After the data on these phases were assembled the influence of the mesophyll characteristics on photosynthetic activity was studied.

PART I. ANATOMICAL STUDY OF APPLE LEAVES

Historical

Pick (1882) and Stahl (1883) concluded that sun leaves are thicker than shade leaves, but that the air spaces of the shade leaves are larger.

de Lamarliere (1892) observed that for equal surfaces, sun leaves have greater intensity of respiration, assimilation, and transpiration than those grown in the shade, the well known structural differences thus having a corresponding physiological significance.

Bonnier (1894) compared the same species of plants at various elevations in the Alps and Pyrenees mountains and found that the Alpine leaves had a better developed palisade tissue, due to larger cells or an increase in the number of rows, and that there were more stomata per unit of leaf surface than on the leaves of plants grown in the lowland.

Eberhardt (1900) reported that humid air favored the production of larger leaves, and greater amounts of chlorophyll and root development. Dry air caused the cuticle to become thicker, more stomata to be formed, and the palisade tissue to be thicker.

Hesselman (1904) studied the influence of light on the leaf structure of forest trees. Those leaves grown in the stronger light had more palisade cells than leaves in the poorer light. Shade leaves produced more starch than sun leaves of the same species when the light is equal.

Bergen (1904) found that sun leaves transpired more than shade leaves because the greater thickness of the former affords a larger evaporating surface.

Clements (1904) came to the conclusion that decreased light and increased water cause an increase in leaf surface and a decrease in thickness; decreased light causes a somewhat looser arrangment of the palisade; increased light and decreased water both cause a reduction in leaf surface and an increase in thickness; strong light causes a closer arrangement of the palisade; and, finally, that no laws can be laid down as to the exact amount of change taking place in the histology of the leaf in response to a definite difference in the physical factors.

Haller and Magness (1925) reported that in order to obtain apples of good size and quality, from 30 to 40 medium sized leaves were necessary per apple for Grimes and Ben Davis but for Delicious even more leaves were required.

Pfeiffer (1928) studied the influences of light of various wave lengths upon plant growth and found that full insolation produced thicker leaves than those in the limits of the visible spectrum.

Magness (1928) correlated leaf area and number of leaves with sugar content and size of apples and concluded that with smaller leaf area per fruit, the leaves seem to function more efficiently.

Vyvyan and Evans (1932) observed that the size of a leaf varies with the nature of the growth bearing it, and the number of leaves and position of the leaves on the growth.

Turrell (1934) computed formulae for the computation of the ratio of the internal exposed surface to the external exposed surface of mesomorphic, and succulent leaves. Though this method may be satisfactory for comparative determinations for leaves which differ as much as xeromorphic and succulent leaves, it was not found satisfactory for studies of varieties of apple leaves. The differences between the structure of apple leaves of different varieties are not sufficiently great to permit the use of this method

Pickett (1934) reported that 20 leaves per fruit on Delicious gave larger fruit than the largest of other varieties with 40 or even 50 leaves per fruit. The York ratio of 50 leaves per fruit produced apples which were larger than either Winesap or Jonathan, but smaller than Delicious at this ratio. The York trees however, had not produced fruit the preceding year, but the other varieties had borne.

The weight of fruit produced per 100 square centimeters of leaf area was determined by Fisher (1934) for McIntosh, Delicious, Rome, and Newtown. This weight was least with McIntosh and greatest with Rome.

Chandler (1934) reported fruiting does not reduce wood growth, in proportion to leaf surface, by as much as the dry matter of the fruit. One of the possible causes suggested was that the presence of the fruit accelerated photosynthesis by removal of the products which otherwise would accumulate in the leaves and inhibit photosynthesis.

Anatomy - procedure

Seven varieties of apples have been used in this study. The selection was based on the supposed efficiency of the leaves in promoting tree vigor and fruit production, rather than upon any previous knowledge of their internal structure. Livland and Wealthy were chosen because the fruit of these varieties ripens from early summer to mid-summer under Kansas conditions, Livland is rather a dwarfish tree, and Wealthy trees are of standard size. (Plates 1, 2, 3, and 4). Jonathan and Delicious trees are vigorous, and Jonathan is of major importance in the Missouri river valley district as a commercial variety. These varieties are usually harvested between the first and fifteenth of September. Delicious produces such vigorous young trees that they are extremely difficult to prune properly and wind deformity is a common characteristic of the variety. Winesap, Gano, and York were the winter varieties used. In central and eastern Kansas, Winesap is one of the most profitable varieties, especially in those orchards where adequate cross pollination is provided. Winesap trees are good in vigor on good sites but the fruit on the older trees is below medium in size and the leaves are smaller than those of most other varieties. Of the varieties

Plate 1. A Livland apple tree, representative of those used in this study.

Plate 2. A Jonathan apple tree, representative of those used in this study.

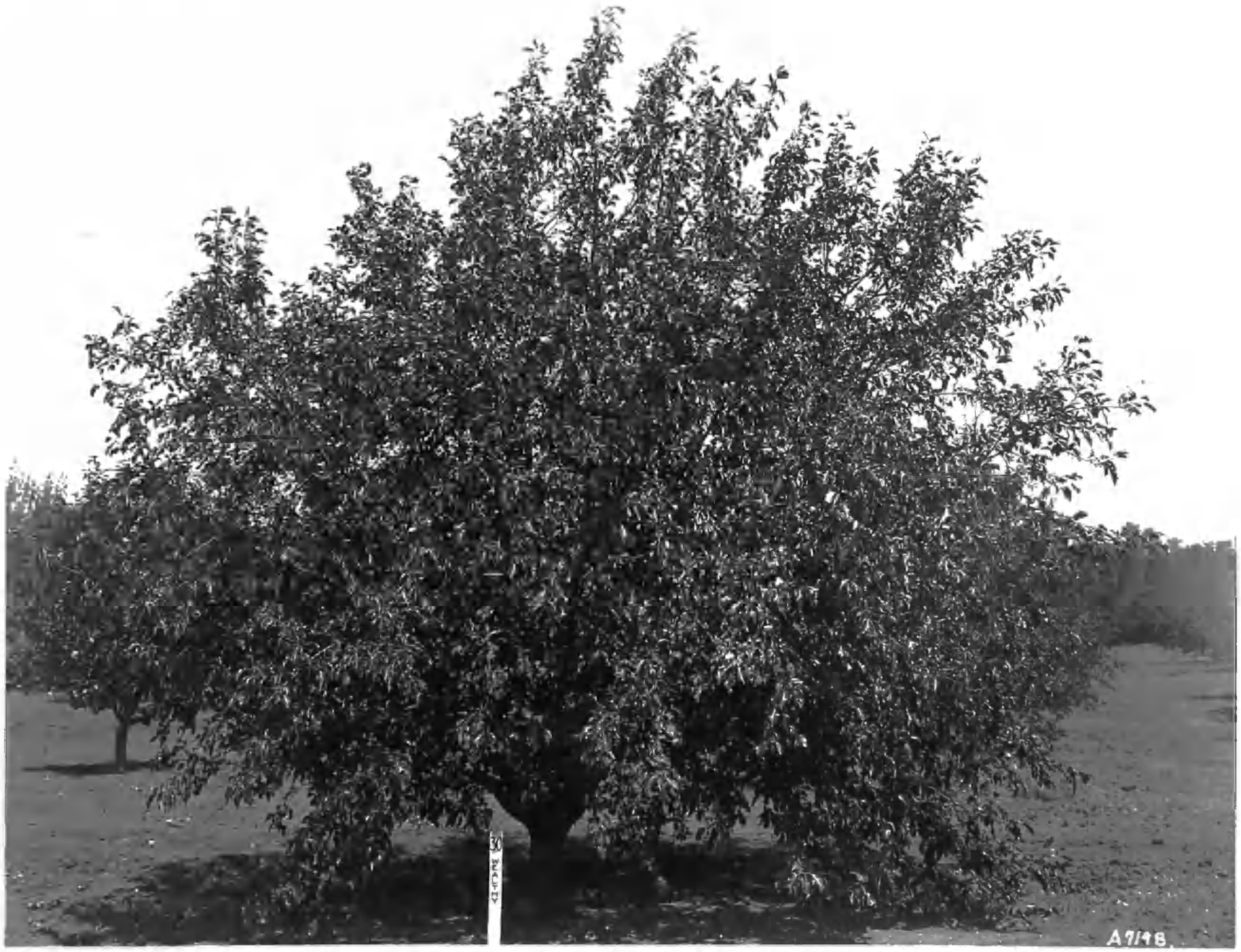


Plate 3. A Wealthy apple tree, representative of those used in this study.

Plate 4. A York apple tree, representative of those used in this study.

selected, Gano is one of the most profitable for the commercial grower. It is an annual bearer and the fruit and leaves are usually of good size. York is distinctly biennial in its fruit bearing habit. The tree is above average in vigor.

The trees in the orchard on the grounds of the Kansas Agricultural Experiment Station at Manhattan were used for this study. The average yearly rainfall at Manhattan is 31.49 inches. The soil on this site belongs to the Derby silt loam series. Considerable soil erosion has taken place and most of horizon A is gone. The trees are eighteen to twenty years old and are fair to good in vigor. The soil management method for the past fourteen years has been a combination clean cultivation, cover crop plan. Frequent scarcity of soil moisture is probably the greatest hazard in growing an orchard in this locality, and this factor becomes of major importance for a period varying from a few days to several weeks during most growing seasons when drouth conditions are aggravated by extremely hot weather and high, hot winds. During these periods the evaporating power of the air is great.

Leaves for the anatomical studies were selected from the middle portions of new shoots on the south side of the outer periphery of the trees. Pieces about one centimeter square were cut from near the mid-section of the leaf blades, only one piece being removed from a leaf, and plunged in a chromo-acetic acid killing solution containing one per cent acetic acid. The leaf portions were left in the killing solution over night, after which the killing agent was washed from the pieces with running tap water.

Dehydration of the samples was accomplished by using the following series of water, ethyl and N-butyl alcohols as reported by Zirkle (1930).

Water, cc	95	89	82	70	50	30
Ethyl alcohol, cc	5	11	18	30	40	50
N-butyl alcohol, cc	0	0	0	0	10	20

The samples were allowed to stand for one hour in each step except the last, which contains a total of 70 per cent alcohol, where the material was allowed to stand over night.

The dehydration was completed by the following steps. One hour was allowed for each except the last, when a 24-hour period was used:

Water, cc	15	5	0	0	0
Ethyl alcohol, cc	50	40	25	0	0
N-butyl alcohol, cc	35	55	75	100	100

Paraffin dissolves extremely slowly in N-butyl alcohol and a simple method of infiltration is to fill a vial two-thirds full of paraffin, allow it to harden, put the leaf portions upon it, cover them with N-butyl alcohol and place in the oven. When the paraffin melts the leaf portions sink and the N-butyl alcohol stays on the surface because it is lighter than the paraffin. The paraffin is changed two or three times and with the leaf samples used in this study, 18 to 24-hours were sufficient for the infiltration. N-butyl alcohol was more satisfactory for this purpose than xylol in that the infiltrated material was less brittle and much time was saved.

During the imbedding the leaf portions were arranged with the lower epidermis toward the bottom of the tray. A rotary microtome was used to cut the sections 10 microns in thickness. The imbedded material was so arranged on the microtome disc that the knife cut through the lower epidermis first; the palisade tissue and greater thickness of paraffin above the upper epidermis prevented tearing of the upper epidermis. Reversing the direction of the cut sometimes resulted in the spongy mesophyll and lower epidermis being torn.

A weak mixture of powdered egg albumen suspended in water was used as the fixative. After the ribbon was floated on this mixture under an electric light bulb, nearly all of the mixture was removed by blotting paper, and the sections thoroughly dried under the overhead heat. The staining was done by carrying the slides through the following series:

xylol 10 minutes

xylol 1 part, alcohol 1 part, 10 minutes

100 per cent alcohol 10 minutes

95 per cent alcohol 10 minutes

75 per cent alcohol 10 minutes

50 per cent alcohol 10 minutes

2 per cent Safranine O in 50 per cent alcohol 4 to 6 hours

50 per cent alcohol 2 minutes

75 per cent alcohol 10 minutes

95 per cent alcohol 10 minutes

100 per cent alcohol 10 minutes

xylol 1 part, alcohol 1 part, 10 minutes

xylol 10 minutes

balsam and cover glass

To permit a study of the looseness or compactness of the mesophyll, the slides were mounted on a microscope so arranged that it served as a microprojector. A spot light was used as the source of light and the images from the projector were focused on a screen 43 inches from the outer end of the microscope barrel so that the leaf sections were magnified approximately 900 diameters.

Fifty tracings of representative samples of the mesophyll of each variety were made on paper, not more than five tracings being made of sections from any one leaf.

To secure mathematical descriptions of the relative compactness of the mesophyll, the cross sectional areas of the intercellular spaces, as traced on paper, were computed with a planimeter. By use of a chartometer the total linear perimeter measurement of each tracing was determined. These data are presented in Table 1.

During the season 1934, for the orchard studies, only four varieties were used in contrast with seven varieties in 1933. The reduction in the number of varieties was feasible because of the great similarity between certain varieties and the reduced number made it possible to determine photosynthetic activity more frequently and thereby secure more representative records of the daily performance of the leaves. The varieties used in 1934 were Jonathan, York, Delicious, and Livland. Delicious and Livland were selected because during 1933 they probably had the most compact and the most open mesophyll structure, respectively, of the varieties studied. Jonathan and York were retained because the leaves are large and hence better suited to photosynthetic studies by the modified Sachs' punch method than the smaller leaves of Winesap foliage.

Table 1. Intercellular measurements of apple leaf mesophyll.*

Variety	Average cross sectional area of intercellular spaces, cm^2		Average perimeter measurements of intercellular spaces, cm	
Orchard grown	1933	1934	1933	1934
Delicious	56.76±5.03	86.96±9.67	148.31±12.88	205.74±19.30
Jonathan	61.15±8.64	77.85±11.74	158.80±15.42	202.51±26.03
Gano	67.27±7.67		154.43±11.45	
Winesap	79.85±7.16		179.93±6.17	
York	81.78±9.35	70.82±7.48	190.02±17.55	196.39±18.62
Wealthy	82.17±6.90		191.62±17.80	
Livland	114.36±5.29	107.97±7.22	254.51±17.48	212.55±17.30
Greenhouse grown				
Delicious		60.44±6.64		123.25±9.27
Livland		63.40±11.74		128.65±22.05

* Measurements for each variety were made from 50 projected images, 11 inches long, at a magnification of X900.

The differences between measurements of the intercellular spaces in the mesophyll of some of these varieties are highly significant statistically. Between others, however, the differences are not significant.

The differences between the means of the intercellular area measurements for 1933 are presented in Table 2 and their perimeter differences in Table 3. As shown by Table 2, the differences between the cross sectional area measurements of orchard grown Livland and those of orchard grown Delicious, Jonathan, Gano, and Winesap are significant to the extent that the differences are at least four times their probable errors. The differences between the means of Livland and the other varieties vary, in terms of their own probable errors, from 7.8 for the Livland - Delicious comparison to 3.04 for the Livland - York comparison. (Pickett 1933).

Considering the 1934 data, (only four varieties were used) there was no significance between any comparison of orchard grown Delicious, Jonathan and York, (Table 4). However, between orchard grown Livland and orchard leaves of the other three varieties, the differences in terms of their own probable errors vary from 3.57 for the Livland - York comparison to 2.19 for the Livland - Jonathan comparison. The 1934 orchard data do not show as great differences in the cross sectional area measurements as those of 1933.

When comparisons between greenhouse grown and orchard grown foliage are made the differences are greater. The difference between orchard grown Livland and greenhouse grown Delicious in terms of its own probable error is 4.85. The formula used for calculating the probable error of the difference between two means is:

$$PE_{M_1 - M_2} = \sqrt{(PE_{M_1})^2 + (PE_{M_2})^2}$$

Table 2. Differences between means of cross sectional area measurements of intercellular spaces in apple leaf mesophyll. Square centimeters. 1933. Orchard grown.

	Delicious	Jonathan	Gano	Winesap	York	Wealthy
Jonathan	4.39±9.99					
Gano	10.51±9.17	6.12±11.55				
Winesap	23.09±8.75	18.70±11.22	12.58±10.49			
York	25.02±10.62	20.63±12.73	14.51±12.09	1.93±11.78		
Wealthy	25.41±8.54	21.02±11.06	14.90±10.32	2.32±9.94	0.39±11.62	
Livland	57.60±7.30	53.21±10.13	47.09±9.32	34.51±8.90	32.58±10.74	32.19±8.69
	Orchard Delicious	Orchard Livland				
Greenhouse Delicious	16.52±11.77					
Greenhouse Livland		50.96±12.88				

Table 3. Differences between means of perimeter measurements of intercellular spaces in apple leaf mesophyll. Centimeters. 1933. Orchard grown.

	Delicious	Jonathan	Gano	Winesap	York	Wealthy
Jonathan	10.49±20.09					
Gano	6.12±17.23	4.37±19.21				
Winesap	31.62±14.28	21.13±16.61	25.50±13.01			
York	41.71±21.77	31.22±23.36	35.59±20.95	11.09±18.60		
Wealthy	43.31±21.97	32.82±23.55	37.19±21.16	11.69±18.84	1.60±25.00	
Livland	106.20±21.71	95.71±23.31	100.08±20.90	74.54±18.54	64.49±24.77	62.89±24.95
	Orchard Delicious	Orchard Livland				
Greenhouse Delicious	66.49±10.55					
Greenhouse Livland		14.29±22.68				

Table 4. Differences between means of cross sectional area measurements of intercellular spaces in apple leaf mesophyll. Square centimenters. 1934.

	Orchard Delicious	Greenhouse Delicious	Jonathan	York	Greenhouse Livland
Greenhouse Delicious	4.11±1.82				0.46±2.08
Jonathan	1.41±2.36	2.70±2.09			
York	2.50±1.84	1.61±1.55	1.09±2.15		
Greenhouse Livland	3.65±2.36	0.46±2.09	2.24±2.58	1.15±2.15	
Orchard Livland	3.26±1.93	7.37±1.52	4.67±2.13	5.76±1.61	6.91±2.13

Photomicrographs of representative portions of cross sectional and tangential sections of the apple leaves are presented in Plates 5 to 14 inclusive. The cross sections were prepared from the seven varieties used in 1933. The tangential views are from the four varieties studied in 1934. The most important feature of the tangential sections is the distinct looseness of the Livland mesophyll when contrasted with that of the York, Delicious and Jonathan sections. The tangential illustrations show a portion of the lower epidermis with a few stomata clearly visible, the lower parts of some of the palisade cells with the spongy tissue constituting most of the field.

Plate 5. Cross section (X 200) of a greenhouse grown Livland apple leaf.

