THE BEHAVIOR OF STOMATA

BY

J. V. G. LOFTFIELD

Published by the Carnegie Institution of Washington
Washington, 1921

11575

CARNEGIE INSTITUTION OF WASHINGTON

Publication No. 314

PRESS OF A. B. GRAHAM CO.
WASHINGTON, D. C.

CONTENTS.

LIST OF ILLUSTRATIONS.

PLATES.

FIGURES.

THE BEHAVIOR OF STOMATA.

INTRODUCTION.

This investigation was undertaken to discover what changes take place in the apertures of stomata throughout the day and night, the influence of physical factors upon such changes, and the final effect upon transpiration. Hence the investigation falls naturally into three subdivisions which form the sections of this volume. As previous investigators have usually worked with few plants and in one region, it was felt that considerable light could be thrown upon the subject by using many plants and working in several regions of diverse climate. Experiments which were first carried out at the American Smelting and Refining Company's laboratory near Salt Lake City were therefore continued at the Desert Laboratory at Tucson, Arizona, and at the University of Minnesota, Minneapolis, Minnesota, as well as at Salt Lake City. A wide range of climate was thus available, since the spring climate of Minneapolis is rather humid, the summer climate of Salt Lake City dry and sometimes hot, and the winter in Tucson dry and warm. For a number of reasons, most of the experiments were made at the Salt Lake laboratory, where the water-content could be controlled readily and the range of humidity and temperature from day to day was almost as great as in the desert.

At the outset, careful consideration was given the methods hitherto employed to determine changes in stomatal apertures. The horn and yucca hygroscopes used by Darwin (1898) were properly criticized by Lloyd (1908) as measures of transpiration rather than of the condition of the stomata. Cobalt-chloride paper is open to the same objection, and therefore only indirectly throws light upon the opening of the stomata. Petroleum ether, when dropped upon a leaf, passes through it if the stomata are open and sometimes gives it a water-soaked, translucent appearance. Hence this reagent may be used as a qualitative test for stomatal opening. These methods, however, are no longer in use, and this left but three methods to be considered for the purpose of the investigation.

The two methods used by Lloyd (1908, 1913) may be regarded as reliable in a high degree. The first method consists in observing the degree of opening in stomata in properly fixed strips of epiderm, the second in observing the stomata in their natural state on the leaves while these are still on the plant. Naturally, the latter method is preferable, since it shows the stomata on leaves which are undisturbed until they are placed under the microscope. But the impossibility of doing this at night or in poor light reduces the applicability of this method very considerably. The porometer method, devised by Darwin and Pertz (1911) and modified and improved by Laidlaw and Knight (1916), was not considered favorably for several reasons, chief among which were the unnatural conditions introduced by pulling a current of air through the intercellular spaces of the leaf, and a doubt as to what porometer readings actually represented. To the best of the writer's knowledge, no comparison has been made between a series of direct observations upon the condition of the stomata and porometer readings made at the same time. Until the method is checked in this manner, it is felt that its reliability is questionable.

For the reasons given, it was felt that the most desirable method to employ was the first one used by Lloyd (1908). This consists in stripping the epiderm from the leaf and quickly plunging it into absolute alcohol, which immediately fixes the epidermal cells, keeping them in the shape they were in when immersed. The guard-cells can not lose an appreciable amount of water in the fraction of a second between stripping and immersion in alcohol. The effect of the alcohol is to dehydrate the cell-wall before penetrating the cell proper to any extent. The dehydration of the cellulose wall causes it to become very stiff and hard. As a consequence the cells retain their original form in spite of the fact that the alcohol next removes the water from within the cell. This form is maintained for some time, provided the walls are kept dehydrated. Theoretically, therefore, the method seemed sound, but it was evident that it could not be considered reliable until checked by direct observation of the stomata of living leaves, not only for each species and variety of plant studied, but for a wide range of stomatal apertures for each as well. Not only was this done, but in addition several parallel series were made with the more important plants, one series being the usual set of epidermal strips collected each hour, the other a set of measurements of stomatal apertures on the living leaves of the same plant, also made each hour.

The check upon the method was ordinarily made in the following fashion: A stand for the microscope was erected in such a manner that a leaf could be brought into the field with the least possible change of position and consequent disturbance. This leaf was then lightly clamped between a long cover-glass and slide, and about 10 stomata quickly measured. Not more than 2 minutes was allowed for this operation, in order to prevent any serious change while the leaf was exposed to these unnatural conditions. Then the leaf was released and stripped in the usual manner, great care being exercised to secure the area just examined. After the customary two days of staining, a similar number of stomata were measured in the strip and the results compared. Table 1 gives in millimeters the results obtained for alfalfa, which are typical of those obtained with other plants.

TABLE 1.—*Results obtained from alfalfa.*

Leaf 11, upper surface.		Leaf 22, upper surface.		Leaf 22, lower surface.		Leaf 25, upper surface.		Leaf 25, lower surface.	
Leaf.	Strip.	Leaf.	Strip.	Leaf.	Strip.	Leaf.	Strip.	Leaf.	Strip.
8.5 × 2.2	8.0 × 2.5	8.5 × 2.5	8.0 × 2.5	7.0 × 2.5	6.0 × 1.3	8.5 × 0.1	8.0 × 0.0	7.0 × 2.1	7.0 × 0.0
8.0 × 3.0	7.0 × 3.0	8.0 × 3.0	7.0 × 3.0	7.0 × 2.4	7.0 × 2.9	8.0 × 0.0	9.0 × 0.5	7.5 × 0.0	7.0 × 0.5
6.3 × 3.0	6.5 × 2.4	6.5 × 3.0	9.0 × 4.0	7.0 × 2.1	7.0 × 2.6	7.4 × 0.0	8.5 × 0.0	7.2 × 0.0	6.0 × 0.2
8.1 × 2.5	8.2 × 2.0	8.0 × 2.4	8.0 × 2.0	8.0 × 2.9	6.0 × 1.1	7.0 × 0.4	7.5 × 0.0	7.2 × 1.0	6.4 × 1.0
8.0 × 2.5	7.0 × 2.9	8.0 × 2.9	7.0 × 3.0	7.0 × 2.6	6.5 × 1.8	8.0 × 0.2	8.0 × 0.2	7.5 × 0.2	7.0 × 0.2
6 0 × 2.3	7.7 × 3.2	6.0 × 2.0	8.0 × 2.9	5.0 × 2.0	8.5 × 3.0	8.0 × 0.2	6.5 × 0.5	8.0 × 0.5	7.0 × 1.0
7.0 × 2.5	8.0 × 3.5	7.1 × 2.4	8.0 × 2.9	8.0 × 2.3	8.2 × 2.8	8.0 × 0.4	6.5 × 1.0	7.0 × 0.4	6.0 × 0.2
7.0 × 3.0	6.0 × 2.0	9.0 × 3.0	6.0 × 2.2	6.0 × 2.1	5.0 × 2.0	6.5 × 0.4	8.0 × 0.4	7.0 × 0.3	6.5 × 0.4
8.0 × 3.0	9.0 × 2.8	7.9 × 2.9	8.0 × 2.0	7.0 × 2.0	8.0 × 3.0	9.0 × 0.4	7.5 × 0.5	7.0 × 0.6	8.0 × 0.2
8.5 × 3.0	8.0 × 3.0	8.5 × 3.1	8.2 × 3.0	8.0 × 2.0	7.0 × 2.6	6.0 × 1.0	8.0 × 0.2	8.0 × 0.4	7.0 × 2.0
								7.6 × 0.0	8.0 × 0.0
								6.0 × 0.2	8.0 × 0.2
75.4 27.0	75.4 27.3	77.5 27.2	77.2 27.5	70.0 22.7	69.2 23.1	76.4 3.1	77.5 3.3	93.0 6.0	91.9 6.1
Aver	age	Aver	age	Aver	age	Aver	age	Aver	age
7.5 × 2.7	7.5 × 2.7	7.7 × 2.7	7.7 × 2.7	7.0 × 2.3	6.9 × 2.3	7.6 × 0.3	7.7 × 0.3	7.2 × 0.5	7.1 × 0.5
Lower surface.									
Leaf.	Strip.								
Closed.	Closed.								

The results from alfalfa are given, since most of the experiments discussed were performed upon alfalfa. At first this was due to the fact that it was the most important plant susceptible to SO_2 injury. Later, its sensitive stomata and great availability made it very useful in many of the series as a basis of comparison in the study of the daily march of stomatal movement in other plants. The results from the checks did not always agree as well as in the above tables, but the agreement became greater as the number of stomata measured was increased. The reason for trying to get as nearly the same area as possible in stripping was not because of any hope of getting the same stomata measured in the strip as on the leaf, but because the effect of the great variation that often exists in the stomata on the same leaf can best be avoided in this manner. Table 2, showing the least amount of agreement in the two sets of measurements of those made on alfalfa, will give the reader a clearer idea of what is meant.

In table 2 the agreement is not at all close. The second set of 10 measurements of stomata in the strip is closer than the first 10, but when the two sets are averaged together, the result is 7.1 by 0.39, which is still nearer the average for width of pore. Had 20 stomata been measured in the leaf, the agreement would probably have been quite close. Where there was much variation in size of stomatal apertures the number of measurements was usually increased, when the agreement was invariably much better.

Many objections have been raised to the stripping method since its development by Lloyd, most of them made without sufficient reason. The essence of the method is the almost instant dehydration by the alcohol and consequent hardening of the cellulose walls

TABLE 2.

Leaf 13, lower epiderm.		
Leaf.	Strip I.	Strip II.
7.3 × 0.2	8.0 × 0.5	6.5 × 0.4
6.0 × 0.2	7.0 × 0.4	8.0 × 0.2
6.2 × 0.2	7.5 × 0.0	7.0 × 2.0
6.0 × 1.0	7.0 × 0.2	6.0 × 0.2
7.0 × 0.4	7.0 × 0.0	7.0 × 1.0
6.4 × 1.0	7.0 × 0.0	7.0 × 0.2
7.0 × 0.2	7.5 × 0.2	6.4 × 1.0
7.2 × 0.2	7.5 × 0.2	6.0 × 0.2
7.4 × 0.2	8.0 × 0.2	7.0 × 0.5
7.6 × 0.0	8.0 × 0.4	7.0 × 0.0
68.1 3.6	74.5 2.1	67.9 5.7
Av. 6.8 × 0.46	7.5 × 0.21	6.8 × 0.57

of the guard-cells and surrounding epidermal cells, which fixes them in the shape in which they were when plunged into the alcohol. The dilution of alcohol in the cell-wall caused by the extraction of the water within the cell is not sufficient to soften these walls or to cause them to lose their form. The error, if error there be, must occur during stripping or immediately following. The objection made by Laidlaw and Knight that the jarring due to the operation causes closure may be dismissed, for, if stomata of plants growing in the open field closed each time they were jarred, they would be permanently closed in a region of almost constant wind, such as the Great Plains or the Great Basin. Moreover, stripping itself would make it impossible to find any strips with open stomata.

The more common objection that shrinkage and distortion occur is to some extent true. A large number of strips were measured to find the amount of this shrinkage, and it was found to be less than 1 per cent when it occurred at all. Immediately after stripping, the width and length of the stripped area upon the leaf were measured with dividers and compared with the length and width of the strip. In most cases the shrinkage was not perceptible, but in the case of wilted leaves there was very slight shrinkage. But as this was always less than 1 per cent, the greatest error possible was not more than 0.04 micron in the width of an alfalfa stoma, while the smallest division that can be accurately measured is 0.2 micron.

The distortion which occurred as a result of the epidermal strip rolling or curling was usually caused by keeping the strip exposed to the air too long. As this shrinking was greater on the one side than on the other, and greater in one direction than the other, it was thought possible that the stomata lying in the direction of the axis of rolling would tend to close, and those lying at right angles to this direction would tend to open slightly. If this occurred it was too slight to be detected. Nevertheless, such strips were not used, except in rare cases, not so much because of doubt in regard to their accuracy as because of difficulty in photographing them. The curled strips apparently checked just as closely as the others with measurements of the stomata *in situ.*

The chief reason for confidence in the method lies in the agreement of the stomatal openings observed in the undisturbed leaves with those measured in the strips removed at the same time. Any serious objection to the method must come through showing that there is no such agreement between results from properly performed stripping and those obtained by carefully conducted observations of stomata *in situ.* Except for the impossibility of observing them during the hours of darkness and poor light without the use of strong artificial light, this latter method might have been used throughout. However, other objections were decisive, in view of the objects sought. These were the impossibility of photographing the stomata in such leaves, and the difficulty of finding enough leaves on a single plant in such position that they could be slipped under the microscope without undue disturbance.

It must be emphasized that an examination of a leaf in this manner does set up a disturbance which sooner or later appears in the stomata, and hence such a leaf can be used but once in the series. This was tested several times by comparing such a leaf with some undisturbed leaf half an hour or an hour later. The usual effect was to produce closure of the stomata. Leaving a leaf under the microscope would in extreme cases cause closure in 3 minutes in some stomata, but as a rule 10 minutes or even more elapsed before a change occurred. As the experiments demonstrated that the stripping method was as reliable as direct observations, it was used because of its greater convenience, and direct examination merely for checking and comparison.

The accuracy of the method depends greatly upon the process of stripping. To gain speed, the scalpel used in making the cut to start the strip, and the forceps used in seizing the epiderm and in stripping, were bound together with rubber bands, the end of the forceps projecting about an inch beyond the tip of the scalpel blade. The cut once made, the instrument was twirled half a turn to bring the forceps into position, the cut end seized, and the epiderm plunged into the previously opened vial of alcohol well within a second. A good flash-light was usually used for night work, or at times an electric light from a field socket nearby. This light was sufficient to select the leaves desired and to strip them, the process taking about a minute, an interval too short to cause opening by the light used.

A number of precautions must be observed in stripping if the results are to be reliable. Stomata vary greatly in sensitiveness in response to stripping. Those of cereals and grasses generally are very sensitive to exposure to air and must be plunged into the alcohol in less than a second, while those of potato or *Rumex patientia* may be kept out for a much longer time. A strip of this latter was kept exposed to the air for a full minute in one experiment without change of stomatal opening. Corn is not so sensitive as wheat or barley to such exposure, but even with this plant it is best to reduce the process to less than 2 seconds when stripping. Another precaution is not to strip a wet leaf. If rain or heavy dew has fallen, the moisture should be removed with filter-paper or absorbent cotton, since the water carried with the strip may dilute the alcohol seriously. The water on the strip may also affect the stomata before the strip reaches the alcohol, especially when, as is usually the case, capillarity causes the water to be pulled to the under side of the strip. This latter objection depends a good deal upon the kind of plant stripped. The epiderm of alfalfa, which has sensitive stomata, has been kept in water for an hour or more after stripping and no appreciable difference found as compared with a similar strip plunged into alcohol in the usual manner. However, even with alfalfa, the water carried with the strip will often dilute the alcohol in contact with the strip long enough to cause partial or complete collapse of the guard-cells.

Great variation in the size of stomatal apertures on various leaves may be found at any one time, not only on leaves of different individuals, but also those of the same plant. They even vary much at times on the same leaf. The causes for this are many. No two plants are found in exactly similar situations, nor are the leaves on the same plant under identical conditions. The usual cause for difference in stomatal openings in the various leaves on the same plant lies in their age or, more properly, their degree of maturity. A leaf does not have functional stomata until it is fully grown, and young stomata do not open so soon or stay open so long as the stomata on the more mature leaves. The same is true in even greater measure of an aging leaf. Long before an old leaf turns yellow and falls, the stomata have ceased to function and remain permanently closed. With experience one learns which leaves are mature and actively functional, and these alone should be used in a series. Another cause of variation in the leaves on a plant is due to the fact that the stomata of leaves that are shaded are often closed when those of unshaded leaves are open, or open when the ones on the upper leaves are closed. But when enough stomata are measured to insure checking out the extremes, the degree of opening in the mature leaves of the same plant exposed about equally to the light agree very well. Usually three leaves of each set of plants were stripped each hour, giving three strips of epiderm for each leaf-surface for each hour. This insured against loss due to inadvertently stripping an immature or aged leaf. Such an occurrence was very rare after some experience had been gained in selecting the proper leaves. It also served as a check upon similarity of stomatal behavior in mature leaves. The causes of variations of the stomatal opening of different plants will be dealt with later.

Twenty-four hour series were made of more than 60 species of plants, including crop, garden, and forage plants, weeds, native herbs, trees, and shrubs. The more important of these were checked in the same manner as alfalfa, in order to determine the reliability of the stripping method. A great many were used in but one series, which was made, however, at the same time as an alfalfa series, in order to determine the daily march of its stomatal movement in relation to a plant whose reaction to the environmental factors was more or less known. Except in the case of leaves with but one stomatal surface, each series consisted of two sets of epiderm, one for each surface.

Few investigators seem to have made long, continuous series. The discovery that the variation in the march of stomatal movement from day to day was considerable made it evident that only a long, continuous series would be of any real value. A group of short series covering the entire period of the day and night, but not made within the same 24-hour period, fails to reveal the actual sequence, but makes an artificial one having slight relationship to the stomatal movement upon any such day. Because of this, the usual series was of 24 hours' duration. A number of shorter ones were made for special purposes, especially for what may be termed a reconnaissance of the field and for investigations on the effect of certain factors. Longer series were also made quite frequently, the longest being continuous for 68 hours, but the strain placed upon the investigator by so long a series is not justified by the results obtained. Even a 24-hour series called for at least 36 hours of continuous labor, as it required many hours of work to get everything in readiness, and several hours after the series was finished to complete the work.

The question of the time of starting and ending a series was also of importance. It was especially desirable that no gap should occur between the first and last strip collected. If a series began with the stomata closed and ended with them closed, the course of events leading to the opening and final closing could be followed throughout, but if it began with them closed and ended with them open, the time of closure would be in doubt, and the series left without a definite ending. At first it was thought that the best time to start a series would be just before dawn, as the stomata would have all night to reach closure. Experience showed, however, that just after sunset was the best time, for if the stomata closed at all they usually closed at this time.

Several days prior to photographing, the strips were transferred to vials containing a saturated solution of Congo Red in absolute alcohol. This stains the cell-walls chiefly, making them stand out very clearly. A number of other stains were tried, but none proved as satisfactory as Congo Red. When ready for photographing, the strip was trimmed and placed upon a special slide with two spring clips which held the cover-glass in place, and then mounted in absolute alcohol. This type of slide was not absolutely necessary, but it was very convenient, because it prevented the strip from floating and the consequent loss of a clear, sharp image. Because of the alcohol mount, an upright camera was used. This consisted of a stand for the microscope, a hood which was tied around the top of the microscope, a camera-box taking a 5 by 7 plate-holder, and a frame which supported all of these. A Spencer microscope was used with a 4 mm. objective, a Zeiss projection ocular No. 2 being used to project the micrograph upon the photographic plate. The same microscope and camera were used for all microphotographs made. The magnification (345 diameters) was the largest that could be employed and still have the entire field upon a 5 by 7 plate.

The photographs of a series were mounted for ready comparison on charts 54 inches square, in the form of a 24-hour clock. Those reproduced are reduced charts made by cutting out an average stoma or group of stomata from each photograph and mounting them on a chart 8 inches in diameter. The light, temperature, and relative humidity curves are given in a circular graph inside the circle of microphotographs. Whenever this circle is a double one, the outer circle shows the upper epiderm and the inner circle the lower epiderm of the leaves of the plant under consideration. In the greater number of series the strips were not photographed, but about 20 stomata of each were measured and averaged to get the material for stomatal graphs. All the charts are not reproduced here, since that would involve needless duplication and bring in species that can not be discussed at this time.

The physical data were obtained chiefly by means of simple instruments. A stop-watch photometer was used for the light data, a cog psychrometer to determine the relative humidity, a compensated aneroid barometer for obtaining the pressure, a group of ordinary chemical thermometers checking within a tenth of a degree centigrade to determine the temperature at various levels of the soil and air, and a Tycos anemometer to measure the velocity of the wind. The water-content was determined by taking soil samples at the depths described, weighing, drying them on a water-bath, and weighing again after they had become constant in weight. Samples were always taken in duplicate.

The stop-watch photometer devised by Clements may need a fuller description. It consists of a drum on which a strip of Solio paper is wound and inclosed in a case which is light-proof. This case has a rectangular opening directly over the strip of Solio. This opening is covered and light-proof until an exposure is desired. The case can be revolved one twenty-fifth of the circumference over the drum and paper, exposing a new area on the strip each time. The slide is so arranged that when the opening in the case is uncovered the stop-watch attached to the front of the photometer is started, and stopped when the slide is closed. In this manner the time of exposure can be measured accurately to a fifth of a second—in fact, much more accurately than a fifth, for the operator soon learns to snap the slide shut just at the moment that the hand of the watch jumps to the proper fifth of a second. The time is probably accurate to a fiftieth of a second. The slide is attached to a spring which instantly covers the opening the moment it is released.

The standard was made at noon on a clear day at the time of the summer solstice. This consisted of a Solio strip exposed 1 second at the first area, 2 seconds at the second area, 3 seconds at the third, etc., the last area being exposed 25 seconds. As a rule, several of these were made, one for each clear day during the proper period. The one showing the deepest color was selected as the standard. When a light reading was desired, an exposure was made and the time and number of the exposure recorded. When the 25 exposures were exhausted, the photometer was taken to the dark-room, the strip removed, and each area of exposure compared with the standard. When an area exposed 20 seconds has the same depth of color as the 3-second area on the standard, the light at that time was three-twentieths of the maximum for that region, or 15 per cent.

With the exception of Lloyd, no investigator seems to have used plants growing in a natural environment. In spite of this, many of these workers have tried to apply their data to plants growing naturally, without due allowance for the conditions under which their experiments were performed. Gray and Peirce (1917) experimented with plants grown in a greenhouse under shaded glass and in wooden boxes or flats, which they state was necessary in their climate, and assumed that such plants compare in reaction with similar plants grown in the field. The use of a photometer would have shown this assumption to be unwarranted, as fully 50 per cent of the light is removed in a greenhouse with clean panes. In the case of panes only lightly frosted, it is probable that less than 15 per cent of the light incident actually reached the plants. As their experiments

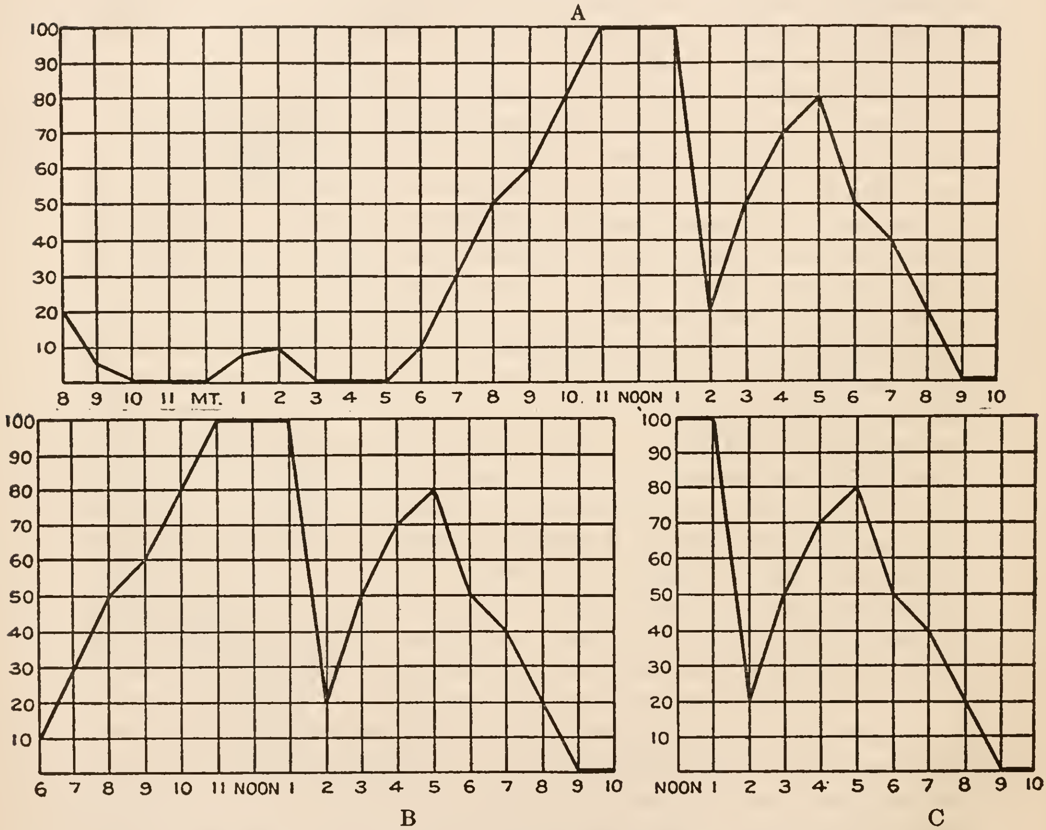


FIG. 1.—Three plants of alfalfa series 14, stripped for 27, 17, and 11 hours respectively, showing that the loss of a few leaves does not affect the stomatal movement.

were carried out with a view of discovering the effect of light upon the stomatal movement of their plants, they can apply only to plants grown under similar conditions as to light intensity. With plants growing in a desert, water is the factor which determines their existence. In shade, even if only partial, the determining factor is light; hence, in experiments conducted as were theirs, it is to be expected that the stomata would respond to light much more quickly than to any other factor. Moreover, their terms "light," "hazy," "rather dark," "too dark to see clearly," are merely descriptive and can not serve as measures of light.

In these studies, field plants grown under natural conditions were used exclusively. All possible factors were accurately measured and the correlations were drawn with due regard to the limits placed by lack of data on other reactions by the plant. Too much emphasis can not be placed on the importance of having the plant under as normal conditions as possible. Of course, a plant on which one leaf after another is removed is not entirely under normal conditions, but an alfalfa plant with approximately 1,000 leaflets is not very much affected by the removal of 50 during a 24-hour series. This is shown by a series started at 8 p. m. on June 26, 1916. A large plant of alfalfa was stripped during the night as well as the next day at 6 a. m., June 27; another set was added to the series by stripping a plant close by, and hence under as nearly the same conditions as possible to the first plant. At noon on June 27 a third plant was added to the series. If the loss of the leaves made any appreciable difference in the stomatal movement there should be no agreement in the curves of the three sets; if these curves coincide, the effect of continued stripping is not appreciable. As the graphs show, no difference can be detected. When a large proportion of the leaves of a plant are removed, there can be no doubt that stomatal movement in the remaining leaves will not be normal, but this was avoided by removing comparatively few leaves from each plant.

In the following descriptions and graphs, stomatal apertures are expressed in percentages of the maximum. This checks out the effect of variations in size of stomata and readily allows comparison of stomatal movement in the various plants investigated. It also allows the relation of light and humidity to be more clearly shown than is possible in any other manner. As it was not possible to measure fractions of 0.1 micron, the degree of opening is given to the nearest twentieth of the maximum. In the earlier experiments the diffusion capacity $n\sqrt{ab}$ was also calculated, but the curve produced was essentially similar and had no advantage not found in the method used.

Acknowledgment is due Dr. P. J. O'Gara, of the American Smelting and Refining Company, for aid and assistance throughout this investigation. As the 1916 work was carried on for the American Smelting and Refining Company, the use of all material and photographs from that year is by their permission. At various periods since that time, the company, through Dr. O'Gara, has extended the use of its laboratory and given material assistance in the continuance of the work. Acknowledgment is also due Dr. Clements for making possible the further prosecution of the work, especially in connection with the effect of stomatal movement upon transpiration.

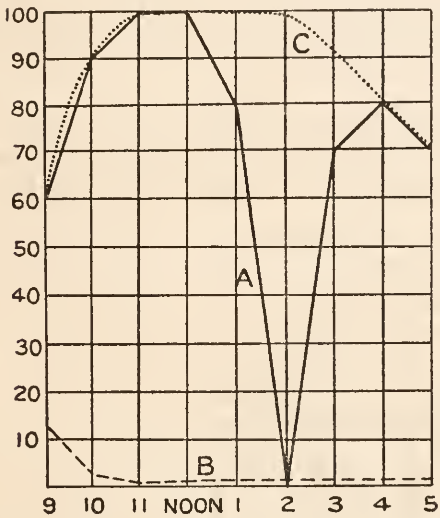
I. THE DAILY MARCH OF STOMATAL MOVEMENT.

These experiments were originally undertaken to determine when the stomata of the more important crop-plants of the Great Salt Lake region were open. Experiment had shown that usually five times the SO_2 concentration required to injure plants during the day was necessary to produce a like degree of injury at night. This was explained as a result of the condition of the stomata, but exact information was needed as to when stomata opened and closed during a 24-hour period. This was especially desirable, as prevention of smelter injury to vegetation was at that time based upon the "sea captain" plan of smelter operation, by which the manager kept informed of the approach of unfavorable conditions when plants were especially susceptible to SO_2 injury, and reduced operations within the smelter accordingly.

At the outset it was thought probable that for each species the daily march of stomatal movement was the same from day to day as long as conditions were not extreme. Lloyd (1908 : 108) states that in *Fouquiera* "the stomata open and close rather slowly and maintain a maximum opening for about 3 hours from 9 a. m. to 12 day. This maximum can not be said to be strictly constant, but the differences are slight and within the personal error of observation. Stomatal closure occurs in the early afternoon, advancing steadily until nightfall. It is difficult to correlate this with changes in the surrounding media." Nevertheless, it was felt that such similarity of behavior on days of unlike weather conditions required demonstration for each species concerned. Largely for this reason, the same plants were used in successive series on days of different weather conditions. By this means it was soon found that similarity of behavior was by no means the rule, and that stomatal movement differed in the same species from day to day in accordance with the physical conditions.

THE DAILY MOVEMENT IN ALFALFA.

The first series, consisting of strips of epiderm collected hourly, was begun at 9 a. m. May 8, 1916, and finished at 5 p. m. the same day. Light, temperature, and humidity readings were made during this period, as well as in all the other series. The curve for stomatal movement in the upper epiderm of alfalfa follows that of sunlight, except for the sharp dip commencing at noon. The curve for sunlight shows that the day was cloudless and totally free from haze. At 9 a. m. both were at 60 per cent of maximum; at 10 a. m. they reached 90 per cent; at 11 a. m. they were at 99 per cent; and at 12 noon both reached maximum. Sunlight continued at maximum for the next hour and a half, and was still 98 per cent at 2 p. m. In the meantime the stomata started to close and reached total closure at 2 p. m. At 3 p. m. they had begun to open again, and at 4 p. m. were 80 per cent open, the maximum for the afternoon (fig. 3). This closure at 2 p. m. was very puzzling. At the time it was believed that some mistake had been made, that inadvertently a functionless leaf



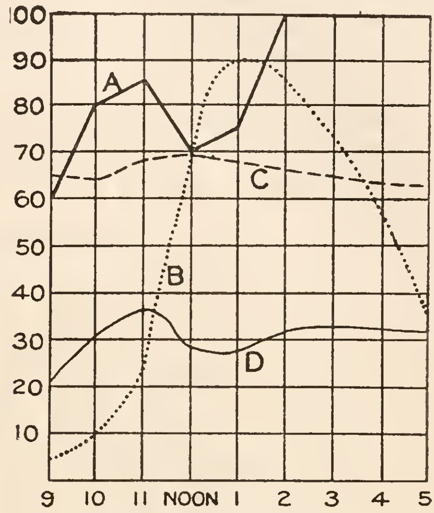


Fig. 2.—Series 1, showing movement in upper stomata of alfalfa (A), lower stomata of barley (B), sunlight (C).

Fig. 3.—Series 2, showing movement in upper stomata of alfalfa (A), sunlight (B), temperature (C), humidity (D).

had been stripped at 2 p. m. or a vial of badly diluted alcohol had been used. No other explanation seemed probable, as the plant was not wilted, and an examination of the soil showed that there was sufficient moisture.

The next day another series was made of the same plants and for the same length of time. The morning started very cloudy, but it slowly cleared during the late forenoon. However, in the early afternoon a haze appeared which thickened until it was rather cloudy again at 5 p. m., when the series ended. An entirely different behavior was observed, and one that had no direct relationship to changes in light and only a superficial one to changes in relative humidity. The stomata were 60 per cent open at the start of the series, 80 per cent at 10 a. m., and 85 per cent at 11 a. m. At noon they had closed to 70 per cent, but had opened slightly the next hour and reached maximum at 2 p. m., remaining in this condition to the end of the series (fig. 2). The one similarity in the two series is the mid-day closure. However, this occurred 2 hours earlier in the second than in the first and involved only 15 per cent change in comparison with total closure found the preceding day. In series 1 maximum opening occurred in the forenoon, and the widest opening in the afternoon was 80 per cent, while in series 2 maximum opening occurred during the afternoon and only 85 per cent was reached in the forenoon. The conclusion could not be escaped that stomatal movement was not uniform each day and that weather conditions probably caused great variation in the movement from day to day. It seemed significant that the stomata did not open fully during the cloudy morning of May 9, and not until an hour after the maximum of sunlight had occurred.

Another series was made of the same plants on May 12 in order to discover whether the mid-day drop in the curve was due to the failure of the stripping method or was an actual occurrence. The day was

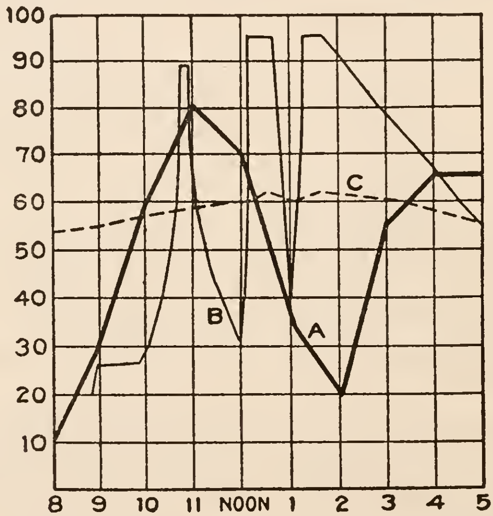


Fig. 4.—Series 3, showing movement in upper stomata of alfalfa (A), sunlight (B), temperature (C).

one of passing clouds, and a certain amount of haze prevented the light from reaching maximum at any time. A severe frost on the 10th had injured the vegetation to some extent, but a preliminary examination showed that the stomata of the alfalfa plants were functioning in spite of the low temperatures. The upper stomata were 40 per cent open when the series started and opened gradually to the morning maximum of 80 per cent at 11 a. m. Again mid-day closure was found, the stomata closing to 70 per cent at noon and to 35 per cent at 1 p. m., reaching a minimum of 20 per cent at 2 p. m. At 3 p. m. they had opened to 55 per cent and to 65 per cent at 4 p. m., in which condition they remained until the end of the series (fig. 4). At 9 a. m., 11 a. m., 2 p. m., and 4 p. m. a direct examination of the leaves on the plant showed the stomatal apertures to check with those found in the strips taken at the same time. This left no doubt as to the actual occurrence of the mid-day closure. Moreover, since this curve does not resemble either of the previous curves, it adds to the evidence that, in alfalfa at least, the course of stomatal opening varies greatly from day to day.