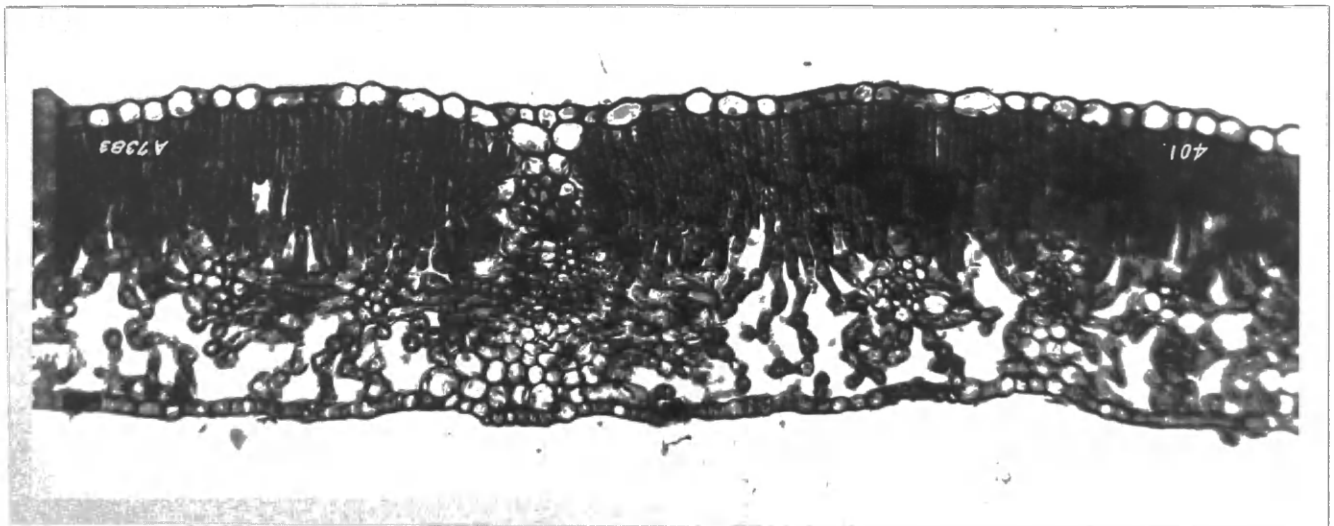


Plate 6. Cross section (X 200) of a greenhouse grown Delicious apple leaf.

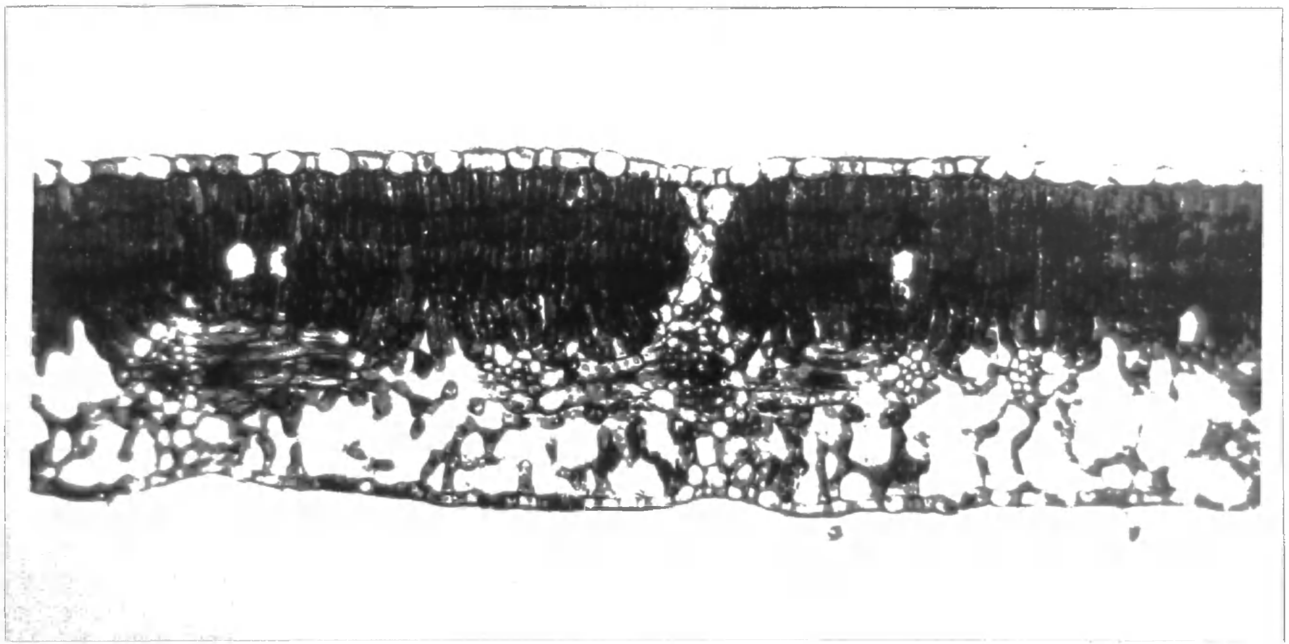


Plate 7. Cross section (X 200) of an orchard grown Jonathan apple leaf.

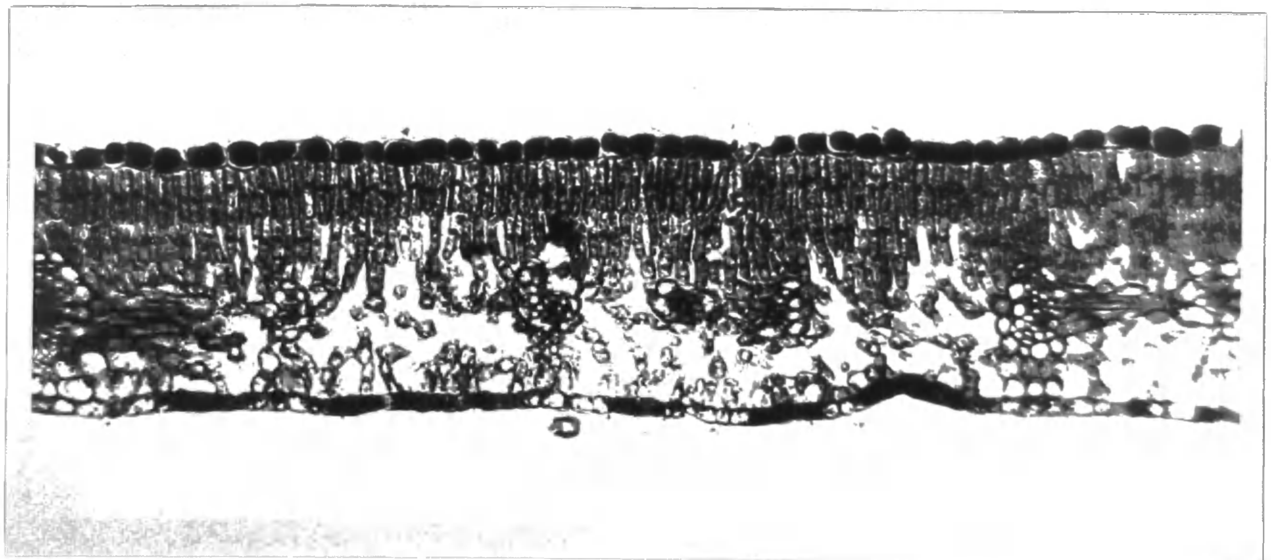


Plate 8. Cross section (X 200) of an orchard grown York apple leaf.

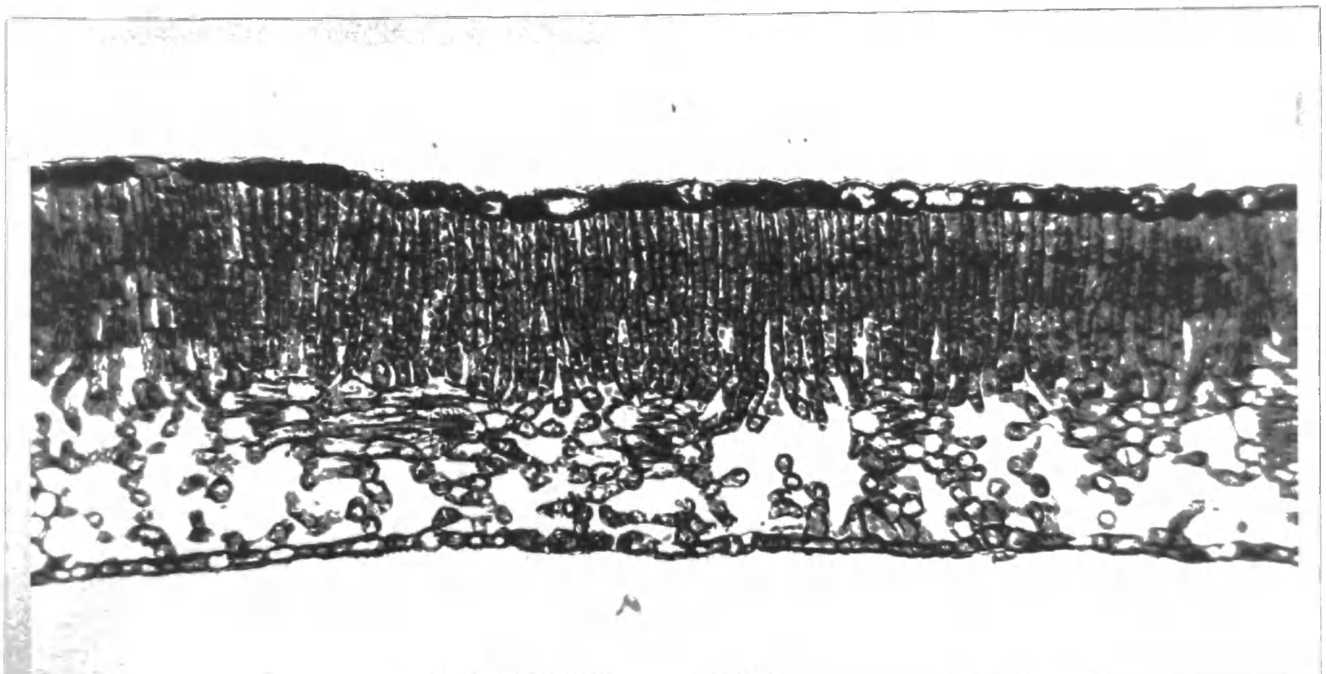


Plate 9. Cross section (X 200) of an orchard grown Delicious apple leaf.

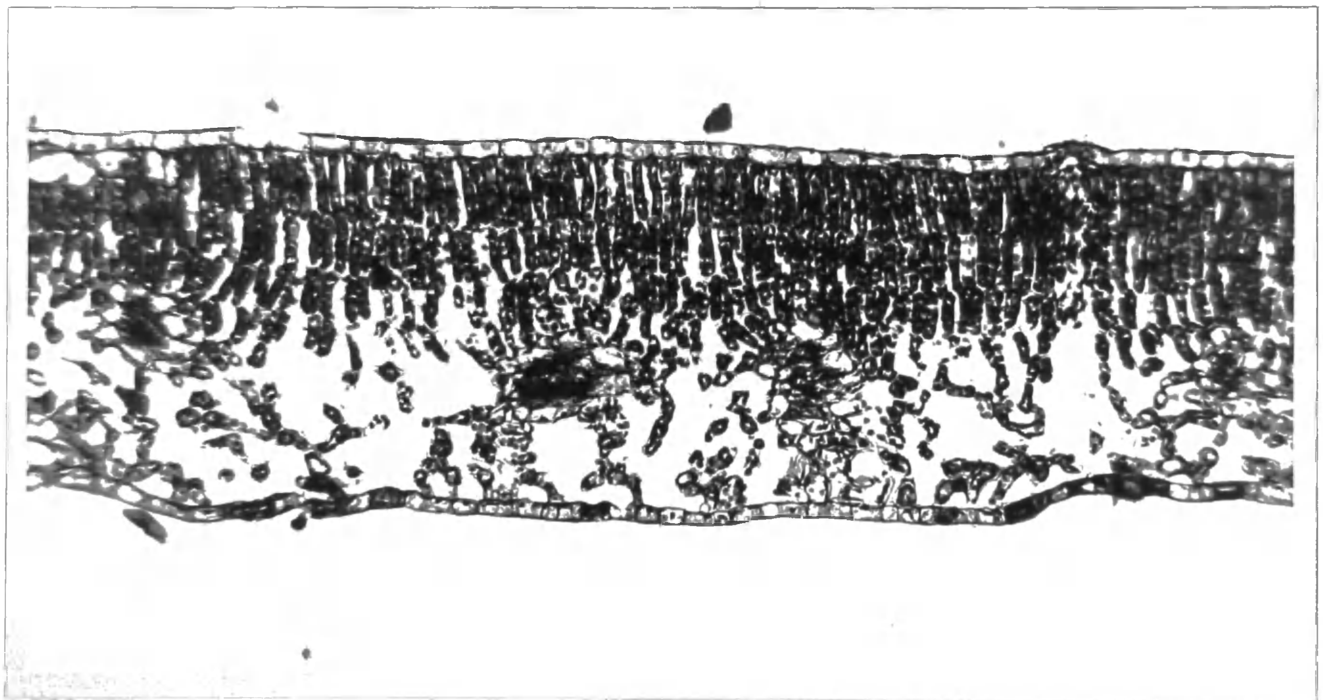


Plate 10. Cross section (X 200) of an orchard grown Livland apple leaf.

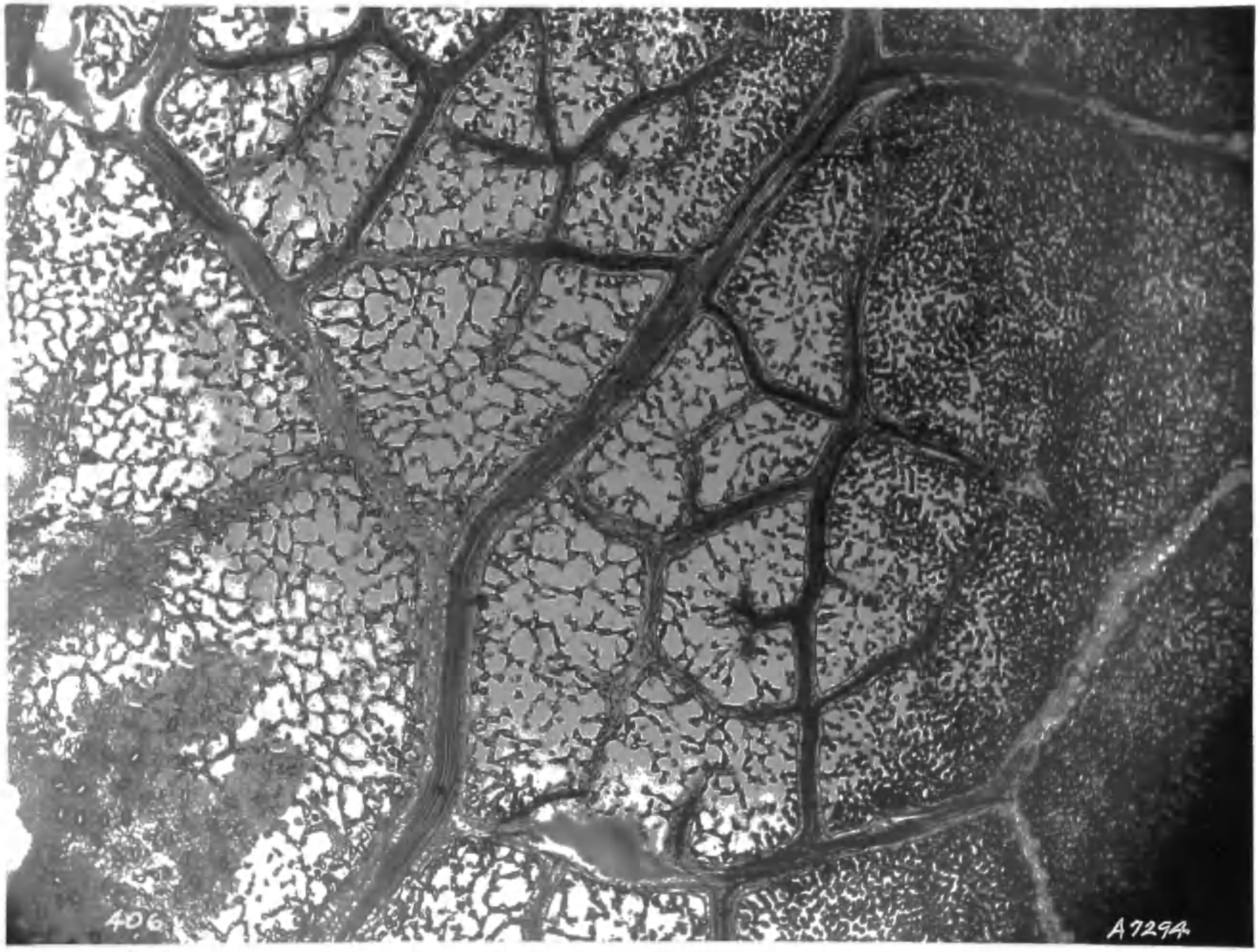


Plate 11. Section (X 80), cut parallel or slightly diagonal to the surface of an orchard grown Jonathan apple leaf. Note stomata at lower left and palisade cells on right.

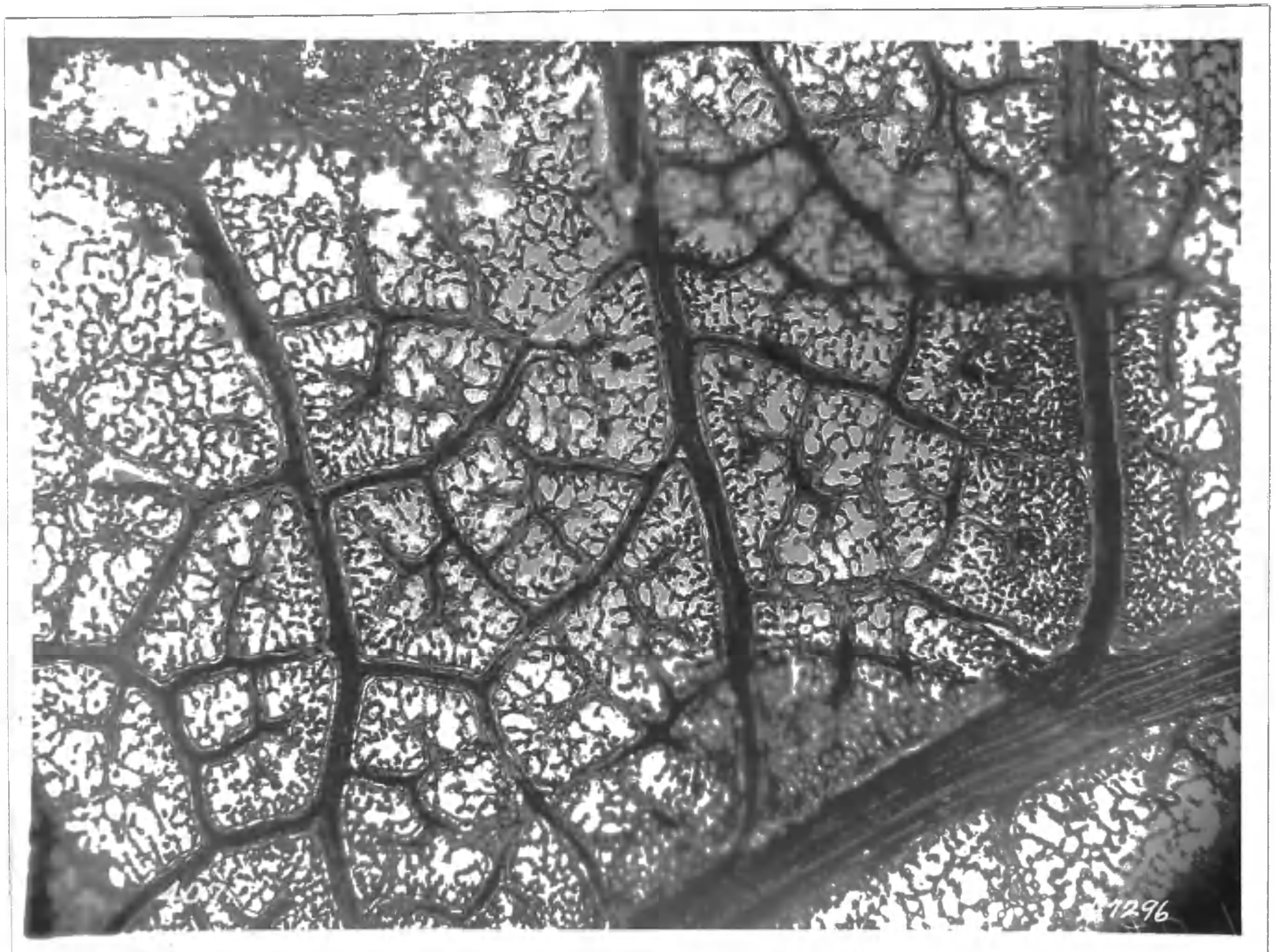


Plate 12. Section (X 80), cut parallel or slightly diagonal to the surface of an orchard grown York apple leaf.