On May 23, 1916, another series was made with these same plants, starting at 10 p. m. and ending at 8 a. m. the next day, while on May 29, 1916, a complementary series was begun at 8 a. m. and ended at 10 p. m., thus furnishing a composite 24-hour series. It was hoped to find from the first whether the stomata remained closed all night, and at what time they opened in the morning. The night of May 23 was cold, clear, and rather windy. The humidity was not high, averaging only 45 per cent. The day of May 29 was clear, sunny, and warm, reaching 83.5°F. at 3 p. m., but

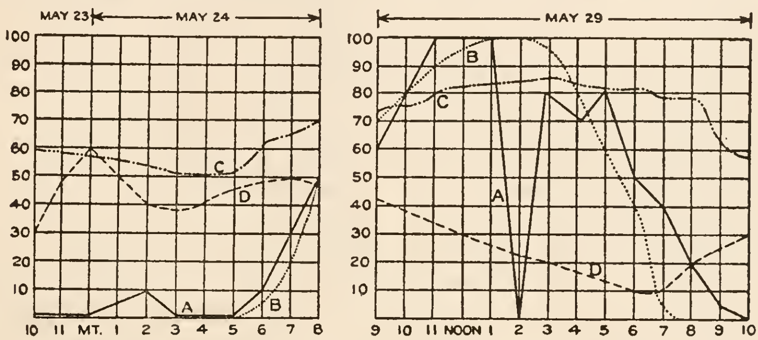


Fig. 5.—Composite series 4–5, showing movement in upper stomata of alfalfa (A), sunlight (B), temperature (C), humidity (D).

after sunset the temperature dropped quickly to 58° F. at 10 p. m. The stomata were closed at 10 p. m. when series 4 started and remained closed until midnight. At 1 a. m. they were 5 per cent open; at 2 a. m. they were 10 per cent open; but they closed again the following hour. The sun appeared at 5^h 20^m a. m. and no further opening was observed before this time. At 6 a. m. they had opened to 10 per cent, at 7 a. m. to 30 per cent, and at 8 a. m., when this series closed, they were half open. At the beginning of series 5, on May 29, they had opened to 60 per cent at 9 a. m. and continued opening uniformly to maximum at 11 a. m., in which condition they remained until 1 p. m. At 2 p. m. they were closed, a change of 100 per cent occurring within the hour. At 3 p. m. they had opened to 80 per cent and, except for a slight closure at 4 p. m., remained in this condition until 5 p. m. They then started to close gradually for the day, the process being gradual and rather uniform and not completed until after 9 p. m. (fig. 5).

Mid-day closure had now come to be expected, but the speed with which the stomata closed and opened was startling, as none of the preceding series or any other investigations had given any hint of it. Another surprise was the night opening at 1 and 2 a. m. in series 4. If the stomatal mechanism depends upon light alone for its action, neither night opening nor day closure should occur. The only conclusion possible was that some other factor than light also played an important rôle in the behavior of the stomata.

Some light is thrown upon this point by series 26, made at the University of Minnesota greenhouse at Minneapolis, Minnesota. These plants were not growing in the field, but had been transplanted into pots in the greenhouse while dormant during the early winter. On May 1, 1917, they were moved out of doors to a sheltered corner. The series was started at 4 a. m. on May 5 and concluded at 3 a. m. the next day. On the whole, conditions for growth were very good. The humidity was rather high, averaging 41 per cent during the day and 72 per cent during the night. The sunlight was almost normal, but the temperature was at times unfavorable. The stomata began to open before 6 a. m. and continued very slowly and uniformly to the maximum at noon. They remained open until 3 p. m. and then closed just as slowly and uniformly, closure being completed between 8 and 9 p. m. There was no night opening (fig. 6). Such behavior of the stomata in the upper epiderm of alfalfa is probably typical of the movement under favorable conditions, save in one respect. The low morning temperatures had an inhibiting effect upon the stomata, and caused the very gradual opening which occurred in the forenoon. The absence of both day closure and night opening seemed to indicate that the two were related in some manner. The following series strengthens this hypothesis.

Series 10, beginning June 8, 1916, at 4 a. m. and ending at 4 a. m. June 9, was the first 24-hour series made. It included, among others, sets of upper and lower epiderm from the leaves of alfalfa and also the stem epiderm. The day was clear and warm, the sunlight nearly normal, and the temperature 88° F. at 2 p. m. The relative humidity reached 74 per cent at night and dropped to 13 per cent in the afternoon, an average of 65 per cent for most of the night and 20 per cent for the greater part of the day. Conditions were typical of the usual clear warm summery day in the region (fig. 7). At the start of the series the upper stomata were 20 per cent open and the lower 15 per cent. At 5 a. m. the upper had opened to 90 per cent and the lower to 60 per cent. At 6 a. m. the upper were wide open. The lower stomata remained at 60 per cent until 7 a. m., after which they gradually closed, the process being completed by noon. In the meantime, the upper stomata closed to 50 per cent at 7 a. m. and to 40 per cent at 8 a. m., and they opened to 60 per cent at 9 a. m. They remained in this condition an hour before beginning to close, and became completely closed at 2 p. m. At 3 p. m. they had opened 30 per cent and were still in this condition at 4 p. m. They gradually closed to 10 per cent at 6 p. m., remained stationary for two hours, and closed completely by 9 p. m. The lower stomata remained closed from noon until 5 p. m., after which they opened slowly to 10 per cent at 7 p.m. At 8 p.m. they again closed, to remain so until 9 p. m. By 10 p. m. the stomata of both surfaces opened 5 per cent, the upper remaining in this condition to the next hour, while the lower opened to 15 per cent. At midnight the lower were closed, but the upper commenced opening again, reaching 15 per cent at 1 a. m., after which they closed slowly once more, this being

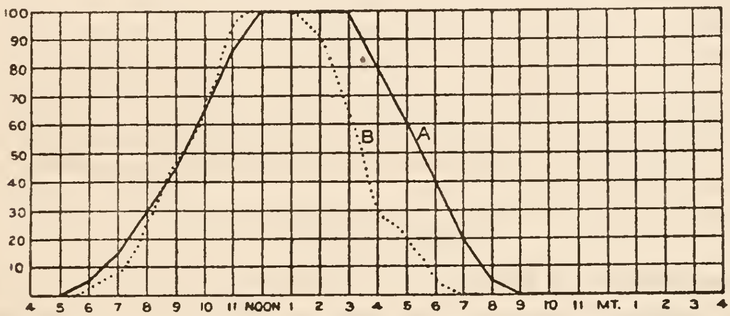


FIG. 6.—Series 26, showing movement in upper stomata of alfalfa (A), sunlight (B).

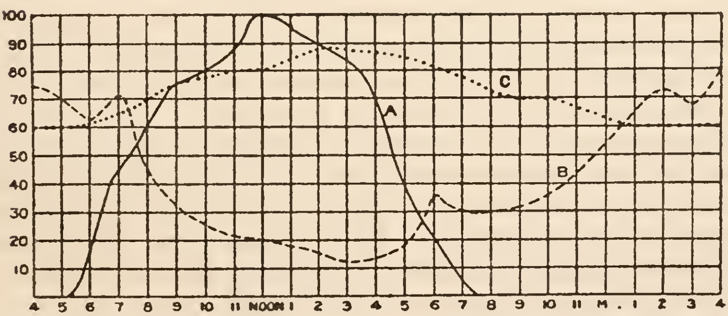


FIG. 7.—Series 10, weather data for June 8–9, 1916, sunlight (A), humidity (B), temperature (C).

completed by 3 a. m. The lower stomata continued closed from midnight to 3 a. m. After this hour the stomata of both surfaces opened, reaching the same degree of opening at 4 a. m., as on the preceding day. The stomata in the epiderm of the stems were somewhat longer and narrower than those of the upper epiderm of the leaves. They seemed to run lengthwise on the stem in all cases. Their behavior agreed with that of the upper stomata, except that maximum morning opening continued until 8 a. m. when they closed gradually to 60 per cent at 10 a. m. and for a time again showed the same movement as did the upper stomata. At 3 p. m., however, they continued to open, reaching 50 per cent at 4 p. m., while the upper stomata remained at 30 per cent. From this time they closed slowly and uniformly, coinciding at 8 p. m. with the 10 per cent opening found in the upper stomata (fig. 8). From this time to the end of the series there was no appreciable difference between the upper and the stem stomata.

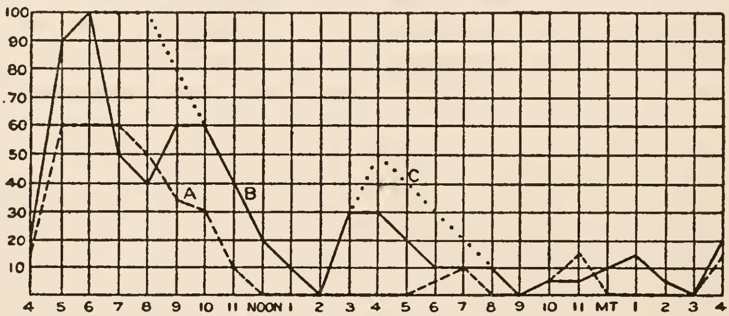


Fig. 8.—Series 10, showing movement of lower (A), upper (B), and stem stomata (C) of alfalfa.

An examination of the previous series showed that the movements of the stomata in the upper and lower epiderms of the same leaf did not necessarily have any relation to each other. In series 10 the general differences in behavior are quite clearly shown. The stomata in the lower surface neither opened as widely nor stayed open as long as the stomata of the upper epiderm. One reason for this is that the lower surface of the leaf receives much less light than the upper, but it seems largely to be a matter of water-supply to the guard-cells. The slower opening in the morning and earlier closing in the evening typical of the lower surface are due to the difference in light, but this is not true of the prolonged mid-day closure and slight afternoon opening in the lower stomata. Experiments made by turning the leaf over and keeping it upside down during the day seem to bear this out, but have not been conclusive at all times. In barley and other plants with leaves that may have either side up, the stomata on both surfaces behaved alike for the most part. In the cases where they did not, the difference was clearly due to difference in lighting, and had no relation to the side on which the stomata were found.

Another point of importance was the behavior of individual stomata. Direct observation showed that individual stomata may become functionless from one cause or another and remain permanently closed. Some seemed to have the ability to open much more widely than others. Moreover, those that opened very widely also opened earlier, reached maximum opening sooner than the others, and remained widely open for a somewhat longer time. Complete closure occurred at approximately the same time, and hence these more active stomata closed more rapidly than the others, as well as opening more widely. In most sets of epiderm examined were found complete series of stomata, from these very functional ones to others which seemed entirely functionless. By keeping a leaf under the microscope, held on a slide without a cover-glass, and shutting off the light between periods of observation, this individual variation in the action of the stomata could be followed in detail. Naturally, the behavior of the stomata of a leaf treated in this manner is not like the behavior of the stomata on the other leaves of the plant. Still it may be safely assumed that when there is a more or less individual reaction by each stoma to rather unnatural conditions, there is also similar variation under more natural conditions. This is the only assumption that will explain the differences in the degree of opening found in the various stomata of a strip or at the moment of observation in a living leaf. However, most of the stomata act alike; at least 80 of each 100 in alfalfa are alike within the accuracy of measurement, and the greater part of the remaining 20 are sufficiently near the average, so that they have no effect. Approximately 2 per cent of all the stomata in an alfalfa leaf are functionless, because of incomplete development or a more obscure cause, and 3 per cent are superfunctional, opening often to nearly twice the normal maximum and usually changing the degree of opening with remarkable speed. These erratic ones counterbalance each other, and have no real effect upon the opening found in a leaf, except during a period of stress. In cereals, however, this variation is important at all times.

From June 8 to September 1, 1916, 16 additional series were made, each continuing over a period of 24 hours or more, while 1 was 68 hours in length; 12 of these series contained a set of alfalfa strips, largely as a basis for comparison with the stomata of other plants. Figure 9 shows the curves of the stomatal movement in the upper epiderm of alfalfa, plotted so as not to intersect each other. In addition to the 5 already given, 6 have been selected to show the progressive changes in movement as the soil became drier, the weather hotter, and the evaporating power of the air increased. The last two curves are of special interest. Edith B. Shreve (1916: 114) states:

> "Under typical conditions the stomata [of *Opuntia versicolor*] begin to close soon after sunrise and they appear to be completely closed by noon. They begin to open at 5 to 6 o'clock in the evening and continue to open throughout the night, the maximum size occurring between 3 and 6 a. m."

This statement applies almost word for word to the behavior of alfalfa stomata under conditions of low water-content, high temperture, continuous brilliant sunshine, and very dry air; in other words,

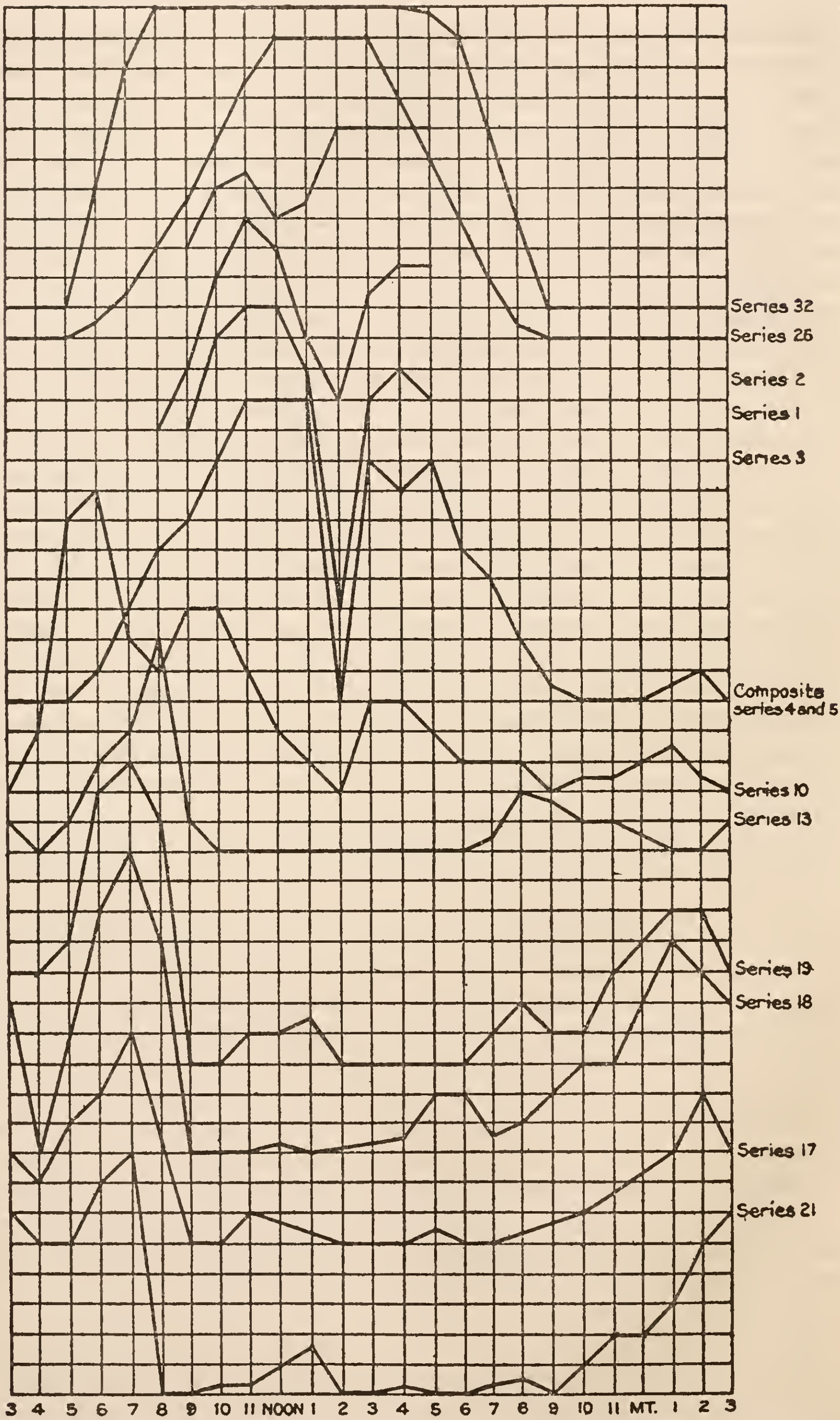


FIG. 9.—Movement in upper stomata of alfalfa, showing change from day opening and night closure to night opening and day closure, as evaporation became more intense.

when conditions approach those under which this *Opuntia* grows. The latter has adapted itself to such conditions, and hence such movement is typical of its stomata. Alfalfa, under favorable conditions, has a stomatal curve typical of the ordinary mesophyte, but it can adjust itself to changing conditions until its stomatal curve is like that of an extreme xerophyte.

This naturally raised a question as to the factors concerned. The accepted view is that light is the all-important factor in stomatal movement. Where the plant is not subjected to extreme conditions this is largely true, as many investigators have shown. However, night opening and day closure of the stomata can not possibly be attributed to light, but must be due to some factor or group of factors which counteract the effect of sunlight. Moreover, night opening and day closure must be related, since in every series an increase in one was accompanied by an increase in the other. The most promising explanation seems to be that both result from the same factor changes, and a study of the data for the group of series pointed to two factors, in which changes were parallel to those in the daily movement of the stomata of alfalfa. These two factors, evaporation and water-content, act upon the plant singly or together to produce a water shortage or "incipient wilting," which brings about closure of the stomata during the day. Changes of no other factor could be correlated with day closure of the stomata, but in all series increased evaporation or decreased water-content, or the combination of the two, increased day closure and night opening.

THE DAILY MOVEMENT IN POTATO.

A set of upper and lower epiderm from the leaves of potato was included in series 10, June 8 and 9, 1916, in order to compare the stomatal movement of this crop-plant with that of alfalfa. The potato plants were still very young and it was difficult toward the end of the series to find leaves sufficiently mature to strip. The soil conditions were not the same for the two plots, as the potato plot had a water-content of 29 per cent, which was very high for the type of soil, while the alfalfa plot had but 16 per cent at 2 feet. However, the roots of the alfalfa plants undoubtedly reached to the moister soil just above the water-table, which was at that time only 6 feet below the soil surface. The weather conditions were the same for both plots (fig. 7).

The stomatal behavior in potato was entirely different from that found in alfalfa. At the start of the series the stomata of both surfaces of the leaves were wide open and remained open throughout the day. After sundown the lower stomata were closed for 3 hours from 9 p. m. until after 11 p. m. At midnight they were half open and reached the maximum again at 1 a. m The upper closed to 50 per cent at 9 p. m., but opened at once and were wide open the following hour. Hence, as in alfalfa, the behavior of the stomata was different in the upper and lower surfaces of the leaves (fig. 10). The striking difference between the two species lies in the widely open stomata of potato throughout the day and most of the night. This behavior indicates that light does not produce opening of stomata in this species. Moreover, though the greatest difference in environment of the upper and lower stomata is one of light intensity, this can hardly explain the behavior of the lower stomata, since these did not close until darkness set in. If lack of light caused closure, the upper stomata should have closed to even a greater degree, since these were exposed to a greater change in lighting.

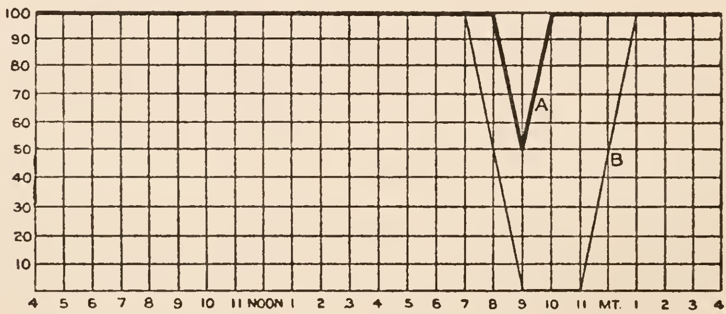


FIG. 10.—Series 10, showing movement in upper (A) and lower (B) stomata of potato.

The tendency of potato stomata to remain continually open is again shown in series 12, started at 1 p. m. on June 21 and continuing to 5 p. m., June 22, 1916. In this case the series was begun on a rainy day and ended on a clear day. The light intensity was not over 6 per cent on the afternoon of June 21 until 6 p. m., when the stopping of the rain caused a rise to 13 per cent. The temperature was also low, averaging 46° F. on this afternoon, but rising to 53° F. after 6 p. m. The humidity averaged 85 per cent during the afternoon and night. The next day was clear, except for a large passing cloud at 10 and 11 a. m., although some haze due to the rain of the previous day prevented the sunlight from reaching a maximum. The temperature was 40° F. just before daylight, but rose quickly after sunrise, averaging 62° F. during the forenoon. The humidity dropped to an average of 40 per cent during the forenoon, which, however, was high for the region (fig. 11). The behavior of the stomata was essentially identical with that found in series 10. Comparison with the behavior found in alfalfa stomata shows some interesting facts.

The low light intensity during the rainy afternoon of June 21 caused the alfalfa stomata to be almost closed. At 6 p. m., with the increase of light, they opened to 80 per cent, remaining in this condition until 7 p. m. They then closed gradually until 9 p. m. They remained closed until 4 a. m., when they showed slight opening. At 5 a. m. they were 20 per cent open and at 6 a. m. half open. At 7 a. m. this had increased to 90 per cent and at 8 a. m. to maximum. The stomata remained in this condition for an hour, but at 10 a. m. they had closed to 60 per cent, at 11 a. m. to 50 per cent, and at

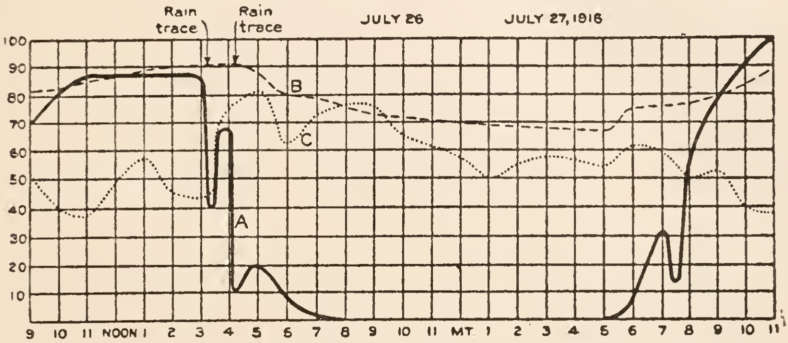


FIG. 11.—Series 12, weather data for June 21–22, 1916; sunlight (A), humidity (B), temperature (C).

noon they opened again to maximum. This forenoon closure is coincident with decrease in light caused by the passing cloud. The stomata remained in this condition 3 hours, closing to 70 per cent at 4 p. m. and to 45 per cent at 5 p. m., when the series ended (fig. 12).

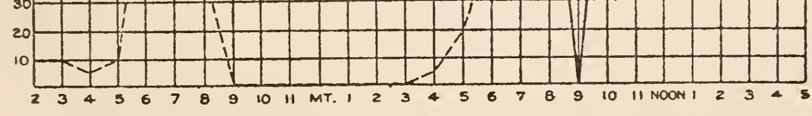


FIG. 12.—Series 12, showing movement in upper (A) and lower (B) stomata of potato, and upper stomata of alfalfa (C).

The relation of stomatal movement in alfalfa to changes in light is distinct in this series. Evaporation was low and the water-content high as results of the rain, which explains why movement in the stomata of alfalfa was related to light alone. But neither the low light intensity on the afternoon of June 21 nor the darkness of night caused any closure whatever in the upper stomata of potato. The lower closed for an hour at 9 p. m., this being the only closure observed. The conclusion is inescapable that light has no direct effect upon stomatal movement in the potato.

The tendency of potato stomata to remain constantly open may be ascribed to two causes, namely, to a large amount of leaf-water in the thick and rather fleshy leaves and to efficient roots and high root-pressure. To show the difference in root-pressure, two mercury manometers were attached to potted plants of alfalfa and potato and the plants were heavily watered. The potato reached its maximum in 5 hours, while alfalfa took 12 hours, and the maximum for potato was three times that of alfalfa. When tried with plants growing in the open, the same experiment failed, as both plants showed negative pressure, probably due to inability to raise the water-content sufficiently during the experiment. While too much reliance can not be placed upon this experiment, it is believed that it is fairly indicative of the relative efficiency of the roots of the two plants. Although conditions during the two series described were very unlike in most respects, the stomata of potato behaved in essentially the same manner. This was due to the high water-content of the soil during both experiments. When the water-content drops below the amount necessary under given conditions of humidity and temperature, potato stomata, as well as alfalfa stomata, react to conserve water. When the water-content is but little below the amount required, closure of the lower stomata occurs in the late afternoon, followed by partial or total closure of the upper stomata. As soon as the leaves regain turgor, the stomata again open and stay open the remainder of the night. In all cases studied the upper stomata remained open longest and were the first to reopen when the lost leaf-water was replaced.

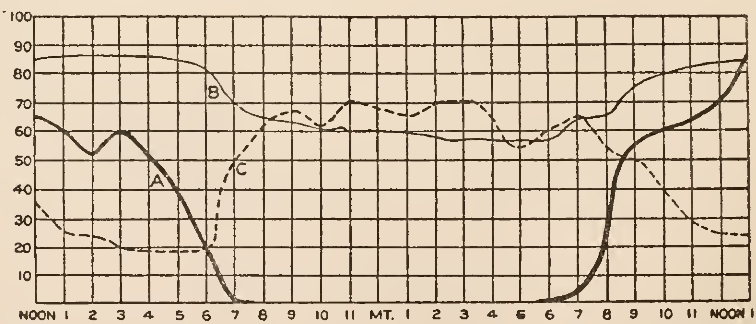


FIG. 13.—Series 20, weather data for August 25–26, 1916; sunlight (A), temperature (B), humidity (C).

This is shown by series 20 (fig. 14), which was begun at noon August 25, 1916, and ended at 1 p. m. the following day. On both days the light was strong, although a certain amount of haze was present, the temperatures high, and humidity low. The soil had dried to a water-content of 15 per cent, which was not quite sufficient, in view of the high evaporation. At noon, when the experiment started, the stomata of both surfaces of the leaves were wide open. At 1 p. m. the lower had closed to 30 per cent, although the upper were still open. At 2 p. m. the lower stomata were closed and the upper were but 40 per cent open. By 3 p. m. the stomata of both surfaces were closed and so remained until 9 p. m. when the upper stomata showed a slight amount of opening. At 10 p. m. the lower stomata also started to open; the upper had then opened to 30 per cent of maximum. At 11 p. m. the upper stomata were 60 per cent open and the lower 15 per cent. At midnight the upper stomata were wide open and the lower were 40 per cent open. At 1 a. m. the lower stomata were 80 per cent open and fully open at 2 a. m. The stomata in both surfaces were open until 11 a. m., when the lower stomata suddenly began to close. At 12 noon the lower were but 10 per cent

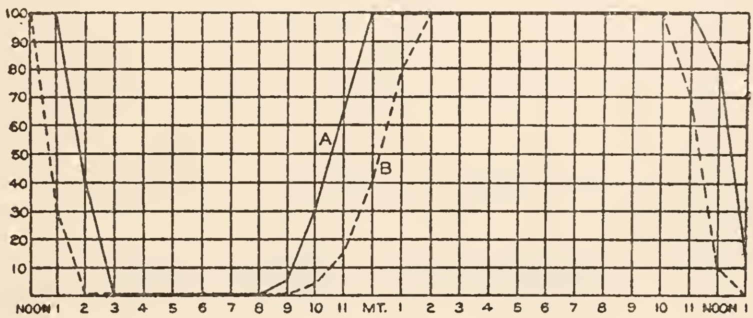


FIG. 14.—Series 20, showing movement in upper (A) and lower (B) stomata of potato, plot 7.

open and the upper stomata started to close. At 1 p. m., when the series ended, the lower epiderm showed all stomata closed and the upper but 15 per cent open. The stomatal movement in the upper epiderm of alfalfa for the same time shows that potato is not so susceptible to evaporation as alfalfa. Nevertheless, the stomata of potato, like those of alfalfa, are influenced by these factors.

Less available water tends to increase the day closure. When it is very much less, the stomata close very early and the entire plant shows signs of wilting. During the night such a plant will recover and its stomata again open. When the small reserve of water gained overnight begins to disappear, the stomata again close and the plant assumes once more a semi-wilted appearance. Hence, as in alfalfa, the stomata of potato react to excessive water-loss by closure. This cuts down the period for the absorption of carbon dioxide and naturally the amount of photosynthesis which can occur in such a plant, without regard to other effects due to water-loss.

The stomata of potato show other interesting differences as compared with those of alfalfa. One variety, the name of which was unknown, had hypostomatal leaves. The variety used in these experiments (Russet Burbank) had three-twentieths as many stomata on the upper surface as on the lower. Unlike alfalfa, the lower stomata were more active than the upper. All the stomata of an alfalfa leaf become functional at practically the same time. In potato, however, the first stomata may begin to function long before the last have formed. Two weeks have elapsed in most cases between the time the first stoma on a leaf opened and the last stomata became functional.

The curves made by the lower stomata of potato under three conditions of water-content are shown in figure 15. The last curve is much the same as that of alfalfa under similar conditions, as it

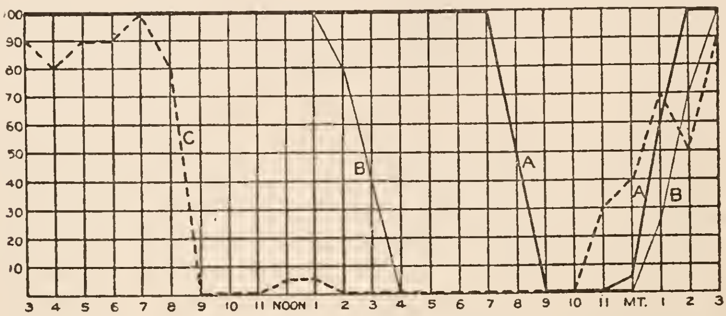


FIG. 15.—Movement in lower stomata of potato under high water-content and moderate evaporation (A), low water-content and moderate evaporation (B), low water-content and excessive evaporation (C).

shows day closure and night opening. But where alfalfa under very favorable conditions has stomata open all day and closed at night, potato has stomata open continuously, save for the 3 hours following sundown. Then, as the conditions for obtaining and retaining water become less favorable, this period of closure begins earlier, until finally it includes all of the day except an hour or two at sunrise. There was in no case the mid-day closure and afternoon opening so often found in alfalfa stomata. This may occur possibly in other varieties of potato or under other conditions, but was not found in this investigation. This would seem to indicate that a larger proportion of the leaf-water was lost by the potato before its stomata closed than is the case in alfalfa. The slightly wilted appearance of the plants at the times when their stomata are closed would confirm this, as there is no sign of such wilting of alfalfa plants during the midday closure of their stomata. The more ready closing of stomata in alfalfa may in part explain its greater drought-resistant qualities as compared with potato. It is obvious that a plant which waits until the last moment to cut down water-loss must waste much more than the plant which is so adjusted as to cut down this loss at the first signs of shortage. On the other hand, it seems reasonable that when the water-supply is adequate, but evaporation is high, the potato with its stomata open throughout the day can produce much more photosynthate than alfalfa, which has the stomata closed for a considerable portion of the working period.

THE DAILY MOVEMENT IN SUGAR-BEET.

The stomata and epidermal cells of sugar-beet are unique in being similar on both surfaces of the leaf. This is in marked contrast to the epiderm of alfalfa or potato leaves, where the stomata and cells of one surface are distinctly unlike those of the other. For this reason, as well as because of its importance as a crop plant in the region about the Great Salt Lake, this plant was included in a number of series. The first of these was No. 11, started at 11 a. m. June 19, 1916, and ended at noon June 20. The weather conditions of these two days were characterized by increasing humidity, high temperature, and cloudy nights, which culminated in a rain on June 21. Throughout this period passing light clouds cut down the sunlight, but at no time to the point where the stomata were affected. The temperature ranged from 80°F. at 3 p. m. June 19 to 55°F. just before dawn on June 20. A dense layer of clouds formed during the evening of the 19th and persisted until after sunrise. This condition was responsible for the warm night and resulted in the high humidity of the following day (fig. 16). The light was not as great or the temperature as high this second day as on the first.

At the start of the series the upper stomata had just started to close, while the lower showed but 30 per cent opening. The lower surface reached a minimum of 5 per cent for the afternoon at 1 p. m. and remained in this condition for an hour. The upper had closed to 10 per cent at 2 p. m. At 3 p. m. both surfaces showed the stomata opening; at 4 p. m. the lower had opened to 40 per cent, while the upper had only reached 20 per cent. The next hour the lower had opened to 50 per cent, but the upper, increasing the rate of opening very much, reached 80 per cent. This was the time of greatest opening in either surface that afternoon. The following hour the lower stomata were unchanged, but the upper had closed slightly, and then more rapidly until nearly all were closed at 10 p. m. The lower stomata closed to 10 per cent at 8 p. m. and then began to open, reaching a maximum for the night of 70 per cent at 11 p. m. At this hour the upper stomata had opened slightly and reached 15 per cent at 1 a. m. After 11 p. m. the lower stomata had gradually closed, this change being completed at 3 a. m. The upper stomata reached the minimum of 7 per cent at the same time. The next hour, although the light of approaching dawn was very faint, the upper stomata had opened 60 per cent and were wide open at 5 a. m., about 20 minutes before the sun rose over the mountains. The lower stomata, however, were still closed at 4 a. m. and showed only 5 per cent opening at 5 a. m., when the upper had reached the maximum. The next hour the lower opened swiftly to 60 per cent and then gradually to 90 per cent at 10 a. m., which was the forenoon maximum of these stomata. The upper continued wide open until 11 a. m. and then closed to 90 per cent at noon, when the series ended. The lower started to close after 10 a. m., reaching 65 per cent at 11 a. m. and 35 per cent at noon (fig. 17).

The lower stomata opened 2 hours later in the morning than did the upper, and closed 2 hours earlier in the evening. The smaller amount of light received by them affords ready explanation of this. However, the greater mid-day closure and much greater opening found in these stomata at night can not be explained on the basis

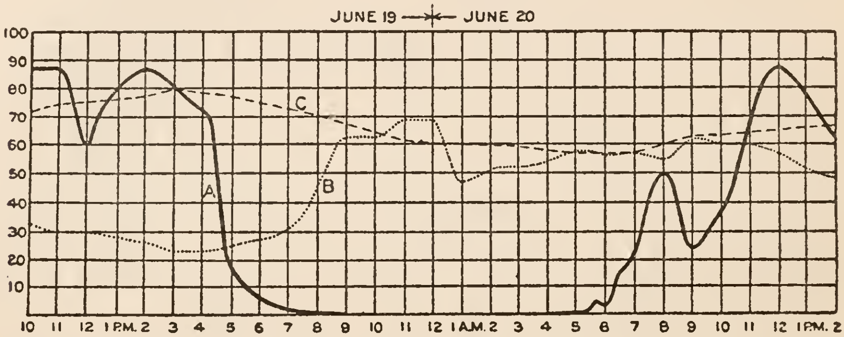


Fig. 16.—Series 11, weather data for June 20–21, 1916; sunlight (A), humidity (B), temperature (C).

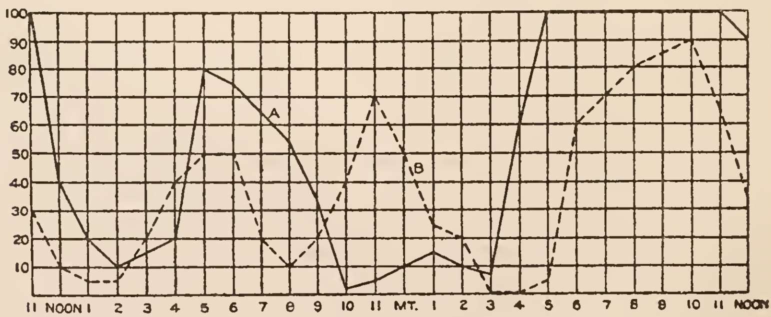


Fig. 17.—Series 11, showing movement in upper (A) and lower (B) stomata of sugar-beet.

of light intensity, in spite of the fact that this is the greatest difference in the environment of the two surfaces. In fact, the alfalfa series would indicate that the upper stomata should show greater night opening and day closure than the lower. On the other hand, the shaded soil in the alfalfa plots did not give rise to convection currents of great evaporating power striking the lower surfaces of the leaves, such as rose from the hot, bare surface of the cultivated soil of the sugar-beet field.

On July 20, 1916, thirty leaves of sugar-beet were carefully twisted so as not to injure them and left clamped with their lower surfaces turned upward. In the following discussion upper and lower surface are used to describe the respective surfaces of the leaves, regardless of the actual position in which they were placed. On July 26, 1916, series 16 was started at 9 a. m. and continued until 11 a. m. the following day. Among the sets of epiderm collected were two of sugar-beet, one from the upper and lower surfaces of normal leaves, and one from the two surfaces of the reversed leaves. This was to determine how much of the difference in the behavior of the stomata in the two surfaces of the leaves was due to physical factors and how much to the internal structure of the leaves.

The weather conditions were rather favorable for growth. Two light showers occurred on the afternoon of the 26th at 3^h15^m and 4^h10^m p. m. Each lasted but a minute or two, and there was not sufficient precipitation to measure. They did not reduce the light sufficiently to produce any change in the behavior of the stomata. The temperature and humidity were high on both days. The 26th

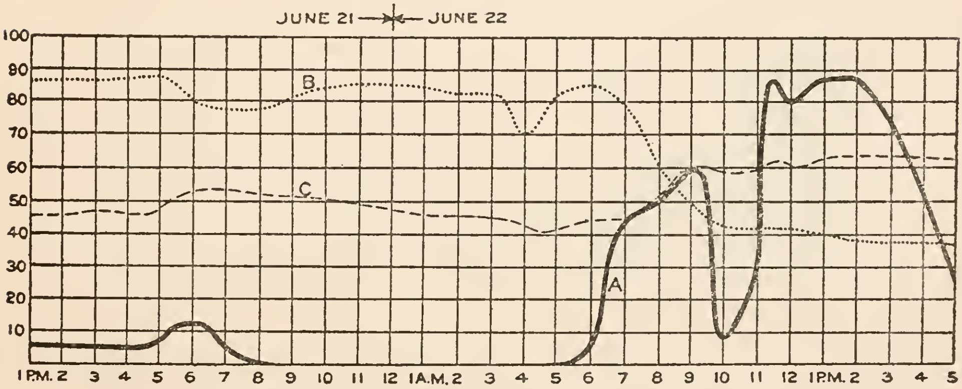


FIG. 18.—Series 16, weather data for July 26–27, 1916; sunlight (A), temperature (B), humidity (C).

especially was a still, hot day, oppressive because of the high humidity, with clouds hanging over the mountains and the valley in brilliant sunshine. There were fewer clouds on the day following, and these disappeared as they drifted away from the mountains (fig. 18). The curves for the two sets of sugar-beet leaves are shown in figure 19. The upper epiderm of the reversed leaves produced the same stomatal curve as the lower epiderm of the normal leaves. The lower epiderm of the reversed leaves did not show exactly the same stomatal movement as in the upper stomata of the normal leaves, but the behavior was essentially similar, demonstrating that most of the difference between the upper and lower stomata of the normal leaf is the result of a different environment. The slightly slower opening and earlier closing of the lower stomata of the reversed leaves may be attributed to a less ready supply of water to their guard-cells. Reversing the leaves of alfalfa did not cause their stomata to show the results found in sugar-beet, but the stomata of each surface still behaved differently from those of the other surface of normal leaves. Earlier opening and later closing of the lower stomata were often induced by reversing the leaves, and at times greater midday closure was brought about.

It is thus evident that the difference in stomatal behavior of the upper and lower epiderms of alfalfa is due to differences in the structure of the leaf, and, in particular, of the stomata and surrounding epidermal cells. The position of the veins and the number of cells through which the leaf-water must pass before reaching the guard-cells of the stomata of each surface must have considerable influence upon the functioning of the stomata, at least under conditions of high evaporation. The air-spaces in the spongy chlorenchyma and fewer paths or bridges of cells through these air-spaces from the veins to the epidermal cells must play a part in the rate at which water passes to the lower epiderm and in the loss by evaporation

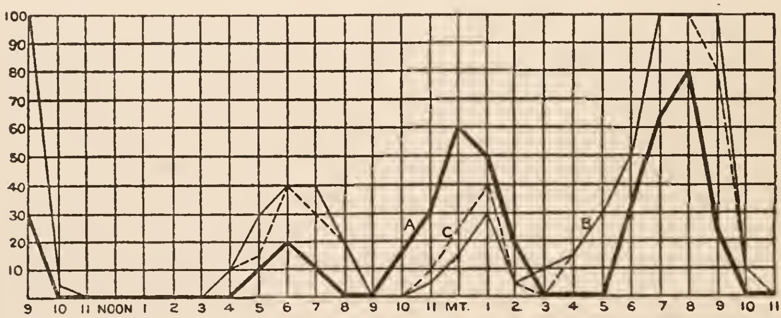


FIG. 19.—Series 16, showing effect on movement of reversing leaves of sugar-beet; normal leaves, lower stomata (A), upper stomata (B); reversed leaves, lower stomata (C), upper stomata (A), as in lower stomata of normal leaves.

during its passage. As leaf sections show, the water on its way to lower and upper epiderm guard-cells passes through the same number of cells in sugar-beet, and the air-spaces in the sponge tissue are not nearly so large or numerous as in alfalfa (plates 5 and 6).

No series was made of sugar-beet under conditions where normal stomatal behavior could be expected, nor was this found. However, it can not be doubted that since the general behavior of the stomata of this species resembles that found in alfalfa, sugar-beet stomata would show essentially the same behavior under conditions of low evaporation, medium temperature, bright sunshine, and high water-content, as found in alfalfa, series 26 (fig. 6). The type of curve shown in each series is like the type found in alfalfa of the same series, in that the stomata respond to changes of physical factors in essentially the same manner, although not to the same degree. The outstanding difference lies in the fact that the stomata of sugar-beet are alike, while those of alfalfa differ on the two surfaces of the leaves.

THE DAILY MOVEMENT IN ONION.

As the leaves of onion are essentially hollow cylinders, with no distinct upper and lower epiderm, but one set of epidermal strips was collected in each major series that included this plant. However, one special series 6 hours in length was made to find whether the difference in lighting on the different sides of the leaf had any effect upon the behavior of the stomata. In addition, a number of isolated tests were made for the same purpose. The results showed that the difference of lighting did cause variation in the stomata affected at certain times, and under certain conditions especially. On this account, at each stripping during a 24-hour series, three to four strips were collected from the different sides of the leaf, so that the average degree of opening for that leaf could be found.

There are almost as many stomata in onion epiderm as unspecialized epidermal cells, as the end of each such cell is usually separated from the next by a stoma (plate 4). However, because of the size of these cells, there are about the same number of stomata to a square millimeter of epiderm as in a sugar-beet leaf. The stomata are rather large and unusually simple in form and mechanism.

The general type of behavior found in the stomata of onion is shown in series 16, started 9 a. m. July 26, and ending 11 a. m. July 27, 1916. As usual, a set of alfalfa epiderm was collected in the same series for comparison. Because of the high humidity, neither the onion nor the alfalfa stomata showed the degree of day closure that would be expected in view of the high temperature and brilliant sunshine (fig. 18). At the outset, the onion stomata were 70 per cent open and those of alfalfa 80 per cent. At 10 a. m. both species had stomata 80 per cent open, and these began to close immediately. By the following hour, alfalfa showed complete closure and remained closed until 3 p. m. The stomata of onion closed much more gradually, reaching 40 per cent at 1 p. m., when they opened as gradually to the maximum at 5 p. m. The alfalfa stomata opened in the meantime and also reached maximum at this hour. At 6 p. m. the onion stomata had closed to 40 per cent, remaining in this condition for an hour, then to 30 per cent at 8 p. m. for an hour, finally closing to 10 per cent at 10 p. m. The alfalfa stomata closed uniformly to 10 per cent at 9 p. m., but showed no further closure the following hour. At 11 p. m. night opening had set in for both species, onion reaching 15 per cent, remaining there for an hour and then closing slowly and completely by 2 a. m. Alfalfa stomata, on the other hand, opened gradually to a maximum of 50 per cent at 1 a. m. and then closed slowly and completely by 3 a. m. After 3 a. m. the onion stomata began to open very slowly to 20 per cent at 5 a. m. As a result of sunrise, however, they opened within the next hour to an unstable maximum of 80 per cent for the morning. This maximum continued with several fluctuations until 11 a. m. when the stomata closed to 60 per cent, as in the preceding day. In alfalfa the stomata were closed until after 4 a. m., when they began to open slowly, reaching 10 per cent at 5 a. m., and then more rapidly to 80 per cent at 8 a. m. They continued in this condition for an hour, and then closed to 60 per cent at 10 a. m. By 11 a. m. they had closed completely.

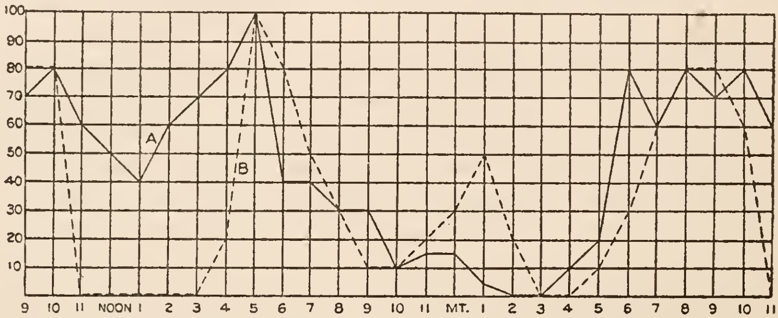


Fig. 20.—Series 16, showing movement in stomata of onion (A) and upper stomata of alfalfa (B).

The stomatal movement in onion resembles that of alfalfa in a number of respects. Both open and close and exhibit day closure and night opening at much the same time. On the other hand, the stomata of alfalfa showed a much greater day closure and a correspondingly greater night opening. The leaves of onion have a larger amount of water relatively and are less affected by the factors of evaporation in consequence, with the result that the stomata show less day closure. The speed of day closure and secondary opening is greater in the stomata of alfalfa, as well as the degree of opening. The curve of stomatal movement in onion is more irregular than that in alfalfa, as the stomata open or close rapidly for a time, then slowly or not at all, and then more rapidly again. The forenoon maximum of 80 per cent on July 27 is referred to as unstable, because of a similar irregularity. The stomata opened to 80 per cent at 6 a. m., closed to 60 per cent the next hour, opened again to 80 per cent at 8 a. m., closed to 70 per cent at 9 a. m. and opened once more to 80 per cent at 10 a. m. Such behavior occurred to some extent in all plants under certain conditions, but most commonly in succulent plants, such as onion and *Portulaca oleracea,* and to a lesser extent in sugar-beet, cow-beet, *Rumex patentia,* salsify, cabbage, and others. However, under certain conditions, such as high evaporation counteracted by high water-content, alfalfa, sweet-clover, corn, nasturtium, and similar thin-leaved plants exhibited striking irregularities in their stomatal movement.

Under extreme conditions of temperature, evaporation, and usually low water-content, the stomata of the onion, as well as those of alfalfa, were open all night and closed during the day. On the other hand, a very high water-content causes the stomata of onion to remain continuously open throughout a 24-hour period, opening somewhat wider upon sunrise and closing very slowly during the night, but not to less than 75 per cent as a rule. Upon the appearance of sufficient light at dawn such closure ceases, and the stomata again open to maximum just after sunrise. However, even such a plant, during a very hot and dry day, will show considerable midday closure, this sometimes being complete. Hence, evaporation may cause the stomata of onion to be closed during the day and open only at night, more or less regardless of the water-content, but as this decreases the effect of evaporation increases, and this reversal of normal behavior in the stomata becomes the more usual occurrence.

THE DAILY MOVEMENT IN CEREALS.

Cereals are alike not only in the structure and mechanism of their stomata, but in the behavior of these as well. Certain peculiarities which distinguish them from other plants must be dependent upon the unique structure of their stomata, but other characteristics found in the behavior are perhaps due to differences in the plant as a whole. The failure to show night opening must be largely a matter of stomatal mechanism, but the rare occurrence of maximum opening of all the stomata of a cereal is probably the result of peculiarities in other parts of the plant. Moreover, the rarity of maximum opening is perhaps a regional phenomenon, as the conditions under which wide opening does occur show that it is probably common in a more humid region, such as the northeastern States.

The stomatal movement described is the average of that found in the upper and lower surfaces. The difference between upper and lower stomata was slight when it did occur and was clearly due to the light intensity. Hence, when a leaf was blown about and alternately illuminated on the two sides, as usually was the case, no difference in the movement of stomata on the two surfaces could be detected. The two sets of epiderm were collected from each plant and the apertures were calculated from both surfaces to furnish the graphs. In photographing, however, only the lower surface was used, since this represented the apertures for both surfaces.

A set of barley epiderm as well as of alfalfa was collected in series 1 (fig. 2). The stomata of barley were but 12 per cent open at 9 a. m., when the series started, and were closing, while those of alfalfa were opening rapidly. At 10 a. m. barley showed less than 3 per cent opening and complete closure at 11 a. m., when the stomata of alfalfa reached maximum. The stomata of barley remained closed to the end of the series. The 12 per cent opening at the start of the series is the average of 200 stomata, several of which were at maximum and a great many closed. It is not definitely known whether a few stomata with more accessible water-supply do the opening on days of unfavorable conditions, or whether groups of stomata open and shut very rapidly and at different times. Direct observations on the same leaf would indicate the former, but the fact that open and closed stomata occur in groups, and that the stomata of cereals can open and close with amazing rapidity, makes the latter hypothesis possible.

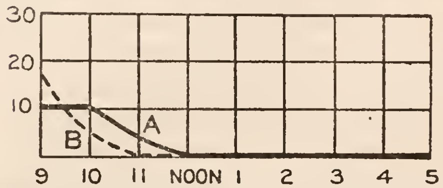


Fig. 21.—Series 2, showing movement in lower stomata of oats (A) and of barley (B).

Oats and barley epiderms were collected in series 2 as well as that of alfalfa. The weather data of this series are shown in figure 3. Because of the cool, cloudy forenoon, the barley stomata were 17 per cent open at 9 a. m. and 10 per cent the following hour. As in the first series made the day before, they closed before 11 a. m. and remained closed to the end of the series. The stomata of oats did not open as widely as those of barley, but remained open longer. They were 10 per cent open at 9 a. m. and remained in this condition an hour. At 11 a. m. they closed to 4 per cent and were completely closed by noon. Like the stomata of barley, they remained closed to the end of the series (fig. 21). The difference in the weather conditions in the two series, although made on successive days, caused the stomata of barley as well as those of alfalfa to act differently. The same plants were included in series 3, made 3 days later, May 12, 1916. It was a day of passing clouds, and was distinctly cool, as the temperature did not rise above 63° F. Barley had the same type of stomatal movement as in the first series. Oats

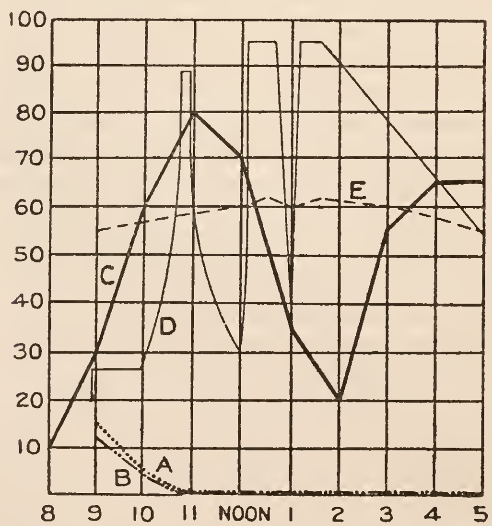


Fig. 22.—Series 3, showing movement in lower stomata of oats (A) and of barley (B), and upper stomata of alfalfa (C); sunlight (D), temperature (E).

showed 16 per cent opening at 9 a. m., 6 per cent at 10 a. m., and showed closed stomata after this time, as in barley (fig. 22). Although conditions were different, the stomata of barley behaved in the same manner as in the first series. However, it is improbable that such similarity would have been found throughout had the two series been complete. Other experiments have shown that low morning temperature has an inhibiting effect upon opening of stomata.

Wheat, oats, and barley were included in series 10, June 8 and 9, 1916, together with alfalfa and potato, the stomatal movements of which have already been described in detail. The day was clear and fairly warm, the highest temperature (88°F.) being reached at 2 p. m. (fig. 7). Because of the low humidity, the evaporating power of the air was high, and in consequence all the plants faced the danger of excessive water-loss. The stomata of alfalfa were closed for a longer period during the day and hardly showed maximum opening at all. Potato, on the other hand, had stomata open widely all day and most of the night. The cereals did not have the high water-content in their plots that the potato had, and it is doubtful whether their roots had been able to follow the rapid dropping of the water-table to 6 or more feet. Hence the water-content of 16 per cent found at a depth of 2 feet probably represented the highest amount within reach of the roots. Barley showed only a small fraction of maximum stomatal opening during the first 3 hours of daylight and closure during the remainder of the series. The greatest opening occurred at 7 a. m., but was only 9 per cent. The stomata of wheat remained open until after 10 a. m., as did the stomata of oats, but while wheat reached 22 per cent opening at 7 a. m., that of oats was

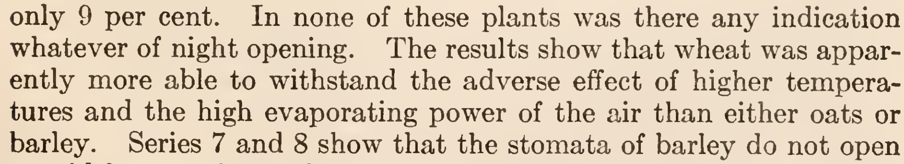


FIG. 23.—Series 10, showing movement in lower stomata of wheat (A), barley (B), and oats (C).

only 9 per cent. In none of these plants was there any indication whatever of night opening. The results show that wheat was apparently more able to withstand the adverse effect of higher temperatures and the high evaporating power of the air than either oats or barley. Series 7 and 8 show that the stomata of barley do not open as widely or as long when the temperature rises above 75° to 80°F.

Further evidence of this is found in series 11, begun at noon June 19 and ended at 2 p. m. June 20, 1916. Among the sets of epiderm collected was one each of wheat, oats, barley, corn, and millet. The maximum temperature reached on June 19 was 79°F. and on June 20, 76.5°F. The humidity was rather high for the region, as it at no time dropped below 20 per cent. Except for the passing of some thin and hazy clouds, the sunlight was nearly normal, and at no time was the light reduced to an extent sufficient to affect the stomata. The weather data are shown in figure 16. At the beginning of the series the stomata of wheat, barley, and oats were closed and showed no opening until 6 a. m., June 20. Maximum opening for the morning occurred at 7 a. m., and was 21 per cent in wheat, 16 per cent in oats, and 17 per cent in barley. Total closure occurred in all three at nearly the same time and was complete at 11 a. m. (fig. 24). In

FIG. 24.—Series 11, showing movement in lower stomata of wheat (A), oats (B), and barley (C).

comparing these results with those obtained in series 10, it will be observed that the stomata of wheat showed very little difference in behavior, those of oats opened more widely than on June 8, while those of barley opened to twice the width and remained open 2 hours longer. The chief difference in weather conditions during the two series was in temperature, the maximum on June 20 being 76.5° F., while the maximum of June 8 was 88°. In addition, the humidity was not as low and the evaporating power of the air was correspondingly less. Wheat was therefore but little affected by the higher temperature of the preceding series, barley showed the greatest effect, and oats occupied an intermediate position.

The stomatal movement in millet (*Setaria italica germanica*) was essentially the same as in wheat, the only difference being a slightly wider opening at 7 a. m. in millet. The stomatal movement in corn,

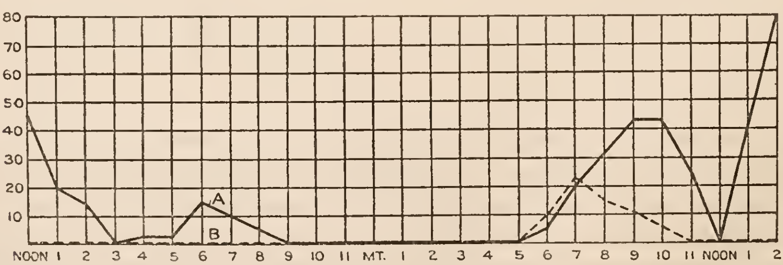


FIG. 25.—Series 11, showing movement in lower stomata of corn (A) and millet (B).

however, was distinctly different. The stomata were 50 per cent open at noon on June 19 when the series started. They closed gradually and completely by 3 p. m. However, they immediately began to open and at 6 p. m. reached 15 per cent, the maximum for the afternoon. They then closed as slowly as they had opened, completing the movement by 9 p. m. As with the other cereals examined, the stomata of corn remained closed throughout the night, but opened directly after sunrise. The maximum opening of 42 per cent for the morning occurred at 9 a. m. They remained in this condition until 10 a. m. and then became closed by noon. At 1 p. m. they had again opened and were 80 per cent open at 2 p. m. when the series ended (fig. 25).

The stomatal movement in corn during this series was remarkable in several respects. One was the much greater opening compared with that of the other cereals, and the comparatively little complete closure during the hours of daylight. The stomata of corn differed from those of alfalfa in opening for a much longer period, but never to a maximum. Another important point is the difference shown on the two successive days of the series. As the maximum opening for the morning usually occurred about 9 a. m., the opening of 50 per cent observed at noon, June 19, indicates that the stomata were more widely open some hours previous to the start of the series. At all events, the stomata showed considerable opening during the forenoon of June 19 and but slight opening on the afternoon of that day. The next day this course was reversed, the stomata showing but moderate opening during the forenoon and twice as great opening in the early afternoon. This can not be fully explained by the physical factors recorded, but was probably in part the result of wind conditions.

When conditions become more unfavorable, corn stomata close for increasing periods during the day. Such midday closure is often accompanied by rolling of the leaf and other evidences of wilting. Under extreme conditions the stomata open with the first light of dawn and close shortly after sunrise, to remain closed until the next morning. If conditions are less extreme they stay open until late morning and then close until evening, when they open for a few hours before darkness sets in. Even under these circumstances, leaf-rolling is very noticeable during the early afternoon, but when the stomata are more or less open throughout the day, as in series 11, no trace of rolling occurs.

A collection of epiderm from potted plants of wheat growing in the greenhouse at the University of Minnesota was made during the course of series 26, on May 5, 1917. Owing to the remains of a coat of whitewash and to soot from the trains passing just below, only 17 per cent of the light outside penetrated through the glass. This light was a diffused sunlight and much more than could pass through a canvas covering, such as used by Gray and Peirce in their experiments, or through a north window, such as used by Darwin. The humidity was high, ranging from 45 per cent to 85 per cent and averaging 70 per cent. The lowest temperature recorded was 55° F. at 4 a. m. and the highest 72° F. at 2^h 30^m p. m. The day being clear and cloudless, the behavior of the stomata was practically normal, in spite of the low light intensity in the greenhouse, and the fluctuations in opening and closing coincide with those of light intensity. At the outset, the stomata were closed and did not open until after sunrise. At 6 a. m. they were only 5 per cent open, but the next hour they had opened to 50 per cent and at 8 a. m. were wide open. They remained at maximum until noon and then started to close slowly. At 2 p. m. they were still 80 per cent open, but with the sudden drop in light intensity at this time they closed to 50 per cent in the next hour. Then, as the light decreased very little for a time, the stomata closed only to 30 per cent in the next 2 hours. At 6 p. m. they closed to 10 per cent, as there was less than 1 per cent light in the greenhouse, and completely by 7 a. m. They remained closed all night.

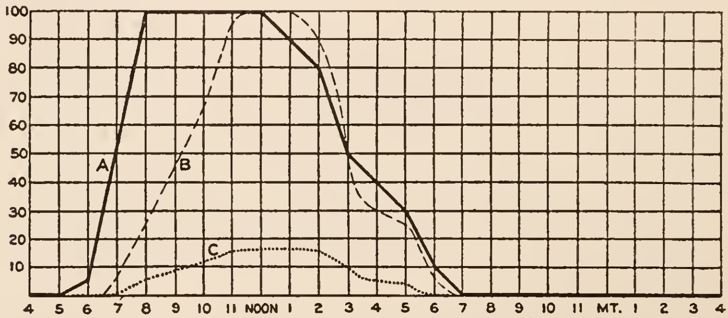


FIG. 26.—Series 26, showing movement in lower stomata of wheat growing in the greenhouse (A); light intensity outside (B), inside (C).

The stomatal movement of wheat in this series may be considered somewhat representative of all cereals under the most favorable conditions. The small amount of light causing opening is not remarkable for plants grown in a greenhouse, and hence adjusted to operate under conditions of reduced light intensity. The plants in the Great Salt Lake region did not show this type of movement, which is not strange, as the average daily minimum humidity for June, July, and August 1916 was only 10 per cent. The behavior of the individual stomata was different in this series. In those made at Salt Lake City only part of the stomata opened, thus making the average opening for all rather low. Then, as closure began, some stomata closed slightly, others completely, and some not at all. As time went on, a larger proportion of closed stomata were found, until finally all were closed. In the greenhouse series, however, far the larger number showed the same degree of opening in each strip, and less than a fifth of their number varied from this average, and most of these only a little. This adds evidence to the belief that, in the series made during less favorable conditions, some remained closed throughout the 24-hour period, while others showed all the opening that occurred.

DISCUSSION OF RESULTS.

The plants described are but a few of those investigated, but they are representative in some measure of all. Their stomatal behavior provides a basis for classifying the stomata of all the species into three general groups, typified by barley, alfalfa, and potato. Naturally, the stomatal movement in each species varies in some respects from that in the type, as has been shown. Barley represents the cereal type with stomata of peculiar construction and great sensitiveness, which show no opening at night, no matter how slight the day opening. Alfalfa represents the group which, under normally favorable conditions, have open stomata during the day and closed stomata at night, but as conditions become less favorable show increasing night opening and extended mid-day closure. Potato belongs to the group of plants that normally have open stomata at night under favorable conditions, and close them only as water-content decreases or evaporation becomes greater. Naturally, it is possible by an unusual grouping of factors to vary the behavior in many plants to such an extent that they will show almost any kind of movement. For this reason it is unsafe to carry out experiments in the greenhouse alone, as the factors present differ greatly in degree from those in the field, and the stomatal movements of greenhouse plants, in consequence, are distinctly different. Hence greenhouse experiments are of value only in connection with similar ones made in the field.

Barley has never been found in the course of the experiment to have open stomata at night correlated with day closure. Many series indicate that highly favorable conditions would probably cause barley or corn to show stomatal behavior resembling that of wheat in series 26, i. e., opening with the appearance of daylight, closing gradually during the afternoon, and remaining closed all night. Under conditions only slightly less favorable, the stomata of barley have been found to close in the afternoon, and on a hot, dry day were but partially open an hour or two after sunrise. In all cereals the tendency seems to be to operate with many closed stomata at nearly all times. Even under very favorable field conditions all the stomata are wide open for only an hour or two. In the case of corn, sorghum, and Sudan grass, very warm or even hot weather, brilliant sunshine, and a high water-content seem to be the optimum conditions. For wheat, oats, and barley, cool and rather humid weather and less sunshine are best. This has a practical bearing on the spread of wheat rust, as the parasite gains entrance to the leaves of the host through the stomatal openings and in part explains how it can make most headway during such weather. Millet occupies a position between barley and corn in respect to optimum conditions. The cereal type of stomatal behavior may be characterized as showing no night opening correlated with mid-day closure, and as rarely showing maximum opening of all the stomata.

The alfalfa type of stomatal movement is characteristic of most thin-leaved mesophytes. The normal light-curve of alfalfa (fig. 6) shows stomatal movement under the most favorable conditions, and is the same kind of curve as that produced by a cereal under such conditions. Then, as these become progressively less favorable, mid-day closure appears, increasing to complete closure, which in turn becomes more and more prolonged until the stomata are closed all day. With the appearance of mid-day closure, night opening also develops and increases with increase of day closure. The final result is a partial opening of stomata all night and complete closure all day.

The plants studied with this type of movement are:

Melilotus alba, sweet clover.
Trifolium pratense, red clover.
T. repens, white clover.
Pisum sativum, garden pea.
Lathyrus odoratus, sweet pea.
Phaseolus nanus, navy bean.
P. lunatus, lima bean.
Beta vulgaris, sugar-beet.
B. vulgaris, blood-beet.
Chenopodium album, lamb's-quarters.
Rumex patientia, western dock.
R. crispus, curly dock.
Brassica napus, rape.
B. campestris, turnip.
Raphanus sativus, radish.
Brassica campestris, rutabaga.
Papaver somniferum, poppy.
Tropæolum major, nasturtium.
Amaranthus retroflexus, pigweed.
A. græcizans, little pigweed.
A. blitoides, carpet-weed.
Polygonum aviculare, knotweed.
Malva rotundifolia, cheeses.
Pastinaca sativa, parsnip.
Daucus carota, carrot.
Lycopersicum esculentum, tomato.
Citrullus vulgaris, watermelon.
Cucumis melo, muskmelon.
C. sativus, cucumber.
Helianthus annuus, sunflower.
Arctium lappa, burdock.
Lactuca scariola, prickly lettuce.
L. sativa, lettuce.
Taraxacum officinale, dandelion.
Ribes aureum, golden currant.
R. rubrum, common currant.

The trees whose stomatal movement was investigated were apple (*Malus sylvestris*), pear (*Pirus communis*), Elberta peach (*Prunus persica*), sweet cherry (*Prunus cerasus*), and Lombardy poplar (*Populus nigra italica*). These, as well as California privet (*Ligustrum japonicum*), must be classified with the alfalfa group, since night opening does not occur under favorable conditions. On the other hand, no mid-day closure was found in any of the series, as they seemed to be little affected by evaporation. It is impossible to state the water-content about their roots, but there is good reason to believe that the soil was moist at all times. The behavior of their stomata resembled the normal light-induced movement in alfalfa at the times when the stomata of alfalfa showed considerable day closure. This was probably due in large measure to the great balance of water on hand in the trunk and branches of the tree, and to the high water-content within reach of the roots.

The third group contains the larger number of fleshy-leaved plants as well as some thin-leaved ones. Under conditions of high water-content and low evaporation, the stomata are continuously and often widely open all day and night. As the evaporating power of the air increases beyond a certain point, the stomata tend to close. Based upon the time of such closure, three subgroups may be distinguished. Potato stomata at first close just after sunset for a time, but as the water-content decreases this time extends backward into the afternoon. Then, as conditions become extreme, the stomata close a short time after sunrise, accompanied by visible wilting of the entire plant, which persists throughout the day. After sunset the plant recovers, the stomata open slowly about midnight, and at sunrise are fully open. A second variation is found in cow-beet, where, under favorable conditions, the stomata are wide open during the day and close very little during the night. At sunrise this closure is arrested and shortly afterward the stomata again become wide open. As conditions grow less favorable, night closure becomes more rapid and is completed before sunrise. Progressively as evaporation increases and water-content decreases, such closure is begun at an earlier time, becoming complete at midnight and then at sunset. Finally, the stomata open widely only an hour after sunrise and close gradually and completely during the forenoon or even before morning is over. The third variation of this form of movement occurs in onion, where the stomata are wide open at night under conditions of high water-content and low evaporation. If water-content alone becomes low, the stomata close at night, but if the evaporation increases instead, the stomata tend to close during the day. Hence, with medium or low water-content, onion stomata react to increased evaporation like the plants in the alfalfa group, showing increasing mid-day closure correlated with increasing night opening.

The plants having stomatal movement like that of the cow-beet and potato are cabbage (*Brassica oleracea*), tulip (*Tulipa gesneriana*, Red Prince), *Portulaca oleracea*, and probably *Encelia farinosa*. This latter plant has not been found with open stomata at night in any series, since the latter were not made under the necessary conditions. The general behavior is essentially like that found in cow-beet, and it is probable that under favorable conditions this plant would show open stomata at night. *Verbena ciliata* is also included here, as plants which were heavily watered showed the stomata 20 per cent open all night. *Fouquiera splendens* exhibits a rather bewildering behavior, the stomata of the primary leaves of heavily watered plants showing movement like that of cow-beet, while the secondary leaves showed mid-day closure and correlated night opening, such as found in alfalfa.

The plants with behavior similar to that of onion are salsify (*Tragopogon porrifolius*), Hubbard squash (*Cucurbita maxima*), crook-neck squash (*C. moschata*), pumpkin (*C. pepo*), plantain (*Plantago major*), lily (*Lilium speciosum*), and leek (*Allium porrum*).

Such plants as *Scirpus validus*, *Equisetum hiemale*, and *E. palustre* showed the stomata continuously wide open, and this seems to be their normal state. *Equisetum* was found with wide-open stomata during wilting and even after the death of the stems from water-loss.

SUMMARY.

1. The daily march of stomatal movement varies more or less from day to day. It is as unusual to find movement identical on two successive days as it is for the weather to be the same. This variation is correlated with changes in weather and water-content, and does not occur when these are sufficiently alike.

2. In nearly all plants, stomatal opening is correlated with the presence of light when conditions are favorable. When they become unfavorable, the influence of light is modified by the action of other factors, and finally nullified. In a few plants, light seems to have little or no part in producing stomatal opening.

3. Plants fall into three groups, according to their stomatal behavior, each of which has several subdivisions.

4. The first group includes the cereals in which night opening does not occur under ordinary conditions, favorable or unfavorable. Day opening is dependent in duration and degree upon favorable conditions of evaporation, temperature, and water-content.

5. The second group includes most thin-leaved mesophytes, such as alfalfa. Under favorable conditions their stomata are open all day and closed all night. Alfalfa stomata open in 2 to 6 hours after daylight, remain open from 3 to 6 hours, and then gradually close during a period about twice as long as required for opening. When conditions become less favorable, the stomata close partially or completely for a time during the middle of the day, the period increasing to include the whole day under very unfavorable conditions. Night opening appears when mid-day closure occurs and increases in degree and extent with it. Finally, when conditions become extreme, the stomata are closed all day and open all night, the degree of opening being dependent upon the water-content.

6. The third group of plants, which includes the potato, tends to have stomata open to a greater or less degree throughout the day and night under optimum conditions, especially of water-content. If evaporation increases to a critical degree, the stomata close for a time during the day, when this is greatest. If water-content decreases moderately, the stomata not only become more responsive to evaporation, but in most plants to light changes as well. Hence the stomata open with the appearance of daylight and close at night, unless evaporation has become serious. If the water-content decreases to a critical degree, the effect of sunlight can be modified or

even nullified by increase of evaporation, thus producing day closure and night opening, as in the alfalfa group grown under similar conditions.

7. The stomatal movement of each plant studied tends to follow a regular course under optimum conditions, opening and closing progressing smoothly and uniformly until complete. As conditions become less favorable, however, the rate of movement becomes more and more irregular, opening, for example, progressing rapidly one hour, slowly or not at all the next, and rapidly again the third. When evaporation becomes extreme, movement consists of alternate opening and closing, the one following the other at hour or even shorter intervals. If the general trend is toward opening, the degree of each opening exceeds that of closing, but if toward closing, the reverse is true. The amplitude of these changes is rarely great and a difference of 40 per cent from the smoothed curve is unusual.

8. The stomata of the upper and lower surfaces of the leaves in most plants are different in their structure or in their relation to the rest of the leaf. Hence the stomatal movement is dissimilar, even though each surface be exposed to the same environment. However, in some plants, such as the cereals studied and the sugar-beet, the stomata are sufficiently alike in structure and in relation to the other tissues to produce like behavior under identical conditions. In some of the cereals with leaves blown about and alternately illuminated on each surface, the stomatal behavior is similar under most conditions. In other cereals and in sugar-beet, differences in illumination and in exposure to other factors normally cause considerable divergence in the behavior of the upper and lower stomata, although this is not as great as in those plants with dissimilar stomata.

9. The stomata on the stems, when such are present, usually differ materially from those of the leaves in structure and relation to water-supply, and as a consequence in their behavior as well.

10. The marsh plants studied have permanently open stomata.

II. THE EFFECT OF PHYSICAL FACTORS AND PLANT CONDITIONS UPON STOMATAL MOVEMENT.

The effect of stimuli upon stomata has received by far the greatest attention from investigators in this field. The problem is so vast that no attempt was made to cover it in detail, but certain facts have been brought out in the course of the experiments which aid in explaining the behavior of stomata. Hence, the discussion is confined to the previous series described in their relation to physical factors, and to other series and experiments designed to afford an explanation of some of the phenomena observed. It is in this connection that greenhouse experiments are particularly valuable in supplementing field observations.