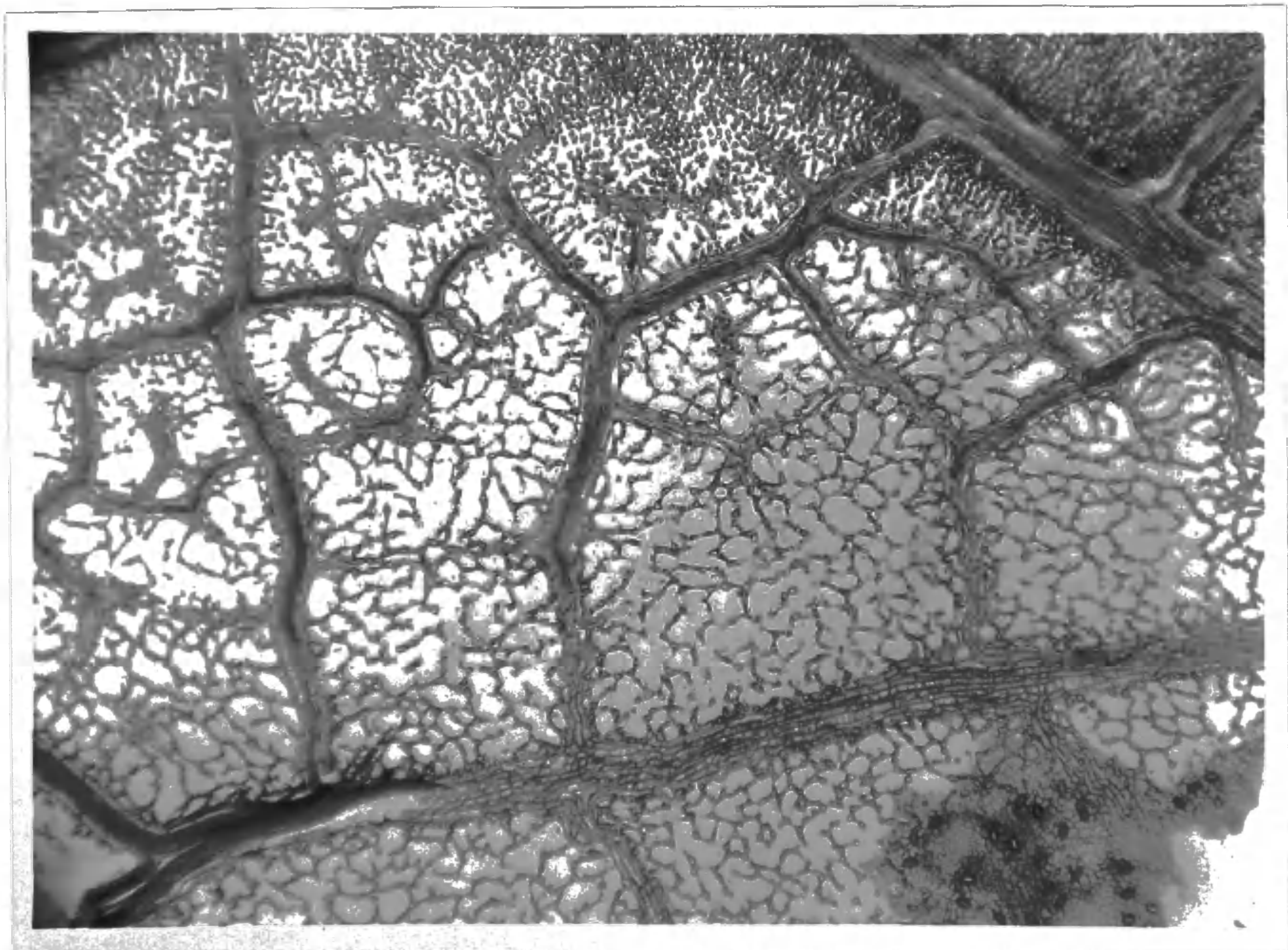


Plate 13. Section (X 80), cut parallel or slightly diagonal to the surface of an orchard grown Livland apple leaf. Note stomata at lower right and lower parts of palisade at top. Compare intercellular spaces in the spongy mesophyll with those in Plate 12.

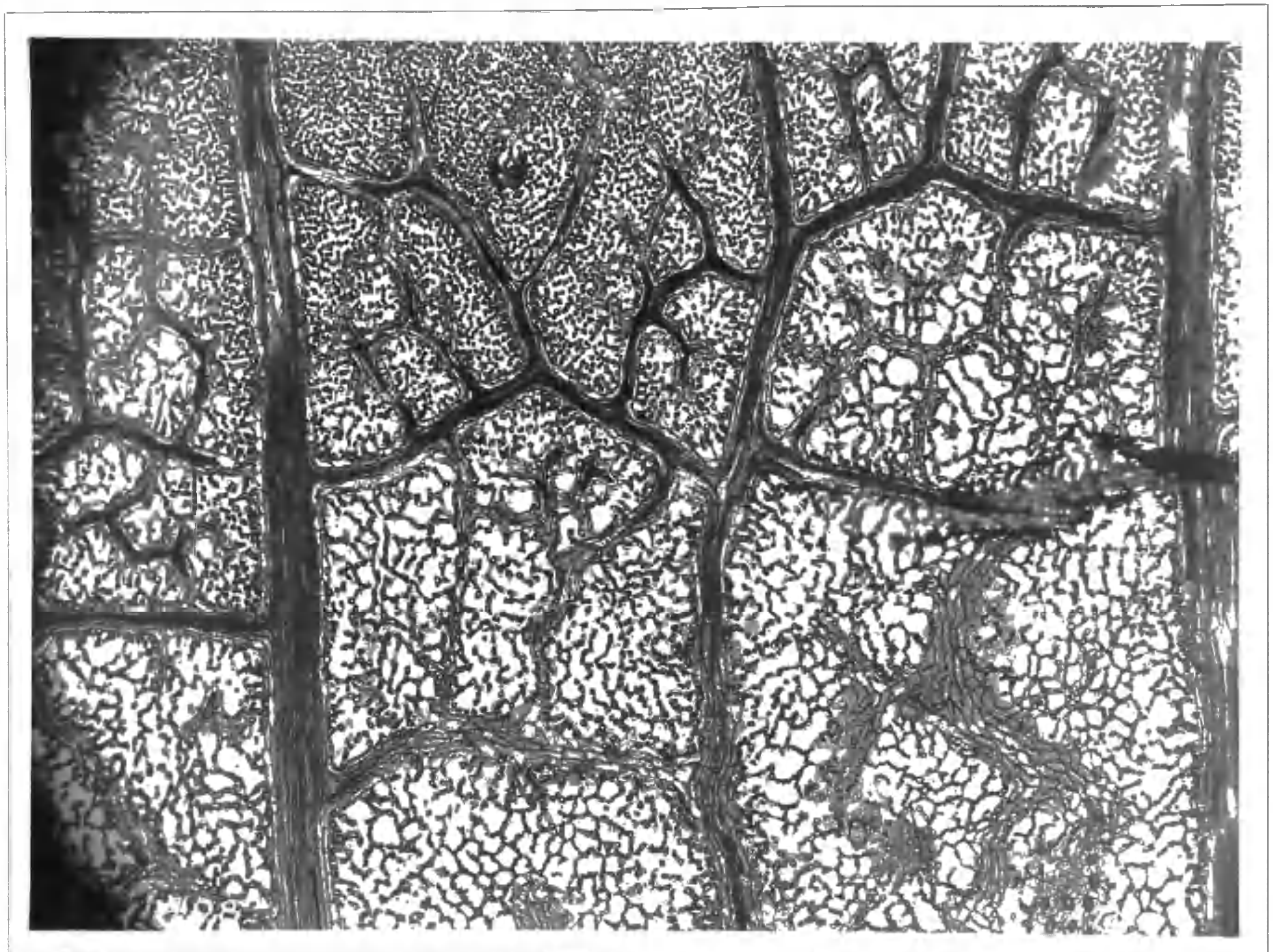


Plate 14. Section (X 80), cut parallel or slightly diagonal to the surface of an orchard grown Delicious apple leaf.

PART II. STOMATAL BEHAVIOR

Introduction

Stomata on apple leaves are found only on the lower surfaces, and are directly involved in the process of photosynthesis since the carbon dioxide enters the leaves, for the most part, through them. Cuticular absorption of carbon dioxide is probably not sufficient to be of importance in photosynthesis in apple leaves.

Historical

No attempt here will be made to give an extensive review of the literature on stomatal behavior or of methods for making these studies. A critical review of the latter together with a bibliography is given by Lloyd (1908). He concluded that, if a section of leaf epidermis be removed and plunged at once into absolute alcohol, the tissue is dehydrated so rapidly that no measurable change in the dimensions of the stomata takes place. Sachs had long before pointed this out to de Vries. The material thus treated may be studied in detail at a subsequent time. Lloyd, working with Fourquieria splendens and Verbena ciliata, concluded that stomatal regulation of transpiration does not occur, except that conservation of the contained water follows on complete closure of the stomata. Lloyd believed that light was the most important of the environmental factors that cause stomata to open in the morning.

Loftfield (1921) studied the daily march of stomatal movement at Salt Lake City, Utah; Minneapolis, Minnesota; and Tucson, Arizona, and classified the apple with the group of plants, typified by alfalfa, in which the stomata are closed during the night and open from day break until midday or until nightfall. He presents data to show that stomata regulate the water loss from plants. The rate of transpiration is closely governed by stomata when they are nearly closed and by the factors affecting evaporation when they are wide open. He also gives a bibliography on stomatal behavior, especially as it is involved in transpiration. The number and size of the stomata on any given area of leaf are influenced by the conditions under which they were formed. A leaf developed in the shade has fewer and larger stomata per unit than one produced in light.

Maskell (1928) presented evidence to show that diurnal rhythm of assimilation is closely associated with the diurnal behavior of stomatal opening.

Furr and Magness (1930) found that the duration of stomatal opening was closely allied with soil moisture content and the evaporating power of the air. Favorable soil moisture content caused the stomata to remain open longer than when the amount of moisture in the soil is low or near the wilting point. The period of daily opening was approximately twice as long on the irrigated plots as on the dry plots. These workers found that there was a close correlation between the daily period of stomatal opening and the growth of the fruit.

Penfound (1932) working with the castor bean, found that the number of stomata varied with the amount of light and indirectly with soil moisture content. Sawyer (1932) studied the stomatal apparatus of the cultivated cranberry, Vaccinum macrocarpon, and found no chloroplasts in the guard cells. The stomata never open widely and the apparatus seemed poorly adjusted to changes in light, temperature, and moisture. He reported no differences of taxonomic or functional significance were found in four varieties.

Procedure

Three items involving stomatal studies were investigated in this project; namely, the number of stomata per unit area of leaf surface for each variety of apple used, the size of the stomata as judged by the length of the stomatal slit or opening, and the diurnal behavior of opening and closing in a few instances.

In Table 5 are presented the data on the number of stomata per square centimeter of leaf surface for the season of 1933 for both greenhouse grown and orchard grown trees. Livland, a Russian variety, has the smallest number of stomata per unit area of leaf surface for both the greenhouse and orchard trees, but has the longest stomatal opening of any variety used.

In measuring the length of the stomata (Table 5) the opening was measured and not the length of the adjacent guard cells.

Table 5. Number and size of stomata.

Variety	Orchard grown foliage. Average number of stomata per cm^2 leaf area.		Orchard grown foliage. Average length of stomata. Microns.		Greenhouse grown foliage. Average number of stomata per cm^2 leaf area. Cool house		Greenhouse grown foliage. Average number of stomata per cm^2 leaf area. Warm house	
	1933	1934	1933	1934	1933	1934	1933	1934
Livland	27,100	26,000	14.25	14.55	20,000	21,000	20,100	21,500
York	30,800	30,000	12.23	12.50	23,000	......	25,500	......
Gano	31,900	......	13.99	.....	25,000	......	23,100	......
Jonathan	33,000	30,500	12.76	12.53	25,300	......	23,100	......
Winesap	33,000	......	12.16	.....	22,200	......	23,000	......
Delicious	33,500	33,500	12.08	12.27	......	27,500	......	27,000
Wealthy	37,290	......	13.91	.....	......	......	......	.....

Stomatal counts and measurements were made from strips of the lower epidermis torn from the apple leaves and mounted in water. At least twenty-five counts and measurements were made for each variety, representing one strip from each of twenty-five leaves located on the south side of the outer periphery of the trees. Only mature leaves were used. Later in this report, data will be presented on the photosynthetic activity of orchard grown leaves compared with that of greenhouse grown leaves. It is of interest to note that greenhouse grown leaves have fewer stomata per unit area than orchard grown leaves of the same variety.

Stomatal opening and closing was studied for the seven apple varieties used in 1933 and for the four used in 1934. No attempt was made to determine the cause or causes of stomatal behavior, since the objective of this work was to determine if there might be differences in this regard among the several varieties.

Two methods of studying the stomatal behavior were employed. In one method, the microscope was taken to the orchard and the lower epidermis of the leaf was examined while still attached to the tree. This required a strong light to reflect against the leaf from the sub-stage mirror. There was much difficulty in using the higher power objectives for this purpose because of the uneven surface of the leaves and the presence of great amounts of pubescence on some varieties, especially Jonathan. Livland has little pubescence on the lower epidermis. The low power objective was not satisfactory because of insufficient magnification. This method was found to be too cumbersome to be satisfactory. Too much time is required for each observation and no permanent material is on hand for subsequent confirmation of the data.

The method which was more satisfactory was to tear off strips of the lower epidermis and plunge them into absolute alcohol. Pieces of the lower epidermis of the leaves were taken by making a small cut under the epidermis, near the midrib, with a safety razor blade, after which an assistant quickly jerked with a pair of tweezers as much of the epidermis as would tear. The section secured by starting near the midrib and tearing the epidermis laterally toward the leaf margin, was triangular in outline because the epidermis tore along the smaller veins. The epidermal strip was plunged immediately into absolute alcohol, the entire operation requiring only two or three seconds. Later the strips were stained in two per cent safraine O in absolute alcohol, rinsed in absolute alcohol, and permanently mounted in euparal. The preparation of permanent slides was necessary because considerable time was required to determine which stomata were open, due to the fact that with most varieties the stomata were seldom more than partly open. It is entirely probable that with any method of observing stomatal behavior there is sufficient error to make it practically impossible to determine if a stoma is closed or only <u>nearly</u> so. The author has had extreme difficulty in making determinations of this kind.

On August 3 and 4, stomatal behavior was studied on a King David apple tree at Manhattan, Kansas. This 20-year old tree was growing in a heavy clay soil and had been in a wilted condition for three weeks. During the few days preceding August 3, the foliage was wilted during the entire 24-hour period, while during the first part of the 3-week period, the leaves wilted every day but recovered their turgidity over night. The condition of the stomata was determined by removing strips of the lower epidermis as described above. In addition the lower epidermis of the leaves was examined with a microscope without detaching the leaves from the tree. At every inspection all of the stomata were judged to be closed.

The same type of experiment was repeated on August 21 and 22, 1934, with a Jonathan tree. (Plates 15 and 16). Here again all the stomata were judged to be closed at each inspection. The tree was badly wilted. The data for both the King David and Jonathan trees are presented in Table 6. The procedures of judging apparent photosynthesis according to the modified Sachs dry weight method and of determining the total acid hydrolyzable carbohydrates are described later in this publication.

Plate 15. Wilted Jonathan foliage. August 21, 1934.

Plate 16. Wilted Jonathan foliage. August 21, 1934.

Table 6. Stomatal behavior and photosynthetic activity wilted apple foliage, 1934.

Variety	Date - Time	Temperature. centigrade	Relative humidity. per cent	Total dry weight per M^2 leaf area. gms	Ash. per cent	Ash per M^2 leaf area. gms	Combustible material per M^2 leaf area. gms	Acid hydrolyzable carbohydrates as glucose per M^2 leaf area. gms
King David	Aug. 3, 6:00 a.m.	22	42	81.01	....	....	.....	11.72
	Aug. 3, 2:00 p.m.	40	16	83.50	....	....	.....	12.16
	Aug. 4, 6:00 a.m.	29	76	84.47	....	....	.....	12.14
Jonathan	Aug. 21, 6:00 a.m.	15	89	82.72	5.74	4.75	77.97	13.01
	Aug. 21, 2:00 p.m.	31	46	83.75	5.77	4.83	78.92	12.28
	Aug. 22, 6:00 a.m.	21	85	89.25	5.66	5.05	84.20	11.78
	Aug. 22, 2:00 p.m.	31	30	90.46	5.80	5.25	85.21	11.49

Although for the duration of the experiments, there was a gain in combustible organic matter with the samples represented in Table 6, it is considered that it was too small to represent any appreciable photosynthetic activity and probably falls within the limits of the error of sampling. Likewise, the variations in carbohydrate content are not significant.

Stomatal behavior of greenhouse grown apple foliage was studied on May 22, and June 5, 1933, and May 2, 1934. Similar observations on orchard grown leaves were made on June 12, July 6, and July 10, 1933, and on July 18, 1934. (Tables 7, 8, and 9). No experimental work was attempted to determine which factors governed stomatal opening and closing. Neither were the trees subjected to any special treatment to prolong the period of stomatal opening. The primary purpose of the investigations was to determine whether there were any varietal differences in stomatal behavior. It is assumed that stomatal opening and closing largely governs the rate of photosynthesis in leaves, other environmental factors being favorable for this process. A few general statements summarize these studies:

a. Stomata on apple foliage in the greenhouse usually remain open longer each morning than those in the orchard.

b. With all varieties under observation, many stomata were partly open to wide open until noon in the greenhouse.

c. In the orchard, the stomata were seldom open after 8 or 9 o'clock in the morning and on some days they were closed during the entire twenty-four hour period. There is probably a certain amount of error in this last statement, because on one or two occasions the leaves were active photosynthetically to a greater degree than shown in Table 6, although the stomata were judged to be closed. The author is of the opinion that it is impossible to determine with certainty by means of microscopic examination of epidermal strips or of the attached leaves whether stomata are closed sufficiently to be gas tight or only nearly closed.

d. Livland has the smallest number of stomata per unit of leaf area of any of the varieties observed, but the stomata are longer than any others.

e. Wealthy has the greatest number of stomata per unit of leaf area.

f. Fewer Livland and Wealthy stomata are open in the morning than those of the other varieties.

Table 7. Stomatal behavior, orchard grown apple foliage, 1933.