Some factors, such as light, have a direct effect upon stomatal movement, others, like relative humidity, have an indirect effect by acting upon the leaf as a whole, or like wind by increasing the effect of some other factor. The rate of change in a factor may have an effect when rapid, and little or none at all when slow. Minor changes of a factor have little or no effect, except at the critical point. Thus, changes in light intensity have no observable direct effect if they occur above 50 per cent of maximum for the region, but produce corresponding changes in stomatal movement in many plants growing where the light is less than 10 per cent. There is always a lag between the impact of a factor and the resultant effect upon the stomata. This lag is of variable length and depends largely upon temperature and degree of impact, but often upon other conditions as well, such as degree of maturity, fatigue, momentum, and structure and condition of the leaf. As the structure and condition of the plant and its parts also have an effect upon stomatal movement, these are considered as well as the physical factors.

LIGHT.

The importance of light in causing stomata to open has been known for many years, but the manner in which it operates is not yet thoroughly understood. Lloyd (1908) has shown that in *Verbena ciliata*, as well as in other plants, starch almost wholly disappears during the early forenoon, when the stomata are at their widest, and increases toward evening with the closing of the stomata. Iljin (1914) found, by means of the plasmolysis method, that when the stomata were open the guard-cells had a much higher osmotic pressure than the surrounding cells, and attributed this to the conversion of the starch. The appearance of light was considered by him to initiate enzymatic change of starch into sugar. The greater concentration of sap resulted in endosmosis, which increased the turgor of the guard-cells and in consequence caused opening. Then, after a time, reconversion into starch was assumed to take place and the stomata closed. In many cases the starch is manufactured in the guard-cells, but in some it is not, when they have no chloroplasts. In the latter type of stomata the carbohydrates are produced in the chlorenchyma and transported to the guard-cells.

The starch-content of the guard-cells was studied in several of the series, in order to determine its relationship to light intensity and stomatal movement. After several trials, the following method was adopted as showing most clearly the relative amount of starch present in the plastids: The strips in which the stomatal openings had been previously measured were washed free of alcohol and left in distilled water one-half hour. They were all trimmed to the same size and shape, and each placed for exactly 5 minutes in 1 c. c. of N/5 solution of KI and I. The strips were then washed immediately in a large amount of slightly alkaline water to stop the action of the iodine, and mounted in glycerine jelly. The slides were compared under the microscope and arranged in a series according to the amount of starch estimated to be present, taking into consideration the size and color of grains. They were then assigned numbers from zero to 100, based upon differences observable in the slides, and these numbers were used for the starch index. The system was crude in many respects, but was the most satisfactory one available, and is believed to give a general estimate of the starch present in the guard-cells.

One attempt was made in the case of the stomata of cow-beet to use a colorimeter process. Fifty stomata were dissected from a strip of epiderm and placed in a capillary tube 4 cm. long and 1 mm. in diameter. A solution consisting of 2 grams KI and 1 gram of iodine in 1,000 c. c. of water was used to stain the grains, the action going on for 2 hours. For standards, a set of 11 similar tubes were made up, ranging from a KI+I solution without starch, a 1/10 dilution, a 2/10 dilution, etc., to 10/10 suspension of soluble starch and KI+I solution. The two solution-tubes nearest in color to the guard-cell suspension were compared several times and the difference in color estimated in tenths. It was hoped by this means to get a truly quantitative measure of starch, since the amount in the index tubes was known. But the method was abandoned, because it was very difficult to get the guard-cell starch into suspension, and because the differences between these suspensions were mostly too slight to be detected.

The starch index determined in this manner did not show the close correlation to either light changes or stomatal movement that Iljin and Lloyd have found. Lloyd observed a clear and definite relation between changes in the starch-content of the guard-cells and stomatal movement, but, as his graphs show, no midday closure occurred and his curves are of normal light-induced movement only. This type of movement has also been found correlated with changes in the starch index, as the following series shows: Series 6 was begun at 8 a. m. June 1, 1916, and ended at 9 p. m. the same day. At the start the stomata were but 40 per cent open and did not reach maximum until noon. This slow morning opening was due to the heavy clouds and a drizzling rain which lasted until $11^h 30^m$ a. m. The stomata remained at maximum until 3 p. m. At 4 p. m. they closed to 75 per cent and at 5 p. m. to 50 per cent. No strips were then collected until 8 p. m., when the stomata were found closed; they remained closed to the end of the series. At the outset the starch index dropped rapidly to the minimum between 11 a. m. and noon and then rose slowly until 3 p. m. It then rose rapidly as the stomata closed and continued rising to the end of the series. It still showed a rapid increase at 8 and 9 p. m., although the stomata were closed (fig. 27). The stomatal movement in Lombardy poplar was

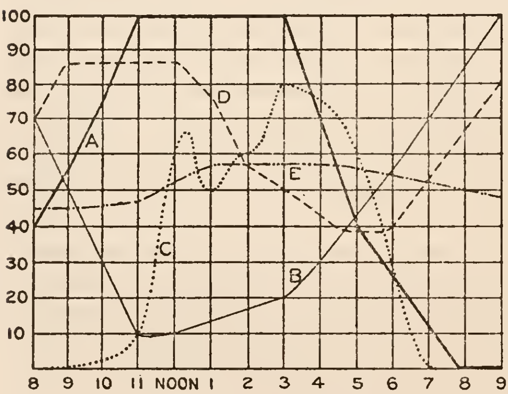


FIG. 27.—Series 6, showing movement in lower stomata of Lombardy poplar (A), and starch index of the guard-cells (B), sunlight (C), humidity (D), temperature (E).

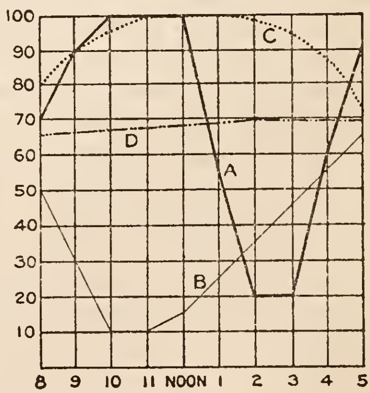


FIG. 28.—Series 7, showing movement in lower stomata of *R. patientia* (A), and starch index of the guard-cells (B), sunlight (C), temperature (D).

light-induced, not modified by any other factor, and was correlated with changes of the starch index as Lloyd found in *Verbena*.

Iljin found that when stomatal closure was induced by wilting, the osmotic pressure of the guard-cells dropped and the amount of starch in them increased. No such definite agreement could be found, however, between midday closure and changes in starch-content. In some series the amount of starch increased slightly during midday closure, and this was quite noticeable when closure was protracted. In others, no corresponding increase whatever was found. Series 7, *Rumex patientia,* illustrates this. The upper stomata were 70 per cent open at 8 a. m., 90 per cent at 9 a. m., and at maximum the following hour. At noon they were still wide open, but closed to 20 per cent at 2 p. m., remaining at this opening 1 hour. Then they opened to 90 per cent at 5 p. m., when the series closed (fig. 28). The starch index resembles that of poplar series 6, no change having occurred because of mid-day closure. The amount of starch in the guard-cells was decreasing rapidly at the start of the series and reached a minimum at 10 a. m. No change was observable the next hour, but after this the amount increased uniformly and gradually to the end of the series. Starch was on the increase while the stomata were closing, but hardly fast enough to produce as great closure as found. It was still increasing at the same rate when the stomata reopened, contrary to expectations. It is evident that reduced concentration of the surrounding cells, or increased density in the guard-cells due to some other factor, caused this behavior.

In some series there was at times a certain relationship between changes in the starch index and mid-day closure, while at other times no relation existed. Thus, in alfalfa during series 10, the stomata were opening rapidly at the start and the amount of starch was decreasing almost as rapidly. The observable minimum in the starch index was reached at 7 a. m. and persisted until 10 a. m. In the meantime, the stomata opened to the maximum at 6 a. m., closed to 40 per cent at 8 a. m., and reopened to 60 per cent at 9 a. m. changes in starch-content showing no relation to this. After 10 a. m. the stomata closed gradually, and completely at 2 p. m. This is correlated with a similar slow rise in the starch-content of the guard-cells. They opened to 25 per cent at 3 p. m., accompanied by a slight drop in the amount of starch, and then closed to 10 per cent at 6 p. m., without change of starch-content. They remained stationary for 2 hours, while the starch index increased perceptibly. It was still increasing, though more slowly, when night opening started, but between 11 a. m. and 1 p. m., when the stomata showed the greatest increase, it remained stationary. Between 1 and 3 a. m., when they closed again, the starch index increased rapidly once more, and decreased very much the next hour, when morning opening started (fig. 29). Hence, at times there existed a good correlation between the changes in starch-content and stomatal movement and at other times none whatever. It seems clear that the entire subject is more complex than either Iljin or Lloyd has supposed, and must be the subject of renewed investigation.

The time required to produce response to light of various intensities was first studied at Salt Lake City in June and July 1916. In order to determine the reduction necessary to induce closure of the stomata, 4 control cabinets, used in the sulphur-dioxide experiments, were set up on as many plots. These cabinets were 6 feet square, 4 feet high, and consisted of a light wooden framework covered with sheets of celluloid. A constant current of air was driven through a 6-inch sheet-iron pipe from an electric blower to the top of the cabinet, and given a whirling motion by stationary radial vanes at the vent of the pipe (plate 10). The air escaped from the cabinet through an adjustable slot 3 inches wide around the bottom. The first cabinet was used unchanged, the second shaded by a large muslin-covered screen, the third covered with muslin tacked on above the celluloid, and the fourth with oil fabric or imitation leather, the black side turned in. Of the light incident, 60 per cent entered the first cabinet, 8.5 per cent the shaded cabinet, and only 2 per cent entered the muslin-covered one. The fourth cabinet showed only the faintest traces of light.

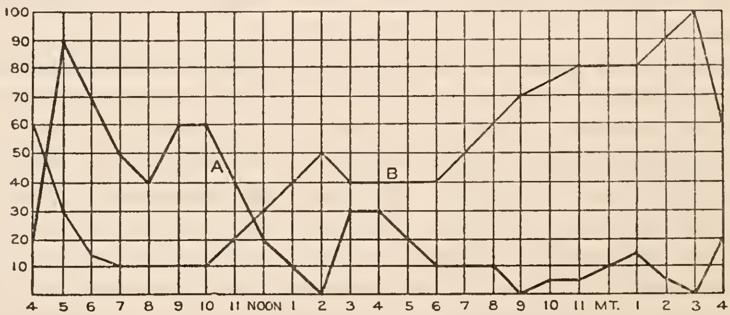


Fig. 29.—Series 10, showing movement of upper stomata of alfalfa (A) and starch index of guard-cells (B).

The cabinets were placed on alfalfa plots to determine the readiness with which the stomata would respond to reduction of light during the forenoon when they had just opened, and again in the afternoon when they were closing. On July 3, 1916, the cabinets were placed upon well-watered plots for 90 minutes, at 3 p. m., when the stomata were 80 per cent open. After half an hour the opening was 75 per cent in all except the dark cabinet. At the end of an hour it was 60 per cent in the open and in the unshaded and shaded cabinets, 40 per cent in the muslin-covered cabinet, and 15 per cent in the dark cabinet. At the end of the experiments the opening was 50 per cent outside as well as in the first cabinet, 45 per cent in the shaded cabinet, 20 per cent in the muslin-covered one, and closed in the dark cabinet. The reduction of the light to 60 per cent had no effect and to 8.5 per cent but little. Reduction to 2 per cent caused noticeable increase in the rate of closure, while darkness caused the stomata to close in less than an hour and a half. Long-period experiments of 6 to 24 hours made at various other times showed that the reduction of light in the unshaded cabinet was not sufficient to cause changes.

The difference in the degree of reduction produced a difference in the time necessary to show an observable response, as well as in the degree of response. In the first half hour only the plants inside the dark cabinet showed a response; at the end of an hour, the plants in the muslin-covered cabinet showed a response as well; at the end of an hour and a half the plants of all the cabinets except the first showed a response. Hence, with light, at any rate, the difference in time between the impact of a factor and the response decreases as the degree of change increases.

Three days later the cabinets were placed on the plots at $9^h\ 30^m$ a. m. and removed at 11 a. m. The stomata of the plants outside were wide open throughout the period. No change of behavior was observed, except in the dark cabinet, in which some closure had begun as the experiment ended. It seems evident that the time of day also makes a difference in the response, and that stomatal movement may be hastened more easily than reversed. This was shown with even greater clearness in the experiments with night illumination and in those carried on in April 1917 at the greenhouse of the University of Minnesota. Potted plants of wheat and corn kept 38 hours in darkness showed stomatal opening in 40 minutes when exposed to the light of a 40-watt mazda light hung 2 dm. above the plants, while others kept in darkness 3 hours showed no opening after 2 hours of such illumination.

Potted plants of wheat which had been kept outside were taken inside on the evening of May 4, 1917, and placed near those that had been grown in the greenhouse for series 26. At the start of the series, 4 a. m. May 5, all the plants were stripped at hour intervals until 9 a. m. as were the plants of the series. The plants brought into the greenhouse showed 5 per cent opening at 6 a. m., as did the plants which had been grown inside. At 7 a. m. the greenhouse plants had stomata half open, while those brought in were but 10 per cent. The next hour the greenhouse plants showed stomata at maximum, and the out-door plants 35 per cent opening. At 9 a. m. both groups of plants had wide-open stomata. It is evident that the stomata of wheat plants accustomed to strong light do not open as readily in diffuse light as those of plants adjusted to such light conditions.

To find the effect of night illumination upon alfalfa plants growing in the field, a series was made on August 13, 1919, from midnight to 2 a. m. A 250-watt mazda light was suspended 1 meter above a plot of alfalfa and a similar one above a plot of cow-beet. The night was clear and moonlight, the average temperature during the experiment being 60° F. No difference in stomatal movement could be detected between the lighted and unlighted plots, the stomata of alfalfa being 40 per cent open throughout the experiment and those of cow-beet remaining 75 per cent open during the 2 hours. Hence, it seems that the additional light was not sufficient to cause further opening in either plant, and that the amount of opening found was caused by moonlight alone.

Another series was made August 23, 1919, to find the effect of such illumination on a dark night. A plot of alfalfa and one of cow-beet were illuminated from 10 p. m. to midnight and strips collected every 30 minutes. The stomata of alfalfa were closed at the start and remained closed until $11^h 30^m$ p. m. in the dark plot. In the illuminated plot the upper stomata showed slight opening at 11 p. m. and by 12 had opened to 25 per cent. Night opening also occurred in the dark plot, but was only 5 per cent at midnight. No opening occurred in the lower stomata of either plot. In the cow-beet, the stomata were 40 per cent open at the start of the experiment and closed in the unilluminated plants to 25 per cent at midnight. They closed slightly in the upper surface of the plant in the lighted plot as well, since they were 35 per cent open at midnight. The lower stomata of the lighted plants were like those of the unlighted ones. Three hours later, from 3 to $4^h 30^m$ a. m. August 24, the experiment was repeated on plots that had not been used previously. The upper stomata of alfalfa were closed and remained closed in the dark plot, while they opened 30 per cent in 90 minutes in the light plants, the first slight appearance of opening being produced in half an hour. The upper stomata of cow-beet were 20 per cent open at the outset and closed to 15 per cent in the unlighted plants, but opened to 50 per cent in 90 minutes in the plants illuminated.

These results indicate that night illumination is effective on a dark night, but not on a night which is brightly lighted by the moon. The additional light was not sufficient in the earlier experiment to produce increased opening. The time of night likewise plays an important part in the effectiveness of illumination, since in alfalfa only 25 per cent opening occurred in 2 hours during the earlier part of the night, while 30 per cent was produced in an hour and a half toward morning. In cow-beet no opening occurred in the first part of the night, but in the later part rapid opening was found. This may be due either to fatigue or more probably to the fact that storing of starch had to be reversed during the first part of the night, but was practically complete and everything ready for the reverse process during the later part of the night.

TEMPERATURE.

Temperature is manifest to a plant in three forms, radiant energy, air-temperature, and soil-temperature. As radiant energy it tends to raise the temperature of the leaf above that of the surrounding air, and this must be met by the cooling action of transpiration. As air-temperature, it affects the evaporating power of the air, and determines in large measure the speed of the reactions occurring in the leaf. As soil-temperature, it affects the direct water-loss from the soil and the growth and functioning of the roots. In all these cases temperature plays an important though indirect part in the behavior of the stomata.

In one respect, however, temperature acts directly upon stomatal movement, affecting the rate of morning opening. Such opening is light-induced and hence dependent upon starch conversion, which is undoubtedly an enzymatic process. It is to be expected that such conversion will therefore follow the same law in regard to rate of reaction as any other chemical reaction. No rigorous study of this could be carried out, but inspection of the various series led to the conclusion that possibly humidity changes and fluctuations of light intensity within certain limits would not seriously affect the results of the experiments attempted. These consisted in taking pots of alfalfa kept in the dark-room over night into the sunlight on days of different temperatures. It was realized that it was impossible to allow for the effects of changes in light, humidity, wind, and other factors, and hence the experiments were liable to serious error, but on the whole the experiments agreed very well, and are at least indicative of the general effect of temperature upon opening (fig. 30).

The plants were well-watered the evening before and carried into the dark-room. If conditions were of the kind desired, they were carried out and placed in the sun the next day about 9 or 10 a. m. after stripping a leaf for evidence that the stomata were closed. Strips were then removed at hour and half-hour intervals on cold days and at 5-minute intervals on very hot days, in order to determine the time of maximum opening. There was rarely more than 2° F. difference between the temperature of the dark-room and the air outside, and hence no error could be expected from this source. As the light in each experiment was at least 80 per cent of the maximum for the region, light differences could hardly introduce any serious error. Apparently, evaporation had little or no effect upon this opening when it was not excessive, causing closure before or shortly after maximum opening occurred. In all, 23 experiments

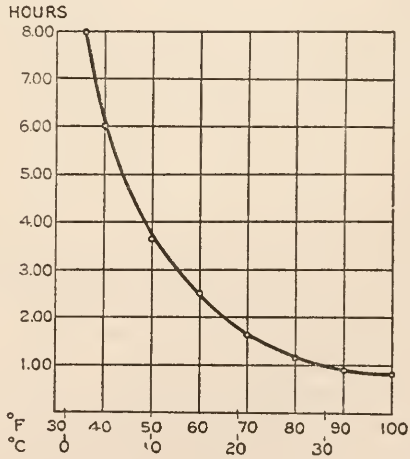


Fig. 30.—Relation of speed of total opening to temperature.

were made, 6 of which were discarded because too great a change of temperature occurred while the stomata were opening or because the stomata were not closed at the start.

Just above freezing, it required 8 hours to produce opening. At 10° C. approximately 4 hours were required to reach maximum. At 20° C. slightly less than 2 hours was required, and at 30° C. nearly an hour. The highest temperature, 34° C., caused complete opening in less than 50 minutes. At freezing and above 40° C. opening does not occur or is very erratic. Hence, upper and lower limits for stomatal movement must be near these temperatures. The results obtained correspond with the time required for opening by the plants of the series, these plants, however, requiring a somewhat longer time, since the light intensity at the start is low. This also varies, as apparently at higher temperatures it requires less light to initiate opening. If this be true, it explains why plants show opening at the first traces of approaching dawn when the weather is hot, and no opening until after sunrise on cold mornings.

The effect of changes in soil-temperature on stomatal movement is difficult of determination for several reasons. During the course of a 24-hour period, the superficial layer of dust in a cultivated field undergoes greater changes of temperature than the air if the day is clear, but at a depth of 1 dm. these fluctuations are not nearly so great and consist of a steady, slow rise after sunrise and a gradual decrease from evening through the night. The amplitude of this variation increases as the soil becomes drier, since the conductivity of dry soil is greater than that of moist. At greater depths this slow

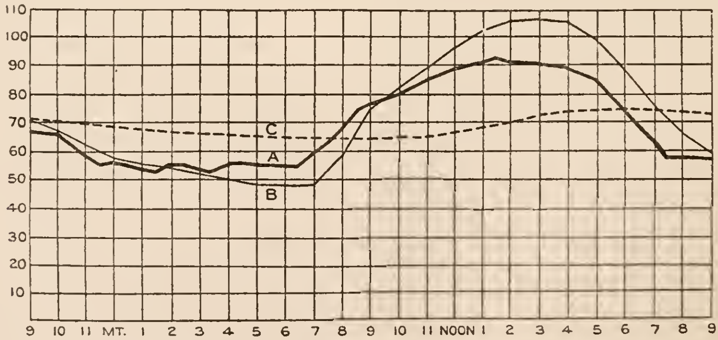


Fig. 31.—Series 34, factor data for September 10–11, 1919; temperature 2 dm. above soil surface (A), at the surface (B), at 1 dm. in the soil (C).

rise and fall becomes less, until at 5 dm. it is very slight. At such a depth only a series of cold days following hot weather, or the reverse of this, causes any distinct changes in temperature. Naturally, there is a gradual rise at this and greater depths during the spring, and as slow a decrease toward winter, but these seasonal changes do not come within the scope of this discussion. The changes observed in the temperature of the air, the upper centimeter of soil, and at a depth of 1 dm. during the course of series 34 are shown in figure 31.

Since the temperature of the soil differs so much at various depths, the effect upon shallow-rooted plants can not fully be determined, but must play some part in the functioning of the roots. The temperature of the superficial layers also has an effect upon the carpet-weeds, like *Portulaca oleracea* and *Amaranthus blitoides*, as these plants are subject to higher working temperatures than the more erect plants. Deep-rooted plants, such as alfalfa, have their roots in soil of uniform temperature and hence only seasonal changes affect them.

To determine the reaction of the stomata to changes in soil-temperature, a number of alfalfa plants were exposed to sunlight in metal containers, containing the same soil with the same water-content. Some of these were blackened to absorb more heat, some left bright, and others were placed in moist earth for protection. A chemical thermometer was placed in the soil of each container. The results were indefinite, as sudden rises in temperature caused an effect at lower temperatures than slow rises did. Low water-content increased the effect or caused closure at lower temperatures, and other factors which could only be guessed at entered into the experiments. Closure was caused by temperatures ranging from 21° to 30° C. at different times. It was found, however, that a rise of temperature in the soil would first cause stomatal closure and then wilting, even when the humidity was very high.

The temperature of the leaves of a plant, while rarely the same, usually differs but slightly from that of the air. This difference is the result of equilibrium between the cooling effect of transpiration and that of radiant energy. A number of readings of leaf-temperature were made upon alfalfa, potato, and sugar-beet with a chemical thermometer graduated to tenths of 1° C. The temperature of the air was read, then that of a leaf wrapped about the bulb, and the air-temperature again. An unwilted alfalfa leaf did not vary more than 0.2° C. above air-temperature, but a wilted leaf was at one time found to be 1.9° C. above the temperature of the air. Usually the leaves with open stomata were found to be lower than the air, and those with closed stomata during the day to be higher. Compared with the results obtained by E. B. Shreve (1919a) with far more accurate methods, these readings are too low, but are indicative of the effect of stomatal movement upon leaf-temperature.

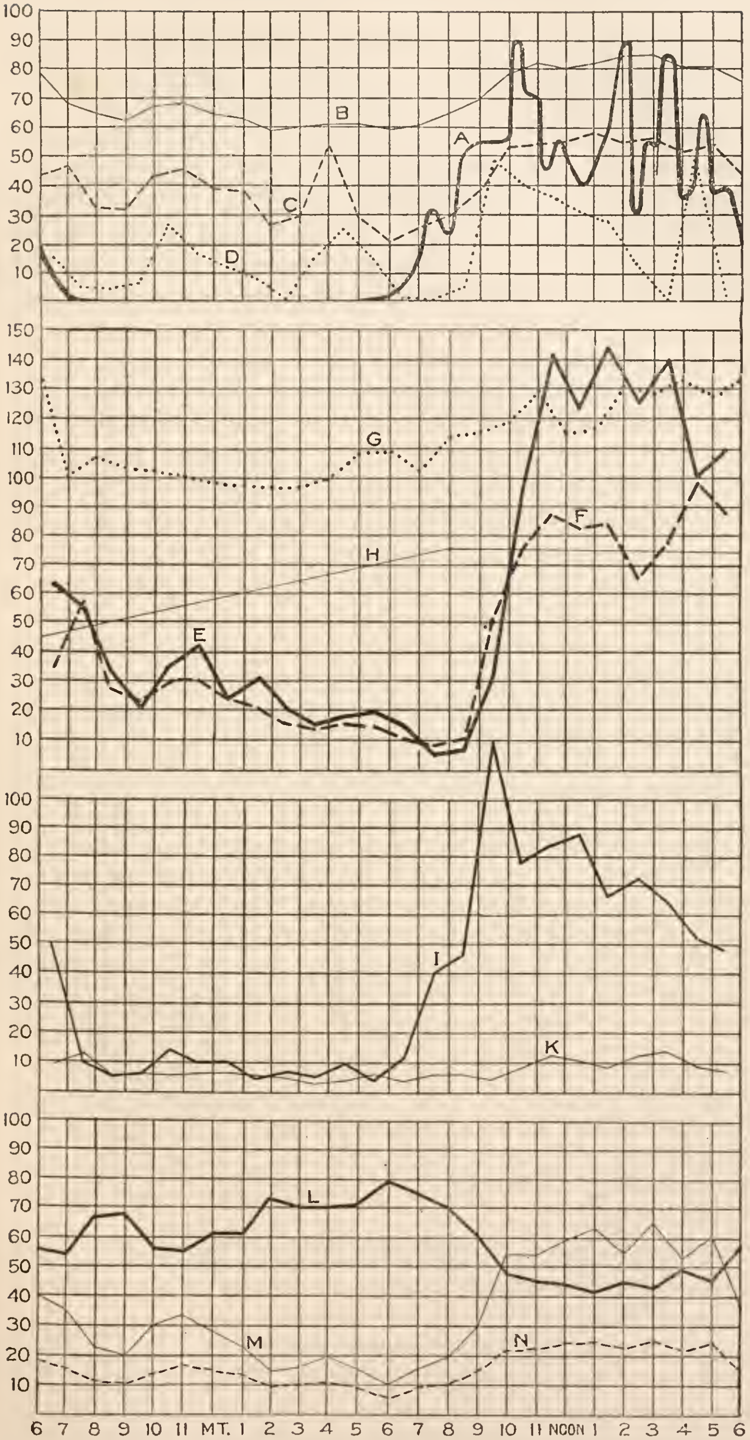
EVAPORATION AND CAUSAL FACTORS.

Evaporation is a product of several factors, the most important of which are absolute humidity, air-temperature, barometric pressure, wind, and sunlight. It is usually measured as the water-loss from a free water-surface or from some type of evaporimeter, the most important of which is the Livingston porous-cup atmometer.

Unfortunately, none of these agree very closely with the water-loss from a plant or even with each other, as Briggs and Shantz (1917) have shown. This is due to the fact that they are not affected in the same manner or to the same extent by each of the factors concerned. In consequence, attempts to calibrate these with one another have not met with much success. Even in the case of free water-surfaces it has not been possible to correlate the evaporation of one with that of another, except within certain limits (Thomas and Ferguson, 1917). Since the porous-cup atmometer is standardized and in widespread use, it is useful as a measure of relative conditions in various habitats, but can not be used in studies of hourly water-loss from the plant.

Until an evaporimeter is devised the water-loss of which will correlate accurately with the transpiration of a plant having open stomata, it is advisable to measure each factor by itself. It will then be possible to determine the effect of changes in one factor upon the plant under various constant combinations of other factors. It is also possible to arrive at some conclusion concerning the effect of any one factor when all are measured at hourly intervals during a 24-hour period, or oftener when necessary, and compared with the plant responses. This, in short, was the manner of carrying out the later and more elaborate series. The factors concerned in evaporation measured were the temperature of the air, soil-surface, and soil at a depth of 1 dm., the wind velocity, wet-bulb depression, barometric pressure, evaporation from a white-cylinder porous-cup atmometer, from a special blotting-paper evaporimeter standardized in darkness or dim light against a free water-surface, and from a free water-surface in a flat pan blackened inside. The atmometers were standardized by the maker and given the factors 0.68 and 0.73. The readings from these were reduced to unity.

The water-loss from the free water-surface was determined by weighing. The pan was a large Petri dish painted black on the inside, and was filled to a depth of 6 mm., which brought the surface to 9 mm. of the edge. This level was not permitted to drop below 2 mm. from its original height, when it was replenished from an inverted flask with burette tips and stopcock, which permitted water to be added drop by drop to the pan until the exact original weight was reached. The atmometers were not weighed, but were calibrated for changes of temperature, a thermometer running through the cork into the reservoir bottle. The water-level of each was brought to the original level marked on the open tube once an hour, the water being added from burettes. This method was checked carefully by weighing and found accurate to 0.02 c. c. The blotting-paper first used was green in color, matching the shade of alfalfa leaves as closely as possible. Later it was found that a standard blue-green paper had practically the same rate of absorption of radiant energy, and this was used.



A. Sunlight.
B. Temperature.
C. Saturation deficit.
D. Wind velocity.

E. Evaporation, blotting paper atmometer.
F. Evaporation, porous cup atmometer.
G. Absolute humidity.
H. Barometric pressure.

I. Transpiration of potted plant (Cow-beet).
K. Transpiration of cut leaves in potometers (Cow-beet).

L. Relative humidity.
M. Vapor pressure deficit.
N. Dew-point depression.

Fig. 32.—Series 33, showing evaporation, transpiration, and the factors concerned for September 8–9, 1919.

The evaporation from the two types of atmometers, transpiration from potometers and phytometers, relative humidity, absolute humidity, saturation deficit of the air, vapor-pressure deficit, dew-point depression, barometer pressure, wind velocity, air-temperature, and sunlight are shown in figure 32. These data were taken from series 33 started at 6 p. m. September 8, and ended 7 p. m. September 9, 1919. The evaporation from the two types of atmometers does not agree, and a study of the maxima and minima of the curves shows that the Livingston white cylinder is much more responsive to wind

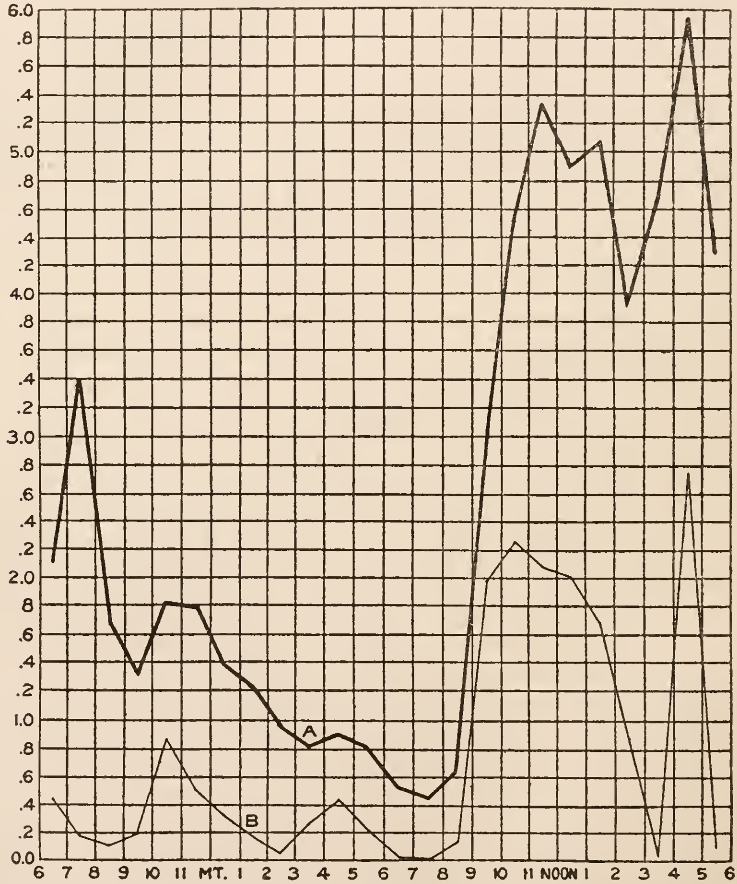


Fig. 33.—Series 33, showing evaporation from white-cylinder porous cup in cubic centimeters per hour (A), compared with product of vapor-pressure deficit and wind velocity (B), calculated by Johnston's method.

and much less to sunlight than the blotting-paper type. Neither type of atmometer showed any correlation whatever between its water-loss and transpiration from potted plants or cut leaves. The lack of any relationship in the curves representing water-loss from potometers and phytometers shows the entire unreliability of cut stems in determining the normal hourly transpiration from rooted plants. The data from the various series were used to calculate the theoretical water-loss of the white-cylinder atmometers according to the method employed by Johnston (1919), following the precaution of averaging the vapor-pressure deficit for the beginning and end of each hour as prescribed. The results calculated in this manner for series 33 with those actually obtained with the atmometer are shown graphically in figure 33. As the curves show, the relation is not at all close. But when the wind velocity is divided by a factor to reduce its effect upon the results, and a constant added to represent the replacement by diffusion of still air around the evaporating surface, the calculated results are brought into much closer accord with the observed rate of evaporation. Even then there are discrepancies that can not be explained fully by radiant energy, as suggested by Johnston. The method may be of value, however, as a first step toward an analysis of the part played by each factor in producing water-loss from the atmometer, and thus lead the way to a new attack upon the problem of transpiration.

In all the series made in the Great Salt Lake region, low relative humidity and strong sunlight were found associated, and usually higher temperatures as well. For this reason it was not easy to separate the action of each, but a study of the precise effect of each was beyond the scope of the investigation. The general effect of increased evaporation as determined by these three factors is shown for alfalfa in figure 9. This represents essentially what occurs in most of the mesophytes studied, low relative humidity and intense sunlight producing day closure. On the other hand, no closure was found taking place during the day if the humidity was high. If the water-content had become critical, no opening occurred at all, even under a condition of high humidity, but if the plant could obtain sufficient moisture to produce opening, the stomata would show no mid-day closure. The increased irregularity in the rate of movement following increased evaporation is shown in figure 9.

Each factor concerned plays at times a rôle independent of evaporation in producing changes of stomatal movement. Thus, light which acts through radiant energy in producing water-loss from the plant has been shown to act independently of air-temperature. The latter, which is a very important factor in evaporation, also plays an important part in the speed at which opening occurs. Humidity is more closely related to evaporation, but, when high, precipitation occurs in the form of dew or rain, and this causes changes in stomatal movement. Wind increases the effect of low humidity, but also carries dust, and when high probably also causes currents of air to flow through the leaf.

The effect produced upon stomatal movement by the wetting of the leaves by dew or rain is most clearly shown in series 35, begun at 5 a. m. September 19, and ended at 6 a. m. September 20, 1919. A heavy dew had fallen during the night before and the leaves were wet, the upper surfaces much more than the lower. The humidity was very high during the series, being 98 per cent at the start, decreasing slowly to a minimum of 49 per cent at 3^h 40^m p. m. and increasing again to 80 per cent or more during the following night. The day was only moderately warm, and, while there were no clouds, the large amount of water-vapor and the time of the year prevented sunlight from approaching maximum. The stomata of both surfaces were closed at the outset, but the upper opened 10 per cent at 6 a. m. and 30 per cent the following hour. At this time the lower stomata, which had been closed, opened 50 per cent. By 8 a. m. the dew had dried on the lower surfaces of the leaves and in response the stomata closed to 20 per cent. They then began to reopen, the maximum occurring at 1 p. m. The stomata of the upper surface continued opening rather uniformly until 9 a. m., when they reached 90 per cent. In response to evaporation of the heavy dew upon the surfaces, the stomata closed to 70 per cent the next hour. Maximum opening was reached, however, at 11 a. m. The stomata of both surfaces remained wide open until 5 p. m., when they started to close, the lower closing only slightly more rapidly than the upper. Closure was complete in the lower stomata and nearly so in the upper at 7 p. m. At this time dew again began to form, and at 8 p. m. was

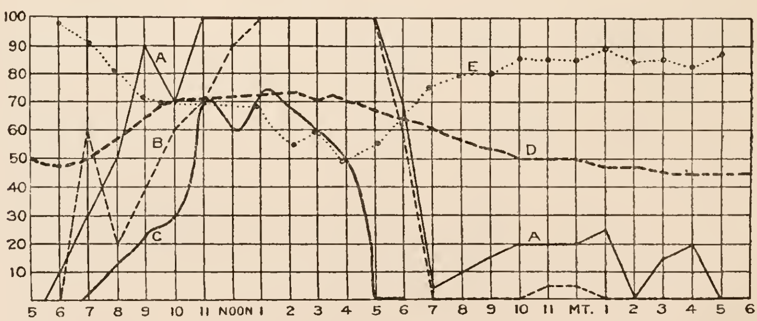


FIG. 34.—Series 35, showing stomatal movement in heavily watered plants of alfalfa, the partial closure at 8 and 10 a. m. following disappearance of dew; upper stomata (A), lower stomata (B), sunlight (C), temperature (D), humidity (E).

distinctly noticeable on the upper surfaces of the leaves. In consequence, the upper stomata began to reopen very slowly, reaching 25 per cent at 1 a. m. At this time a breeze sprang up, causing closure the next hour, but this died away and the stomata again opened, reaching 20 per cent at 4 a. m. The next hour they closed, and remained in this condition until 6 a. m., when the series ended. The lower stomata were closed all night, except for 5 per cent opening at 11 p. m. and midnight, when the dew first affected the lower surfaces (fig. 34). When the leaves and stems of alfalfa, sweet clover, and nasturtium were wetted experimentally the stomata opened. Closure, however, did not always follow the drying of this moisture as rapidly as it did in this series.

Wind carries fine particles of dust to coat the leaves, thus reducing light penetration and chlorovaporization and indirectly affecting stomatal movement. Dust particles are also found wedging open many of the stomata after a violent wind. A stoma of wheat kept from closing in this manner is shown in plate 1. In one leaf of sugar-beet, 17 per cent of all the stomata observed were wedged open by dust particles. This is an unusually large proportion, but at many times sufficient stomata in each leaf are found permanently held open in this manner to produce a serious effect upon the plant during times of stress. High winds which arise suddenly seem to force currents of air through the leaves before the stomata close, and wedge the grains into the slit in this manner. This is also the most plausible explanation of the immediate great increase of transpiration which occurs at once upon the sudden appearance of a high wind. Under ordinary circumstances, however, wind probably only removes shells of nearly saturated air from the outer ends of the stomatal pores (Dixon, 1914).

The attempt to correlate evaporation from atmometers and free water-surfaces with transpiration has met only with negative results. A porous cup can hardly be expected to respond more freely to its environment than a free water-surface or saturated blotting paper,

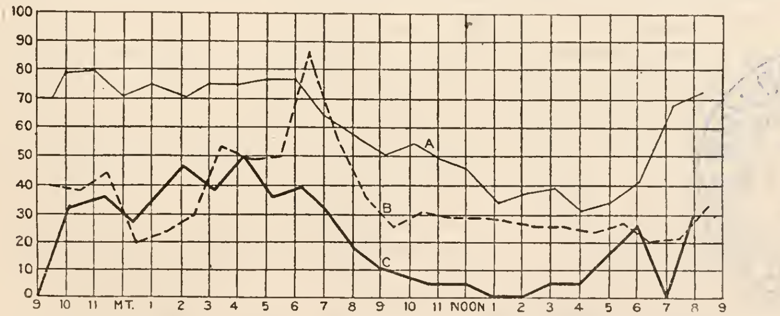


Fig. 35.—"Relative transpiration" based on evaporation from blotting-paper atmometer (A) and from white-cylinder porous cup (B), compared with stomatal movement in onion (C).

and hence the results from a white-cylinder atmometer have no advantages over those from the other types. Until an atmometer is devised which responds in the same manner and degree to each of the factors concerned, the ratio of transpiration to evaporation is meaningless. Thus, in series 34, "relative transpiration," calculated from the data obtained by the blotting-paper atmometer, shows a certain degree of correlation with stomatal movement, while that calculated from the evaporation from the porous cup shows practically none (fig. 35). There is, in consequence, no warrant for the view that lack of agreement beween stomatal movement and "relative transpiration" based upon evaporation from the porous-cup atmometer indicates that stomata are non-regulatory. Nor, on the other hand, can the lack of close agreement between "relative transpiration" based upon the blotting-paper type of atmometer be attributed to incomplete regulation by the stomata with as much justice as that this evaporimeter fails to respond in the same manner and degree to the various factors as the plant, aside from the effect of stomatal movement. The only conclusion permissible is that the blotting-paper atmometer represents with somewhat greater fidelity the water-loss from the plant when it responds freely to its environment than does the porous cup.

WATER-CONTENT.

Water-content acts upon stomatal movement by changing the rate at which water is supplied to the leaves. When humidity is high, therefore, a moderately low water-content may not produce any significant difference in movement as compared with that found in a plot of highly watered plants. But if humidity becomes low, temperature high, and sunlight intense, the same water-content may be critical and produce striking differences in movement. The Great Salt Lake region was peculiarly adapted to studies dealing with the divergence of movement caused by differences of water-content in relation to evaporation, as the rainfall is deficient, permitting the water-content to be readily controlled, and very humid days are found as well as very dry ones. Attempts to conduct similar experiments at the greenhouse of the University of Minnesota failed to show anything conclusively, since the humidity could not be lowered materially, or the much greater changes produced by deficient light be discounted. Hence, to be conclusive, such experiments must be conducted in the field in a dry, irrigated region.

Four plots of alfalfa plants were used in series 32, started at 7 p. m. August 25 and ended 7 p. m. August 26, 1919. The plants of the first plot were water-logged; the second was irrigated with 2 inches of water daily, beginning a week before the start of the series; the third had been irrigated the week before with 4 inches of water; and the fourth had not been irrigated for more than a month. The soil of plot 1 was saturated, plot 2 had a water-content of 32 per cent at the start of the series, plot 3 had 24.3 per cent, and plot 4 had 10.6 per cent. The plants of plot 1 presented a wilted appearance throughout the series, those of plots 2 and 3 were normal, while those of plot 4 wilted somewhat during the day and recovered at night. The weather conditions during the series were rather favorable, although the temperature rose to 89° F. at 2 p. m. August 26. A haze formed after sunrise and thickened into light clouds at 10 a. m., which floated away and disappeared by 1 p. m. At 2, 3, and 5 p. m. passing clouds reduced the light for short periods of time. The humidity was not extremely low, as often happened in the region, since the minimum was 25 per cent at 3 p. m., while the average for the day was over 35 per cent. The wind was slight, except between 8 and 9 a. m., when it rose to 2.16 miles per hour.

The stomata of the plants of plot 1 were closed throughout the series, as was expected from the appearance of the plants. Those of the plants in plot 3 were 17 per cent open at the start and closed completely by 9 p. m. They remained closed until 4 a. m., when the first light of dawn caused them to open slightly, reaching 10 per cent at 5 a. m. Sunrise then caused them to open smoothly and uniformly to maximum at 8 a. m., in which condition they remained until 4 p. m., when they started to close. They had decreased only to 85 per cent at 6 p. m., but the following hour they closed to 20 per cent. The stomata of plot 2, which had nearly saturated soil, showed stomata open from 10 to 25 per cent throughout the night, but closing completely at 4 a. m. The following hour they opened 20 per cent and then more rapidly until maximum was reached at 7 a. m. They remained wide open until 2 p. m., when, as usual, the plot was given 2 inches of water. In consequence, they commenced closing immediately and smoothly to 60 per cent at 4 p. m. By this time the water had soaked into the ground and the upper surface was drying slightly. The stomata remained at 60 per cent for an

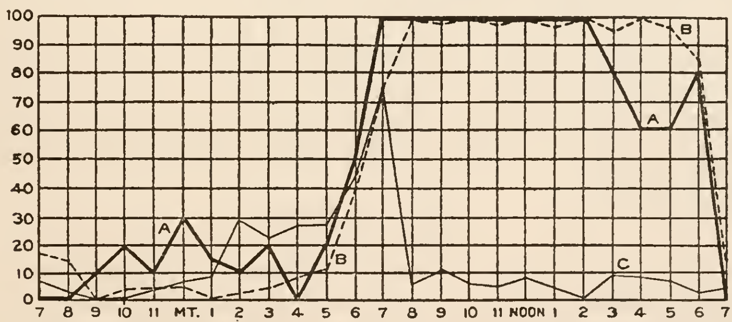


FIG. 36.—Effect of water-content on movement in upper stomata of alfalfa; irrigated daily with 2 in. of water (A), irrigated 7 days before with 4 in. (B), not irrigated (C).

hour and then opened to 80 per cent at 6 p. m. The next hour they closed completely. The stomata of the plants of plot 4 were closed at the start of the series and remained closed until 10 p. m. Then they opened very slowly, reaching 9 per cent at 1 a. m., thereafter more rapidly and irregularly to 28 per cent at 5 a. m. As a result of daylight, they opened to 45 per cent at 6 and 70 per cent at 7 a. m. The next hour they closed and remained closed until the end of the series, except for slight irregular openings characteristic of plants operating with deficient leaf-water (fig. 36).

The movement shown by the plants of plot 3 is characteristic of the normal light-induced behavior of alfalfa stomata under favorable conditions, save in one very minor point; the slight irregularity in maintaining maximum opening, especially toward the last, shows that evaporation was just on the verge of becoming critical. The higher water-content of plot 2 caused the stomata to show partial opening throughout the night. This is not characteristic of stomatal movement of the alfalfa group of plants under normal conditions, as found in the regions in which these experiments were conducted. It may be characteristic of such plants, however, in regions where there is a heavy rainfall each day. Maximum opening was reached by the plants of this plot an hour earlier than those of plot 3. The closure induced by irrigation may be attributed either to the cutting off of air from the roots, which seems improbable, or to chilling or shock inhibiting their functioning for a time. The wilting and continued closure of stomata of the plants of the first plot, however, was undoubtedly due to the cutting off of air to the roots, causing them to fail to function.

The stomata of the plants in the fourth plot showed the effect of very low water-content. The plants began to recover turgor after sundown, and the first effect was observable at 11 p. m., when slight opening of the stomata occurred. Not until after 1 a. m., however, was the water lost during the day fully replaced in the leaves. With the appearance of daylight the stomata of these plants began to open, as well as those of the other two plots, but much more slowly. At 7 a. m. the water-loss was becoming critical and the following hour the stomata closed. The water-content of this plot was so low that recovery of turgor was incomplete, as well as very much delayed. As shown in figure 9, when evaporation is great and water-content only moderately low, night opening occurs much earlier and is considerably greater.

LEAF TURGOR.

The amount of water in leaves normally changes throughout a 24-hour period. The changes are slight when water-content is high and evaporation low, but they are usually great with moderate water-content and high evaporation. The percentage of water present in the leaf at any time is determined by the rate of transpiration on the one hand and the rate of water-supply on the other. When the stomata open at daylight and the factors concerned in evaporation become more intense, the rate of water-supply soon falls behind that of transpiration. In consequence, the amount of water in the leaf begins to decrease, until the loss often becomes critical. At this time the stomata close partially or completely, and transpiration is diminished. If this reduces the rate of water-loss sufficiently, the leaves begin to regain turgor, and after a time the stomata reopen; otherwise, they remain closed until nightfall brings on a decrease in the intensity of the factors producing evaporation, and the turgor again rises.

A certain amount of the water present in a turgid leaf may be regarded as the working margin. The loss of part of this margin does not seem to interfere in any manner with the functioning of the leaf, and it is probable, even when it is wholly lost and the stomata are closing, that photosynthesis and other functions are still carried on, in part at least. Its presence, therefore, permits the stomata to remain open and carbon-dioxid absorption to go on for a time, in spite of excessive evaporation. As the amount of leaf-water changes not only during a 24-hour period but also from one rain or irrigation to another, this working margin also changes. The content of water in alfalfa leaves has been found to change from a maximum of 410 per cent of dry weight after several days of rain and high humidity to 290 per cent of the dry weight when on the verge of wilting as a result of low water-content a month later. As the critical minimum of leaf-water at which stomatal closure occurs varies in lesser proportion, there must be some means of adjustment to decrease in water-content by reduction of the amount of water with which a leaf can operate.

The failure to recognize the presence of this working margin of water in the leaf led Lloyd (1912) and Knight (1917*b*) to conclude that the stomata had no active part in keeping up leaf turgor. Lloyd concludes from his experiment that "decrease in water in the leaf occurs during the opening of the stomata. These organs are, therefore, not closely regulatory of the loss of water and are ineffectual in maintaining a constant supply of leaf-water." Knight states that "the experiments in the present paper have confirmed Lloyd's results," and later that "the stomatal aperture is not reduced by slight water deficiency in the leaf; hence the ordinary view that the stomata, by their response to incipient drying, are the chief factors in maintaining the water-content of the leaf is not tenable. On the other hand, the stomata are very sensitive to light changes, so that with increasing light intensity the stomata may continue to open, whilst the water-content of the leaf is decreasing."

The assumptions made by both writers seem to be that if it were not for the ineffectiveness of the stomata, the water present in the leaves would be constant, and that any decrease in the water contained in the leaf inhibits its normal functioning. The first assumption would then mean that the normal leaf water-content is constant, and that the same per cent of water is found night after night, and departure from this occurs only as a result of excessive transpiration during the day. This has not been found to be the case, however, as the nightly maximum fluctuates greatly from one rain or irrigation to another. The second assumption would mean that the plot of alfalfa plants, for instance, having the highest average of water in their leaves during each 24-hour period would exhibit the greatest growth and would produce the most dry matter. This again has not been found to be the case. The plants of plot 2 used in series 32, which was discussed in connection with water-content, showed a higher average per cent of leaf-water than did the plants of plot 3, but the plants of the latter showed the greatest increase of dry matter during the two weeks' observation. The greater decrease of leaf-water during the day of the plants in plot 3 can not be assumed to have interfered in any manner with their functioning. Hence, it may safely be said that fluctuations of leaf-water are normal and universal. When not so great as to induce closure of the stomata, they do not inhibit or in any other manner affect photosynthesis, translocation, or other functions of the leaf. Such fluctuation may be said to occur only in the working margin of the leaf-water.

Changes of leaf turgor offer the best explanation regarding the mechanism of mid-day closure and night opening. It has always been found that mid-day closure occurs when the leaf-water has been reduced to a point which is the safe minimum for a given water-content. The stomata do not reopen until the per cent of water rises once more above this point and the leaf again has a margin with which to safely operate. It is not known definitely whether this is due to the effect of loss of turgor causing reconversion of starch and thus producing closure, or whether evaporation concentrates the sap in the adjacent cells to a sufficient degree to cause exosmosis from the guard-cells. Inspection of the relation between the starch index curve and stomatal curve for series 10 (fig. 29) would indicate that both are possibly involved. Night opening occurs only in those leaves in which turgor is recovered faster than starch is stored in the guard-cells. It therefore seems plausible that the sap of the parenchymatous and adjacent epidermal cells is diluted more rapidly by this increase of turgor than that of the guard-cells is by the removal of sugar. This would then produce a relatively higher concentration of sap in the guard-cells and consequent opening of stomata, in spite of the fact that such concentration was decreasing. The great difference found by Iljin (1914) between the concentration of sap in the guard-cells and the surrounding epidermal cells would make it impossible for night opening to occur in this manner, but his results are undoubtedly too high. Wiggins (1921), repeating this work with apparently much greater care, found considerably less difference. Because he failed to disrupt the neighboring epidermal cells, however, the solutions producing plasmolysis of the guard-cells were probably diluted by passing through these adjacent cells, and hence his findings are also too high. It seems hardly possible, for example, that the guard-cells can lose their turgor, and cause closure while they still have a distinctly higher concentration than the adjacent cells. The solution of this problem therefore requires further investigation.

PLANT HABIT AND CONDITION.

The growth habit of a plant influences the stomatal movement by introducing differences in the relation of the rest of the plant to the stomata. Thus, the trees investigated did not exhibit mid-day closure, even on days when this was extreme in all the herbs studied. The greater depth to which tree roots penetrate would permit these to draw upon supplies of moisture not available to many other plants. This, however, does not explain why the Lombardy poplar showed no mid-day closure on a day when alfalfa exhibited extended and complete mid-day closure, since the roots of both plants reached the moist soil just above the water-table. As this type of behavior was found in all the trees studied, it is undoubtedly the result of their growth habit and may be attributed to the great amount of water present in the trunk, which acts as a reserve. Dendrograph studies carried on with *Pseudotsuga taxifolia* and *Pinus ponderosa* at the Alpine Laboratory show that the water present in the trunks probably moves with great rapidity up to the leaves.

Stomatal movement in herbaceous plants is subject to great variation as a rule, because the leaf has no reserve of water to draw upon. Hence, when the working margin has disappeared, it is necessary for such a leaf to restrict water-loss and reduce its functions accordingly. The thin-leaved plants naturally have a smaller margin of water than the thicker-leaved ones, and hence show earlier and more extended closure as a rule. With a moderate water-content such leaves may show several periods of day closure under extreme conditions of evaporation. Fleshy-leaved forms, on the other hand, rarely show more than one, and mid-day closure is accompanied by visible wilting in all the cases studied. All of the plants in the group with normally open stomata at night under optimum conditions are rather thick-leaved. As the stomata of alfalfa opened at night after normal day opening only when the water-content was abnormally high, night opening generally is due probably to a greater available supply of water in the thicker leaves. Some of the shrubs showed the same kind of behavior as the trees, but others exhibited some degree of mid-day closure at times, and are intermediate in behavior between trees and herbs.

The age or degree of maturity, as well as the growth habit of a plant, affects its stomatal movement. Young cabbage plants showed greater and longer continued opening during the day than plants ready to head. Ripening barley showed no movement at the time when plants started later and not yet headed out showed 4 hours opening during the morning. In series 32, two other plots were included with essentially the same water-content as plot 3, but in different stages of growth. In plot 6, the plants were 0.8 dm. high, in 7, 2.0 to 2.5 dm. high, and in plot 3 they had reached a height of 3.5 to 4.5 dm. and were beginning to flower. The differences found in stomatal movement were insignificant and readily explained by the slight differences in water-content and error of measurement. In series 34 the experiment was repeated. The new growth of the first plot averaged 0.7 dm., the second, 2.0 dm. and in the third plants were just starting to blossom. As the water-content was lower and evaporation higher, 2 hours of mid-day closure was found in the second plot, none in the first, and slight closure in the third. The greater proportion of roots to leaf area in the first plot explains its lack of closure, but the reason for the greater opening in the blossoming plants as compared with the half-grown plants is not so clear.

The age of the leaf also affects its stomatal movement. This was investigated in alfalfa, barley, corn, *Rumex patientia*, sweet clover, turnip, potato, and sugar-beet. In all these the stomata are the last of the epidermal cells, and probably of all the cells in the leaf, to be formed. With most of the plants they are formed at nearly the same time, potato being the one conspicuous exception. In consequence, they also begin to function at the same time, a few starting a day or two earlier than the others. At first, opening is slight and very brief, but after a few days it becomes more prolonged. However, a certain number are badly formed, resulting in mechanically or physiologically defective stomata which never open. About the time that the stomata approach maturity the granular protoplasm of the epidermal cells begins to disappear, and at maturity these are rather transparent and clear. The length of life of a mature leaf depends upon the species of the plant and the treatment to which it is subjected. Bruising by wind, injury by insects and fungi, exposure to extreme evaporation when the water-supply is deficient, drying out because of stomata wedged open by dust grains and other vicissitudes, often shorten its life. The larger number wither in the natural course of growth, the first visible indication of which is a slight yellowing. But it is usually unsafe to strip the next younger leaf on the stem, since its stomata have begun to function poorly and, before any very evident change of color occurs, they have closed permanently.

Other conditions in the leaf affect stomatal movement. The relation of the stomata of the two surfaces to the water-supply affects this movement, as shown in connection with sugar-beet. The number of stomata on the leaf and their size is a result of the degree of expansion of the leaf, which in turn was determined in part by weather conditions during development. Thus, a leaf of *Malva rotundifolia* had 241 stomata per square millimeter and averaging 6.1 μ. long, when developed during the hot, dry weather of the first part of July 1916, while leaves produced a month earlier had only 173 stomata per square millimeter, averaging 7.9 μ long. The ratio of stomata to other epidermal cells was the same, and hence the difference was merely one of expansion or the size of the cells. This expansion does not affect guard-cells and epidermal cells equally, since the average increase of area was 1.4 greater in the June leaves than those formed in July, while the area of each pore when wide open was 1.7 greater.

The diffusive capacity, $n\sqrt{ab}$, of the stomata when wide open was very nearly the same, that of the July leaves being 1,103 and the June leaves 966, a difference of less than 5 per cent. As this was the greatest difference found, and the diffusion capacity of the leaves usually checked within 1.6 per cent, there was no advantage in using this instead of the percentage of maximum opening, except where it was desired to compare the diffusion capacity of the different species other than stomatal movement. Hence, the difference in expansion has little or no effect upon the diffusion through the stomata, or upon the effect of stomatal movement upon such diffusion. The stomata of these larger leaves, however, are more sensitive to low humidity than those of the smaller leaves. It may be pointed out in this connection that Eckerson's figures for the number and size of stomata of various species apply only to greenhouse plants, as field plants differ greatly from these in most cases examined.

The effect of hairs, wax, and sunken stomata is to protect the leaves from water-loss to a certain degree. In the case of waxy leaves and leaves with sunken stomata, it obviously is impossible to determine the effect of these by direct comparison, but in the case of hairy leaves the hairs can be removed, at least in part. Two experiments were performed upon *Encelia farinosa* in March 1918, at Tucson, Arizona, comparing the movement in "shaved" and in normal leaves. The woolly hairs of a number of leaves were reduced as much as possible with a safety razor. On the following day a short series was made, beginning at 8 a. m. and ending at noon. The stomata of the shaved leaves were wide open when the series started and remained at maximum 1 hour. At 10 a. m. they had closed to 60 per cent, at 11 a. m. to 20 per cent; at noon closure was complete. The stomata of the normal leaves were only 70 per cent open at 8 a. m., opened to maximum at 9 a. m., and remained in this condition to the end of the series. The second experiment showed essentially similar results. The removal of most of the hairs increased the amount of light reaching the stomata; this accounts for the earlier opening in the shaved leaves. The closure before noon may be ascribed to a greater water-loss from the leaves when the hairs were removed. Inspection of the leaves supported this view, but unfortunately no determination could be made of the percentage of water in the shaved and normal leaves at the time. Nevertheless, the stomatal closure and less turgid appeareance at noon of the shaved leaves is evidence that hairs, especially when as dense as on the leaves of this plant, are of considerable value in reducing water-loss.

Plants adjust and adapt themselves to their environment in a number of ways, one of which is stomatal behavior. The hydrophytes studied had permanently open stomata, since they usually have no need of the mobile kind. In fact, night closure would probably be disadvantageous to a plant such as *Scirpus validus*, since diffusion of gases through the air-passages to the submerged parts must necessarily be slow and is therefore continued throughout the night.

Plants which grow normally in a very humid region, where the weather rarely becomes hot and dry and the water-content low, do not show the rapid response to evaporation that a plant developed in a more arid habitat does. Thus, alfalfa shows a greater response to low humidity than potato or sugar-beet, and a greater response than even the white-flowered sweet clover (*Melilotus alba*), which has very similar leaves and stomata. The plants of the arid regions have generally adapted themselves in one of two ways, some functioning at all times, and others only during the rainy season. *Opuntia versicolor* belongs to the former class (E. B. Shreve, 1916), its stomata functioning normally in the manner that alfalfa stomata do under extreme conditions. *Fouquiera splendens*, on the other hand, produces a crop of leaves when opportunity permits, which function until the water-supply runs low, in the same manner as a mesophyte under optimum conditions. When the water-content runs low the stomata begin to show less and less opening, until toward the last, when the leaves begin to change color, they open very little, and for only a short time each day. This behavior duplicates that of an aging leaf of alfalfa. *Encelia farinosa*, which tends to retain some leaves at all times, shows behavior much like that of *Fouquiera* when conditions are favorable, and somewhat the same behavior as *Opuntia versicolor* when these are unfavorable. Hence, it is intermediate between the two, especially since it drops most of its leaves upon approach of unfavorable conditions, and those that remain probably show opening only at night, if at all.

SUMMARY.

1. Light induces opening of the stomata after daybreak by initiating conversion of the starch within the guard-cells into sugar. This increases the osmotic pressure of the guard-cells, which in turn causes the increase of turgor necessary to produce opening. The starch-content of the guard-cells never wholly disappears, but usually is at its lowest about 10 a. m. When no other factor influences the movement, the starch-content rises from this time until just before daylight the next morning. During the middle part of the day and until shortly before the stomata start to close, the rise in the starch-content is very slow, but is rapid during closure. After

closure the rate of increase again becomes slow and is further retarded during the night.

2. Changes of movement caused by factors other than light are not necessarily accompanied by corresponding changes in the starch-content of the guard-cells.

3. Reduction of light to less than half of normal is usually necessary to produce any effect upon the stomata of plants growing in the open. When the stomata are closing, they respond to decrease of light more rapidly than when opening.

4. Stomata open at night as a result of moonlight or a strong artificial light of much less intensity than 1 per cent of the sunlight maximum. They open more readily toward morning than before midnight, and hence at night also the reactions which tend to produce opening or closure are more readily hastened than reversed.

5. The temperature of the air affects the speed at which the stomata open during the morning. The length of time required for opening is reduced by approximately one-half for every 10° C. rise in temperature. This, of course, occurs only within the limits of temperature at which protoplasm functions.

6. When the temperature of the soil rises too much the stomata close, and in extreme cases the plant wilts.

7. The temperature of the leaves was usually found to be lower than that of the air when the stomata were open, and higher when closed in sunlight.

8. A high humidity of the air permits the stomata to open wider and remain open longer than a low humidity under most conditions. This is especially true when the water-content has decreased seriously and to a point where the plant has great difficulty in obtaining sufficient water to meet evaporation during the day.

9. No atmometer or evaporimeter was found that would measure at all accurately the effect upon the plant of all the factors concerned in evaporation.

10. When the leaves of a plant were wet by dew or rain, or wet artificially, the stomata usually opened if closed, or opened more widely if partially open. When the water dried the stomata closed wholly or partially.

11. Wind caused increases of transpiration unlike the increases of evaporation as measured by atmometers. In the main, the plant showed much less response to wind than the atmometer, but with the sudden advent of a high wind it often showed greater response.

12. Wind carries dust to the leaves, coating them and often wedging particles into the open stomatal slits, so that they remain permanently open.

13. Water-content is the chief of the factors that determine the rate at which the leaves can be supplied with water. When the soil is dry the rate of supply is slow, causing turgor to be lost early in the day and the stomata to close.

14. Water-logging the soil causes closure of the stomata and subsequent wilting by inhibiting the functioning of the roots.

15. The maximum of leaf turgor in most cases is reached about midnight. After stomatal opening at daybreak, or shortly afterward, it begins to decrease, the rate being dependent upon transpiration on the one hand and water-supply on the other. Part of the water present in the leaf at the start is a working margin and the stomata do not close until this is gone. If the roots can keep up a sufficient rate of supply this working margin does not wholly disappear during the day and the stomatal movement is of the normal light-induced type. If the margin is lost, however, the stomata close until it is recovered, at least in part.

16. The nightly maximum of leaf turgor increases after every rain or irrigation and thereafter decreases until the next rain. Such fluctuation between rains is often very great. In lesser degree, the critical leaf-water or percentage at which the working margin disappears also fluctuates between rains or irrigations.

17. The degree of succulence of a leaf determines to a large extent the amount of water present as a working margin, and therefore the readiness with which the stomata close when transpiration is high.

18. The growth habit of a plant often affects stomatal movement by reserves of water to increase the rate of supply during periods when loss is greatest and by roots which penetrate to permanently moist soil.

19. The age or degree of maturity of the plant affects its stomatal movement, and the age of each leaf influences the readiness with which the stomata on it function.

20. 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 area than one produced in sunlight.

21. The removal of the hairs from the leaf of a plant with very hairy leaves caused the stomata to open somewhat earlier upon the appearance of light and close very much sooner, showing that the water-loss from such a leaf was probably much higher than from the normal leaves.

22. Plants of desert regions have adapted themselves to their surroundings in two ways, so far as stomatal movement is concerned. Some produce a crop of leaves when opportunity permits, which wither and disappear when the water-supply becomes critical. Such leaves are mesophytic in type as a rule, and show no specialization for desert conditions. Other plants have persistent leaves, or stems adapted for photosynthetic work, which show considerable adaptation to desert conditions, hairs, wax, sunken stomata, and other means being used to reduce water-loss. Such plants often normally show a reversal of the usual behavior of the stomata, opening occurring at night and closure during the day, as in many mesophytes when exposed to similar arid conditions.

III. THE EFFECT OF STOMATAL MOVEMENT UPON TRANSPIRATION.

Indirect evidence that stomatal movement affects the water-loss of plants had gradually accumulated during the course of the experiments. This evidence, however, was not conclusive and in some instances was capable of several interpretations. For this reason a number of experiments were made to discover the relationship between stomatal movement and transpiration, and to find some reason for divergence of views concerning the effectiveness of the stomatal regulation of water-loss. It seemed inconceivable that complete closure of the stomata should fail to reduce the rate of transpiration to a considerable extent, as Buscalioni and Polacci (1902) have confirmed the earlier results as to the relative insignificance of cuticular transpiration by showing that a collodion film placed on a leaf clouded quickly just above the stomata only. In the course of several series, plants were often found to wilt visibly during the early forenoon, and the stomata to close, as a consequence of which they recovered turgor by afternoon, when the stomata again opened. The recovery of turgor during the part of the day when evaporation was greatest must be ascribed to closure of the stomata. The commonly accepted view that stomata are not regulatory was not in accord with these facts, but further investigation was imperative to fully explain the divergence of views.

As a result of the researches of Mohl (1856), Merget (1873), Schwendener (1881), Leitgeb (1888), Stahl (1894), Kohl (1895), Darwin (1898), and many others, the mechanism of the stomata was demonstrated, and they were shown to be the outlets for water-vapor and hence of the greatest importance in transpiration. In consequence, the view came to be held that the stomata by their movements completely controlled the water-loss from the plant, except for the inconsiderable amount evaporated from the cuticle. It was apparently believed that the air-spaces of the leaf contained a water-saturated gas with but little CO_2 and the vapor diffused out through the stomata as the CO_2 passed in. Under such conditions the evaporating power of the air, especially when moving, would have little effect upon transpiration and the stomata would be wholly regulatory. Brown and Escombe (1900), however, showed that the theoretical diffusion of water-vapor from a leaf of *Helianthus annuus* was about six times as great as that actually lost by the plant. The full significance of this discovery was overlooked, however, in view of the expressed doubt that the stomata were of sufficient size and number to permit such large amounts of CO_2 and water-vapor to pass through by diffusion alone. The authors showed that no theory of streaming currents into the leaf or from it was needed. Lloyd (1908) apparently was the first to point out that since this rate of diffusion is much greater than the rate of transpiration, the gas inside the leaf can not be fully saturated:

"The actual rate of diffusion through stomata will depend upon the length of the tube, gradation of density of the water-vapor between the surface of the cells of the chlorenchyma and the outer air, modified by air-currents. It seems clear from Brown and Escombe's experiment that there exists such a gradation of density, from which it follows that under conditions of rapid transpiration the vapor-pressure within the leaf cavities is less than when a low rate occurs, assuming the evaporation capacity of the cells to be constant. As in the case of CO_2, the pressure of the water-vapor within the leaf, though much greater, must vary with the size of the stomatal pores, but here the relative humidity without is a very variable factor and will, therefore, modify the rate of transpiration independently of the pores."

In this Lloyd summed up the entire problem of the water-loss from a plant-leaf.

The problem of stomatal regulation is therefore complicated by several conditions, the chief of which is the changing vapor-pressure of the air-spaces under different conditions of stomatal opening, relative humidity, wind, radiant energy, and air-temperature. Trelease and Livingston (1916) believe that changes in the chlorenchyma reduce transpiration before stomatal closure occurs. As this belief is based upon the "relative transpiration" calculated from the evaporation-rate of the porous-cup atmometer, it has not been demonstrated conclusively. Nevertheless, it is probable that this condition also enters into the problem and adds its complications to it. The problem of stomatal regulation of transpiration, therefore, can not be confined to the thesis that small changes of apertures produce like changes in the absolute rate of transpiration. Stomata must be considered definitely regulatory, if changes in their openings produce similar changes in the effectiveness upon the leaf of the evaporating power of the air.

The problem was attacked from another angle by Muenscher (1915), who compared the water-loss from 9 species of plants with the size and number of their stomata. While the investigation apparently showed no relationship between transpiration and stomatal opening, the results are vitiated by the failure to take into account the length of time the stomata of each plant were open. The following table is taken from his paper.

Table 3. *Relation between amount of transpiration and stomatal aperture (from Muenscher).*

	Helianthus annuus.	Zea mays.
Linear units of stomatal pore in microns per sq. mm...	2,056	1,530
Amount of transpiration per sq. dm. per hour in milligrams.	156	80
Area of stomatal apertures per sq. mm. leaf surface.....	6,433	3,864

The ratio of 2,056 linear units in sunflower to 1,530 linear units in corn is approximately 4 : 3, while the transpiration per unit surface of sunflower is nearly twice that of corn. Only when the assumption is made that the stomata of both species of plants are open all the time, or are open equally for the same period, can this be taken as evidence that the diffusion capacity of the stomata does not regulate transpiration. Muenscher's plants were under far different conditions of environment from those used in this investigation, but a recalculation of results based upon the present findings is of interest. A series containing both *Zea mays* and *Helianthus annuus* growing under the same conditions showed the stomata of corn open approximately 8 hours and those of sunflower 12 hours. If this was true for Muenscher's plants, disregarding cuticular water-loss, the amount transpired during a 24-hour period must have been lost largely during 12 hours in *Helianthus* and 8 hours in *Zea mays*. The hourly water-loss for *Helianthus* would then be 312 mg. and for *Zea mays* 240 mg. per hour per square decimeter. The ratio of linear units for sunflower and corn is approximately 20 : 15 and the ratio of hourly transpiration recalculated in this manner 312 : 240, both of which reduce approximately to 4 : 3. Correction for cuticular water-loss would undoubtedly make the agreement very close. However, this explanation is only of theoretical interest; the preceding sections have shown the futility of attempting to estimate the stomatal behavior of a plant in an unknown environment. It does show, however, why Muenscher can not be considered to have produced valid evidence to the effect that "the amount of transpiration is not governed entirely by stomatal regulation."

In spite of the care with which Lloyd (1908) carried out his experiments, and the clearness of his analysis of the problem, he can not be said to have proved the case against stomatal regulation with any greater success. He realized that the use of potometers to measure transpiration was the weak part of his investigation, but did not check them sufficiently to ascertain where this weakness lay. His discovery that the amount of water lost from the stem and leaves was not the same as that absorbed possibly caused him to overlook the very important fact that the stomatal movement in the leaves of a cut stem is not at all like that of the leaves of a field plant or even a potted plant. Hence Lloyd assumed inadvertently the very thing he attempted to prove, namely, that stomatal movement had no effect upon transpiration.

To gain a clearer idea of the reliability of cut stems as a measure of water-loss from rooted plants, an alfalfa series was made on August 8, 1916. A battery of potometers was set up and a preliminary experiment carried out on August 5 to determine whether alfalfa stems treated in this manner would check with one another in regard to their transpiration (plate 11). The burette in each case was connected through a glass tee to the plant on one hand and a flask used as a reservoir on the other. A pinch-cock permitted the refilling of the burette from the reservoir after each reading; this constantly maintained the water-pressure upon the cut end of the stem at nearly the same level. Other precautions were observed; the entire apparatus was sterilized with 4 per cent formalin, and the water used was recently distilled and boiled just before using. The stem of the plant used was sterilized at the point desired, and cut under water with a sterile knife. The cut end of the stem was protected by a loose plug of absorbent cotton. These potometers were run 28 hours and read at half-hour and hour intervals during the two days and at the end of a 10-hour night interval. Since the readings ran in parallel series, the test was considered satisfactory. The stems used were then discarded and the apparatus resterilized.