Time	5:00 a.m.			9:00 a.m.			1:00 p.m.			3:30 p.m.			7:00 p.m.		
	June 12	July 6	July 10	June 12	July 6	July 10	June 12	July 6	July 10	June 12	July 6	July 10	June 12	July 6	July 10
Temperature, C	22	20	24	29	27	29	31	33	32	36	38	34	32	37	32
Relative humidity per cent	85	71	96	80	67	76	50	60	65	27	30	57	30	36	52
	Stomata open, per cent*														
Delicious	15	19	20	6	2	10	0	0	0	0	0	1	0	0	1
Jonathan	12	8	10	10	5	96	0	0	0	0	0	0	0	0	0
Gano	20	21	55	8	0	23	0	0	2	0	0	1	0	0	1
Winesap	18	20	25	6	1	2	0	0	0	0	0	0	0	0	1
York	12	10	20	9	9	5	0	0	0	0	0	0	0	0	0
Wealthy	5	0	1	1	0	11	0	0	0	0	0	0	0	0	0
Livland	6	1	11	0	0	4	0	0	0	0	0	0	0	0	0

*In compiling this table no distinction was made between the stomata which were only partly open and those open to a greater extent; both conditions were listed as being "open."

Table 8. Stomatal behavior orchard grown foliage, July 18, 1934.

Time	5:00 a.m.	6:00 a.m.	7:00 a.m.	8:00 a.m.
Temperature, C	28	29	30	31
Relative Humidity per cent	42	40	37	35
	Stomata open, per cent*			
Jonathan	19	20	—	—
York	16	18	—	—
Delicious	11	16	—	—
Livland	2	7	—	—

*No stoma recorded as "open" in this table was open more than one-fourth of what would be considered as being wide open.

Table 9. Stomatal behavior greenhouse grown foliage, May 2, May 22, and June 5, 1934.

Time	8:00 a.m.			9:30 a.m.			11:30 a.m.			1:30 p.m.			3:30 p.m.		
	May 2	May 22	June 5	May 2	May 22	June 5	May 2	May 22	June 5	May 2	May 22	June 5	May 2	May 22	June 5
Temperature C	22	21	20	24	20	19	24	20	24	23	25	27	23	23	31
Relative humidity per cent	77	75	70	66	72	67	76	68	65	69	69	62	69	65	60
	Stomata open, per cent*														
Livland	90	75	77	100	90	89	100	76	72	10	5	10	--	--	--
Delicious	85	80	75	100	93	84	100	70	85	10	15	7	--	--	--

PART III. PHOTOSYNTHETIC ACTIVITY OF APPLE LEAVES

Introduction

No entirely satisfactory method for accurately determining the rate of photosynthesis has been devised. The reasons for this are obvious. Concomitant with the phenonomenon of photosynthesis are the processes of respiration and translocation and it is difficult, if not impossible, to measure the first and exclude the effects of the latter two. There are several methods for measuring the apparent rate of photosynthesis; that is, the amount of photosynthetic material produced in excess of the amount of substances used in respiration and translocation. One of these methods considers the quantitative determination of the gaseous exchange. This method has two distinct types of study: in one the amount of oxygen liberated is measured and in the other the intake of carbon dioxide is determined. The second general method is based on the determination of organic substance formed or the dry matter accumulated.

In the present study, apparent photosynthetic measurements were made in three ways: (a) by determining the amount of carbon dioxide absorbed by a known amount of leaf area, (b) the weight of the dry matter accumulated per unit of leaf area during a definite period, (c) comparisons of the total acid hydrolyzable carbohydrates at different periods of the day. Each of these methods will be discussed separately because the results are conflicting. No two of them measure the same plant activities. In each of the three methods the process of respiration complicates the photosynthetic determinations. Not all plant cells carry on photosynthesis, but all cells involved in photosynthesis also respire. In photosynthesis, the carbon dioxide is reduced and oxygen is released, but in respiration exygen is absorbed, plant foods are used up and carbon dioxide and water are the end products. Respiration is essentially an oxidation process. Thus, in the cells containing chlorophyll, two different kinds of chemical processes are taking place simultaneously; one in which oxygen is released, the other in which oxygen is absorbed. Since carbon dioxide is utilized in the former and released in the latter, the amount of this gas absorbed from the outside atmosphere will be less than the total amount used in photosynthesis. The process of photosynthesis over any considerable length of time proceeds at a greater rate than respiration.

a. Determination of the amount of carbon dioxide absorbed by a known amount of leaf area.

Historical

The method of determining the absorption of carbon dioxide was first used by Kreusler (1885). Other investigators, including Matthaei (1905), Blackman and Matthaei (1905), Brown and Escombe (1905), Willstatter and Stoll (1918), Spoehr and McGee (1923), and Heinicke and Hoffman (1933) have modified and improved the method. Good bibliographies on this topic are found in the works of Stiles (1925), Spoehr (1926), Miller (1931), Gassner and Goeze (1932), and Heinicke and Hoffman (1933). Stiles (1925) is of the opinion that the continuous current method is the most reliable of all methods that have been evolved for measuring photosynthesis. In the continuous current method, a stream of air is drawn past the plant part under test and through an absorbing solution where the remaining carbon dioxide is removed from the air.

Any leaf or group of leaves enclosed within a cellophane envelope, through which a stream of air is drawn is not under entirely natural conditions. Miller (1931) and others have pointed out that the various methods of estimating apparent photosynthesis give conflicting results because different activities associated with this process are measured. It is considered that the intake of carbon dioxide is the first step in photosynthesis and the increase in dry matter is the last.

Miller (1931) points out several details which must be very carefully manipulated if the results of the carbon dioxide absorption method are to be of value. Among these are: (1) the regulation of the air stream, (2) the constant temperature of this stream, (3) the carbon dioxide content, (4) the regularity of the illumination, and (5) the construction of forms of apparatus suitable for the collection of the gas and at the same time placing the plant under favorable conditions for photosynthesis. Heinicke and Hoffman (1933) considered each of these details and stressed the necessity of using great skill and accuracy in manipulating the apparatus. As perfected by Heinicke and Hoffman, (1933), this method, with proper precautions, gives an index of relative amounts of apparent photosynthesis under the artificial conditions to which the leaves are subjected. These investigators have attempted to create natural environmental conditions for their studies.

Procedure

The apparatus used in the series of experiments herein reported is essentially like that described by Heinicke and Hoffman in Science (1933). In this procedure, the author used a series of three absorption towers for each day's run. One tower was used for a Livland leaf, one for Delicious, and the third for a blank, through which a stream of normal air was drawn.

For each determination, the difference of carbon dioxide content of the air drawn through the blank tower and that of the air drawn through the towers connected with the leaves was ascertained by titration with N/10 hydrochloric acid. Phenolphthalein was the indicator used. The suction pressure was secured from an aspirator. The principle on which the determinations are based is that a N/5 or N/10 solution of sodium hydroxide will absorb all of the carbon dioxide from a stream of air passed through it.

The air current is divided into small bubbles by means of a Gooch crucible or a glass crucible with fused-in fritted glass disks. The sodium hydroxide solution is drawn into a glass tube about 3 cm in diameter and 75 cm long, supported in an upright position, the crucible being fastened to the lower end of the tower by a piece of thin walled rubber tubing. Below the crucible is a 500 cc suction flask so arranged that the crucible is fastened to glass tubing which in turn is secured in the large opening of the flask with a rubber stopper. The lower end of this tube touches the bottom of the flask. The flask serves as a reservoir for the sodium hydroxide solution. When the suction is turned on, the liquid is drawn up through the crucible into the tower. The side arm of the flask is attached to the cellophane envelope enclosing the leaf under test by means of rubber and glass tubing.

To the top of the tower is attached a piece of capillary glass tubing so arranged with a manometer that when the rate of air flow is once determined for a given amount of suction, this rate can be duplicated later by adjusting the flow so that the height of liquid in the two arms of the manometer is also duplicated. A precision wet test meter was used to calibrate the apparatus. During the tests of the rate of carbon dioxide absorption by leaves, the air was drawn through the absorption towers at a rate of 2.25 liters of air per square centimeter of leaf area per hour.

Two hundred fifty cubic centimeters of N/10 sodium hydroxide were used in each tower in these experiments. At the end of each run the solution was allowed to flow back into the 500 cc reservoir and approximately 200 cc of distilled water were used to thoroughly rinse the tower, crucible, and other fittings. The dilute solution was transferred to a 500 cc volumetric flask and about 10 cc of a 25 per cent solution of barium chloride were added in order to precipitate the carbonates. The volume is then brought up to the mark with distilled water. After thorough shaking, the flasks are not disturbed until the barium carbonate has settled, after which 50 cc of the clear solution is drawn off through a pipette, a few drops of phenolpthalein are added, and N/10 hydrochloric acid is added until the end point is reached. The duration of the experiment was five hours on each of the three days.

The apple trees used in these tests were growing in a greenhouse and were planted in 12 inch clay pots as one year old budded stock in January, 1934. The tests were made in August, 1934.

At the beginning and at the conclusion of each experiment, leaf punches were taken from 50 leaves of each variety and used as an index of photosynthesis by the dry weight accumulation method. Also entire leaves were collected at these periods, killed by dry heat in an electric oven, and later analyzed for total acid hydrolzyable carbohydrates, expressed as glucose. The procedure for these methods is described later. This plan makes it possible to compare these three methods of judging apparent photosynthetic activity of apple foliage under the same environmental conditions. These data are presented in Table 10. There is no consistency in the results of the three methods, and it would be difficult to select one method as the standard of comparison, by which to judge the accuracy of the other two. Heinicke and Hoffman (1933) found that there was great variation in the rate of photosynthesis shown by adjacent leaves on the same shoot, also on different shoots of the same variety. It seems justifiable therefore to expect to find different photosynthetic values for leaves on different shoots or even different trees of one variety as well as between leaves of different varieties.

Table 10. Apparent photosynthetic activity of greenhouse grown foliage, August 15, 16, and 17, 1934.*

Date 1934	Variety	Time, temperature C, and relative humidity per cent: At beginning of run		Time, temperature C, and relative humidity per cent: At end of run		Total dry weight accumulation per M^2 leaf area. gms	Gain of combustible matter per M^2 leaf area. gms	Accumulation of total acid hydrolyzable carbohydrates as glucose per M^2 leaf area. gms	Carbon dioxide absorbed per 100 cm^2 leaf area per hour. mgm	Carbon dioxide absorbed per M^2 leaf area for 5 hours. gms	Duration of experiment
		9:45 a.m.		2:45 p.m.							
Aug. 15	Delicious	28°	63	39°	34	3.42	2.86	0.39	4.69	2.35	5 hours
	Livland					5.54	5.13	0.40	11.13	5.55	
		8:45 a.m.		1:45 p.m.							
Aug. 16	Delicious	25°	87	30°	70	1.61	1.43	0.23	3.36	1.67	5 hours
	Livland					0.58	0.42	0.42	7.30	3.65	
		8:15 a.m.		1:15 p.m.							
Aug. 17	Delicious	24°	82	25°	55	2.30	2.22	- 0.05	3.36	1.67	5 hours
						3.26	2.88	0.87	10.43	5.22	
Average 3 days	Delicious					2.44	2.30	0.22	3.80	1.89	
	Livland					3.12	2.81	0.56	9.62	4.81	

* See Table 38.

The same two leaves were used for all the experiments. The area of the Delicious leaf was 39.49 cm^2 and that of the Livland leaf, 40.09 cm^2. At the conclusion of these experiments, the two leaves were removed from the trees and mounted sections for microscopic study were prepared, as described before. (Table 11).

The ratio of the average weight of carbon dioxide assimilated per square meter of leaf area for the three 5-hour runs to the average weight of the total acid hydrolyzable carbohydrates accumulated per square meter of leaf area for the same periods is 8.59 for Delicious and 8.58 for Livland. However, the ratio between the increase of the weight of the dry matter per square meter of leaf area and the carbohydrate gain for Delicious is 11.09 and for Livland it is 5.62. The small gain in dry matter recorded for Livland on August 16, is no doubt responsible for part of the discrepancy between these two ratios. The total molecular weight of the six molecules of carbon dioxide entering into the formation of each molecule of glucose is 264, and the molecular weight of glucose is 372. The ratio between these molecular weights is 1.409, which does not agree with the ratios of 8.59 and 8.58 above.

The photosynthetic activity of these leaves is greater when judged by the dry weight accumulation than by the carbon dioxide absorption method. All three methods give higher values for the Livland leaf than for the Delicious leaf, and it is significant that the intercellular spaces in the Livland leaves are more extensive than those of Delicious.

Table 11. Intercellular measurements of the mesophyll of apple leaves used with carbon dioxide absorption towers in greenhouse, August 15 to 17, 1934.*

Variety	Average cross sectional area of intercellular spaces, cm^2	Average perimeter measurements of intercellular spaces, cm
Delicious	87.01±7.01	179.78±14.21
Livland	107.71±12.19	192.79±16.06

* Measurements for each variety were made from 50 projected images, 11 inches long, at a magnification of X 900.

b. The Dry Weight Method.

The weight of the dry matter accumulated per unit of leaf area is the second method used in this study of the photosynthetic activity of apple leaves.

Historical

Sachs (1884) was the first to use the dry weight method of measuring the rate of the formation of carbohydrates. He removed one-half of a leaf blade at the beginning of an experiment and left the other half attached to the midrib. Portions of the severed half were measured for area and the dry weight determined. The half leaf left on the plant was removed at the end of the experiment, measured, and weighed as before, care being taken to avoid large veins in both cases. The gain in dry weight per unit area of leaf surface was attributed to the carbohydrates formed by photosynthesis. Sachs recognized that translocation and respiration should be measured and discovered that detached leaves showed a greater gain when illuminated than leaves which remained attached to the plant. He attributed the smaller gain in the latter case to the translocation of the products of photosynthesis to other plant parts. In order to obtain the corrected photosynthetic value therefore, he added the loss in dry weight per unit area during the night to the gain in dry weight per unit area found during the day.

The dry weight method has been criticised by several investigators. Brown and Escomb (1905) compared the results of measuring photosynthesis by the continuous gas stream method and the dry weight method. Working with Catalpa bignonioides they recorded values two and three times greater for the dry weight method than that calculated from the amount of carbon dioxide absorbed. They concluded that the great objection to Sachs' method is that all of the errors become accumulative in the final result. Table 12 is a partial summary of their study.

Table 12. Direct comparison of the increase in weight during insolation of the leaf lamina of Catalpa bignoniodes, with the intake of carbon dioxide from ordinary air, and the corresponding amount of carbohydrate formed. Dec. 2-hr. Brown and Escombe (1905).

	Increase in dry weight in mg	CO_2 absorbed by leaf cc	Carbohydrate formed calculated from CO_2 absorbed. mg
Experiment 1	9.83	1.41	1.76
Experiment 2	7.14	1.43	1.79
Experiment 3	2.60	2.35	2.94
Experiment 4	7.22	2.33	2.92
Mean	6.69	----	2.35

Ganong (1908) was not convinced that the dry weight method gave results much too high. He designed (1905) a punch which could be used to remove circular discs, having a diameter of 1.128 cm or an area of 1 square centimeter, from leaves. Since that date, most of the dry weight records have been made by using the punch, rather than the half leaf method. Ganong (1908) concluded that the removal of the discs does not interfere seriously with the functioning of the leaves.

Too little is known of the composition of the products of assimilation to make possible an accurate calculation of the carbon dioxide absorbed from a given increase in dry weight, (Thoday, 1909). He recommends that the ash content of the leaf samples be determined and deducted from the increase in dry weight. According to this investigator there are two main errors in the dry weight method. These are: (1) the shrinkage of leaves in area during the experiment, and (2) their lack of symmetry in respect to dry weight per unit area. The latter objection may be overcome by avoiding large veins and using a large number of leaves for each experiment. Thoday concluded that if the dry weight gains are as great as 2 milligrams per square decimeter per hour, the method is capable of yielding useful results for comparative studies of assimilation. In 1910 he found that Helianthus leaves increased in dry weight by 17 mg 100 cm^2 hr., at a time when catalpa gained only 5 mg. 100 cm^2 hr., a fact which is correlated with the absence of stomata from the upper surface of catalpa leaves.

Spoehr (1926) sums up the merits of the dry weight method by stating that it offers the simplest means of estimating photosynthesis and that its very simplicity makes it highly desirable, but in its present form it is not reliable. He emphasizes the necessity of correcting the values for respiration and translocation.

Miller (1931) has used the dry weight method for making photosynthetic comparisons between, corn, milo, and the sorghums under field conditions in the Great Plains.

The dry weight method was used more extensively than any other in these photosynthetic studies because it is the best suited to orchard conditions. Whatever may be said against this method, it remains that the results are comparable, and in the final analysis no method is accepted as absolutely accurate. Every possible precaution to avoid error was adopted in planning these experiments. Chief among these precautions are: (1) many of the samples were corrected for variations in ash content, (2) large numbers, fifty or one hundred leaves were selected for sampling each variety for each experiment, (3) careful measurements showed that the leaves did not shrink in area during the daylight hours, (4) dust, spray residues and similar material were washed from the leaves twenty-four hours before the beginning of each experiment, (5) in the removal of each disc with a Ganong punch, care was exercised to avoid the cutting of large veins, (6) comparable leaves were always selected, only mature leaves on new shoot growth being used, (7) no leaf showing injury caused by any fungus, insect or other pest was sampled,

(8) all leaves from which discs were cut were located on the south and southwest exposure of the outer periphery of the tree and were located at heights ranging from four to six feet from the surface of the ground, (9) none of these leaves was at any time between the hours of 5 a.m. and 2 p.m. shaded by the foliage of other trees, (10) all the trees were growing under practically the same method of soil management.

Plate 17 illustrates the relative sizes of representative leaves of the varieties under test. Most orchard grown apple leaves are not sufficiently large to permit the removal of more than six discs each having an area of one square centimeter without severing large veins or the midrib. Accordingly many of these experiments were so planned that only four or six discs were removed from each leaf. Several preliminary tests revealed that apple leaves in the orchard ceased to gain in weight about midafternoon, that is, the processes of respiration and translocation then proceed at a faster rate than does apparent photosynthesis. The leaves in Plate 17 were pressed between two pieces of glass to aid the photographer to secure a sharp focus. The pressing so flattened the leaves that the holes do not appear to be circular, but in natural position the holes were round.