At 4 p. m. on August 8, the potometers were started with fresh stems, which were gathered with all the precautions observed when making the trial. Readings were made at half-hour intervals until 7 p. m. to insure that the potometers were working properly. As it was found to be impossible to check the transpiration from the cut stems against that of field plants, or even of potted plants, it was decided to compare the stomatal movement of such cut stems with the stomatal movement of field plants. For this purpose, 30 branches of alfalfa were cut under water with the usual precautions. These were placed in flasks of the same kind of water used in the burettes. In order to find whether the head of water against the cut ends of the stems in the potometers had some effect upon the stomatal movement not found in those in the flasks, several stems were treated in the same manner as those in the potometers, using plain glass tubing instead of burettes. A sufficient number of these were set up so that strips could be taken at 4-hour intervals during the series. As may be expected, the increased water-pressure on the cut ends did not have sufficient effect to make a discernible difference in the behavior of the stomata. The leaves of one stem were stripped each hour and the stem immediately discarded to prevent its being used again.

A slight and misty rain occurred between 10 and 11 p. m., but by midnight the weather had cleared. At 1 a. m. the wind had risen and was drying the vegetation. By 2 a. m., when the wind died down, all effects of the rain had disappeared, and the night remained clear and still from that time. The following day was warm, practically cloudless, and more humid than ordinarily, since the relative humidity did not fall below 28 per cent. Although it was a sunny day, haze prevented the light from reaching more than 75 per cent of the maximum intensity for the region. As usual, the lowest temperature, 55° F., was recorded at 5 a. m., just before dawn. The highest was 78° F., occurring at 3 p. m. the same day (fig. 37).

The difference in the stomatal movement of the cut stems and the watered field plants was striking. The watered plants showed 15 and 20 per cent opening from 10 p. m. until 1 a. m., and closure from then to dawn. This was largely the result of the slight rain which

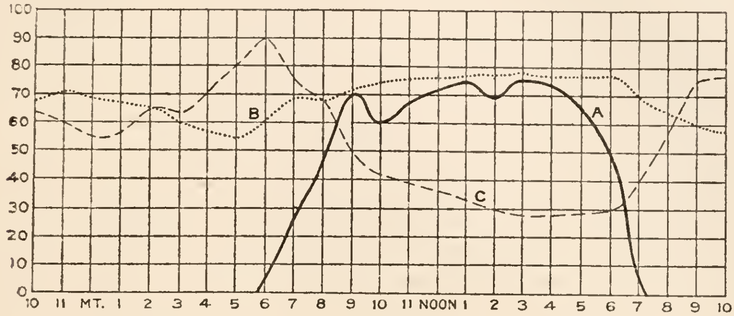


FIG. 37.—Series 17, weather data for August 8–9, 1916; sunlight (A), temperature (B), humidity (C).

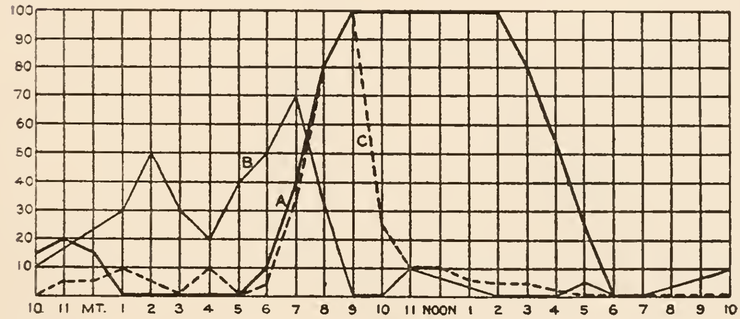


FIG. 38.—Series 17, showing average movement of alfalfa stomata in watered (A) and unwatered (B) field plants, and in cut stems (C).

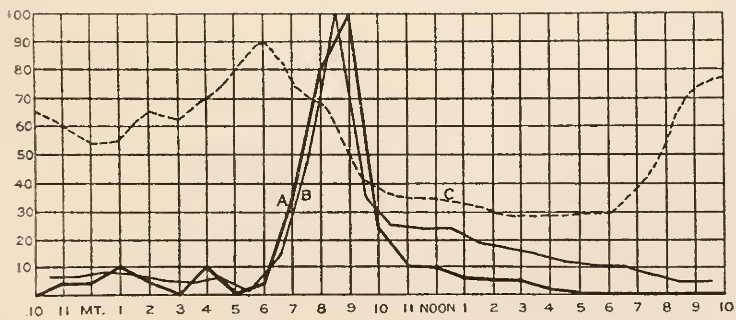


FIG. 39.—Series 17, showing stomatal movement (A), and transpiration (B) in cut stems of alfalfa; humidity (C).

occurred after 10 p. m. The cut stems had closed stomata at 10 p. m., but they opened slowly to 10 per cent at 1 a. m. and then began closing. At 3 a. m. this was complete. The next hour, however, they opened 10 per cent and closed again at 5 a. m. The sun appeared through the clouds over the mountains at 5^h 45^m a. m., but it began to be light shortly after 5 a. m. In consequence, the stomata of the field plants opened 10 per cent at 6 a. m., 40 per cent at 7, 80 per cent at 8, and were wide open by 9 a. m. The stomata of the cut stems acted in practically the same manner, except that opening was somewhat slower at the start. Maximum opening lasted until 2 p. m. in the field plants. The stomata of the cut stems, however, closed immediately to 25 per cent at 10 a. m., 10 per cent at 11 a. m. and noon, and then slowly to 5 per cent at 2 p. m. They remained in this condition until 3 p. m., and then closed very slowly and completely by 5 p. m., remaining closed to the end of the series. The watered field plants started to close after 2 p. m. This continued uniformly throughout the afternoon, and was completed at 6 p. m. (fig. 38).

The stomatal movement of the unwatered field plants showed even greater divergence from that of the cut stems. The considerable night opening found in these plants did not occur in the cut stems, and the maximum opening at 7 a. m. was 2 hours earlier than that found in either the heavily watered field plants or the cut stems. Complete closure occurred at 9 a. m., when the stomata of the cut stems and watered plants had reached maximum opening (fig. 38). It must be concluded, therefore, that the water-loss from alfalfa potometers can not be used as a measure of the water-loss from naturally growing plants, nor can stomatal regulation be judged ineffective by reason of such comparison.

The transpiration of the cut stems as measured in the potometers is distinctly controlled by stomatal movement. For several reasons, the correlation between the rate of water-loss and the degree of stomatal opening is among the best found in these experiments. The complications entering into the problem by sudden changes of wind velocity, wide fluctuations of relative humidity within brief intervals, and sudden changes of sunlight caused by passing clouds did not occur. In addition, the difference between the maximum and minimum temperatures was not nearly as great as usual, changes were slow and gradual, and the temperature remarkably constant during the greater part of the day. The stomatal opening described in connection with transpiration in this section is the average for both surfaces of the leaf, calculated for the number of stomata per unit area on each surface.

Between 10 and 11 p. m. the average water-loss from the alfalfa potometers or, more accurately, the water absorbed, was 7.0 mg. per minute per square decimeter of leaf-surface, which is the upper and lower surface of 0.5 square decimeter of leaf area. During this time the stomata opened to 4 per cent, most of the opening occurring in the upper surface. During the next hour the water-loss was

6.7 mg. per minute, while the stomata remained at 5 per cent opening. From midnight to 1 a. m. the average loss was 8.7 mg. per minute, corresponding to an increase to 10 per cent opening of the stomata. Between 1 and 2 a. m. the rate of loss decreased to 7.5 mg. per minute, while the stomata closed to 5 per cent. A fall in the rate to 4.5 mg. during the next hour occurred as the stomata closed. As the stomata opened to 10 per cent at 4 a. m. the average rate of transpiration was 4.2 mg., but as a result of this opening it rose to 6.7 mg. between 4 and 5 a. m. At 5 a. m. the stomata closed again and the rate of loss dropped to 1.5 mg. This very low rate was also due to the rise of relative humidity to 80 and 90 per cent. Between 6 and 7 a. m. the stomata opened from 5 to 35 per cent and the rate of transpiration rose to 13.5 mg. The following hour opening increased from 35 to 80 per cent, relative humidity fell below 70 per cent, and, in consequence, the rate increased to 47.5 mg. per minute. Between 8 and 9 a. m. the stomata opened from 80 per cent to maximum, and the rate of loss to 100 mg. per minute. The next hour the stomata closed to 25 per cent and transpiration fell to 37.0 mg. This was in spite of the fact that relative humidity had fallen to 46 per cent and sunlight had reached the average maximum for the day. Further closure to 10 per cent at 11 a. m. was accompanied by a fall to 26.7 mg. per minute. From this time slow closure of the stomata, completed at 5 p. m., was accompanied by a similar fall in the transpiration-rate. The stomata were closed from 5 to 10 p. m., at which time the series ended. The rate of water-loss remained rather constant from 5 to 7 p. m., since the relative humidity was also as constant, but after 7 p. m. the humidity rose rapidly and the transpiration-rate again fell. Between 9 and 10 p. m., when the humidity again became fairly stable, changing only from 74 to 76 per cent, transpiration decreased only from 4.7 to 4.5 mg. per minute.

The correlation in this experiment between the stomatal movement and the rate of transpiration of cut stems is remarkably clear. Where there is deviation in the rate of water-loss from the changes of stomatal opening this is clearly due to fluctuations of relative humidity. When the fluctuations are rapid and irregular they introduce complications which are hard to measure. But in this experiment the humidity changed slowly and rather uniformly, and was nearly constant for long periods. Because this did not occur in the other experiments, and because of other reasons, the later series failed to show as clear a relation between stomatal movement and transpiration. The stomatal movement of the field plants, watered and unwatered, did not in any manner resemble that of the cut stems, and the transpiration from the potometers in turn showed no relationship to the changes in stomatal opening of these plants. For both these reasons, in the case of alfalfa at least, potometers can not be used to represent the transpiration of rooted plants.

Another series was made with the Russet Burbank potato instead of alfalfa, in order to determine the reliability of potometers in the case of this species. The potometers were fitted up with the same care observed in the earlier series in which alfalfa was used. In this case the stomatal movement of the cut stems was compared with that of potted plants, as well as with watered and unwatered field plants, to discover if the cut stems would show movement more nearly resembling that found in the potted plants than the field plants. As no scales of sufficient capacity were available to weigh the very large pots closely enough to measure their water-loss, only the transpiration from the cut stems was measured. The experiment (series 20) was started at noon on August 25 and ended at 1 p. m. on August 26, 1916. The potometers were set up and the first readings made at 10 a. m. on the 25th, in order to find whether each was working properly, before the series was begun. As the readings ran in a parallel series, they were considered as functioning properly, and each potometer read to the end of the experiment the next day. As in the case of the alfalfa plants, the leaves were then clipped off and printed on Solio paper, and the total area determined for each stem separately by means of a planimeter. An error enters in at this point, because the stems also have functional stomata, but their number is not great and they can not have much more influence than cuticular water-loss. As the area of stem-surface in proportion to leaf-area was nearly the same in each potometer, the effect of the stem-surface could not be determined. Hence, it would be more accurate to state that the water-loss given was that from 1 sq. dm. of leaf-surface and 1.62 sq. cm. stem-surface. As, however, no attempt is made to compare the transpiration of several plants, the unit of area from which the water-loss occurs is immaterial, providing it is not changed during the experiment. The real problem is whether the changes in the rate of transpiration from some given area, regardless of its size, shows any relationship to changes in the stomatal openings.

The temperature, relative humidity, and sunlight fluctuated a great deal during the experiment. The two days were rather hot, the highest temperatures being 87° F. at 3 p. m. on August 25 and 88° F. at 1 p. m. on the 26th. As usual, the lowest temperature, 57.5° F., was recorded at 5 a. m., just before dawn. The humidity dropped to 19 per cent at 4 and 5 p. m. on the 25th, about one-third lower than that recorded during the alfalfa series. Inconstant winds caused irregular decreases of humidity during the night, and the maximum of 71 per cent occurred several times. During the greater part of the two days there was a gentle breeze, with occasional gusts of strong winds, which increased transpiration during short intervals. There were occasional periods of calm, which also affected water-loss. Sunlight was very variable because of changing haziness. On

August 25 there was 65 per cent light at noon, which decreased to 53 per cent at 2 p. m. At 3 p. m. the intensity had increased to 60 per cent and then decreased uniformly until sundown at 7 p. m. The sun rose above the mountains shortly after 6 a. m. August 26, but clouds prevented the light from reaching more than 4 per cent at 7 a. m. At $7^h\ 30^m$ it was 9 per cent, 28 per cent at 8, and 43 per cent at $8^h\ 25^m$ a. m. At 9 it reached 55 per cent, but deepening haze prevented a rapid increase in intensity for a time. At noon the haze began to clear, and at 1 p. m. the light reached 88 per cent, the maximum during the period of the experiment (fig. 40).

All the plants, except the heavily watered field plants, suffered from excessive water-loss. The cut stems had open stomata from 10 a. m. until 1 p. m., when the lower stomata started to close, reaching 15 per cent at 2 p. m., while no change occurred in the upper. At 3 p. m. the upper closed to one-half of their maximum, while the lower opened to 25 per cent. The upper closed to 40 per cent and the lower to 20 per cent at 4 p. m. At 5 p. m. the lower closed to 10 per cent and the upper to 20 per cent. At 6 p. m. the lower opened

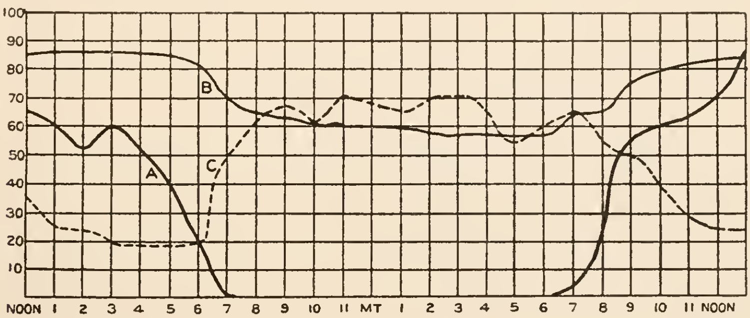


Fig. 40.—Series 20, weather data for August 25–26, 1916; sunlight (A), temperature (B), humidity (C).

to 20 per cent, while the upper remained in the same condition. At 7 p. m. the upper opened to 25 per cent and the lower closed to 10 per cent. At 8 p. m. the lower stomata closed, while the upper were 20 per cent open. At 9 p. m. the upper closed to 15 per cent and at 10 p. m. closed completely. At this time, however, the lower opened 3 per cent, and 5 per cent at 11 p. m. By midnight all closed, except occasional stomata in both surfaces, producing 1 or 2 per cent opening throughout the rest of the night. At 6 a. m. the upper stomata opened 10 per cent, and 20 per cent at 7 a. m. At 9 a. m. the lower opened 5 per cent, while the upper remained in the same condition. At 10 a. m. the lower closed to 2 per cent, in which condition they remained until noon. The upper closed to 15 per cent at this time, then to 5 per cent at 11 a. m. and remained in this condition until noon. At 1 p. m. the stomata of both surfaces closed completely (fig. 41).

The stomata of the potted plants were wide open at the start and remained open an hour. The lower stomata then closed completely by 3 p. m., while the upper stomata closed to 20 per cent at 5 p. m. No further change took place in either surface until after 7 p. m. At 8 p. m. the upper started to reopen slightly, reaching 30 per cent at 10 p. m. At midnight further opening again took place, the upper showing 50 per cent opening. At 1 a. m. the lower showed 20 per cent opening, and the upper 80 per cent; at 2 a. m. the lower were half open and the upper at maximum. At 3 a. m. the stomata of both surfaces were wide open, and so remained to the end of the series.

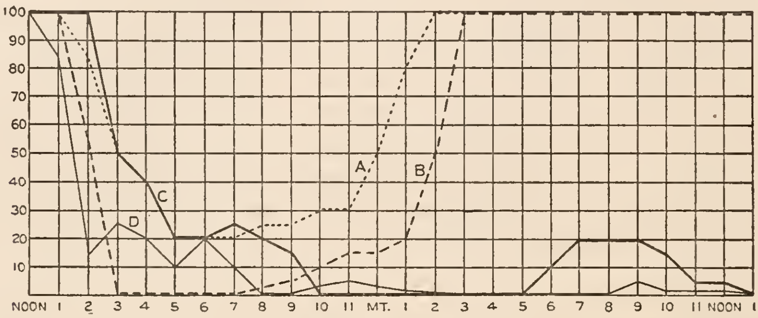


Fig. 41.—Series 20, showing movement in upper (A) and lower (B) stomata of potted potato plants, and in upper (C) and lower (D) stomata of cut potato stems.

The stomata of the watered field plants were wide open until 4 p. m., when the lower began to close. At 5 p. m. the upper also showed closure to 80 per cent, while the lower were but 40 per cent. At 6 p. m. the lower were closed and the upper were 50 per cent open. At 7 p. m. the upper closed to 30 per cent, and at 8 p. m. closed completely. At midnight the upper showed 50 per cent opening, and complete opening the following hour. The lower were but 30 per cent open at midnight, 60 per cent at 1 a. m. and wide open at 2 a. m. They remained at maximum to the end of the series. The unwatered field plants had closed stomata when the series began. At 2 p. m. the lower opened 10 per cent, closed to 5 per cent at 3, and opened to 15 per cent at 4 p. m. At this time the upper opened to 20 per cent, and to 30 per cent at 5 p. m., while the lower closed to 5 per cent at this time. At 6 p. m. the lower closed completely and the upper to 15 per cent. At 7 p. m. both were closed, but at 8 the upper reopened 5 per cent. At 9 p. m. the lower opened 10 per cent, while the upper reached 25 per cent. At 10 p. m. both surfaces showed 20 per cent opening, and closure at 11 p. m. At midnight the stomata of the upper surface opened 25 per cent, those of the lower 10 per cent, and at 1 a. m. the upper reached 60 per cent and the lower 30 per cent. At 2 a. m. the upper were 80 per cent open, but closed to 70 per cent the following hour. In the meantime, the lower opened to 50 per cent at 2 a. m. and 70 per cent at 3. At 4 a. m. both surfaces showed 50 per cent opening, and remained in this condition for an hour. At 6 a. m. the lower were but 40 per cent open, while the upper opened to 90 per cent. At 7 a. m. the lower opened to 70 per cent, while the upper were wide open. At 8 a. m. the stomata of both surfaces were wide open and so remained until 10 a. m., when the lower closed to 70 per cent. At 11 a. m. the upper closed to 90 per cent and the lower to 30 per cent. At noon all the stomata were closed and remained closed to the end of the series (fig. 42).

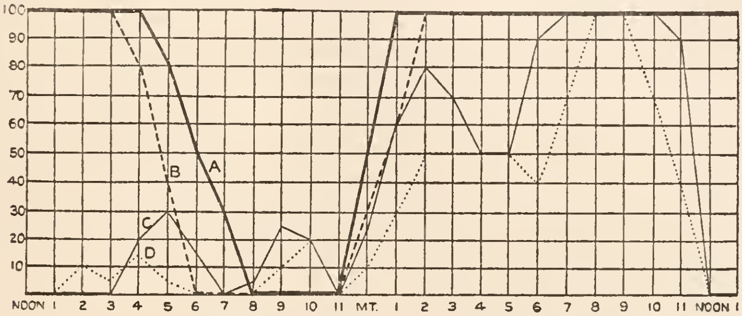


Fig. 42.—Series 20, showing movement in upper (A) and lower (B) stomata of heavily watered potato plants, and in upper (C) and lower (D) stomata of plants in very dry soil.

Since the stomatal movement of the cut stems was very different from that of the other plants, it is clear that the water-loss shown by the potometers can not be considered representative of the transpiration from any field plants or watered potted plant. Moreover, because of the difference found in the stomatal movement of heavily watered potted and field plants, the transpiration from a plant in a sealed pot can not, under these conditions, be considered to be the same as that from a field plant. The plants, however, were under conditions of high evaporation, high temperature, and very high radiation. The potted plants were influenced by the increased temperature of the soil-mass, due to the action of radiant heat. The effect of extreme insolation is shown especially by the cut stems, including those in the potometers, which did not recover turgor during the night. Similar potometers kept in the shade functioned a week before being seriously affected by bacterial action. This experiment and the care taken to prevent bacterial infection of the stems and the consequent occlusion of the vessels tend to show that wilting was not due to this cause.

As in the case of alfalfa, the stomatal openings found on the upper and lower surfaces of the leaves were averaged in proportion to the number of stomata present per unit area for comparison with the rate of transpiration. The changes found in one correspond fairly well with those found in the other, especially when the probable effect of sunlight and relative humidity are taken into consideration. The agreement, however, is not so close as in the alfalfa experiment for several reasons, in addition to those already enumerated. Later experiments as well indicate that changes in the degree of stomatal opening are not nearly so effective in producing changes in transpiration when near the maximum opening as when approaching closure. Moreover, in this as well as in other experiments, the stomata of the two surfaces do not have an equal effect upon the control of transpiration in a leaf having unlike stomata upon the upper and lower surfaces. Hence, the rate of water-loss corresponds more nearly at times to the movement found in the lower surface than to the average stomatal movement. As the ratio of upper stomata to lower was 3 : 20 per unit area in this variety, the effect of the upper stomata upon the calculated average was not large. If, in addition to the use of this ratio, all changes of opening above 50 per cent of the maximum had been ignored, the effectiveness of stomatal movement upon transpiration would probably have been shown with greater accuracy (fig. 43).

The water-loss from noon to 1 p. m. on August 25 was 91.6 mg. per minute. Stomatal opening during this time changed from 100 to 91 per cent. A sudden reduction to 15 per cent in the lower

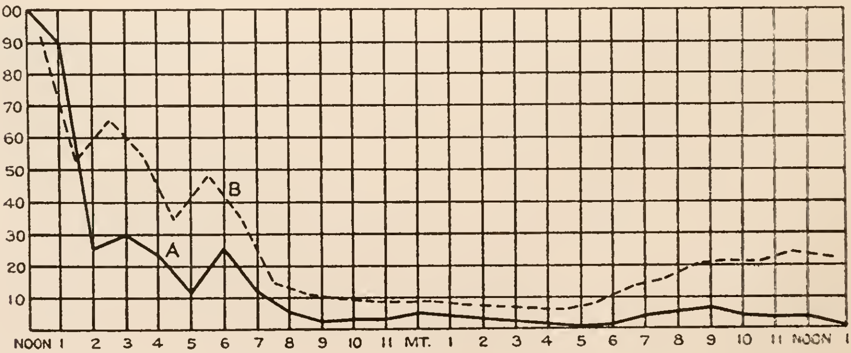


Fig. 43.—Series 20, showing average stomatal movement (A) and transpiration (B) in cut stems of potato.

stomata at 2 p. m. brought average opening to 26 per cent and decreased the water-loss to 52.5 mg. The next hour the lower stomata opened to 25 per cent, while the average of opening rose only to 28 per cent, since the upper stomata closed 50 per cent, The transpiration-rate rose to 64.7 mg., which would indicate that the change from maximum to half opening in the upper stomata did not have nearly the effect upon the rate of water-loss that it had upon the calculated average stomatal movement. At 4 p. m. the average dropped to 22.6 per cent, while during the hour transpiration fell to 55.4 mg. per minute. Closure to 11 per cent was accompanied by a fall in the rate to 35.9 mg. per minute. The stomata then opened to 20 per cent in both surfaces, and the rate rose to 47.8 mg. Closure to 12 per cent at 7 caused a falling off to 36.4 mg. From this time there was a slow closure of stomata and a very slow falling off in the transpiration-rate. A slight increase in opening from 2.6 per cent to 4.3 per cent at midnight probably caused the slight increase in the rate of water-loss from 8.5 mg. to 8.8 mg. at midnight. At 5 a. m. all the stomata were closed, and the minimum rate of 7.2 mg. was recorded at that hour. At 7 a. m. the stomata opened 3 per cent and the rate rose to 12.6 mg., due in part to the decrease in relative humidity. At 8 a. m. average opening increased to 5 per cent and the rate increased to 15 mg. At 9 a. m. the average increased to 7 per cent, the maximum for the morning, while the rate increased to 20.1 mg. per minute. Between 9 and 10 a. m. the stomata closed from 7 to 3.5 per cent, but because of the rapid fall in humidity and of increased sunlight, the rate rose slightly, reaching 21.1 mg. per minute. Reduction to 3 per cent average opening at 11 a. m. brought on a slight decrease of water-loss to 21.0 mg. The stomata remained in this condition another hour, but the rapid fall of relative humidity caused an increase in the rate to 23.9 mg. Closure of the stomata at 1 p. m. brought on a reduction in the rate to 21.7 mg. per minute (fig. 43).

The two series described show that the potometer was useless as an indicator of the water-loss from a field plant, or even a potted plant of alfalfa or potato. One series with apple and peach twigs in the potometers indicated that, with the variety of apple used the water-loss from a twig may represent fairly well the transpiration of the tree, since the stomata of the cut twigs and of the tree corresponded to some extent; but even in this case there was some divergence. The great lack of correlation between transpiration and stomatal movement in *Verbena ciliata* and *Fouquiera splendens* as found by Lloyd supported the view that no more correlation existed between the stomatal movement of cut stems and rooted plants in the case of these plants than in alfalfa or potato. Hence, when the opportunity arose to test this view directly, a series was made with these plants.

This series, carried out at the Desert Laboratory, Tucson, Arizona, was begun at 9 a. m. on March 15, 1918, and ended 9 a. m. the next day. It was conducted in the same manner as the alfalfa series, the water-loss from potometers being measured at hour intervals, and sets of epiderm collected from cut stems, and watered and unwatered field plants. The day was fairly warm, clear, and very dry. The humidity varied from 12 per cent at 3 p. m. to a maximum of 47 per cent at 4 a. m. The temperature varied from 88° F. at noon to 50.5° F. at 4^h30^m a. m. The sunlight curve was rather regular, though a morning haze prevented maximum from being reached until 1 p. m. (fig. 44).

The stomata of the field plants of *Fouquiera* were wide open at the start of the series and remained open until noon. At 1 p. m. they closed to 85 per cent and continued until 20 per cent was reached at 4 p. m. No further change took place until midnight, when they

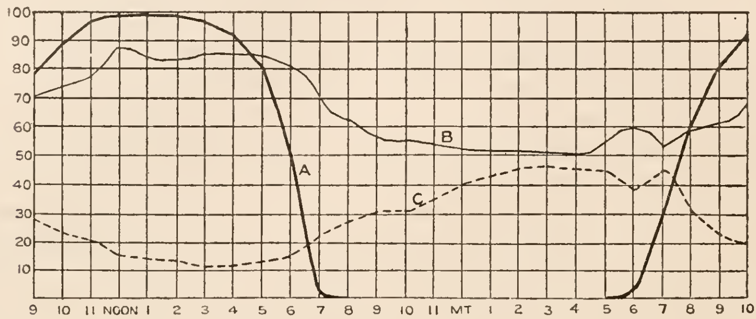


FIG. 44.—Series 29, weather data for March 15–16, 1918; sunlight (A), temperature (B), humidity (C).

closed to 10 per cent. At 1 a. m. they opened to 20 per cent and at 2 to 25 per cent. They remained in this condition an hour and then closed to 5 per cent at 4 a. m. At 5 a. m. they opened to 20 per cent, 35 per cent at 6, 50 per cent at 7, and 80 per cent at 8 a. m. At 9 a. m. they were again wide open. Watering had no effect upon the stomatal movement exhibited. This movement is apparently in full accord with that found by Lloyd for this species.

The stomatal movement found in the cut stems of this plant, however, differed greatly. Unlike the cut stems of alfalfa and potato, there was no complete closure. At no time did the stomata show less than 65 per cent opening. As in the field plants, the stomata were wide open at the start and remained at maximum until 1 p. m., when they gradually began to close. At 4 p. m. they were 70 per cent open and then reopened. At 5 they were 80 per cent open, at 6, 90 per cent, and at 7 at maximum opening again. At 8 p. m. they closed to 90 per cent, remained in this condition 2 hours, and at 11 p. m. closed to 75 per cent. At midnight they were 70 per cent open, at 1 a. m. 65 per cent, and so remained until 4 a. m. At 5 a. m. they reopened to 75 per cent, at 6 to 85 per cent, at 7 to 90 per cent, and to maximum at 8 a. m. They were still wide open when the series closed (fig. 45).

The behavior of *Verbena ciliata* was just as striking. As in *Fouquiera*, the stomata of the field plants were wide open at the start of the experiment and remained wide open until noon. They then started to close, the process being rather rapid and uniform. At 3 p. m. they were closed to 15 per cent. After this the rate of closure became slow, the stomata being 12 per cent open at 4 p. m., 10 per cent at 5 p. m., 8 per cent at 6, 6 per cent at 7, and 3 per cent at 8 p. m. At 9 p. m. closure was complete and the stomata remained closed until after 1 a. m. At 2 a. m. they opened 5 per cent and at 3 to 12 per cent. They remained in this condition until 6 a. m. when they opened 30 per cent. At 7 a. m. they were 50 per cent open, at 8 a. m. they were 80 per cent, and at 9 a. m. they were again wide open. As with *Fouquiera*, watering some of the plants caused no change in stomatal movement. The probable reason was that all the plants had sufficient moisture, and the amount added did not result in enough increase to produce a visible change. This was not checked by sampling the soil; hence it can only be inferred.

As in *Fouquiera*, the cut stems of *Verbena* showed no complete closure of the stomata. They were wide open at the start of the experiment and, like the rooted plants, remained open until noon. At

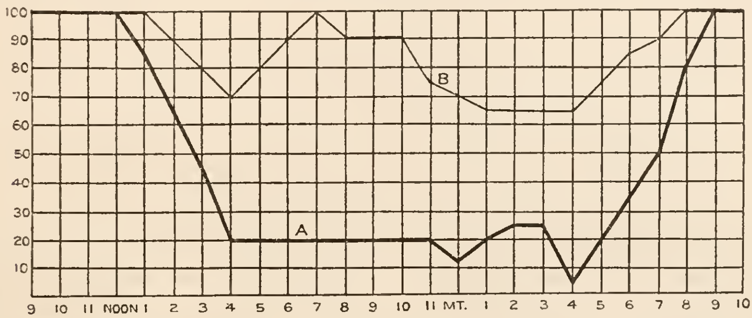


FIG. 45.—Series 29, showing average movement in upper and lower stomata of watered and unwatered field plants (A) and cut stems (B) of *Fouquiera splendens*.

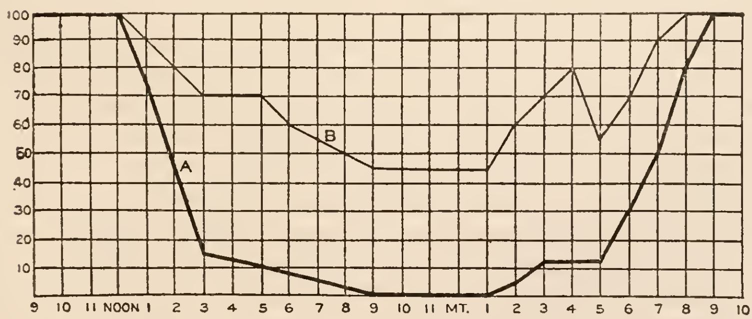


FIG. 46.—Series 29, showing average movement in upper and lower stomata of field plants (A) and cut stems (B) of *Verbena ciliata*.

1 p. m. they closed to 90 per cent, at 2 to 80 per cent, and to 70 per cent at 3 p. m. They remained in this condition until 5 p. m., and then closed to 60 per cent at 6. They closed to 55 per cent at 7 p. m., 50 per cent at 8, and 45 per cent at 9 p. m. No further change oc-

curred until after 1 a. m. At 2 a. m. they opened to 60 per cent, to 70 per cent at 3, and to 80 per cent at 4 a. m. At 5 a. m. they had closed to 55 per cent, but opened to 70 per cent at 6, and to 90 per cent at 7 a. m. At 8 a. m. they were wide open, and remained open to the end of the series (fig. 46).

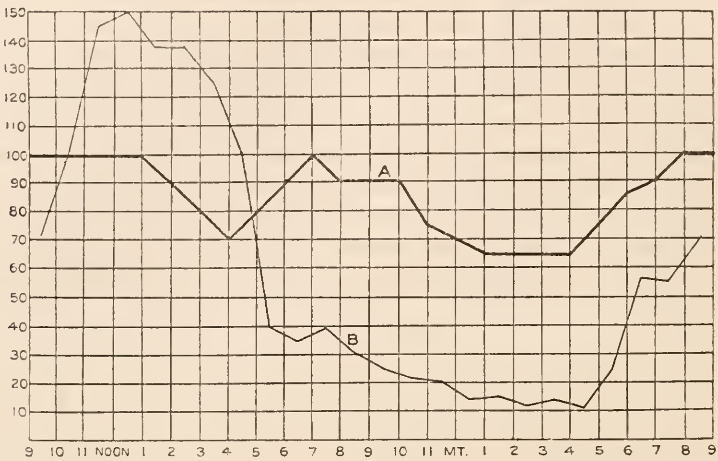


Fig 47.—Series 29, showing stomatal movement (A) and transpiration (B) in cut stems of *Fouquiera splendens.*

There was no close correlation between stomatal movement and transpiration in either species. The maximum transpiration-rate for *Fouquiera* occurred between noon and 1 p. m., and was clearly the result of low humidity and maximum sunlight at the time when the stomata were still open. After this time the rate fell, in spite of the fact that the light was still at maximum and humidity less, owing to partial closure of the stomata. The fall was not rapid, however, until between 4 and 6 p. m., when the humidity rose somewhat and sunlight fell off rapidly. But as the stomata at no time closed to less than 65 per cent, their effect upon the water-loss of the plant was mostly overshadowed by that of humidity and sunlight, as well as air-temperature and wind (fig. 47).

In *Verbena* the maximum rate of transpiration was reached between 11 a. m. and noon, an hour earlier than in *Fouquiera.* The rate was only slightly less on the following hour, as there was but little closure. The rapid fall in the rate occurred at the same time as in *Fouquiera* and was clearly the result of changes in the physical factors and not of stomatal movement. Just as in *Fouquiera,* the fact that the stomata of cut stems never closed sufficiently to cause close regulation of transpiration allowed the water-loss to be controlled almost completely by the factors of evaporation (fig. 48).

Throughout his investigation, Lloyd (1908) apparently made no parallel series of observations upon the stomatal movement in cut stems and rooted plants. But in a later paper (1912), he checked the rate of loss against the rate of absorption of water in the cut stem of *Fouquiera* and at the same time apparently measured the stomatal movement *in one of the cut stems.* As a result, the stomatal movement illustrated is like that found here to be typical of the movement in cut stems of *Fouquiera.* Throughout the period shown in his graph the stomata fluctuated between 50 and 90 per cent of maximum, at no time closing to a point where they would closely regulate the water-loss from the potometer. He states, however, that the stomata were 2.4 microns open at midnight preceding the experiment, but evidently the stem had just been removed, since in none of the present series have the stomata of a cut stem of *Fouquiera* closed to this degree, i. e., 28 per cent. Regarding the failure of the stomata to close during the afternoon, he states: "the behavior during the latter part of the day can not with certainty be regarded as wholly normal, as it does not accord with my previous results."

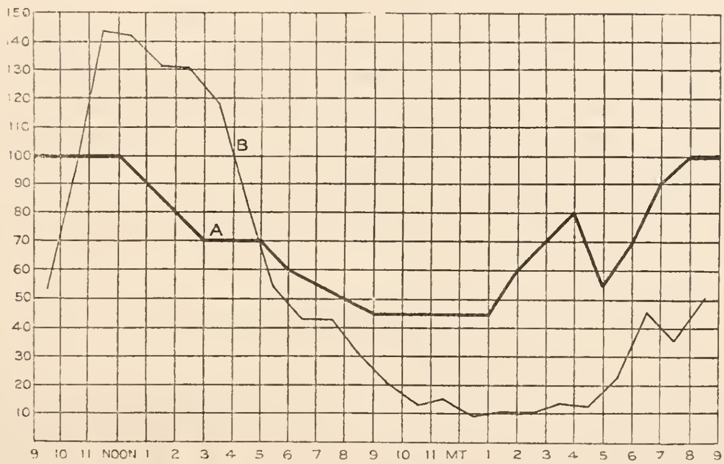


Fig. 48.—Series 29, showing stomatal movement (A) and transpiration (B) in cut stems of *Verbena ciliata.*

Knight's evidence against stomatal regulation is largely based upon "relative transpiration" readings as compared with the stomatal aperture index as measured by the porometer. As previously stated, it is to be doubted whether anyone really knows what this index represents, since apparently no one has compared it with direct observations of the stomatal apertures which it is supposed to measure. Moreover, "relative transpiration" is meaningless until some instrument is devised that will represent with some degree of accuracy the effect upon the plant of each factor concerned in evaporation. This objection applies to Trelease and Livingston's investigation (1916) as well.