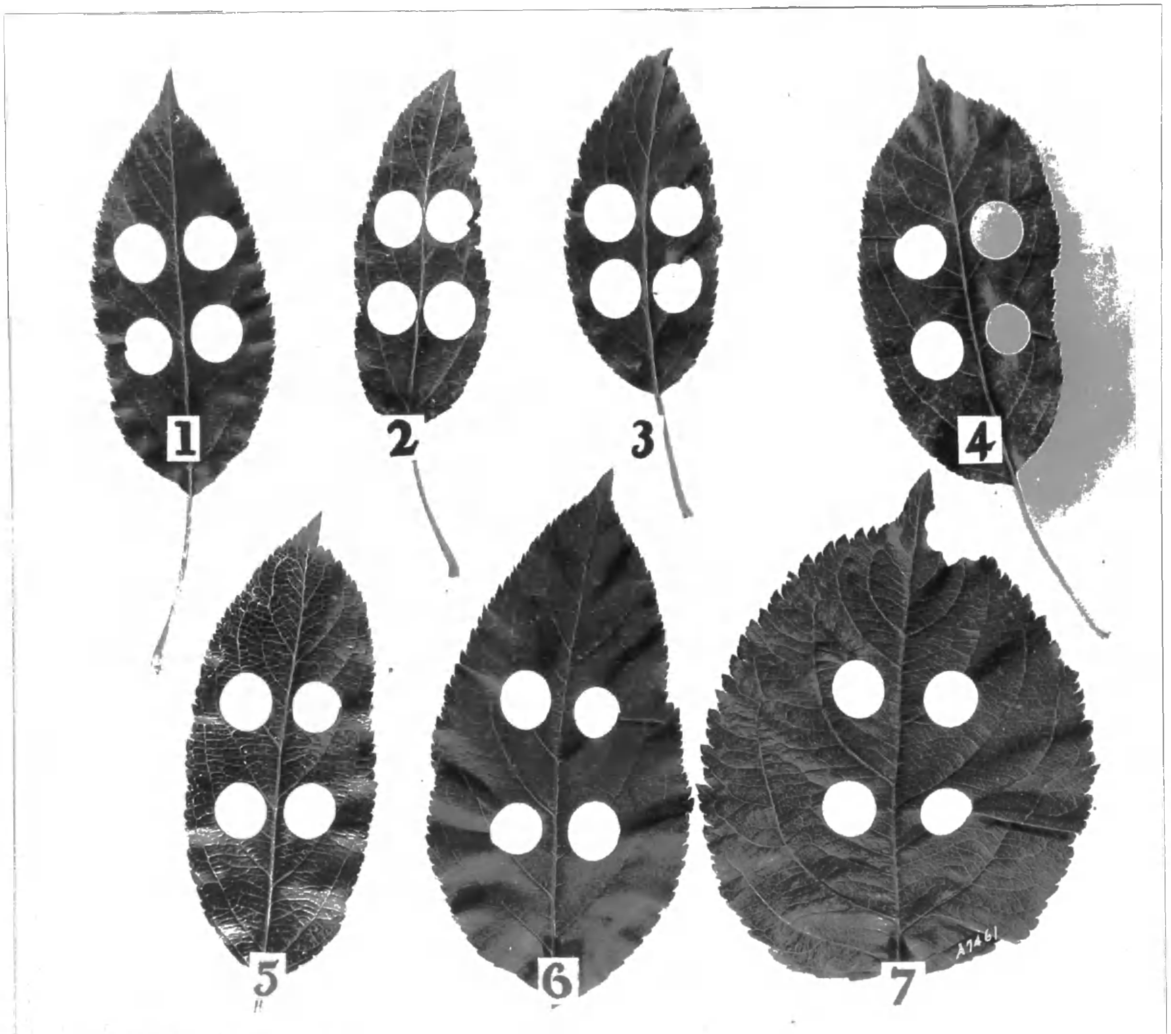


Plate 17. Representative leaves (X 5/8) with punches removed: (1) Jonathan, (2) Winesap, (3) York, (4) Gano, (5) Delicious, (6) Wealthy, and (7) Livland.

In January, 1933, two lots of one year old apple trees were planted in greenhouses in 12 inch clay pots. One lot of trees were plunged in a ground bench in a house designated as Plot A, where the temperatures were kept as high as the heating facilities permitted. The day temperatures usually ranged from 90°F to 110°F and the night temperatures were seldom below 70°F. Effort was made to keep the relative humidity high in this house, by keeping one aisle flooded with water and at times, a small stream of water was directed on the steam heating pipes. The other lot of trees, Plot B, were plunged in a ground bench in a house largely devoted to the culture of lettuce. The day temperatures were kept at about 70°F or slightly lower, and the night temperatures were in the upper forties. The object of growing the trees under these two sets of environmental conditions was to attempt to produce within a variety leaves with variation in the extent of intercellular spaces in the spongy mesophyll. The two greenhouses were adjoining and on the days the leaf punches were collected the door between the houses was open, and the temperatures and relative humidity were kept the same in the two rooms. In Table 13 are presented some data of anatomical and photosynthetic studies on leaves of these two groups of trees.

Table 13. Influence of apple leaf structure on daily increase in total dry matter, in greenhouse, 1933.

Variety	Plot	Intercellular spaces (1)		Gain in total dry matter per M^2 leaf area between 7:30 a. m. and 5:30 p. m.				
		Area cm^2	Perimeter cm	April 5	April 6	April 26	April 27	Mean
Livland	A	116.46	226.39	6.02	5.38	4.66	6.40	5.61
	B	94.59	196.92	5.96	4.08	3.12	6.34	4.87
Jonathan	A	66.52	146.66	6.10	3.68	3.08	5.02	4.47
	B	62.52	124.01	2.82	2.24	2.98	2.28	3.34
Gano	A	60.46	142.90	4.46	3.16	....	....	3.81
	B	64.05	157.95	6.64	5.64	....	....	6.14
York	A	82.91	212.09	....	....	3.48	5.34	4.41
	B	72.78	189.74	....	....	2.52	3.50	3.01

(1) See Table 1 for explanation of measurements of intercellular spaces.

Table 14. Temperature and relative humidity records in greenhouse.

Date - 1933		April 5	April 6	April 26	April 27
Time of day					
7:30 a.m.	Temperature (C.)	15	8	10	16
	Relative humidity per cent.	66	58	56	68
12:00 Noon	Temperature (C.)	27	22	20	34
	Relative humidity per cent.	45	45	42	39
5:00 p.m.	Temperature (C.)	15	15	11	21
	Relative humidity per cent.	61	63	58	50

Many factors are blended together in governing the rate of photosynthesis and in determining the quantity of photosynthate produced. It is extremely difficult to select any one of these factors and attempt to demonstrate the definite role it occupies in a process as complicated at this. As one variable is changed, its effect on the rate of photosynthesis is masked by the overlapping influences of the other variables. Nevertheless, the data in Table 13 indicate certain trends of behavior between the two variables of leaf structure and photosynthetic activity. These are: (a) the leaves of Livland, Jonathan, and York grown on trees in Plot A have a greater extent of intercellular spaces in the spongy mesophyll than leaves of the corresponding varieties grown in Plot B, where relatively low temperatures prevailed when the leaves were growing, while the reverse was true with Gano; (b) within each variety, the leaves possessing the more extensive intercellular spaces produced the greater gain in dry weight per square meter of leaf area between 8:30 a.m. and 5:30 p.m.; (c) the intercellular spaces in the Livland mesophyll from Plot A are larger than those of other varieties in the same plot. The same is true comparing Livland with the other varieties in Plot B, (d) Livland leaves accumulated a greater average weight of dry matter per square meter of leaf surface than the other varieties within each plot.

These records do not isolate leaf structure from the other factors which regulate photosynthesis, but it is likely that variations in intercellular spaces partially influence this activity or accompany it under the dominance of other more powerful factors.

Air temperature and relative humidity data in the greenhouse for April 5, 6, 26, and 27, 1933, are presented in Table 14. In Table 15 the percentage of water in the apple leaves used in photosynthetic studies on the above dates is given.

Table 15. Water content of apple leaves (greenhouse), per cent.

Date - 1933	April 5				April 6				April 26				April 27			
Time -	7:30 a.m.		5:30 p.m.		7:30 a.m.		5:30 p.m.		7:30 a.m.		5:30 p.m.		7:30 a.m.		5:30 p.m.	
Plot -	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Livland	67.89	66.21	63.81	61.55	65.03	64.00	61.09	62.40	66.27	65.10	64.11	64.00	66.00	67.00	64.09	64.40
Jonathan	66.25	64.60	63.05	60.31	61.54	62.43	60.00	61.17	65.14	66.01	63.33	63.90	65.90	66.13	61.01	64.11
Gano	66.49	65.26	62.81	62.01	64.80	63.85	62.31	61.07	65.49	64.98	62.97	62.78	66.20	64.25	63.09	61.19
York	65.33	64.76	63.27	62.50	64.70	63.83	63.11	61.13	64.87	64.50	63.80	63.13	65.46	65.57	63.75	62.39

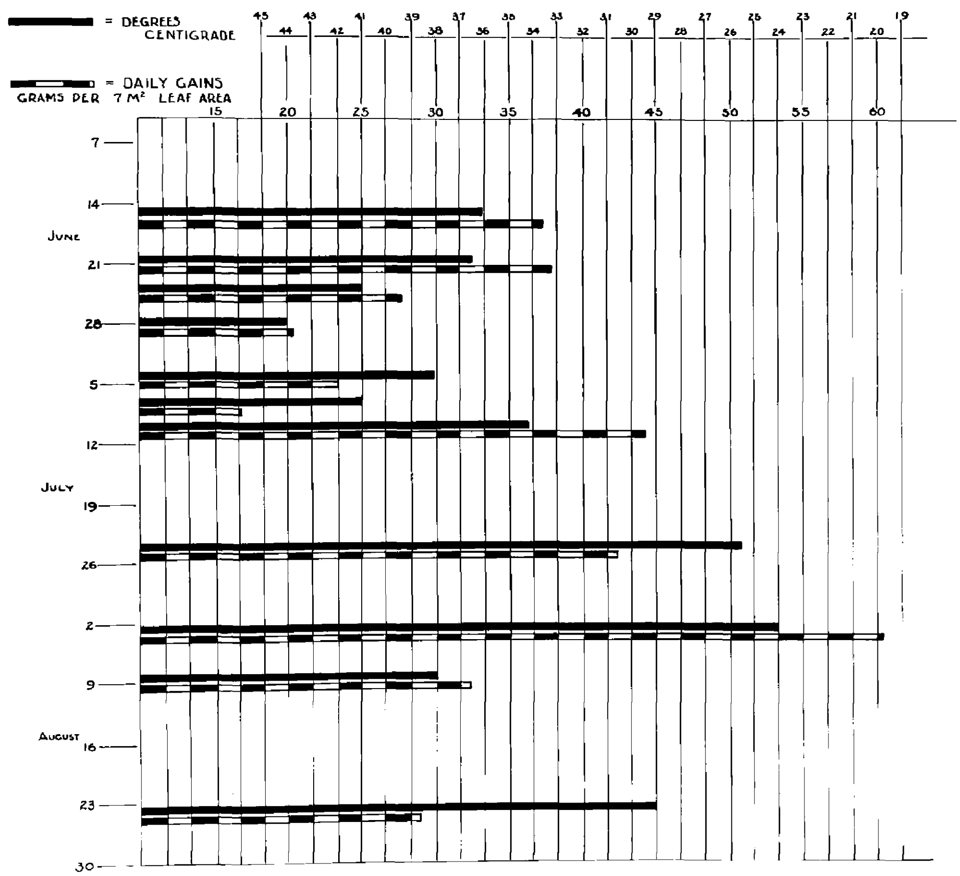
Scarcity of available soil moisture accompanied by high temperatures constitutes one of the hazards of crop production in the Great Plains. The season 1933 in Kansas was characterized by high temperatures and below normal rainfall. Soil moisture determinations were made on 10 days between June 14 and August 23. Between June 14, and July 7, the soil moisture content in non-irrigated plots ranged from 15 per cent to 17 per cent which is not far above the wilting coefficient. Although the apple trees used in these studies did not exhibit wilted foliage, 1400 gallons of water were used to irrigate each tree. Daily maximum temperatures exert a pronounced influence on the amount of dry matter gained per unit of leaf area, according to Plate 18. Generally, the higher the daily maximum temperature, the lower the leaf gain in dry weight. Soil moisture data are not included in Plate 18 because it was impossible to collect representative soil samples in the vicinity of the greatest root concentration, due to the irrigation given each tree.

Bose (1924) concluded that the rate of photosynthesis is the expression of the combined effect of the factors of light and temperature. Photosynthetic activity increased from 7:30 a.m. to 12:00 noon. After 2:00 p.m. both light and temperature underwent a decline, with resulting rapid fall of activity. His findings are in accord with the relationship between temperature and photosynthetic activity as shown graphically in Plate 18. Sunshine prevailed on each day with the

PLATE 18

RELATIONSHIP BETWEEN MAXIMUM AIR TEMPERATURE AND TOTAL DRY WEIGHT GAIN PER SQUARE METER LEAF AREA FOR SEVEN APPLE VARIETIES

1933



exception of August 25, which was cloudy. No records of light intensity were made. Considering only the maximum air temperatures on the eleven days when the dry weight determinations were made these records show the depressing effect of extremely high temperatures on photosynthetic activity.

An experiment was designed in 1934 to compare the efficiency of greenhouse grown and orchard grown leaves of Livland and Delicious apple leaves under orchard conditions (Pickett, 1934). It has been shown in Table 1 that there are marked differences in the extent of the intercellular spaces in the spongy mesophyll of greenhouse grown Delicious and orchard grown Delicious leaves. A similar condition exists with Livland leaves. In January, 1934, several one year old whips of the two varieties were planted in 12 inch clay pots and placed in a greenhouse. In July, 1934, these trees were taken to the orchard, and the pots were plunged in the orchard soil for a few days to bring the soil moisture in the pots to approximately the same point as that in the soil outside the pots. The photosynthetic determinations of the greenhouse grown trees could then be made under the same environmental conditions as the orchard trees.

This experiment was started on the last day of July, 1934, a month which broke all records at Manhattan for the past 75 years for high temperatures of any month. On twenty-six days the maximum temperature was 100 degrees F or higher and during eleven consecutive days the maximum reading was 111 degrees or higher. The rainfall for the month was 0.86 inch, and at the time of this experiment the average moisture content of the top first, second, and third foot samples of soil was 11.6 per cent, dry weight basis. Many trees in the orchard were wilted but the ones used in this test of July 31 to August 1, were apparently turgid.

The rate of apparent photosynthesis was judged by Ganong's modification of the dry weight method. Discs one square centimeter in area were punched from 50 mature leaves, one disc being removed from each leaf at 6:00 a.m., another at 2:30 p.m., a third set at 6:00 a. m. the second day.

In Table 16, data are presented to show the gains and losses in dry weight per square meter of leaf area from 6:00 a.m., July 31, to 6:00 a. m., August 1, 1934. The orchard grown Livland leaves made a net gain of 5.93 gms M^2 leaf area while the greenhouse grown Livland leaves gained 3.48 gms M^2 leaf area. Likewise, the orchard grown Delicious leaves gain more in dry matter than those grown in the greenhouse. These gains were 4.68 gms M^2 leaf area and 1.88 gms M^2 leaf area, respectively. The four groups of leaves rank as follows in the net day gains in dry weight: (1) orchard grown Livland, (2) orchard grown Delicious, (3) greenhouse grown Livland, and (4) greenhouse grown Delicious. The same ranking applies to the measurements of the intercellular spaces in the mesophyll as shown in Table 1. Apparently the differences of the extent of the intercellular spaces are reflected in the photosynthetic behavior of the two varieties. Several factors enter into and govern the rate of photosynthesis and the extent of the surfaces of the exposed cell walls bordering the intercellular spaces is probably one of them.

Table 16. Variation in dry matter of apple leaves.

Variety	Place grown	Time and date 1934	Dry weight per M^2 leaf area. gms	Gain or loss in dry matter per M^2 leaf area. gms	Total gain in dry matter per M^2 leaf area. gms	Water content of leaves. per cent.
Livland	Orchard	6:00 a.m., July 31	91.60	----	----	55.12
		2:30 p.m., July 31	97.53	+ 5.93	----	54.80
		6:00 a.m., Aug. 1	93.15	- 4.38	10.31	57.31
Livland	Greenhouse	6:00 a.m., July 31	85.24	------	-----	55.17
		2:30 p.m., July 31	88.72	+ 3.48	-----	54.94
		6:00 a.m., Aug. 1	87.26	- 1.46	4.94	57.09
Delicious	Orchard	6:00 a.m., July 31	85.46	------	-----	55.21
		2:30 p.m., July 31	90.14	+ 4.68	-----	53.48
		6:00 a.m., Aug. 1	87.70	- 2.44	7.12	54.80
Delicious	Greenhouse	6:00 a.m., July 31	77.14	-----	-----	56.26
		2:30 p.m., July 31	79.02	+ 1.88	-----	54.05
		6:00 a.m., Aug. 1	76.72	- 2.30	4.18	55.95

Temperatures: 6:00 a.m., July 31, 23 degrees C relative humidity 42 per cent.
2:30 p.m., July 31, 38 degrees C relative humidity 21 per cent.
6:00 a.m., Aug. 1, 28 degrees C relative humidity 37 per cent.
July 31, was cloudy, practically no direct sunlight at any time during the day.

Dry weight determinations of orchard grown apple leaves were recorded on these dates in 1933: June 7, June 14, June 17, June 21, June 23, June 29, July 1, July 6, July 7, July 10, July 25, August 3, August 9, and August 25.

Photosynthetic activity as measured by determining the amounts of total acid hydroloyzable carbohydrates expressed as glucose was determined on July 10, July 25, August 3, August 9, and August 25, 1933.

In 1934, both dry weight and carbohydrate determinations were made from samples collected May 2, May 16, May 23, June 5, July 2, July 3, July 9, July 10, July 18, July 19, and August 3, August 6, August 7, August 15, August 16, August 17, August 21, August 22, August 23, and August 24. The data for the two seasons are presented in Tables 16 to 41.

c. Photosynthetic Activity as Judged by the Saccharification Method.

In this procedure, the entire carbohydrate content is hydroloyzed to hexoses and the products thus formed are estimated as glucose by the reduction of Fehling's solution. The method used in these studies follows: Weight 1.5 gm of dry finely pulverized leaf powder, add 150 cc $2\frac{1}{2}$ per cent hydrochloric acid, boil in water bath under reflux condenser for two hours; cool, nearly neutralize with sodium hydroxide solution, clear with a saturated solution of neutral lead acetate solution and filter. Add sufficient solid sodium oxalate to precipitate all the lead and refilter through a dry paper into 250 cc volumetric flask. Test the filtrate for the presence of lead with a little solid sodium oxalate and filter if precipitate forms. Bring to volume with distilled water.

Prepare Soxhlet's modification of Fehling's solution by mixing equal volumes of (a) and (b) immediately before using.

(a) Copper sulfate solution - dissolve 34.639 gms $CuSo_4 \cdot 5 H_2O$ in water, dilute to 500 cc and filter through prepared asbestos.

(b) Alkaline tartrate solution - dissolve 173.0 gms of Rochelle salts (sodium potassium tartrate) and 50 gms of sodium hydroxide in water, dilute to 500 cc allow to stand for two days, and filter through prepared asbestos.

Transfer 25 cc each of the copper sulphate and alkaline tartrate solutions to a 300 cc Erlenmeyer flask and pipette exactly 50 cc of the sample containing the hexoses and heat in hot water bath at 80° centigrade for 30 minutes (Quisumbing and Thomas 1921). Remove, cool and add 25 cc of a potassium iodide-iodate solution (Shafer and Hartman, 1920-1921) prepared by dissolving 60.0 gms of potassium iodide and 5.4 gms potassium iodate in water, adding a few cc of concentrated sodium hydroxide and diluting to 1 liter. Quickly add approximately 16 cc of 5 N sulfuric acid. Shake the flask until all the copper oxide is dissolved. Next add 20 cc of a saturated solution of potassium oxalate and titrate with a standard N/10 solution of sodium thiosulfate to which has been added a small amount of concentrated sodium hydroxide. Record the number of cc of the sodium thiosulfate solution required to oxidize the iodine and substract this from the amount used when 50 cc of water is substituted for the sugar solution. The addition of a small quantity of soluble starch shortly before the end point is reached makes the end point more distinct. The net titration gives the amount of sodium thiosulfate which has been used to reduce the iodine which had been used to oxidize the cuprous oxide and if multiplied by the factor 6.36 will give the number of milligrams of metallic copper reduced by the sugar.