The relationship between the water-loss and stomatal movement of heavily watered plants on the verge of wilting from lack of water, and cut stems, was investigated in alfalfa series 32, begun 7 p. m. on August 25 and ending 7 p. m. August 26, 1919. The night was clear and starlit, no moon showing. During the first part of the evening there was no wind, but one rose toward midnight, characterized by short gusts and intervals of calm. Between 12 and 1 a. m. it rose to a maximum of 3,700 feet for the hour, and then slowly died down, disappearing between 3 and 4 a. m. Relative humidity was not high, but rather variable, the mean rising from 38 per cent at 7 p. m. to a maximum of 63 per cent at 3 a. m. and again at 7 a. m. The temperature fell from 79° F. at 7 p. m. to 63.5 at 11 p. m., rose again to 70° F. at 1 a. m. and then fell again to 64° F. at 3 and 4 a. m. The next day was slightly hazy and with large drifting clouds, which four times during the day reduced the sunlight. Although the sun rose before 6 a. m., it was not until after 7 a. m. that the sunlight reached 10 per cent. The maximum for the day was reached at 2^h30^m p. m., when the light was 85 per cent. Sunset occurred shortly after the series ended. The maximum temperature was 89° F. at 2 p. m.; hence the day was very warm. Between 8 and 9 a. m. the wind rose suddenly to 12,200 feet for the hour, fell to 9,800 feet the next hour, and then averaged 3,000 feet per hour during the rest of the day, dying away at sunset. During the period the barometric pressure fluctuated from 25.4 inches to 25.7 inches from 7 p. m. on the 25th to noon the next day (fig. 49).

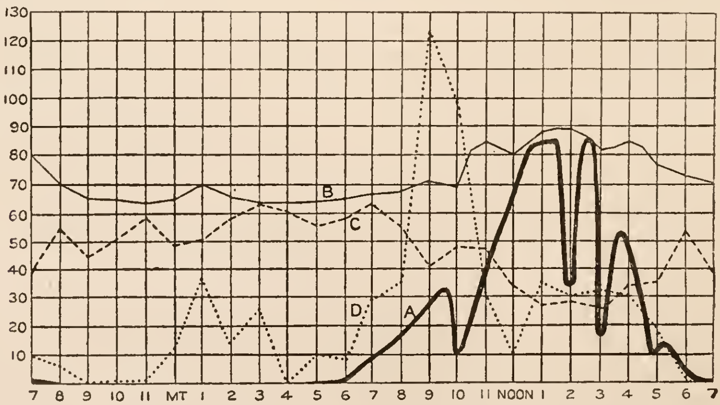


FIG. 49.—Series 32, weather data for August 25–26, 1919; sunlight (A), temperature (B), humidity (C), wind velocity (D).

The water-loss from heavily watered plants was determined by weighing small plants in metallic containers at 2-hour intervals upon a precision balance, weighing them to the nearest milligram. The total weight of each plant and container was approximately 150 grams. The plants stripped were somewhat larger, but of the same age, and were grown in large earthenware pots, an average of 10 plants to a pot. Great care was exercised to keep the soil temperature of the pots and containers the same throughout the day, and the plants under the same conditions in other respects. This was done by embedding the metal containers in the soil of the pots, and keeping the soil from the container by a metal cylinder. A small piece of moist flannel was kept over the top of the container, except when weighed, in order to duplicate as closely as possible the moist soil-surface at the base of the other plants. The containers were removed for about 5 minutes every 2 hours for the purpose of weighing. They were weighed on the half hour, in order that the interval would coincide better with the stomatal observations. Strips were

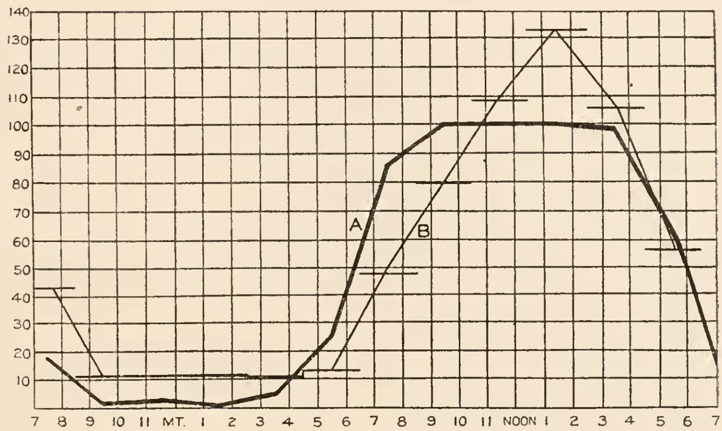


FIG. 50.—Series 32, showing stomatal movement averaged for 2-hour periods (A) and transpiration in milligrams per minute for the same periods (B) in heavily watered potted plants of alfalfa.

collected at hour intervals, but, for purposes of comparison, the two sets of strips collected during each interval between weighings was averaged. This method, of course, produced smoothed curves, but as it was not possible to weigh at shorter intervals, the smaller fluctuations could not be taken into account.

From 7 to 8^h30^m p. m. the water-loss of the watered plants was 44.7 mg. per minute per square decimeter of leaf-surface. During this period the stomatal opening was 16 per cent. From 8^h30^m a. m. to 10^h30^m p. m. the loss was 11.4 mg. per minute and the average stomatal opening 3 per cent. The same rate of loss occurred during the next 2-hour period, while the stomatal opening was 4 per cent. The lack of a corresponding increase is accounted for by the rise of relative humidity and the fall in the rate of evaporation shown by the two types of atmometer. During the next period, from 12^h30^m to 2^h30^m a. m., the average opening was again 3 per cent, but as the rate of evaporation rose again, the rate of water-loss remained at

11.4 mg. as before. During the period between 2^h30^m and 4^h30^m a. m. the average of stomatal opening rose to 6 per cent, but as the humidity increased to the maximum and evaporation fell off to the minimum, the rate of transpiration fell slightly, namely, to 10.2 mg. per minute. During the next period, 4^h30^m to 6^h30^m a. m., the average of stomatal opening rose to 25 per cent, but as evaporation and relative humidity remained the same, this in part explains the slight increase to 12.4 mg. During the period from 6^h30^m to 8^h30^m a. m. the average rose to 85 per cent and the rate of water-loss to 48.3 mg. Evaporation increased somewhat during this period, but was still very low. The average opening rose to 100 per cent during the period from 8^h30^m to 10^h30^m a. m. and the rate of loss to 80 mg. per minute. The stomata were wide open during the following period as well, while the rate further increased to 108 mg. per minute in response to increase of evaporation. From 12^h30^m to 2^h30^m p. m. the evaporation was very high comparatively and the transpiration-rate rose to the maximum of 132.7 mg. per minute, the stomata still being wide open. During the interval from 2^h30^m to 4^h30^m p. m. the rate fell to 106.7 mg., largely in response to a similar fall in the intensity of the factors of evaporation, although at the end of the period the stomata closed slightly. From 4^h30^m to 6^h30^m p. m. the rate fell to 56.8 mg. per minute, in part due to the fall of evaporation and in part to closure of the stomata, the average opening for the period being 62 per cent. The fluctuations of the rate while the stomata were wide open are clearly caused by the changes in the evaporating factors, and it is evident that these will control transpiration while the stomata are at or near their maximum opening (fig. 50).

The plants in the dry containers were treated in the same manner as the preceding ones. The plants stripped were small ones in large earthenware pots, and differed from those heavily watered in having had no water for 10 days. The water-content for this period was 10 per cent, and the echard was 9 per cent. The containers were weighed, as before, but alternating with the watered plants. On this account the intervals overlap those for the other set by 1 hour, and hence the curves alone can be compared for the two.

As before, the stomatal movement was averaged for the 2 hours of the interval between weighings. Between 7^h30^m and 8^h30 p. m. this average was 2 per cent, while the rate of transpiration was 7.91 mg. per minute. Between 9^h30^m and 11^h30^m p. m. the rate fell to 5^h40^m mg. per minute, in spite of the slight increase of opening to 3 per cent. This fall coincides with a great reduction in the rate of evaporation as shown by the atmometers, the average for the first period being 195 mg. per minute and for the second 46 mg. per minute. In the interval from 11^h30^m to 1^h30^m a. m. the rate was practically unchanged, being 5.42 mg. per minute, although the stomata showed again a slight opening. From 1^h30^m to 3^h30^m a. m.

the average stomatal opening increased to 25 per cent, while the rate rose to 8.73 mg. per minute. A slight increase in opening to 28 per cent from 3^h30^m to 5^h30^m a. m. corresponded to slight increase in the rate to 8.75 mg., although the rate of evaporation decreased. The maximum of opening occurred from 5^h30^m to 7^h30^m a. m., the average being 58 per cent, and the greatest transpiration also occurred at this time, the water-loss being 24.21 mg. per minute. Closure to 8 per cent from 7^h30^m to 9^h30^m a. m. caused in part the drop in the rate to 4.75 mg., but the lowest rate of evaporation observed was partly also responsible. From 9^h30^m to 11^h30^m a. m. the average opening decreased to 6 per cent, but the rise in evaporation to three times its former rate caused a slight increase in the rate of transpiration to 5.11 mg. per minute. From 11^h30^m a. m. to 1^h30^m p. m. the average opening and evaporation were unchanged, and the rate of water-loss also remained the same. From 1^h30^m to 3^h30 p. m. the stomata were again unchanged, but the great increase of evaporation from 85 mg. to 268 mg. per minute caused a rise in the rate of transpiration to 10.67 mg. per minute. During the period from 3^h30^m to 5^h30^m p. m. the stomata opened to 8 per cent, and although the evaporation-rate dropped considerably, the rate of transpiration rose to 10.75 mg. per minute. From 5^h30^m to 7^h20^m

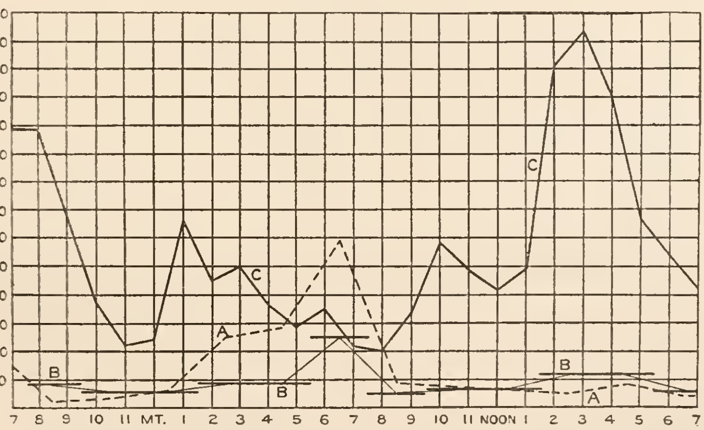


FIG. 51.—Series 32, showing stomatal movement averaged per 2-hour period (A) and transpiration in milligrams per minute (B) of dry alfalfa phytometers; evaporation 1 = 2 milligrams per minute (C).

the stomata closed to 4 per cent and the rate of evaporation fell below 100 mg. per minute, and, in consequence, the average water-loss fell to 5.06 mg. per minute. While this shows that the factors of evaporation play an important part in changing the rate of transpiration, the effect of these factors is as clearly shown to be regulated by the stomata (fig. 51).

A comparison of the transpiration-rates of the two groups of plants brings out certain other interesting facts. At only one time did the rate for the plants sealed in dry soil apparently exceed that of the heavily watered plants. This probably happened between 5 and 6 a. m., although because the two sets were weighed at alternate half hours it was not actually demonstrated. This was due to the much greater stomatal opening at that time in the plants in dry soil. Another point of importance is shown by the comparative rates in the periods including 10 p. m. in both series. At this time the stomata of both sets averaged 3 per cent opening, but the rate for the heavily watered plants was practically twice that of the dry plants. Throughout the experiment the rate was proportionally lower for the plants in dry soil for the given degree of stomatal opening. The most plausible explanation is to ascribe this to the effect of a lower water-margin in the leaf. Moreover, it is very evident that the much lower rate of water-loss in the dry containers during the greater part of the day, when the rate for the wet ones was very high, must be attributed to the control exerted by the nearly closed stomata (fig. 52).

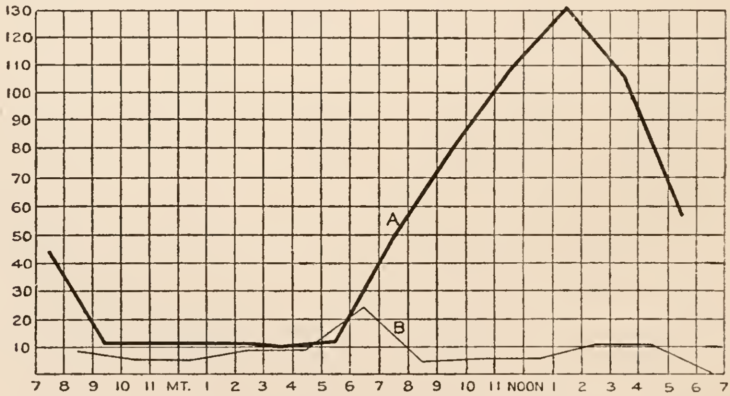


Fig. 52.—Series 32, showing transpiration of wet (A) and dry (B) alfalfa phytometers.

The stomatal movement of plants of cow-beet growing in large pots of dry soil was studied in a similar manner in series 33. This experiment was started at 6 p. m. on August 8, 1919, and ended at 6 p. m. on August 9. The night was not cold, but lowest temperatures were 59° F. at 2 a. m. and 59.5° F. at 6 a. m., just before sunrise. During the day it rose to a maximum of 85° F. at 2 and 3 p. m. The relative humidity fluctuated a great deal, but within unusually narrow limits, since it did not rise above 79 per cent or fall below 42 per cent. Passing clouds caused the sunlight to fluctuate continuously. Like the other factors, the wind was also variable, at times rising to an average of 5 miles an hour and other times dying away completely (fig. 32).

Two weeks before the experiment, three plants were transplanted into the metal containers, watered, and left to establish themselves. Since they were planted in soil of known water-content, and weighed before watering, they were readily brought to the desired water-content at the start of the experiment. Twelve large plants were also placed in earthenware pots with the same water-content. Since the low water-content of the dry containers of the alfalfa series caused more closure than desired, the containers in this case were started at 14 per cent and fell to 10 per cent at the end. No watering was done during the series, since this tends to produce localized regions of high water-content. The containers were weighed each hour, immediately after collecting the strips.

At 6 p. m., when the series started, the stomata were 30 per cent open, and 10 per cent at 7 p. m. During the hour, the rate of water-loss was 50 mg. per minute. At 8 p. m. the stomata closed to 1 per cent and the rate fell to 11.25 mg. At 9 p. m. the stomata practically closed, a few slightly open in the upper surface making the average 0.5 per cent. The rate of water-loss as a result fell to 5.45 mg. per minute during the hour. At 10 p. m. the stomata opened to an average of 5 per cent and the rate rose to 8.00 mg. per minute. At 11 p. m. further opening to 10 per cent occurred and the rate rose to 15.00 mg. At midnight the average opening decreased to 7 per cent and the rate of loss decreased to 10 mg. per minute. At 1 a. m. average opening decreased to 1 per cent, but a great increase of evaporation caused a slight increase of water-loss to 10.91 mg. As the stomata continued at nearly complete closure during the following hour, the rate dropped to 4.62 mg. per minute. At 3 a. m. the average opening increased to 2.5 per cent and the water-loss rose to 7.27 mg. per minute. At 4 a. m. the stomata opened to 10 per cent, and in consequence a great decrease in evaporation caused but a slight decrease in the rate of water-loss to 6.15 mg. The next hour it rose to 10.91 mg. per minute. From 5 to 6 a. m. the stomata closed from 3.0 per cent to 0.2 per cent, the rate of evaporation fell, and hence the water-loss fell to the minimum of 3.65 mg. per minute. The lower stomata then opened, while the upper remained closed, bringing the average to 32 per cent. As evaporation dropped still lower, the increase of water-loss was only 11.67 mg. The stomata remained at this opening another hour and the rate of water-loss rose to 41.43 mg. per minute. Opening to 75 per cent caused a rise to 52.73 mg. per minute, and to a maximum of 120 mg. on the following hour, as the evaporation rate rose very much, in spite of the fact that the stomata began to close again. However, the greatest water-loss occurred during the hour the stomata were at their widest opening for the series. Closure to 15 per cent at 11 a. m. caused the rate to decrease to 78.33 mg. The stomata opened again to 18 per cent at noon, causing the rate also to rise to 83.33 mg.

per minute. Further opening to 25 per cent at 1 p. m. resulted in an increase of water-loss to 88.33 mg. per minute. At 2 p. m. the stomata closed to 7 per cent and the rate fell to 66.67 mg. per minute. At 3 p. m. reopening to 17 per cent was accompanied by a rise in the rate to 72.73 mg. per minute. From this time to the end of the series the stomata closed slowly and the rate of water-loss fell in proportion (fig. 53).

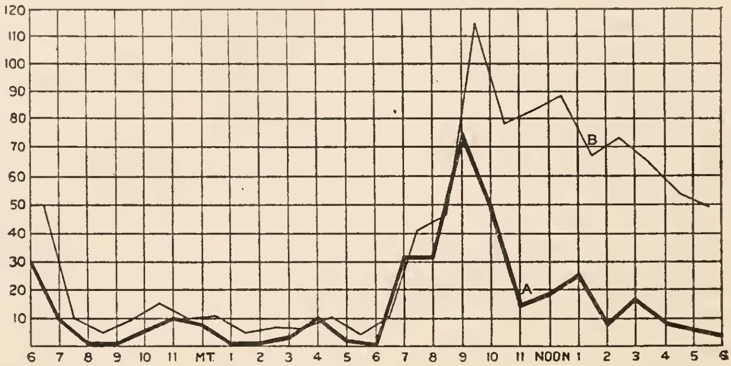


Fig. 53.—Series 33, showing average movement of upper and lower stomata (A) and transpiration (B) of cow-beets in dry pots.

In this series, as well as in the alfalfa, *Fouquiera*, and *Verbena* series, direct evidence was found that as the stomatal movement of cut stems and rooted plants differed considerably, so did their rates of water-loss. In this series the leaves in the potometers showed little or no wilting, but their stomata were closed or nearly closed throughout the experiment. At the start the stomata were completely closed and opened to 7 per cent at 7 p. m. At 8 they closed to 1 per cent and at 9 were completely closed. Thus a comparison shows that the stomatal movement of the cut leaves and the plants in the dry pots was identical at 8 and 9 p. m. and differed not at all in the rates of water-loss at this time. At 1 and 2 a. m. the stomata of the potted plants were practically closed, while those of the cut stems were 2 per cent open; hence at this time the rate of water-loss for the cut leaves was slightly higher than that of the plants. The stomata of the potted plants closed again at 5 and 6 a. m., while the cut leaves showed 4 per cent opening. At this time the transpiration-rate of the potted plants again fell to less than that of the cut leaves. At all other times the transpiration-rate of the cut stems was lower and during the day considerably lower than that of the potted plants. This must be attributed to the closed stomata during the day in the cut leaves (fig. 54).

The experiments presented show that stomata regulate the water-loss from plants and contradict the results of Lloyd (1908). The reasons for this lack of agreement have been demonstrated, and the probable causes for failure of agreement with certain other investigations have been indicated. The regulation of water-loss was found to be controlled closely by the stomata when they are nearly closed and by the factors of evaporation when they are wide open. Brown and Escombe (1900) showed that diffusion of water-vapor through the open stomata is much less than would occur if

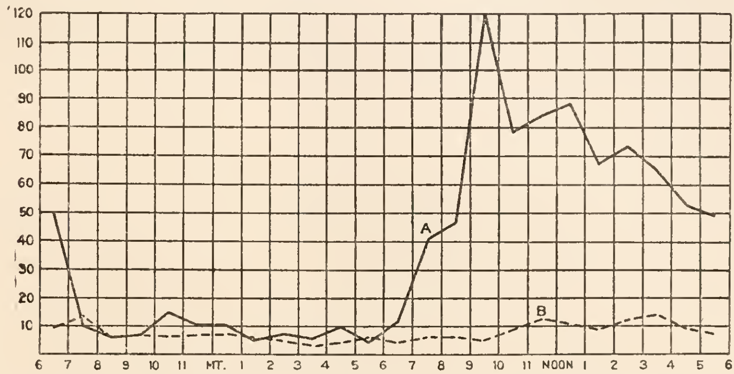


FIG. 54.—Series 33, showing transpiration from potted cow-beet plants in dry soil (A) and from potometers (B).

the air within the leaf were saturated. It therefore appears probable that the air is saturated only in the deeper spaces of the leaf, and that as the outer air is approached through the passages, substomatal chambers, and pores of the stomata, the saturation decreases to become more nearly like that of the air outside. As the stomata close, the amount of saturated intercellular space probably increases, and finally, when closure becomes complete, all the air contained in the leaf becomes saturated. Therefore, water-loss is most clearly affected by changes of stomatal opening when the openings are approaching minimum.

SUMMARY.

1. The stomatal movement of cut stems in 9 species of plants, including *Fouquiera splendens* and *Verbena ciliata*, differs greatly from that found in potted plants and field plants, except in the one experiment with apple. Even in this case there was some divergence.

2. Since the stomatal movement is different, the rate of water-loss is also different from that of potted plants and presumably from field plants.

3. Lloyd's evidence that stomata are non-regulatory is therefore vitiated by his use of potometers to measure the water-loss of field plants.

4. Although the factors concerned in evaporation have great influence upon transpiration, this influence is definitely controlled

by the stomata. When the stomata are wide open or nearly wide open, transpiration is the result of the action of the factors of evaporation alone, since the stomata in nowise interfere with the action. As the stomata close, the influence of the factors is lessened, but until closure has reduced the apertures to 50 per cent or less, stomatal regulation is still largely overshadowed by the control exerted by them. When closure is almost complete, the regulation of water-loss by the stomata is very close and the effect of the factors overshadowed by the effect of even very small changes of the opening.

BIBLIOGRAPHY.

Briggs, L. J., and H. L. Shantz, 1916. Hourly transpiration rate on clear days as determined by cyclic environmental factors. Jour. Agri. Res. 5 : 583.

——— ———. 1916. A daily transpiration during the normal growth period and its correlation with the weather. Jour. Agri. Res. 7 : 155.

——— ———. 1917. Comparison of the hourly evaporation rate of atmometers and free water surfaces with the transpiration of *Medicago sativa*. Jour. Agr. Res. 9:277.

Brown, H. T., and F. Escombe. 1900. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Roy. Soc. London 70 : 397.

Burgerstein, A. 1904. Die Transpiration der Pflanzen.

Buscalioni, L., and G. Polacci. 1902. Alteriori ricerche sull' applicazione delle pellicole di collodio allo studio di alcuni processi fisiologici delle piante ed in particolar modo delle traspirazione vegetale. Att. Ist. Bot. Univ. Pavia. n. s. 7:

Clements, F. E. 1905. Research methods in ecology.

———. 1907. Plant physiology and ecology.

Darwin, Francis. 1898. Observations on stomata. Phil. Trans. Roy. Soc. London B 190 : 531.

———. 1914. On a method of studying transpiration. Proc. Roy. Soc. London B 87 : 269.

———. 1914. The effect of light on the transpiration of leaves. Proc. Roy. Soc. London B 87 : 281.

———. 1916. On the relation between transpiration and stomatal aperture. Phil. Trans. Roy. Soc. London B 207 : 413.

——— and D. F. M. Pertz. 1911. New method of estimating the aperture of stomata. Proc. Roy. Soc. London B 84 : 149.

Dixon, H. H. 1914. Transpiration and the ascent of sap in trees.

Eckerson, Sophia H. 1908. The number and size of stomata. Bot. Gaz. 46 : 221.

Gray, J., and G. J. Peirce. 1919. The influence of light upon the action of the stomata and its relation to the transpiration of certain grains. Am. Jour. Bot. 6:131.

Haberlandt, G. 1904. Physiologische Pflanzenanatomie.

Iljin, W. S. 1914. Die Regulierung der Spaltöffnungen in Zusammenhang mit Veränderung des osmotischen Druckes. Beih. Bot. Cent. 32 : 15.

———. 1914 A. Die Probleme des vergleichenden Studiums der Pflanzentranspiration. Beih. Bot. Cent. 32 : 36.

Johnston, E. S. 1919. Evaporation compared with vapor-pressure deficit and wind velocity. Mo. Weather Rev. 47 : 30.

Knight, R. C. 1915. A convenient modification of the porometer. New Phyt. 14 : 212.

———. 1916. On the use of the porometer in stomatal investigation. Ann. Bot. 30 : 57.

———. 1917. "Relative transpiration" as a measure of the intrinsic transpiring power of the plant. Ann. Bot. 31 : 351.

———. 1917 A. The interrelations of stomatal aperture, leaf water content, and transpiration rate. Ann. Bot. 31 : 221.

Kohl, F. G. 1895. Zum Mechanik der Spaltöffnungsbewegungen. Beiblatt zur "Leopoldina."

Laidlow, C. J. P., and R. C. Knight. 1916. A description of a recording porometer and a note on stomatal behavior during wilting. Ann. Bot. 30 : 47.

Leitgeb, H. 1888. Beiträge zur Physiologie der Spaltöffnungsapparate. Graz Bot. Inst. Mitt. 1:123.

LIVINGSTON, B. E. 1906. Relation of desert plants to soil moisture and evaporation. Carnegie Inst. Wash. Pub. No. 50.

———. 1907. Relative transpiration in cacti. Plant World 10 : 110.

———. 1909. Stomata and transpiration in *Tradescantia zebrina*. Science 29 : 269.

———. 1910. Operation of porous cup vaporimeter. Plant World 13 : 111.

———. 1911. Light intensity and transpiration. Bot. Gaz. 52 : 417.

———. 1913. Resistance offered by leaves to transpirational water loss. Plant World 16 : 1.

———. 1915. Atmometry and the porous cup. Plant World 18 : 21.

——— and W. H. BROWN. 1912. Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. 53 : 305.

——— and A. H. ESTABROOK. 1912. Observations on the degree of stomatal movement in certain plants. Bull. Torr. Bot. Club 39:15.

——— and J. W. SHIVE. 1914. Relation of atmospheric evaporating power to the soil-moisture content at permanent wilting in plants. Plant World 17 : 81.

LLOYD, F. E. 1908. The physiology of stomata. Carnegie Inst. Wash. Pub. No. 82.

———. 1912. The relation of transpiration and stomatal movement to water content of leaves in *Fouquieria splendens*. Plant World 15 : 1.

———. 1913. Leaf water and stomatal movement in *Gossypium*, and a method of direct visual observation of stomata *in situ*. Bull. Torr. Bot. Club 40 : 1.

MERGET, A. E. 1873. Recherches sur le rôle des stomates dans les phénomènes d'échanges gazeux entre la plante et l'atmosphère. Ann. Bot. Soc. Lyon 1:1.

MOHL, H. 1856. Welche Ursachen bewirken die Erweiterung und Verengung der Spaltöffnungen? Bot. Zeit. 14:697.

MOLISCH, H. 1912. Das Offen- und Geschlossensein der Spaltöffnungen veranschaulicht durch eine neue Methode. Zeit. Bot. 4.

MUENSCHER, WALTER L. C. 1915. Relation of transpiration to stomata. Jour. Bot. 2 : 487.

PORSCH, —. 1905. Der Spaltoffnungsapparat im Lichte Phylogenie.

RENNER, O. 1910. Beitrage zur Physik der Transpiration. Flora 100 : 451.

———. 1911. Zur Physik der Transpiration. Ber. Deut. Bot. Ges. 29 : 451.

———. 1911. Experimentale Beiträge zur Kenntniss der Wasserbewegung. Flora 103 : 171.

SCHWENDENER, S. 1881. Ueber Bau und Mechanik der Spaltoffnungen. Monatsber. Berliner Akad.

SHREVE, E. B. 1916. An analysis of causes of variations of the transpiring power of cacti. Physiol. Res. 2 : 73.

———. 1919. A thermo-electrical method for the determination of leaf temperature. Plant World 22:100.

———. 1919A. The role of temperature in the determination of the transpiring power of leaves by hygrometric paper. Plant World 22:172.

STAHL, E. 1894. Einige Versuche über Transpiration und Assimilation. Bot. Zeit. 52:117.

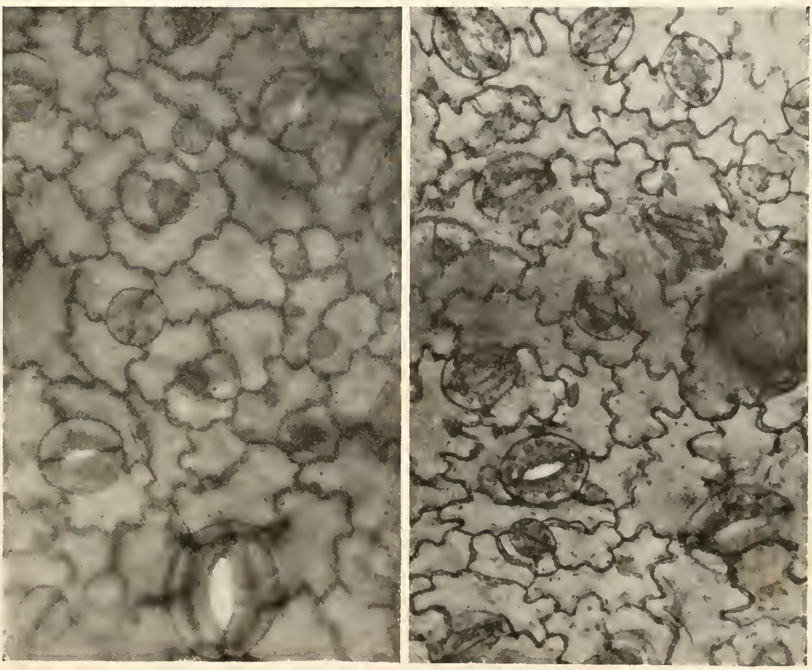
THOMAS, NESTA, and ALLAN FERGUSON. 1917. On the reduction of transpiration observations. Ann. Bot. 122 : 241.

TRELEASE, S. F., and B. E. LIVINGSTON. 1916. The daily march of transpiring power as indicated by porometer and standardized hygrometric paper. Jour. Ecol. 4 : 1.

WIGGINS, R. J. 1921. Variations in the osmotic concentrations of the guard-cells during opening and closing of stomata. Am. Jour. Bot. 8 : 30.

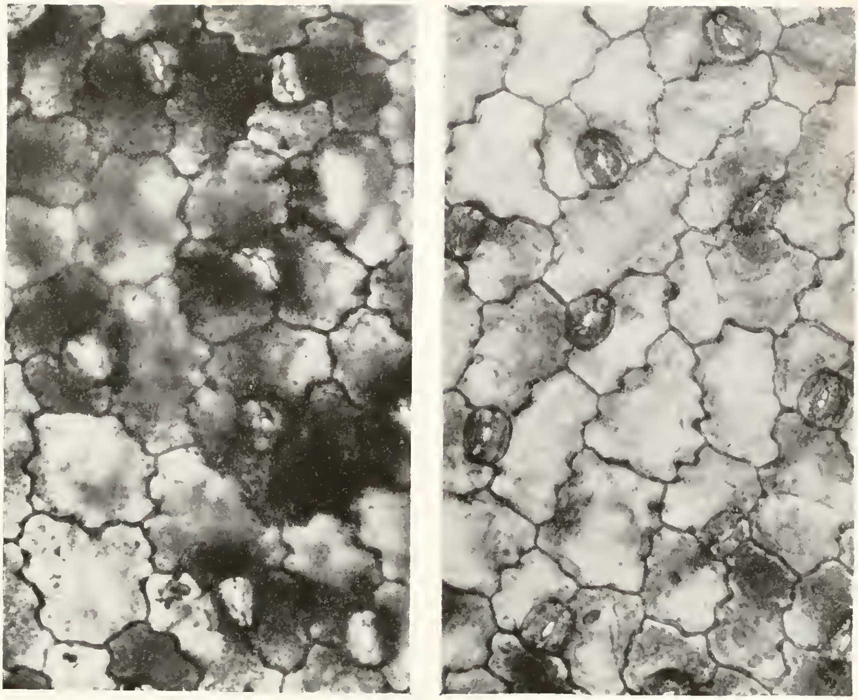
A

B

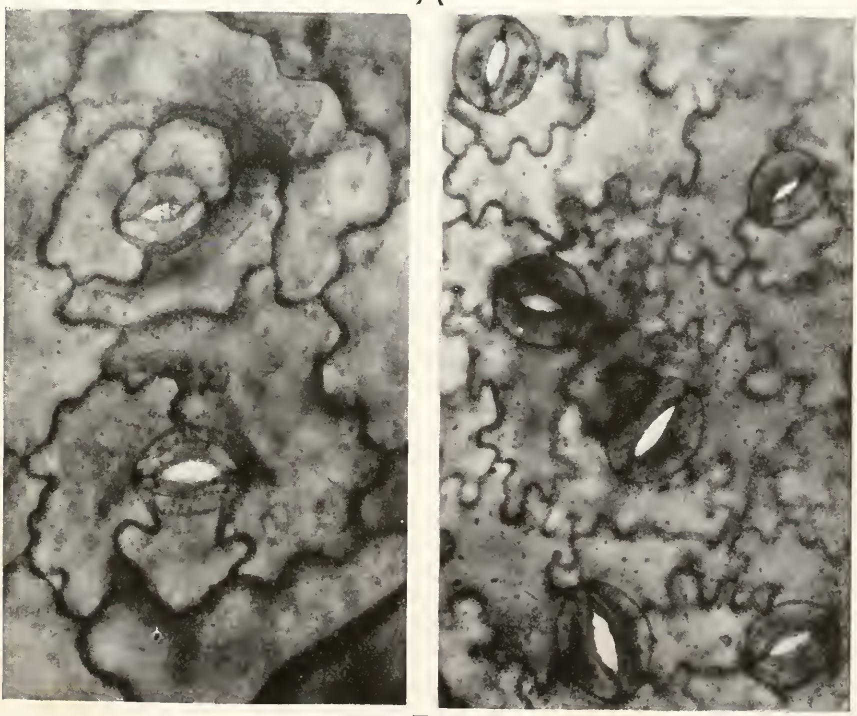


C

A. Wheat stoma wedged open by dust, × 500.
B. Stoma of *Rumex patientia*, × 500.
C. Epiderm of potato showing stomata in all stages of development, × 345.