The acid is added as directed above to release free iodine from the iodide-iodate solution. The potassium oxalate is added to unite with all the divalent copper. The iodine oxidizes the cuprous copper to cupric copper and the sodium thiosulfate reduces the iodine. The tables of Quisumbing and Thomas (1921) were referred to for calculating glucose from the copper value.

It is important to standardize the sodium thiosulfate solution as follows: Prepare a N/10 solution of potassium biiodate. Pipette exactly 50 cc of this solution into a 250 cc Erlenmeyer flask and add to this a solution of 3 gms of potassium iodide dissolved in 25 cc of water. Then add 10 cc of 5 N sulfuric acid and titrate with the N/10 sodium thiosulfate solution. If the number of cc of biiodate used divided by the number of cc of thiosulfate used is more or less than one, the quotient secured is a factor by which all determinations must be multiplied.

The leaves used in these analyses were killed in dry heat in an electric oven. They were placed in convenient trays made of hardware cloth and for the first hour the oven temperature was 70° C. During this hour the door was left open slightly to permit the ready escape of water vapor. The killing was completed at 98° C after which the leaves were ground with a mortar and pestle until the powder would pass through a 40-mesh sieve. This method produced a powder which was a natural green. If samples of leaves were put in the oven at 98° without first going through the 70° step, generally the powder was somewhat darkened. No comparative analyses of the two methods were made. It was assumed that the bright green powder was the better.

None of the trees used in 1933 had apples on them. Trees not in bearing were selected because the presence of fruit probably influence the photosynthetic activity of nearby leaves. In 1934, the photosynthetic behavior of leaves on branches bearing apples was determined. For comparison, leaves on branches from which the fruit had been removed were used. The ratios given in Tables 35, 36, 40, and 41 indicate the number of leaves per fruit on the branches from which the leaves were selected for study.

In Tables 25 to 41 inclusive the photosynthetic activity of apple leaves is presented, based on both the dry weight and saccharification methods.

Table 17. Variation in dry matter and water content of apple leaves.

Variety	June 7 to 8, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 a. m.	71.02	61.55	
Jonathan	6:00 p. m.	73.60	59.64	+ 2.58
	6:00 a. m.	73.02	61.01	- 0.58
	6:00 a. m.	68.80	62.72	
Winesap	6:00 p. m.	71.04	61.58	+ 2.24
	6:00 a. m.	71.42	61.23	+ 0.38
	6:00 a. m.	65.70	61.04	
York	6:00 p. m.	65.82	61.10	+ 0.12
	6:00 a. m.	66.52	61.46	+ 0.70
	6:00 a. m.	65.36	58.44	
Gano	6:00 p. m.	65.60	59.16	+ 0.24
	6:00 a. m.	66.52	60.26	+ 0.92
	6:00 a. m.	75.92	62.21	
Delicious	6:00 p. m.	75.96	60.53	+ 0.04
	6:00 a. m.	74.16	63.55	- 1.80
	6:00 a. m.	66.46	66.74	
Livland	6:00 p. m.	69.26	66.52	+ 2.80
	6:00 a. m.	67.18	66.05	- 2.08

Table 18. Variation in dry matter and water content of apple leaves.

Variety	June 14 to 15, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 p. m.	67.14	58.36	
Jonathan	6:00 a. m.	63.86	60.46	- 3.28
	6:00 p. m.	67.06	59.25	+ 3.20
	6:00 p. m.	74.40	57.79	
Winesap	6:00 a. m.	72.66	58.99	- 1.74
	6:00 p. m.	75.80	59.29	+ 3.14
	6:00 p. m.	59.98	60.96	
Delicious	6:00 a. m.	56.60	62.81	- 3.38
	6:00 p. m.	58.98	62.02	+ 2.38
	6:00 p. m.	74.40	60.12	
Wealthy	6:00 a. m.	70.58	61.67	- 3.82
	6:00 p. m.	75.60	60.62	+ 5.02
	6:00 p. m.	63.96	63.01	
Livland	6:00 a. m.	60.34	64.75	- 3.62
	6:00 p. m.	64.74	64.00	+ 4.40

Table 19. Variation in dry matter and water content of apple leaves.

Variety	June 16 to 17, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 p. m.	72.80	59.19	
Jonathan	6:00 a. m.	71.44	58.80	- 1.36
	6:00 p. m.	73.84	58.47	+ 2.40
	6:00 p. m.	79.12	56.98	
Winesap	6:00 a. m.	75.42	57.50	- 3.70
	6:00 p. m.	79.20	55.46	+ 3.78
	6:00 p. m.	69.72	57.69	
York	6:00 a. m.	69.24	58.95	- 0.48
	6:00 p. m.	72.78	58.37	+ 3.54
	6:00 p. m.	67.16	55.98	
Gano	6:00 a. m.	66.04	56.60	- 1.12
	6:00 p. m.	71.50	55.26	+ 5.46
	6:00 p. m.	81.88	60.16	
Delicious	6:00 a. m.	79.82	61.13	- 2.06
	6:00 p. m.	84.26	60.55	+ 4.44
	6:00 p. m.	91.58	58.58	
Wealthy	6:00 a. m.	86.96	60.59	- 4.62
	6:00 p. m.	92.34	59.18	+ 5.38
	6:00 p. m.	73.00	62.72	
Livland	6:00 a. m.	71.28	66.05	- 1.72
	6:00 p. m.	77.82	63.84	+ 6.54

Table 20. Variation in dry matter and water content of apple leaves.

Variety	June 21 to 22, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 a. m.	71.64	59.33	
Jonatham	6:00 p. m.	74.84	56.94	+ 3.20
	6:00 a. m.	72.80	59.59	- 2.04
	6:00 a. m.	76.38	58.34	
Winesap	6:00 p. m.	81.98	55.87	+ 5.60
	6:00 a. m.	77.84	59.41	- 4.14
	6:00 a. m.	68.14	59.12	
York	6:00 p. m.	71.70	56.99	+ 3.56
	6:00 a. m.	71.26	59.26	- 0.44
	6:00 a. m.	66.44	57.85	
Gano	6:00 p. m.	71.24	56.54	+ 4.80
	6:00 a. m.	69.66	58.31	- 1.58
	6:00 a. m.	75.38	61.60	
Delicious	6:00 p. m.	80.42	59.23	+ 5.04
	6:00 a. m.	75.58	62.20	- 4.84
	6:00 a. m.	82.44	60.23	
Wealthy	6:00 p. m.	88.46	57.92	+ 6.02
	6:00 a. m.	84.32	61.25	- 4.14
	6:00 a. m.	69.12	62.62	
Livland	6:00 p. m.	76.64	61.24	+ 7.52
	6:00 a. m.	69.74	63.36	- 6.90

Table 21. Variation in dry matter and water content of apple leaves.

Variety	June 23 to 24, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 a. m.	72.34	60.13	
Jonathan	6:00 p. m.	75.90	57.51	+ 3.56
	6:00 a. m.	73.40	59.96	- 2.50
	6:00 a. m.	77.12	57.76	
Winesap	6:00 p. m.	79.62	56.14	+ 2.50
	6:00 a. m.	77.92	58.00	- 1.70
	6:00 a. m.	66.64	58.96	
York	6:00 p. m.	69.08	57.54	+ 2.44
	6:00 a. m.	68.60	59.45	- 0.48
	6:00 a. m.	62.70	58.69	
Gano	6:00 p. m.	66.16	57.37	+ 3.46
	6:00 a. m.	66.06	58.40	- 0.10
	6:00 a. m.	79.48	60.80	
Delicious	6:00 p. m.	82.90	59.07	+ 3.42
	6:00 a. m.	80.44	60.43	- 2.46
	6:00 a. m.	84.16	58.15	
Wealthy	6:00 p. m.	90.08	57.10	+ 5.92
	6:00 a. m.	87.90	58.71	- 2.18
	6:00 a. m.	72.30	62.46	
Livland	6:00 p. m.	77.68	61.82	+ 5.38
	6:00 a. m.	72.70	62.46	- 4.98

Table 22. Variation in dry matter and water content of apple leaves.

Variety	June 29 to 30, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
	6:00 a. m.	67.02	59.41	
Jonathan	6:00 p. m.	69.10	57.90	+ 2.08
	6:00 a. m.	69.96	59.36	+ 0.86
	6:00 a. m.	75.40	57.86	
Winesap	6:00 p. m.	79.90	56.00	+ 4.50
	6:00 a. m.	78.70	56.73	- 1.20
	6:00 a. m.	64.96	58.18	
York	6:00 p. m.	67.20	57.11	+ 2.24
	6:00 a. m.	69.96	58.02	+ 2.76
	6:00 a. m.	57.40	58.97	
Gano	6:00 p. m.	59.66	57.01	+ 2.26
	6:00 a. m.	59.54	59.34	- 0.12
	6:00 a. m.	69.80	61.26	
Delicious	6:00 p. m.	72.68	59.92	+ 2.88
	6:00 a. m.	70.78	62.24	- 1.90
	6:00 a. m.	72.66	59.27	
Wealthy	6:00 p. m.	75.70	57.77	+ 3.04
	6:00 a. m.	77.10	58.95	+ 1.40
	6:00 a. m.	63.72	63.17	
Livland	6:00 p. m.	67.66	61.59	+ 3.94
	6:00 p. m.	66.20	63.02	- 1.46

Table 23. Variation in dry matter and water content of apple leaves.

Variety	July 1, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
York	6:00 a. m.	71.14	56.33	
	6:00 p. m.	74.34	54.72	+ 3.20
Livland	6:00 a. m.	69.22	61.19	
	6:00 p. m.	76.82	55.99	+ 7.60

Temperatures - 6:00 a. m., - 85° F
3:00 p. m., - 110° F
6:00 p. m., - 90° F

Table 24. Variation in dry matter and water content of apple leaves.

Variety	July 6 to 7, 1933	Dry matter M^2 leaf area. gms	Water content. per cent	Gain or loss of dry matter M^2 leaf area. gms
Jonathan	5:00 a. m.	80.04	57.47	
	7:00 p. m.	84.18	55.65	+ 4.14
	5:00 a. m.	82.04	57.87	- 2.14
	7:00 p. m.	85.46	56.06	+ 3.42
Winesap	5:00 a. m.	85.14	55.55	
	7:00 p. m.	88.44	54.16	+ 3.30
	5:00 a. m.	85.70	56.68	- 2.74
	7:00 p. m.	87.74	54.59	+ 2.04
York	5:00 a. m.	69.70	57.83	
	7:00 p. m.	71.84	56.80	+ 2.14
	5:00 a. m.	70.78	58.84	- 1.06
	7:00 p. m.	71.82	57.49	+ 1.04
Gano	5:00 a. m.	68.60	56.64	
	7:00 p. m.	72.08	54.48	+ 3.48
	5:00 a. m.	70.12	57.60	- 1.96
	7:00 p. m.	73.12	55.44	+ 3.00
Delicious	5:00 a. m.	79.64	58.80	
	7:00 p. m.	82.74	58.01	+ 3.10
	5:00 a. m.	80.30	59.95	- 2.44
	7:00 p. m.	81.96	58.49	+ 1.66
Wealthy	5:00 a. m.	80.14	57.69	
	7:00 p. m.	82.64	56.95	+ 2.50
	5:00 a. m.	80.12	59.08	- 2.52
	7:00 p. m.	82.38	57.23	+ 2.26
Livland	5:00 a. m.	62.42	60.60	
	7:00 p. m.	66.74	59.81	+ 4.32
	5:00 a. m.	60.58	62.47	- 6.16
	7:00 p. m.	63.76	61.29	+ 3.18

Table 25. Analysis of apple leaves. July 10 to 11, 1933. Variation in dry matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area. Dry matter.	Gain or loss of total dry matter.	Carbohydrates.	Gain or loss of carbohydrates.	Percentage. Water.
	5:00 a. m.	65.88		9.16		60.21
	10:00 a. m.			10.94		
Jonathan	1:00 p. m.	71.20	+ 5.32	10.72	+ 1.56	54.05
	4:00 p. m.			12.51		
	7:00 p. m.	70.68	- 0.52	13.61	+ 2.89	55.12
	5:00 a. m.	64.78	- 5.90	11.47	- 2.14	64.08
	5:00 a. m.	80.66		13.03		58.15
	10:00 a. m.			13.62		
Winesap	1:00 p. m.	89.34	+ 8.68	14.06	+ 1.03	51.54
	4:00 p. m.			14.91		
	7:00 p. m.	86.58	- 2.76	13.75	- 0.31	53.55
	5:00 a. m.	83.48	- 3.10	10.83	- 2.92	63.83
	5:00 a. m.	63.72		10.88		61.23
	10:00 a. m.			11.03		
York	1:00 p. m.	69.08	+ 5.36	11.28	+ 0.40	55.93
	4:00 p. m.			14.66		
	7:00 p. m.	69.60	+ 0.52	13.42	+ 2.14	57.20
	5:00 a. m.	68.48	- 1.12	11.05	- 2.37	66.92
	5:00 a. m.	70.60		11.30		57.14
	10:00 a. m.			14.52		
Gano	1:00 p. m.	74.16	+ 3.56	11.87	+ 0.47	53.50
	4:00 p. m.			14.71		
	7:00 p. m.	76.22	+ 2.06	10.35	- 1.52	54.64
	5:00 a. m.	73.60	- 2.62	9.52	- 0.83	61.44
	5:00 a. m.	83.98		9.85		58.41
	10:00 a. m.			9.78		
Delicious	1:00 p. m.	89.36	+ 5.38	8.79	- 0.06	54.73
	4:00 p. m.			9.05		
	7:00 p. m.	90.74	+ 1.38	11.61	+ 2.62	55.90
	5:00 a. m.	87.68	- 3.06	10.80	- 0.81	61.28
	5:00 a. m.	73.06		10.93		59.48
	10:00 a. m.			11.58		
Wealthy	1:00 p. m.	80.48	+ 7.42	13.89	+ 2.96	56.21
	4:00 p. m.			13.48		
	7:00 p. m.	78.32	- 2.16	12.44	- 1.45	58.09
	5:00 a. m.	82.06	+ 3.74	10.62	- 1.82	60.78
	5:00 a. m.	65.88		8.44		61.07
	10:00 a. m.			8.63		
Livland	1:00 p. m.	71.20	+ 5.32	10.69	+ 2.25	58.64
	4:00 p. m.			10.44		
	7:00 p. m.	70.68	- 0.52	10.15	- 0.54	60.41
	5:00 a. m.	64.78	- 5.90	9.49	- 0.66	63.40

Table 26. Analysis of apple leaves. July 25 to 26, 1933. Variation in dry matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area. Dry matter.	Gain or loss of total dry matter	Carbohydrates.	Gain or loss of carbohydrates.	Percentage. Water.
	6:00 a. m.	81.60		12.85		56.78
	10:00 a. m.			13.51		
Jonathan	1:00 p. m.	86.54	+ 4.94	10.78	- 2.07	53.20
	4:00 p. m.			10.67		
	7:00 p. m.	86.20	- 0.34	11.74	+ 0.96	54.38
	6:00 a. m.	89.82	+ 3.62	10.82	- 0.92	54.96
	6:00 a. m.	91.78		13.24		54.41
	10:00 a. m.			11.22		
Winesap	1:00 p. m.	95.74	+ 3.96	13.49	+ 0.25	51.20
	4:00 p. m.			9.80		
	7:00 p. m.	96.70	+ 0.96	10.33	- 3.16	52.78
	6:00 a. m.	94.12	- 2.58	11.62	+ 1.29	55.83
	6:00 a. m.	71.16		10.83		57.44
	10:00 a. m.			9.89		
York	1:00 p. m.	75.44	+ 4.28	12.37	+ 1.54	53.93
	4:00 p. m.			9.99		
	7:00 p. m.	75.14	- 0.30	11.61	- 0.76	55.41
	6:00 a. m.	76.00	+ 0.86	11.01	- 0.60	58.09
	6:00 a. m.	79.42		12.10		55.74
	10:00 a. m.			12.26		
Gano	1:00 p. m.	85.20	+ 5.78	16.01	+ 3.91	53.32
	4:00 p. m.			13.25		
	7:00 p. m.	84.86	- 0.34	10.46	- 5.55	54.04
	6:00 a. m.	84.48	- 0.38	10.39	- 0.07	55.86
	6:00 a. m.	82.14		11.97		56.86
	10:00 a. m.			12.54		
Delicious	1:00 p. m.	90.32	+ 8.18	12.84	+ 0.87	53.72
	4:00 p. m.			14.45		
	7:00 p. m.	86.56	- 3.76	13.41	+ 0.57	55.54
	6:00 a. m.	87.76	+ 1.20	11.26	- 2.15	57.77
	6:00 a. m.	89.04		9.52		57.32
	10:00 a. m.			9.01		
Wealthy	1:00 p. m.	97.92	+ 8.88	11.41	+ 1.89	53.75
	4:00 p. m.			13.31		
	7:00 p. m.	97.06	- 0.86	10.68	- 0.73	55.61
	6:00 a. m.	95.22	- 1.84	10.69	+ 0.01	58.05
	6:00 a. m.	71.44		11.62		60.75
	10:00 a. m.			13.02		
Livland	1:00 p. m.	77.82	+ 6.38	11.66	+ 0.04	57.46
	4:00 p. m.			12.09		
	7:00 p. m.	76.44	- 1.38	13.75	+ 2.09	58.02
	6:00 a. m.	72.20	- 4.24	10.08	- 3.67	61.17

Table 27. Analysis of apple leaves. August 3 to 4, 1933. Variation in dry matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area. Dry matter.	Gain or loss of total dry matter.	Carbohydrates.	Gain or loss of carbohydrates.	Percentage Water
	7:00 a. m.	77.02		9.86		56.33
Jonathan	4:30 p. m.	86.18	+ 9.16	10.31	+ 0.45	52.88
	7:00 a. m.	81.76	- 4.42	9.62	- 0.69	55.61
	7:00 a. m.	88.48		10.67		54.72
Winesap	4:30 p. m.	98.52	+ 10.04	11.30	+ 0.63	51.92
	7:00 a. m.	93.34	- 5.18	11.13	- 0.17	54.51
	7:00 a. m.	72.46		9.06		56.18
York	4:30 p. m.	80.60	+ 8.14	11.42	+ 2.36	52.84
	7:00 a. m.	78.66	- 1.94	10.53	- 0.89	54.66
	7:00 a. m.	72.30		11.82		56.12
Gano	4:30 p. m.	81.48	+ 9.18	13.90	+ 2.08	52.90
	7:00 a. m.	78.80	- 2.68	12.47	- 1.43	54.61
	7:00 a. m.	71.22		8.38		58.39
Delicious	4:30 p. m.	78.54	+ 7.32	11.20	+ 2.82	56.34
	7:00 a. m.	75.72	+ 2.82	10.45	- 0.75	58.13
	7:00 a. m.	88.88		12.03		53.96
Wealthy	4:30 p. m.	98.76	+ 9.88	13.37	+ 1.34	52.41
	7:00 a. m.	92.86	- 5.90	12.38	- 0.99	54.60
	7:00 a. m.	75.72		11.01		59.79
Livland	4:30 p. m.	82.22	+ 6.50	12.06	+ 1.05	57.56
	7:00 a. m.	76.20	- 6.02	10.62	- 1.44	59.38

Table 28. Analysis of apple leaves. August 9 to 10, 1933. Variation in dry matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area.				Percentage
		Dry matter.	Gain or loss of total dry matter	Carbohydrates.	Gain or loss of carbohydrates.	Water
	7:30 a. m.	86.92		11.25		54.05
Jonathan	4:30 p. m.	92.22	+ 5.30	11.52	+ 0.27	52.50
	7:30 a. m.	90.58	- 1.64	11.86	+ 0.34	55.09
	7:30 a. m.	99.16		12.39		52.55
Winesap	4:30 p. m.	104.28	+ 5.12	13.24	+ 0.85	50.50
	7:30 a. m.	98.44	- 5.84	12.04	- 1.20	54.12
	7:30 a. m.	80.00		10.16		56.94
York	4:30 p. m.	82.38	+ 2.38	10.33	+ 0.17	53.34
	7:30 a. m.	81.08	- 1.30	10.05	- 0.28	55.11
	7:30 a. m.	80.70		10.41		53.57
Gano	4:30 p. m.	87.36	+ 6.66	11.83	+ 1.42	52.66
	7:30 a. m.	84.42	- 2.94	10.97	- 0.86	54.58
	7:30 a. m.	77.90		8.83		55.82
Delicious	4:30 p. m.	80.90	+ 3.00	11.09	+ 2.26	55.19
	7:30 a. m.	78.10	- 2.80	10.42	- 0.67	56.00
	7:30 a. m.	99.56		14.50		54.08
Wealthy	4:30 p. m.	102.68	+ 3.12	14.91	+ 0.41	52.46
	7:30 a. m.	100.00	- 2.68	14.66	- 0.25	54.77
	7:30 a. m.	75.18		10.57		58.25
Livland	4:30 p. m.	79.96	+ 4.78	11.14	+ 0.57	57.88
	7:30 a. m.	81.00	+ 1.04	10.96	- 0.18	55.37

Table 29. Analysis of apple leaves. August 25 to 26, 1933. Variation in dry matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area. Dry matter.	Gain or loss of total dry matter.	Carbohydrates.	Gain or loss of carbohydrates.	Percentage Water.
	7:30 a. m.	93.16		13.66		51.83
Jonathan	4:30 p. m.	100.38	+ 7.22	14.62	+ 0.96	49.76
	7:30 a. m.	91.44	- 8.94	13.14	- 1.48	55.62
	7:30 a. m.	99.10		14.53		50.08
Winesap	4:30 p. m.	102.82	+ 3.72	16.04	+ 1.51	50.39
	7:30 a. m.	98.48	- 4.34	13.72	- 2.32	54.08
	7:30 a. m.	85.20		13.40		52.49
York	4:30 p. m.	88.32	+ 3.12	13.04	- 0.36	51.74
	7:30 a. m.	84.10	- 4.22	12.25	- 0.79	53.95
	7:30 a. m.	97.16		14.86		51.73
Gano	4:30 p. m.	101.36	+ 4.20	15.71	+ 0.85	50.25
	7:30 a. m.	94.30	- 7.06	14.52	- 1.19	54.00
	7:30 a. m.	89.84		15.78		52.80
Delicious	4:30 p. m.	91.24	+ 1.40	15.57	- 0.21	53.16
	7:30 a. m.	86.92	- 4.32	15.41	- 0.16	55.30
	7:30 a. m.	109.88		20.91		49.50
Wealthy	4:30 p. m.	115.24	+ 5.36	20.51	- 0.40	50.11
	7:30 a. m.	105.98	- 9.26	19.61	- 0.90	52.72
	7:30 a. m.	84.56		14.69		54.62
Livland	4:30 p. m.	88.52	+ 3.96	15.03	+ 0.34	56.65
	7:30 a. m.	82.42	- 6.10	13.46	- 1.57	58.03

Table 30. Daily increase in total dry matter and acid hydrolyzable carbohydrates estimated as glucose per square meter leaf area, grams. Partial summary of Tables 19, 20, 21, 22, 24, 25, 26, 27, 28, and 29. 1933.

Date	Time a.m.-p.m.	Wealthy Dry matter	Wealthy Carbohydrates	Jonathan Dry matter	Jonathan Carbohydrates	Livland Dry matter	Livland Carbohydrates	Gano Dry matter	Gano Carbohydrates	Winesap Dry matter	Winesap Carbohydrates	Delicious Dry matter	Delicious Carbohydrates	York Dry matter	York Carbohydrates
June 17	6:00 to 6:00	5.38		2.40		6.54		5.46		3.78		4.44		3.54	
June 21	6:00 to 6:00	6.02		3.20		7.52		4.80		5.60		5.04		3.56	
June 23	6:00 to 6:00	5.92		3.56		5.38		3.46		2.50		3.42		2.44	
June 29	6:00 to 6:00	3.04		2.08		3.94		2.26		4.50		2.88		2.24	
July 6	5:00 to 7:00	2.50		4.14		4.32		3.48		3.30		3.10		2.14	
July 7	5:00 to 7:00	2.26		3.42		3.18		3.00		2.04		1.66		1.04	
July 10	5:00 to 1:00	7.42	2.96	5.32	1.56	5.32	2.25	3.56	0.47	8.68	1.03	5.38	-0.06	5.36	0.40
July 25	6:00 to 1:00	8.88	1.89	4.94	-2.07	6.38	0.04	5.78	3.91	3.96	0.25	8.18	0.87	4.28	1.54
Aug. 3	7:00 to 4:30	9.88	1.34	9.16	0.45	6.50	1.05	9.18	2.08	10.04	0.63	7.32	2.82	8.14	2.36
Aug. 9	7:30 to 4:30	3.12	0.41	5.30	0.27	4.78	0.57	6.66	1.42	5.12	0.85	3.00	2.26	2.38	0.17
Aug. 25	7:30 to 4:30	5.36	-0.40	7.22	0.96	3.96	0.34	4.20	0.85	3.72	1.51	1.40	-0.21	3.12	-0.36
Total		59.78	6.20	50.74	1.17	57.82	4.25	51.84	8.73	53.24	4.27	45.82	5.68	38.24	4.11
Mean		5.43	1.24	4.61	0.23	5.25	0.85	4.71	1.74	4.84	0.85	4.16	1.13	3.47	0.82

Table 31. Analysis of apple leaves. May 2 and 16, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time - Date	Grams per square meter leaf area						Percentage	
		Total dry matter	Combustible matter	Gain of total dry matter	Gain of combustible matter	Carbohydrates	Gain of carbohydrates	Water	Ash
Delicious	7:30 a. m., May 2	59.18	54.38			5.98		68.04	8.11
Delicious	3:30 p. m., May 2	61.18	56.23	2.00	1.85	6.42	0.44	67.46	8.09
Livland	7:30 a. m., May 2	73.16	66.93			8.28		65.05	8.52
Livland	3:30 p. m., May 2	79.84	73.08	6.68	6.15	8.65	0.37	64.13	8.47
Delicious	9:00 a. m., May 16	65.60	60.12			8.59		65.97	8.37
Delicious	11:30 a. m., May 16	66.96	61.42	1.36	1.30	8.77	0.18	65.07	8.28
Livland	9:00 a. m., May 16	82.76	75.72			11.12		62.56	8.51
Livland	11:30 a. m., May 16	83.40	76.23	0.64	0.51	11.29	0.17	62.20	8.60

Table 32. Analysis of apple leaves. May 23, 1934. Variation in total dry matter combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time - Date	Grams per square meter leaf area						Percentage	
		Total dry matter	Combustible matter	Gain of total dry matter	Gain of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
Jonathan	7:00 a.m., May 23	67.88	62.25			8.35		61.53	8.30
Jonathan	3:00 p.m., May 23	68.68	62.78	0.80	0.53	8.52	+ 0.17	63.62	8.60
York	7:00 a.m., May 23	68.82	64.37			10.76		59.54	6.47
York	3:00 p.m., May 23	71.82	67.07	3.00	2.70	10.32	- 0.44	58.12	6.62
Delicious	7:00 a.m., May 23	66.52	61.56			10.04		63.44	7.46
Delicious	3:00 p.m., May 23	71.56	66.41	5.04	4.85	10.50	+ 0.46	60.81	7.20
Livland	7:00 a.m., May 23	69.20	63.96			9.57		64.86	7.57
Livland	3:00 p.m., May 23	74.86	69.18	5.66	5.22	10.43	+ 0.86	62.86	7.59

Table 33. Analysis of apple leaves. June 5, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time - Date	Grams per square meter leaf area.						Percentage.	
		Total dry matter	Combustible matter	Gain of total dry matter	Gain of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
Jonathan	7:00 a.m., June 5	72.12	67.22			9.62		59.75	6.80
Jonathan	3:00 p.m., June 5	77.48	72.13	5.36	4.91	9.45	- 0.17	56.79	6.90
York	7:00 a.m., June 5	72.14	67.34			12.02		57.28	6.65
York	3:00 p.m., June 5	74.26	69.43	2.12	2.09	13.19	+ 1.17	56.06	6.50
Delicious	7:00 a.m., June 5	75.92	70.57			10.98		62.90	7.05
Delicious	3:00 p.m., June 5	79.80	74.13	3.88	3.56	11.47	+ 0.49	60.67	7.10
Livland	7:00 a.m., June 5	68.14	62.83			9.65		64.38	7.80
Livland	3:00 p.m., June 5	72.02	66.19	3.88	3.36	9.29	- 0.36	62.83	8.10

Table 34. Analysis of apple leaves. July 2 and 3, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

		Grams per square meter leaf area.						Percentage	
Variety	Time	Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
Jonathan	6:00 a. m.	77.38	72.46			10.78		57.56	6.36
Jonathan	2:30 p. m.	79.88	74.80	+ 2.50	+ 2.34	11.56	+ 0.78	54.30	6.36
Jonathan	6:00 a. m.	79.14	73.92	- 0.74	- 0.88	10.55	- 1.01	57.45	6.59
Jonathan	2:30 p. m.	80.70	75.54	+ 1.56	+ 1.62	11.16	+ 0.61	55.76	6.39
York	6:00 a. m.	77.32	73.37			12.01		54.96	5.11
York	2:30 p. m.	79.74	75.48	+ 2.42	+ 2.11	12.55	+ 0.54	52.52	5.34
York	6:00 a. m.	79.88	75.59	+ 0.14	+ 0.11	12.30	- 0.25	54.62	5.37
York	2:30 p.m.	82.54	78.01	+ 2.66	+ 2.42	12.55	+ 0.25	52.87	5.49
Delicious	6:00 a. m.	82.46	77.35			11.62		60.25	6.20
Delicious	2:30 p. m.	84.00	78.88	+ 1.54	+ 1.53	12.29	+ 0.67	58.28	6.19
Delicious	6:00 a. m.	84.32	79.17	+ 0.32	+ 0.29	12.20	- 0.09	60.07	6.11
Delicious	2:30 p. m.	85.94	80.78	+ 1.62	+ 1.61	12.43	+ 0.23	58.41	6.01
Livland	6:00 a. m.	80.84	74.80			8.08		60.24	7.47
Livland	2:30 p. m.	85.10	78.85	+ 4.26	+ 4.05	9.93	+ 1.85	59.06	7.34
Livland	6:00 a. m.	80.08	74.34	- 5.02	- 4.51	9.00	- 0.93	58.41	7.17
Livland	2:30 p. m.	83.92	77.89	+ 3.84	+ 3.55	9.56	+ 0.56	59.68	7.18

Table 35. Analysis of apple leaves. July 9 and 10, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

	Variety	Time	Grams per square meter leaf area						Percentage	
			Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
	Jonathan	6:00 a.m.	75.38	70.59			8.54		57.06	6.35
	Jonathan	2:30 p.m.	80.44	75.17	+ 5.06	+ 4.58	9.81	+ 1.27	54.23	6.55
	Jonathan	6:00 a.m.	81.08	75.89	+ 0.64	+ 0.72	10.05	+ 0.24	56.65	6.40
	Jonathan	2:30 p.m.	80.42	75.18	- 0.66	- 0.71	9.28	- 0.77	53.97	6.52
	York	6:00 a.m.	70.02	65.89			10.06		55.00	5.95
	York	2:30 p.m.	70.98	67.35	+ 0.96	+ 1.46	10.58	+ 0.52	53.59	5.12
	York	6:00 a.m.	73.80	69.71	+ 2.82	+ 2.36	10.82	+ 0.24	54.63	5.54
	York	2:30 p.m.	76.68	72.42	+ 2.88	+ 2.71	10.94	+ 0.12	52.27	5.53
No fruit	Delicious	6:00 a.m.	75.02	70.10			11.95		57.92	6.56
	Delicious	2:30 p.m.	77.32	72.40	+ 2.30	+ 2.30	10.62	- 1.33	56.32	6.37
	Delicious	6:00 a.m.	78.06	73.34	+ 0.74	+ 0.94	11.94	+ 1.32	57.59	6.05
	Delicious	2:30 p.m.	78.76	73.94	+ 0.70	+ 0.60	11.74	- 0.20	56.06	6.13
Fruit 15:1	Delicious	6:00 a.m.	68.82	63.91			8.82		61.45	7.13
	Delicious	2:30 p.m.	72.24	67.30	+ 3.42	+ 3.39	9.32	+ 0.50	58.91	6.84
	Delicious	6:00 a.m.	71.92	67.06	- 0.32	- 0.24	11.24	+ 1.92	61.09	6.75
	Delicious	2:30 p.m.	74.66	69.73	+ 2.74	+ 2.67	11.20	- 0.04	58.59	6.60
	Livland	6:00 a. m.	76.78	71.74			11.44		59.61	7.27
	Livland	2:30 p. m.	77.42	71.97	+ 0.64	+ 0.23	10.97	- 0.47	60.00	7.04
	Livland	6:00 a. m.	77.38	71.73	- 0.04	- 0.24	11.04	+ 0.07	58.95	7.30
	Livland	2:30 p. m.	81.50	75.55	+ 4.12	+ 3.82	11.36	+ 0.32	57.93	7.30

Table 36. Analysis of apple leaves. July 18 to 19, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

			Grams per square meter leaf area						Percentage	
	Variety	Time	Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
No fruit	Jonathan	6:00 a. m.	73.14	67.90			9.90		54.90	7.16
	Jonathan	2:30 p. m.	74.24	68.73	+ 1.10	+ 0.83	9.21	- 0.69	52.92	7.42
	Jonathan	6:00 a. m.	75.94	70.43	+ 1.70	+ 1.70	11.14	+ 1.93	53.73	7.25
	Jonathan	2:30 p. m.	78.74	73.12	+ 2.80	+ 2.69	11.55	+ 0.41	52.13	7.14
Fruit 20:1	Jonathan	6:00 a. m.	68.50	63.50			11.14		57.01	7.30
	Jonathan	2:30 p. m.	68.88	63.88	+ 0.38	+ 0.38	10.91	- 0.23	54.70	7.27
	Jonathan	6:00 a. m.	68.70	63.77	- 0.18	- 0.11	11.11	+ 0.20	57.02	7.17
	Jonathan	2:30 p. m.	74.12	68.63	+ 5.42	+ 4.86	11.66	+ 0.55	54.08	7.40
No fruit	York	6:00 a. m.	76.06	71.89			12.63		51.90	5.48
	York	2:30 p. m.	75.06	71.09	- 1.00	- 0.80	11.66	- 0.97	49.84	5.29
	York	6:00 a. m.	78.00	74.04	+ 2.94	+ 2.95	12.95	+ 1.29	50.99	5.07
	York	2:30 p. m.	82.96	78.66	+ 4.96	+ 4.62	13.41	+ 0.46	48.60	5.18
No fruit	Delicious	6:00 a. m.	79.26	74.38			12.47		57.90	6.16
	Delicious	2:30 p. m.	80.38	75.42	+ 1.12	+ 1.04	12.65	+ 0.18	55.88	6.17
	Delicious	6:00 a. m.	80.32	75.16	- 0.06	- 0.26	11.30	- 1.35	57.03	6.43
	Delicious	2:30 p. m.	82.08	76.75	+ 1.76	+ 1.59	12.11	+ 0.81	52.59	6.49
Fruit 15:1	Delicious	6:00 a. m.	68.26	63.12			10.51		59.99	7.53
	Delicious	2:30 p. m.	70.42	65.17	+ 2.16	+ 2.05	10.19	- 0.32	58.03	7.46
	Delicious	6:00 a. m.	70.06	64.94	- 0.36	- 0.23	10.58	+ 0.39	60.22	7.31
	Delicious	2:30 p. m.	73.20	67.73	+ 3.14	+ 2.79	9.99	- 0.59	57.74	7.47
No fruit	Livland	6:00 a. m.	76.56	70.64			9.57		58.81	7.73
	Livland	2:30 p. m.	77.38	71.40	+ 0.82	+ 0.76	10.96	+ 1.39	57.27	7.60
	Livland	6:00 a. m.	77.04	71.21	- 0.34	- 0.19	10.91	- 0.05	58.35	7.57
	Livland	2:30 p. m.	82.94	76.74	+ 5.90	+ 5.53	11.56	+ 0.65	56.24	7.47

Table 37. Analysis of apple leaves. August 3 to 4, and August 6 to 7, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

			Grams per square meter leaf area.						: Percentage	
	Variety	Time	Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
Aug. 3	King David	6:00 a.m.	81.01	76.61			11.72		50.50	5.43
	King David	2:30 p.m.	83.50	76.77	+ 2.49	+ 0.16	12.16	+ 0.44	48.99	5.66
Aug. 4	King David	6:00 a.m.	84.47	79.85	+ 0.97	+ 3.08	12.14	- 0.02	49.99	5.47
Aug. 6	Jonathan	6:00 a.m.	90.72	85.00			13.52		52.17	6.30
	Jonathan	2:30 p.m.	93.70	87.74	+ 2.98	+ 2.74	14.34	+ 0.82	50.64	6.36
	Jonathan	6:00 a.m.	95.16	89.17	+ 1.46	+ 1.43	14.18	- 0.16	52.80	6.30
Aug. 7	Jonathan	2:30 p.m.	95.44	89.35	+ 0.28	+ 0.18	14.92	+ 0.74	50.40	6.38
	York	6:00 a.m.	75.04	71.39			12.13		50.43	4.87
	York	2:30 p.m.	77.92	74.11	+ 2.88	+ 2.72	12.93	+ 0.80	48.63	4.89
	York	6:00 a.m.	80.88	76.95	+ 2.96	+ 2.84	11.94	- 0.99	50.51	4.86
	York	2:30 p.m.	78.94	75.14	- 1.94	- 1.81	12.00	+ 0.06	48.17	4.80
	Delicious	6:00 a.m.	87.30	82.50			12.80		53.54	5.50
	Delicious	2:30 p.m.	91.30	85.94	+ 4.00	+ 3.44	13.88	+ 1.08	54.23	5.87
	Delicious	6:00 a.m.	91.84	86.45	+ 0.54	+ 0.51	13.96	+ 0.08	54.15	5.87
	Delicious	2:30 p.m.	91.62	86.54	- 0.22	+ 0.09	14.11	+ 0.15	51.92	5.55
	Livland	6:00 a.m.	80.72	75.26			11.84		56.83	6.77
	Livland	2:30 p.m.	84.46	78.85	+ 3.74	+ 3.59	12.39	+ 0.55	54.51	6.64
	Livland	6:00 a.m.	83.00	77.46	- 1.46	- 1.39	11.48	- 0.91	57.18	6.68
	Livland	2:30 p.m.	89.16	83.23	+ 6.16	+ 5.77	12.33	+ 0.85	54.75	6.65

Table 38. Analysis of apple leaves. August 15, 16, and 17, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf.area. Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Percentage Water	Ash
Delicious	9:45 a.m.	66.96	61.50			9.06		62.70	8.16
Delicious	2:45 p.m.	70.38	64.36	+ 3.42	+ 2.86	9:45	+ 0.39	60.63	8.56
Delicious	8:45 a.m.	66.05	61.01	- 4.33	- 3.35	9.49	+ 0.04	63.14	7.63
Delicious	1:45 p.m.	67.66	62.44	+ 1.61	+ 1.43	9.72	+ 0.23	62.09	7.71
Delicious	8:00 a.m.	64.04	58.94	- 3.62	- 3.50	10.35	+ 0.63	63.45	7.97
Delicious	1:00 p.m.	66.34	61.16	+ 2.30	+ 2.22	10.30	- 0.05	60.31	7.82
Livland	9:45 a.m.	76.20	69.88			11.18		59.98	8.30
Livland	2:45 p.m.	81.74	75.01	+ 5.54	+ 5.13	11.58	+ 0.40	57.12	8.24
Livland	8:45 a. m.	77.58	71.18	- 4.16	- 3.83	11.38	- 0.20	60.59	8.25
Livland	1:45 p.m.	78.16	71.60	+ 0.58	+ 0.42	11.80	+ 0.42	59.10	8.40
Livland	8:00 a.m.	76.76	70.40	- 1.40	- 1.20	12.23	+ 0.43	60.80	8.28
Livland	1:00 p.m.	80.02	73.28	+ 3.26	+ 2.88	13.10	+ 0.87	58.50	8.42

Table 39. Analysis of apple leaves. August 21 to 22, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

Variety	Time	Grams per square meter leaf area.						: Percentage	
		Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
Jonathan	6:30 a.m.	82.72	78.03			13.01		52.15	5.70
Jonathan	2:00 p.m.	83.75	78.93	+ 1.03	+ 0.90	12.28	- 0.73	50.01	5.75
Jonathan	6:30 a.m.	89.25	84.20	+ 5.50	+ 5.27	11.78	- 0.50	50.83	5.66
Jonathan	2:00 p.m.	90.46	85.23	+ 1.21	+ 1.03	11.49	- 0.29	49.60	5.80

Table 40. Analysis of apple leaves. August 23 to 24, 1934. Variation in total dry matter, combustible matter, water content, and acid hydrolyzable carbohydrates estimated as glucose.

			Grams per square meter leaf area.						Percentage	
	Variety	Time	Total dry matter	Combustible matter	Gain or loss of total dry matter	Gain or loss of combustible matter	Carbohydrates	Gain or loss of carbohydrates	Water	Ash
No fruit	Jonathan	6:00 a.m.	84.12	79.33			13.40		52.47	5.70
	Jonathan	2:30 p.m.	85.22	80.29	+ 1.10	+ 0.96	12.58	- 0.82	50.60	5.79
	Jonathan	6:00 a.m.	90.08	85.26	+ 4.86	+ 4.97	13.99	+ 1.41	52.51	5.91
	Jonathan	2:30 p.m.	92.34	87.00	+ 2.26	+ 1.74	14.93	+ 0.94	50.47	5.79
Fruit 10:1	Jonathan	6:00 a.m.	75.92	70.54			11.13		54.45	7.08
	Jonathan	2:30 p.m.	78.48	72.71	+ 2.56	+ 2.17	11.12	- 0.01	52.25	7.33
	Jonathan	6:00 a.m.	80.28	74.34	+ 1.80	+ 1.63	11.77	+ 0.65	54.53	7.40
	Jonathan	2:30 p.m.	81.70	75.74	+ 1.42	+ 1.40	12.42	+ 0.65	51.62	7.30
No fruit	York	6:00 a.m.	72.16	68.83			12.58		51.10	4.61
	York	2:30 p.m.	72.92	69.52	+ 0.76	+ 0.69	11.62	- 0.96	48.60	4.66
	York	6:00 a.m.	75.08	71.42	+ 2.16	+ 1.90	11.26	- 0.36	51.05	4.87
	York	2:30 p.m.	78.06	74.29	+ 2.98	+ 2.87	11.61	+ 0.35	49.40	4.83
No fruit	Delicious	6:00 a.m.	94.86	89.37			14.42		52.46	5.79
	Delicious	2:30 p.m.	96.80	91.10	+ 1.94	+ 1.73	14.62	+ 0.20	50.04	5.89
	Delicious	6:00 a.m.	95.96	90.36	+ 0.16	- 0.74	15.93	+ 1.31	52.88	5.84
	Delicious	2:30 p.m.	99.28	93.65	+ 3.32	+ 3.29	16.66	+ 0.73	51.32	5.67
Fruit 40:1	Delicious	6:00 a.m.	87.20	81.81			13.08		53.47	6.18
	Delicious	2:30 p.m.	90.60	84.60	+ 3.40	+ 2.79	13.59	+ 0.51	51.26	6.63
	Delicious	6:00 a.m.	91.84	85.84	+ 1.24	+ 1.24	14.05	+ 0.46	53.30	6.50
	Delicious	2:30 p.m.	94.52	88.38	+ 2.68	+ 2.54	14.27	+ 0.22	51.29	6.50
No fruit	Livland	6:00 a.m.	91.14	84.94			14.04		55.30	6.80
	Livland	2:30 p.m.	91.24	85.13	+ 0.10	+ 0.19	14.47	+ 0.43	53.26	6.70
	Livland	6:00 a. m.	89.48	83.45	- 1.76	- 1.68	15.78	+ 1.31	55.96	6.74
	Livland	2:30 p.m.	91.26	85.19	+ 1.78	+ 1.74	16.91	+ 1.13	54.36	6.65

Table 41. Daily increase in total dry matter, combustible matter, and acid hydrolyzable carbohydrates estimated as glucose, grams per square meter leaf area. 1934. (Partial summary of Tables 31 to 40 inclusive).

Date	Time	JONATHAN No fruit Total dry matter	JONATHAN No fruit Combustible matter	JONATHAN No fruit Carbohydrates	JONATHAN With fruit Total dry matter	JONATHAN With fruit Combustible matter	JONATHAN With fruit Carbohydrates	YORK No fruit Total dry matter	YORK No fruit Combustible matter	YORK No fruit Carbohydrates	DELICIOUS No fruit Total dry matter	DELICIOUS No fruit Combustible matter	DELICIOUS No fruit Carbohydrates	DELICIOUS With fruit Total dry matter	DELICIOUS With fruit Combustible matter	DELICIOUS With fruit Carbohydrates	LIVLAND No fruit Total dry matter	LIVLAND No fruit Combustible matter	LIVLAND No fruit Carbohydrates
May 2	7:30 a.m. to 3:30 p.m.										2.00	1.85	0.44				6.68	6.15	0.37
May 16	9:00 a.m. to 11:30 a. m.										1.36	1.30	0.18				0.64	0.51	0.17
July 31	6:00 a. m. to 2:30 p.m.	Greenhouse									1.88						3.48		
Aug. 15	9:45 a.m. to 2:45 p.m.										3.42	2.86	0.39				5.54	5.13	0.40
Aug. 16	8:45 a.m. to 1:45 p.m.										1.61	1.43	0.23				0.58	0.42	0.42
Aug. 17	8:00 a.m. to 1:00 p.m.										2.30	2.22	-0.05				3.26	2.88	0.87
May 23	7:00 a.m. to 3:00 p.m.	0.80	0.53	0.17				3.00	2.70	-0.44	5.04	4.85	0.46				5.66	5.22	0.86
June 5	7:00 a.m. to 3:00 p.m.	5.36	4.91	-0.17				2.12	2.09	1.17	3.88	3.56	0.49				3.88	3.36	-0.36
July 2	6:00 a.m. to 2:30 p.m.	2.50	2.34	0.78				2.42	2.11	0.54	1.54	1.53	0.67				4.26	4.05	1.85
July 3	6:00 a.m. to 2:30 p.m.	1.56	1.62	0.61				2.66	2.42	0.25	1.62	1.61	0.23				3.84	3.56	0.56
July 9	6:00 a.m. to 2:30 p.m.	5.06	4.58	1.27				0.96	1.46	0.62	2.30	2.30	-1.33	3.42	3.39	0.50	0.64	0.23	-0.47
July 10	6:00 a.m. to 2:30 p.m.	-0.66	-0.71	-0.77				2.88	2.71	0.12	0.70	0.60	-0.20	2.74	2.67	-0.04	4.12	3.82	0.32
July 18	6:00 a.m. to 2:30 p.m.	1.10	0.83	-0.69	0.38	0.38	-0.23	-1.00	-0.80	-0.97	1.12	1.04	0.18	2.16	2.05	-0.32	0.82	0.76	1.39
July 19	6:00 a.m. to 2:30 p.m.	2.80	2.69	0.41	5.42	4.86	0.55	4.96	4.62	0.46	1.76	1.59	0.81	3.14	2.79	-0.59	5.90	5.53	0.65
July 31											4.68						5.93		
Aug. 6	6:00 a.m. to 2:30 p.m.	2.98	2.74	0.82				2.88	2.72	0.80	4.00	3.44	1.08				3.74	3.59	0.55
Aug. 7	6:00 a.m. to 2:30 p.m.	0.28	0.18	0.74				-1.94	-1.81	0.06	-0.22	0.09	0.15				6.16	5.77	0.85
Aug. 23	6:00 a.m. to 2:30 p.m.	1.10	0.96	-0.82	2.56	2.17	-0.01	0.76	0.69	-0.96	1.94	1.73	0.20	3.40	2.79	0.51	-0.90	0.19	0.43
Aug. 24	6:00 a.m. to 2:30 p.m.	2.26	1.74	0.94	1.42	1.40	0.65	2.98	2.87	0.35	3.32	3.29	0.73	2.68	2.54	0.22	1.78	1.74	1.13
T (1)		25.14	22.41	3.29				22.68	21.78	1.90	27.00	25.63	3.47				39.90	37.81	7.66
M (1)		2.09	1.86	0.27				1.89	1.81	0.158	2.25	2.13	0.287				3.32	3.15	0.638
T (2)											44.25	35.29	4.66				66.01	52.90	9.99
M (2)											2.33	2.07	0.27				3.47	3.11	0.58
T (3)		7.26	6.22	-0.16	9.78	8.81	0.96				11.14	10.55	0.39	17.54	16.23	0.28			
M (3)		1.81	1.55	-0.04	2.44	2.20	0.24				1.85	1.79	0.06	2.92	2.70	0.047			

T (1) and M (1) indicate total and mean respectively for data from May 23 to August 24 inclusive, except July 31.
T (2) and M (2) indicate total and mean respectively for data from May 2 to August 24 inclusive.
T (3) and M (3) indicate total and mean respectively for data from Jonathan and Delicious on the days when photosynthetic activity of leaves on fruit bearing branches was compared with that of leaves on branches not bearing fruit.

Discussion and Conclusions

Based on the data presented in this paper, the following conclusions may be drawn.

1. Apple varieties differ in their internal structure when the extent of the intercellular spaces in the spongy mesophyll is considered. The extent of these spaces was judged by measuring tracings of projected images with a planimeter and a chartometer.

2. Orchard grown apple leaves possess more extensive intercellular spaces than greenhouse grown leaves.

3. Livland, a Russian variety, has more extensive intercellular spaces than any other variety studied. In 1933, the Delicious mesophyll was the most compact, but in 1934, the mesophyll in York leaves was the most compact.

4. During the abnormally dry and hot seasons of 1933 and 1934, the stomata on outdoor foliage were seldom open after 9:00 a. m. and frequently they were closed or nearly so by 7:00 a. m. or 8:00 a. m. No afternoon opening of the stomata was observed. Stomata on apple leaves in the greenhouse remained open longer each morning than those on outdoor foliage.

5. Extreme difficulty was encountered in attempting to determine whether stomata were closed or nearly closed. It is probable that the stomata which appeared to be closed were not sufficiently closed to make them gas tight.

6. Livland leaves had the fewest stomata per unit of leaf area and the longest pores.

7. Outdoor grown leaves have more stomata per unit of leaf area than greenhouse grown leaves. In some instances this difference amounts to as much as 50 per cent.

8. In January 1933, some one-year-old Livland, Jonathan, Gano, and York trees were divided into two groups. One group was planted in a greenhouse where the day temperatures were usually 15 to 20 degrees C higher than in the house where the second group was planted. The leaves of Livland, Jonathan, and York grown in the warmer house had a greater extent of intercellular spaces than those in the cooler house. The reverse was true with Gano.

(a) Within each variety, the leaves possessing the more extensive intercellular spaces produced the greater gain in total dry matter per square meter leaf area between 8:30 a. m. and 5:30 p. m.

(b) The intercellular spaces in the mesophyll in the Livland leaves from the warmer house were more extensive than those of the other varieties grown in the same house. The same was true comparing Livland with the other varieties grown in the cooler house.

(c) Livland leaves accumulated a greater gain of dry matter per unit of leaf area than the other varieties within each group.

(d) Leaves on potted trees in the greenhouse have a greater amount of water in them than orchard grown foliage.

9. In January 1934, Livland and Delicious trees were planted in 12-inch clay pots in the greenhouse. In July 1934 these trees were transported to the orchard so that photosynthetic studies of the greenhouse grown trees could be made under the same environmental conditions as the orchard trees. There are marked differences in the spongy mesophyll of the leaves of greenhouse grown trees contrasted to orchard grown leaves. The four groups of leaves rank as follows in the measurements of the intercellular spaces in the mesophyll: (1) orchard grown Livland, (2) orchard grown Delicious, (3) greenhouse grown Livland, and (4) greenhouse grown Delicious. The day gain in total dry matter per unit of leaf area stands in the same relative order.

10. In 1933, the amount of total dry matter accumulated per square meter of leaf area of the seven varieties used was apparently markedly influenced by the maximum daily air temperature. On the days when the temperature was extremely high, the gain in dry matter was less than on days when the temperature was lower. In 1934, however, the inhibitory action of high temperatures did not become manifest, probably because of the great deficiency of soil moisture which became a limiting factor in leaf activity. On several days in 1934 dry matter determinations were made when the leaves were partially wilted.

11. Apparent photosynthetic determinations were made on three consecutive days in the greenhouse in 1934 by measuring the amount of carbon dioxide absorbed by a leaf on a potted Delicious tree and by one on a potted Livland tree. Dry weight increments, ash determinations, and carbohydrate analyses were made from other leaves collected from these trees.

(a) On two of the three days the Livland gained more in dry weight per unit of leaf area than the Delicious foliage. After being corrected for ash content the mean gain of the dry matter for the three days was 2.81 gms M^2 5 hrs. for Livland and 2.30 gms M^2 5 hrs. for Delicious. The mean accumulation of total acid hydrolyzable carbohydrates estimated as glucose was 0.56 gm M^2 5 hrs. for Livland and 0.22 gm M^2 5 hrs. for Delicious.

(b) The mean amount of carbon dioxide absorbed by the Livland leaf was 4.81 gms M^2 5 hrs. and for Delicious 1.89 gms M^2 5 hrs.

(c) Subsequently the two leaves used in these continuous gas stream studies were killed, dehydrated, and imbedded in paraffin after which mounts on slides for microscopic study were prepared. As described earlier, the intercellular spaces in the mesophyll were measured. The cross sectional area of these spaces for Livland was 107.71 cm^2 per unit area compared to 87.01 cm^2 for Delicious.

(d) The ratio of the average weight of carbon dioxide absorbed per square meter of leaf area for the three 5-hours run to the average weight of the total acid hydrolyzable carbohydrates accumulated for the same area and time is 8.59 for Delicious and 8.58 for Livland.

12. York leaves possess the following distinguishing characteristics: (a) a lower percentage of ash than Jonathan, Delicious, or Livland, (b) usually a lower water content than Winesap, Jonathan, Delicious, Livland, Wealthy, or Gano, (c) a lower total dry weight per unit of leaf area than any of the other varieties, with the occasional exception of Gano, (d) a more compact mesophyll than any of the other varieties studied in 1934.

13. During both 1933 and 1934, York made lower daily gains in total dry matter per square meter leaf area, and lower gains in total acid hydrolyzable carbohydrates estimated as glucose, per square meter leaf area, than any of the other varieties included in these studies. The varieties used in addition to York were: for 1933, Livland, Gano, Wealthy, Jonathan, Winesap, and Delicious; for 1934, Livland, Jonathan, and Delicious. It may be considered that the distinct biennial bearing habit of York may be due in part, to the fact that the foliage is relatively inefficient, photosynthetically, which in turn appears to be due to some of the items listed in the preceeding paragraph.

14. Livland leaves on the average have greater weight per unit of leaf area than any of the other varieties used in 1934. In many instances during 1933, Wealthy leaves weighed the most per unit of leaf area. This is probably due to the extensive palisade development of these two varieties. The water content of Livland leaves is usually higher than that of the other varieties. These two characteristics in addition

to the great extent of the intercellular spaces in the mesophyll probably account for the fact that Livland ranked first in average daily gains in total dry matter and total acid hydrolyzable carbohydrates estimated as glucose per unit of leaf area, during 1934, and that Wealthy and Livland ranked first and second respectively in total dry matter gains per unit of leaf area in 1933.

15. In 1934, the ranking of the varieties based on the extent of the intercellular spaces and the ranking based on photosynthetic activity as judged by the average daily gains in total dry matter, in combustible matter, and the average daily gains in total acid hydrolyzable carbohydrates estimated as glucose, were identical. This ranking was Livland, Delicious, Jonathan, and York.

16. On six days during 1934 the total dry matter increase of Delicious leaves on branches bearing good crops of fruit was compared with that of leaves on branches bearing no fruit. On five of these days the leaves on the fruiting branches gained more in total dry matter and combustible matter than the leaves on fruitless branches. The mean gain of total dry matter for the six days was: 2.92 gms M^2 leaf area on fruiting branches, and 1.85 gms M^2 leaf area on fruitless branches. The mean gains of combustible matter were 2.70 gms M^2 leaf area and 1.79 gms M^2 leaf area respectively.

17. On four days during 1934, the total dry matter increase of Jonathan leaves on branches bearing fruit was compared with that of leaves on fruitless branches. On two of these days the gain was greater for the leaves on fruiting branches and on two days the leaves on

fruitless branches made the greater gain. The mean gain of total dry matter for the four days was 2.44 gms M^2 leaf area from branches with fruits and 1.81 gms M^2 leaf area from branches from which the fruit had been removed. The ash and water content of leaves near fruits was in general higher than in leaves on fruitless branches. The carbohydrate concentration was usually lower in leaves on fruiting branches than in leaves on branches from which the fruit had been removed.

Summary

1. Apple varieties differ in the extent of the intercellular spaces in the mesophyll of the foliage leaves.

2. This anatomical difference in leaf structure is reflected in photosynthetic activity.

3. York leaves are the least active photosynthetically, a characteristic which may play an important role in the distinct biennial fruit bearing habit of this variety. Low dry weight per unit area, low ash content, low water content, and in 1934 the most compact mesophyll are other characteristics of York leaves.

4. Leaves on branches bearing fruit make greater gains in dry matter and carbohydrates, have a higher ash content, higher water content, and less dry weight per unit area than leaves on fruitless branches.

5. Leaves on fruiting branches usually have a lower concentration of carbohydrates than leaves on branches not bearing fruit.

6. The rate of photosynthesis was determined on 17 days in 1933 and on 20 days in 1934. Both growing seasons were abnormally hot and dry.

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