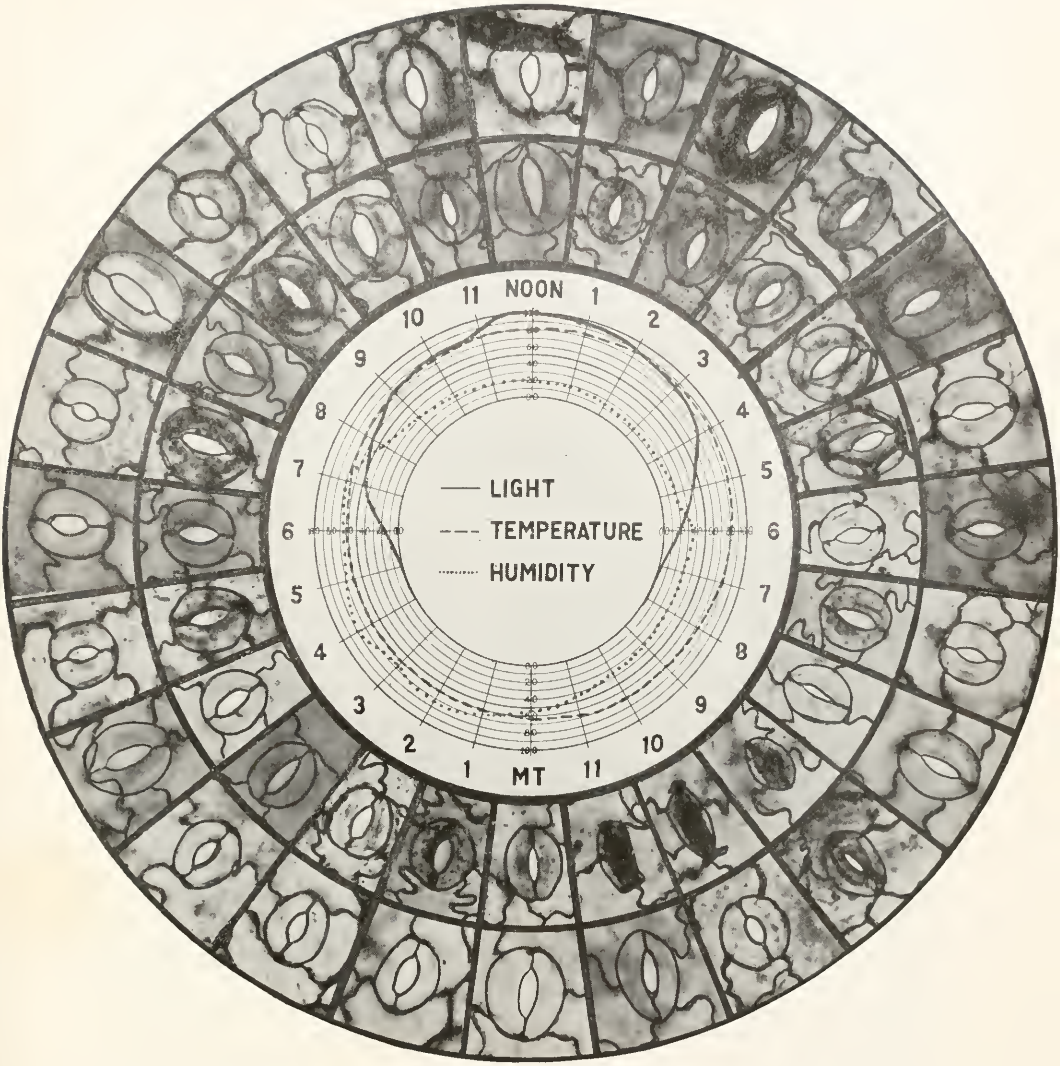


A

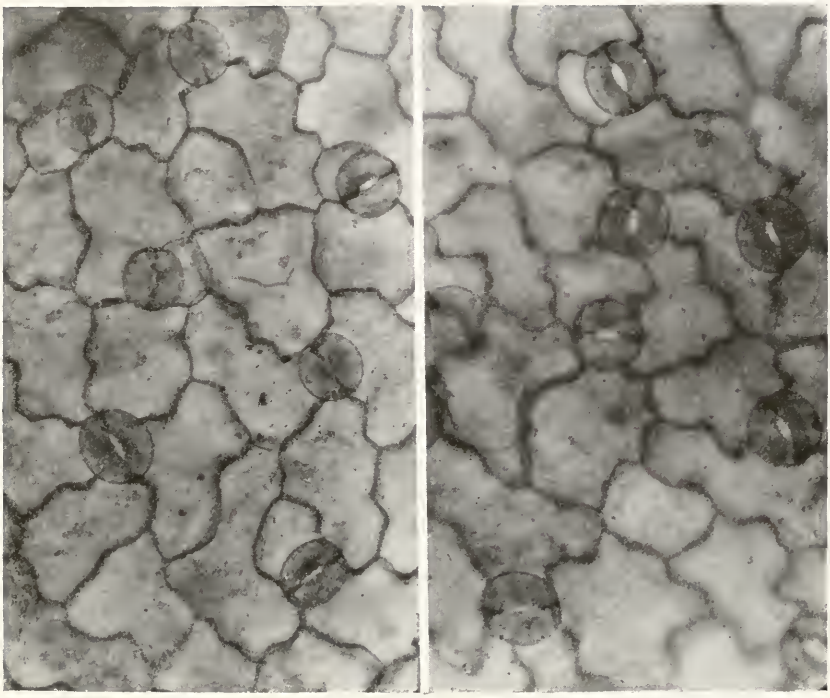


B

A. Upper and lower epiderm of alfalfa, × 345.
B. Upper and lower epiderm of potato, × 345.

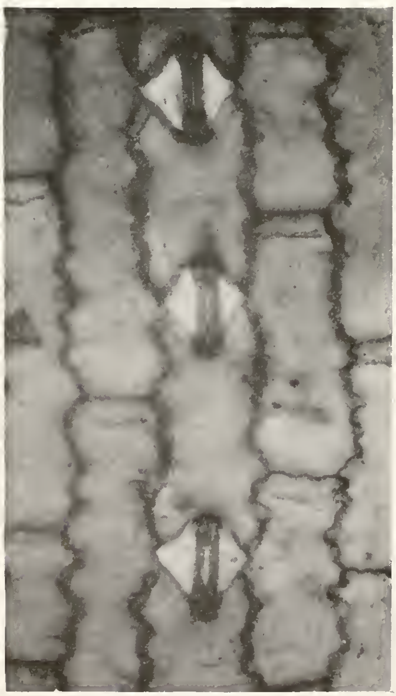


Series 10, showing condition of upper (outer) and lower (inner) stomata of potato, × 240, at each hour of a 24-hour day, together with curves for sunlight, temperature, and humidity.



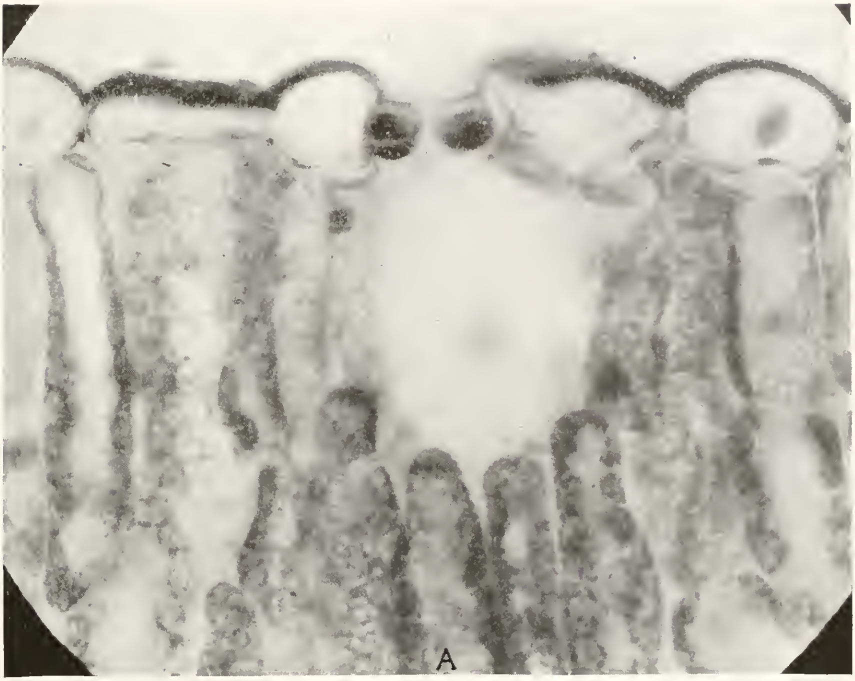
A

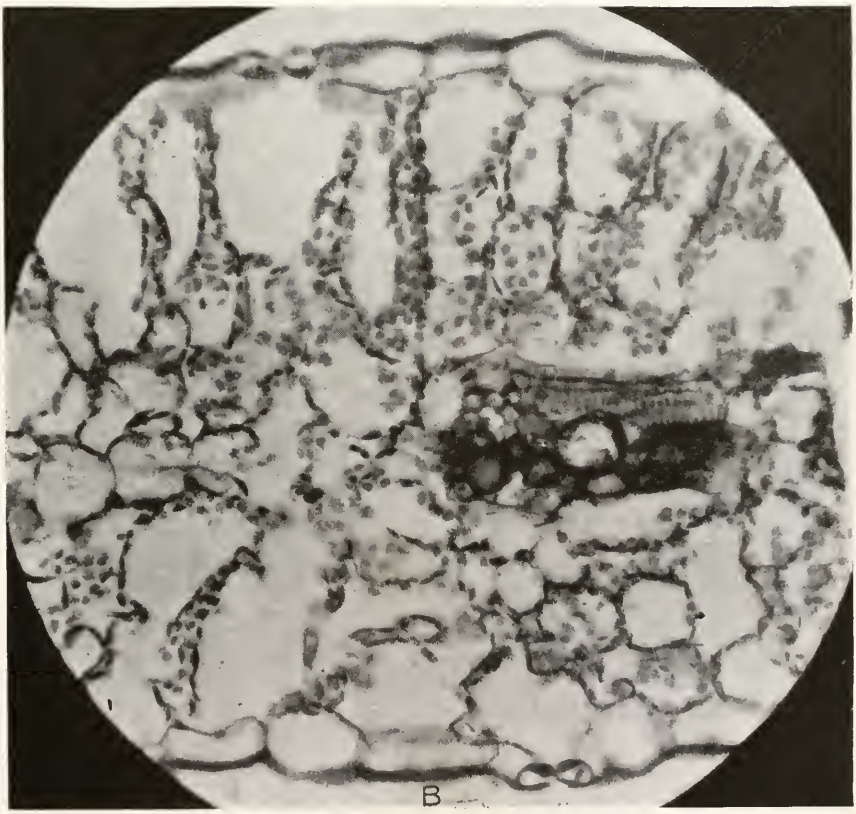
B



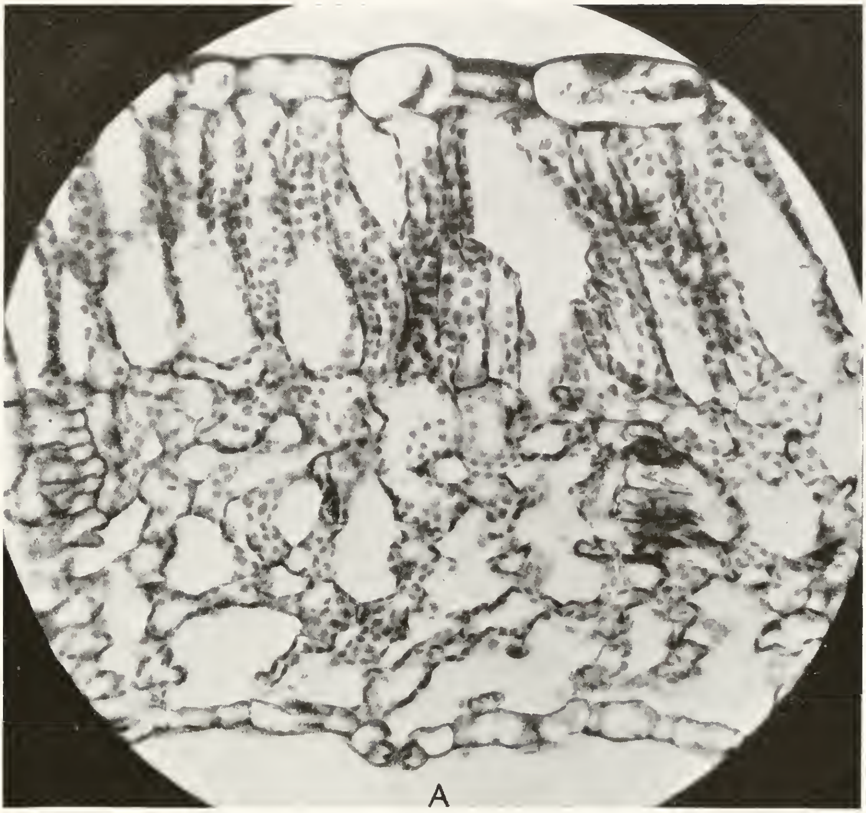
C

A. Upper and lower epiderm of sugar-beet.
B. Epiderm of onion. C. Epiderm of corn.

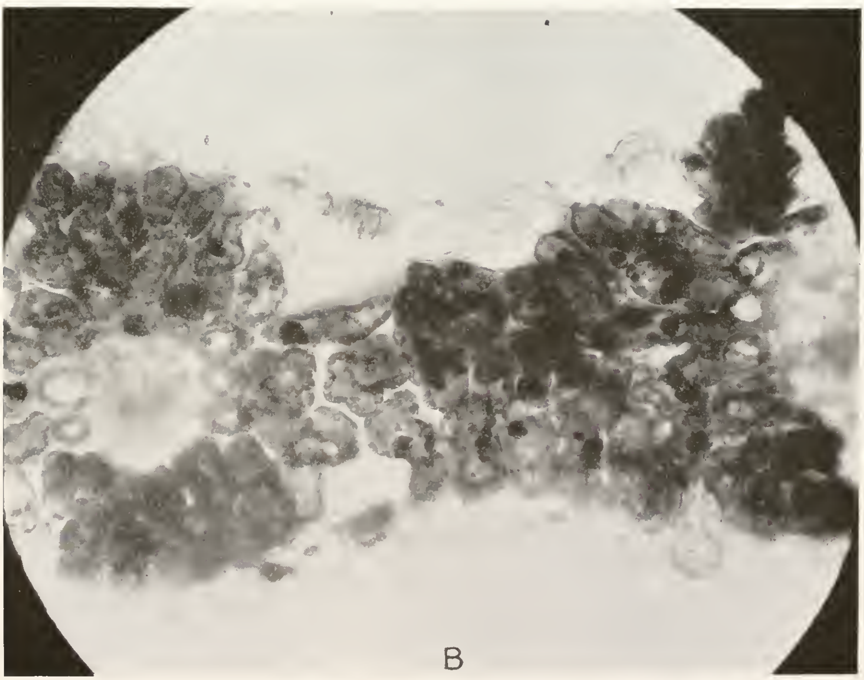




A. Cross-section of alfalfa leaf, showing stoma and chamber, × 500.
B. Cross-section of sugar-beet leaf, showing stomata and chambers, × 345.

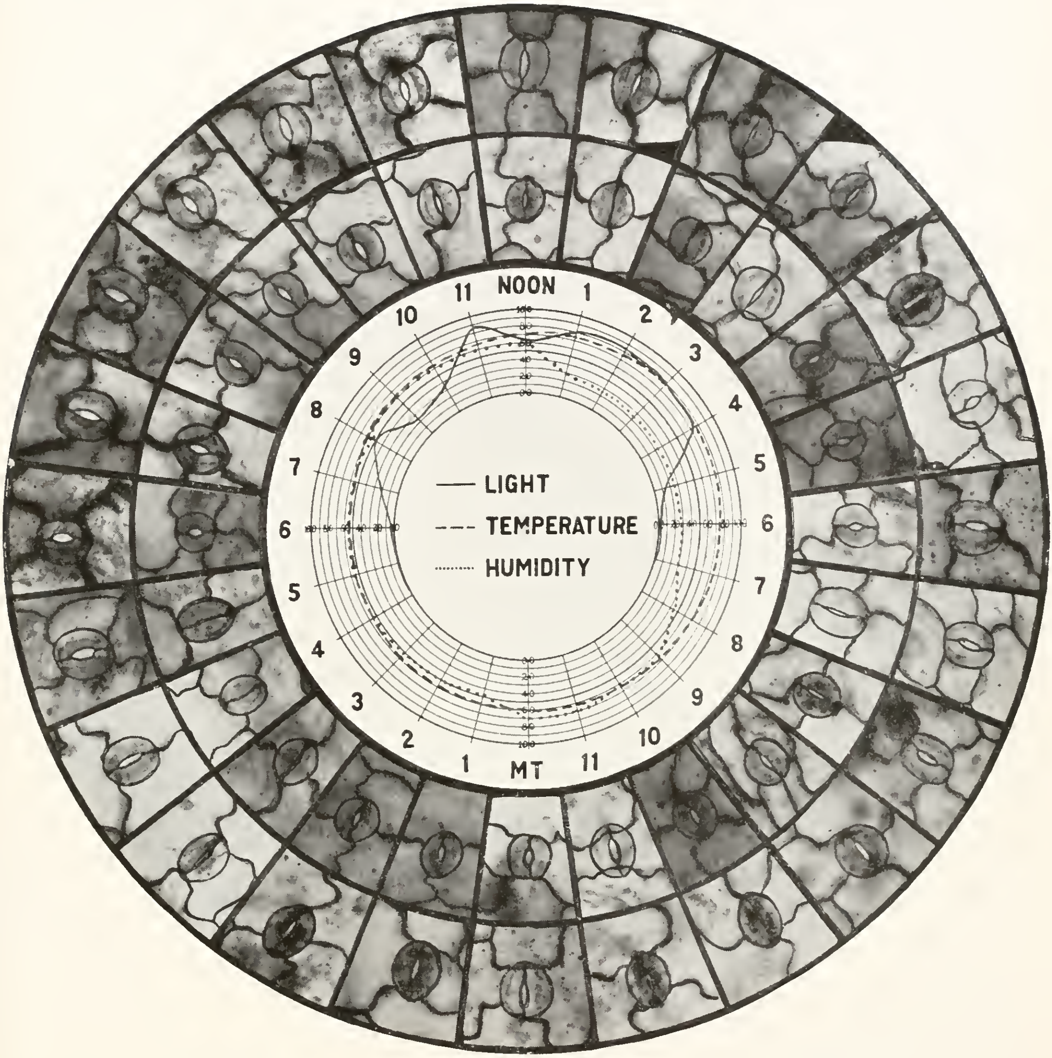


A

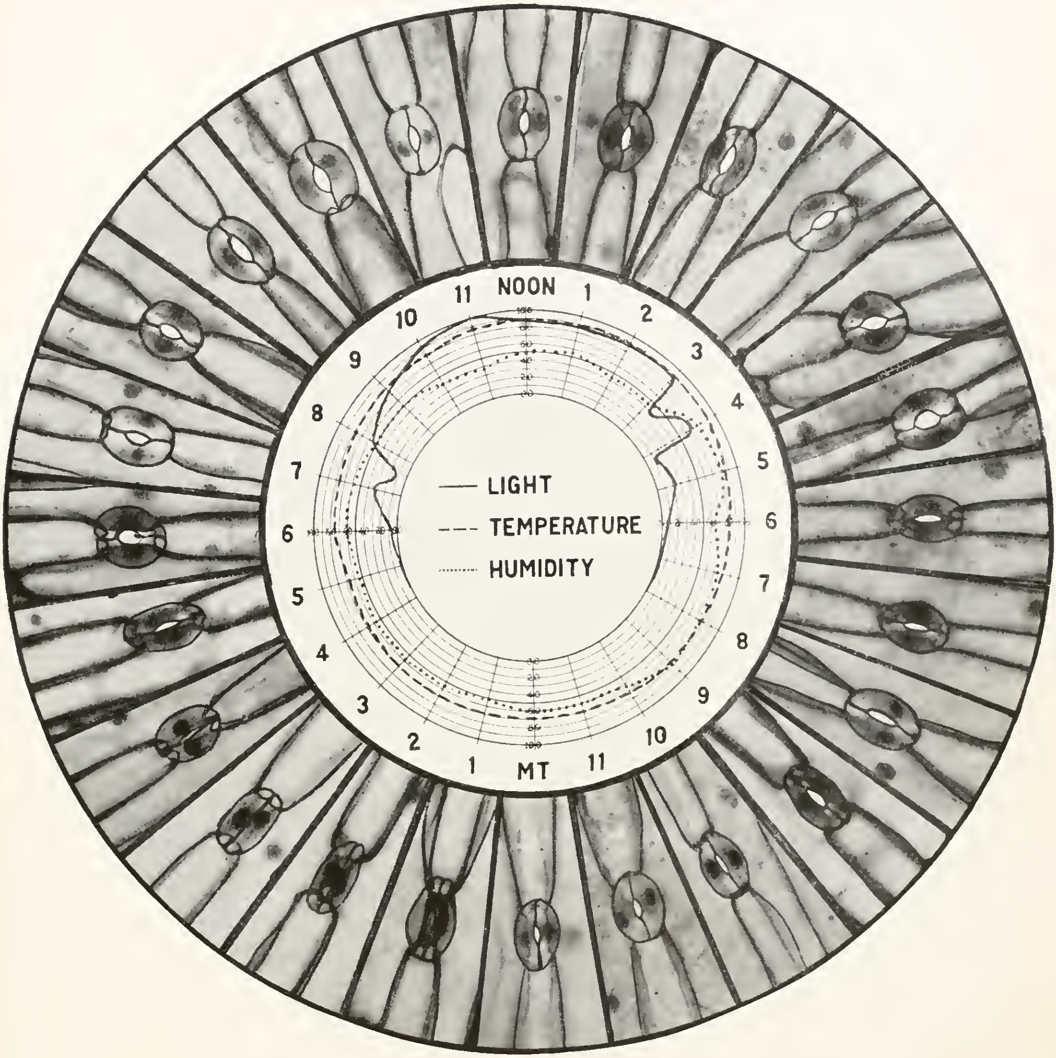


B

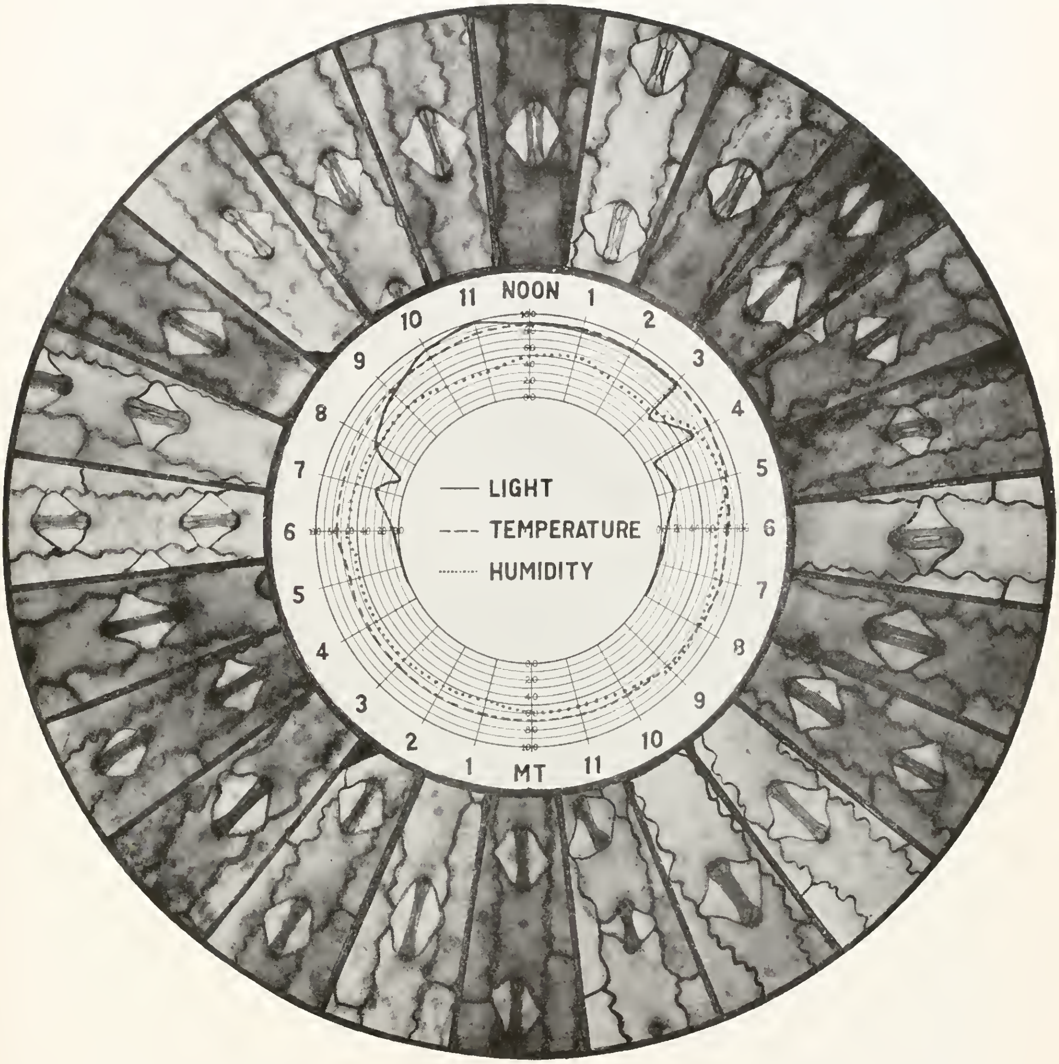
A. Cross-section of potato leaf, showing stomata, chambers, and air-passages, × 345.
B. Cross-section of corn leaf, showing stomata and chambers, × 345.



Series 11, showing upper and lower stomata of sugar-beet, × 240, and factors during a 24-hour day.

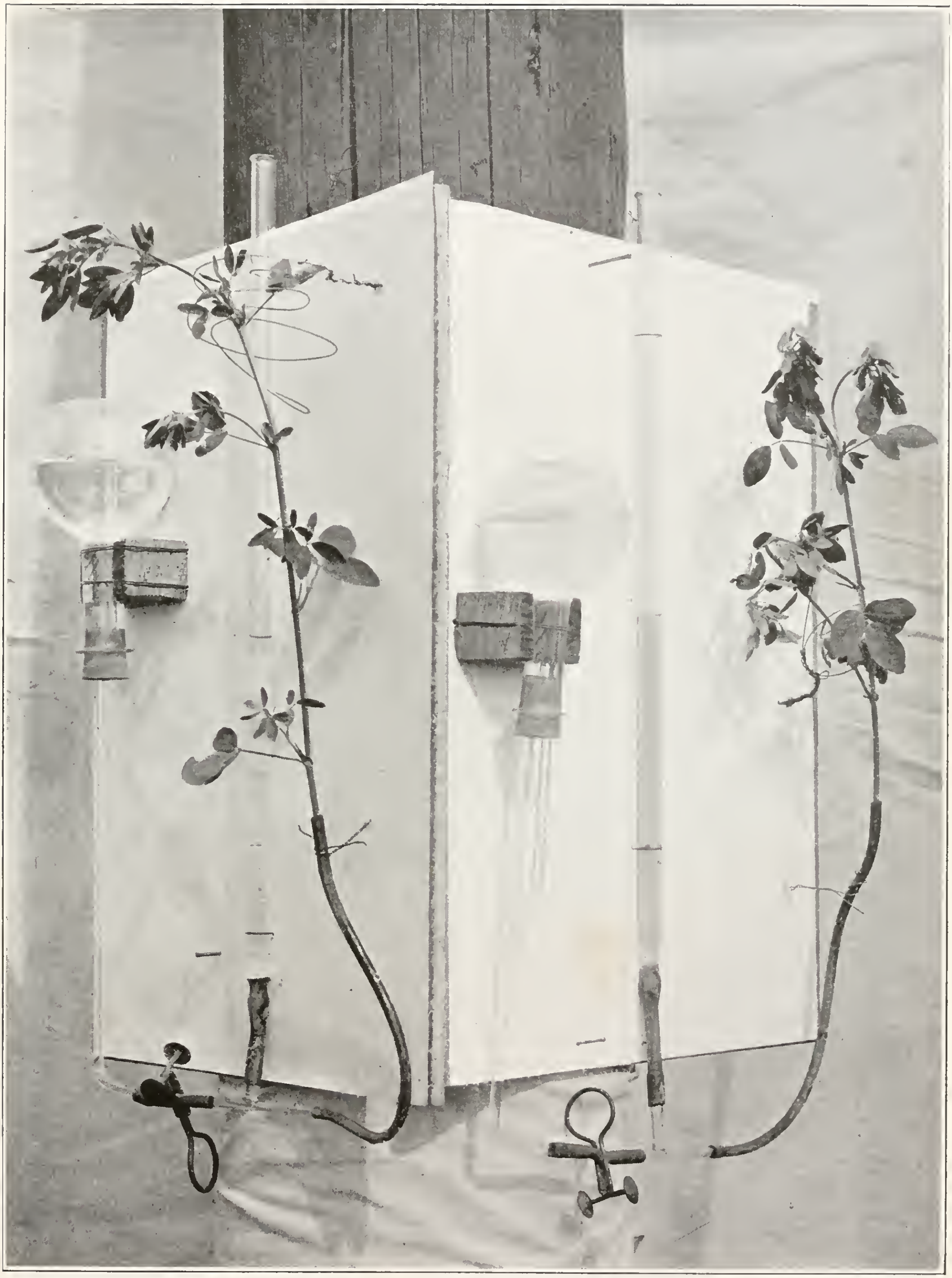


Series 16, showing stomata of onion, × 240, and factors during a 24-hour day.

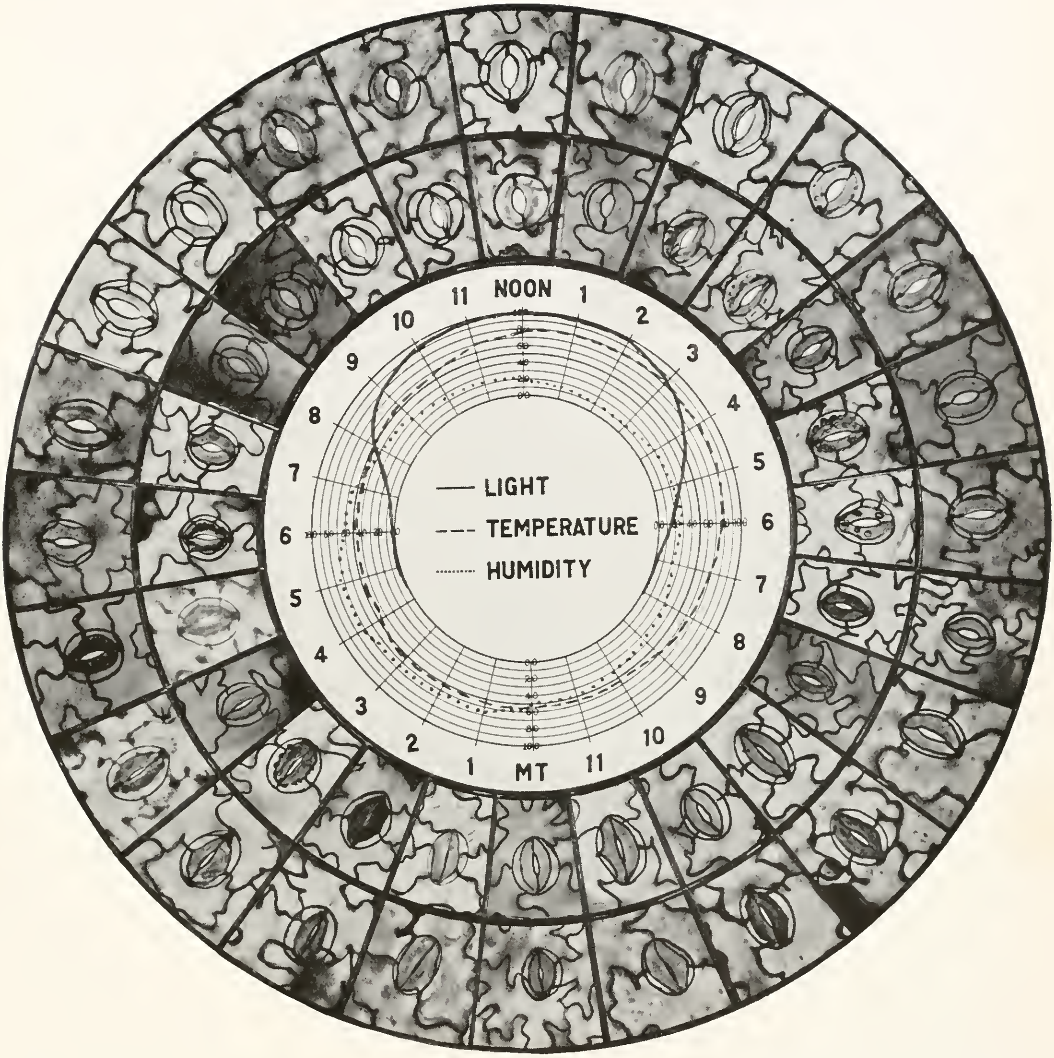


Series 16, showing stomata of corn, × 240, and factors during a 24-hour day.

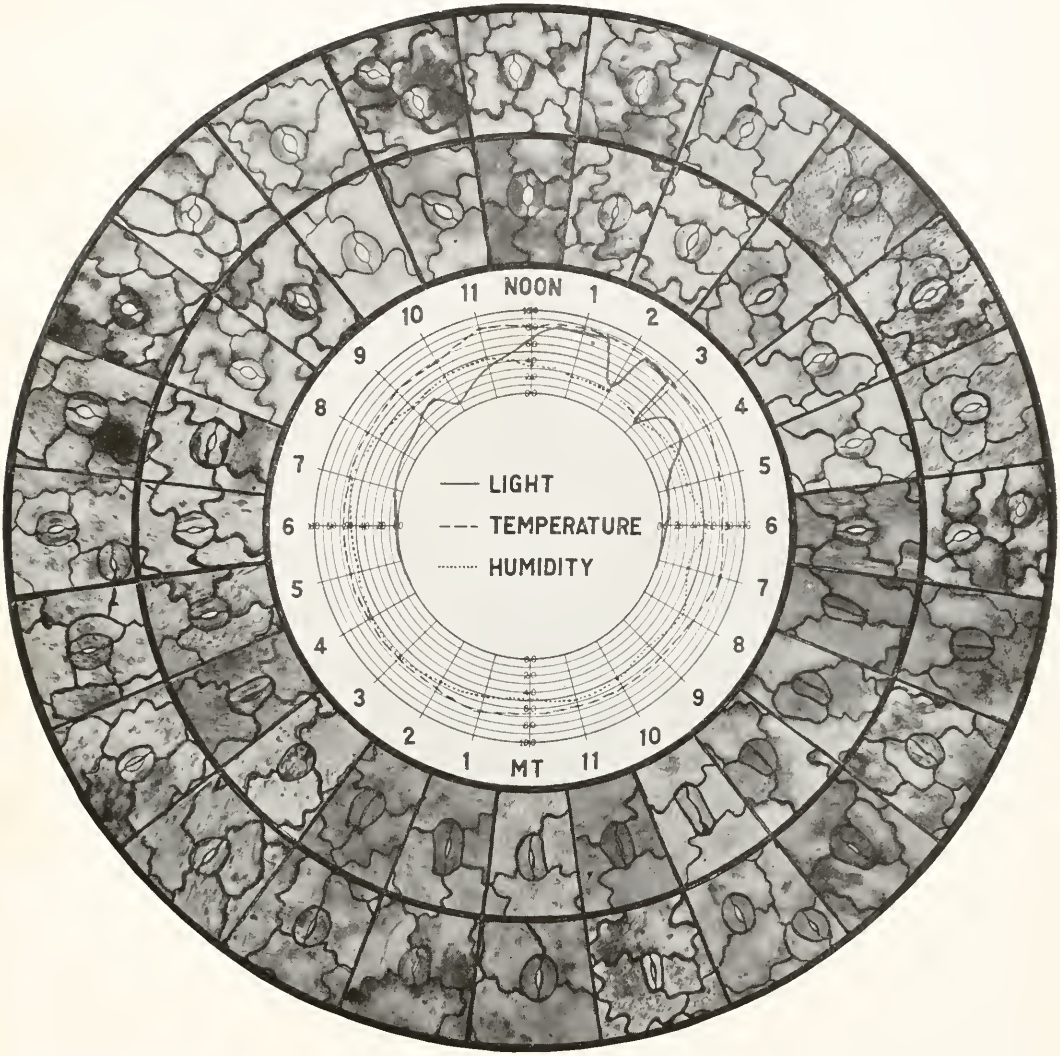
Control cabinets used for light experiments with stomata.



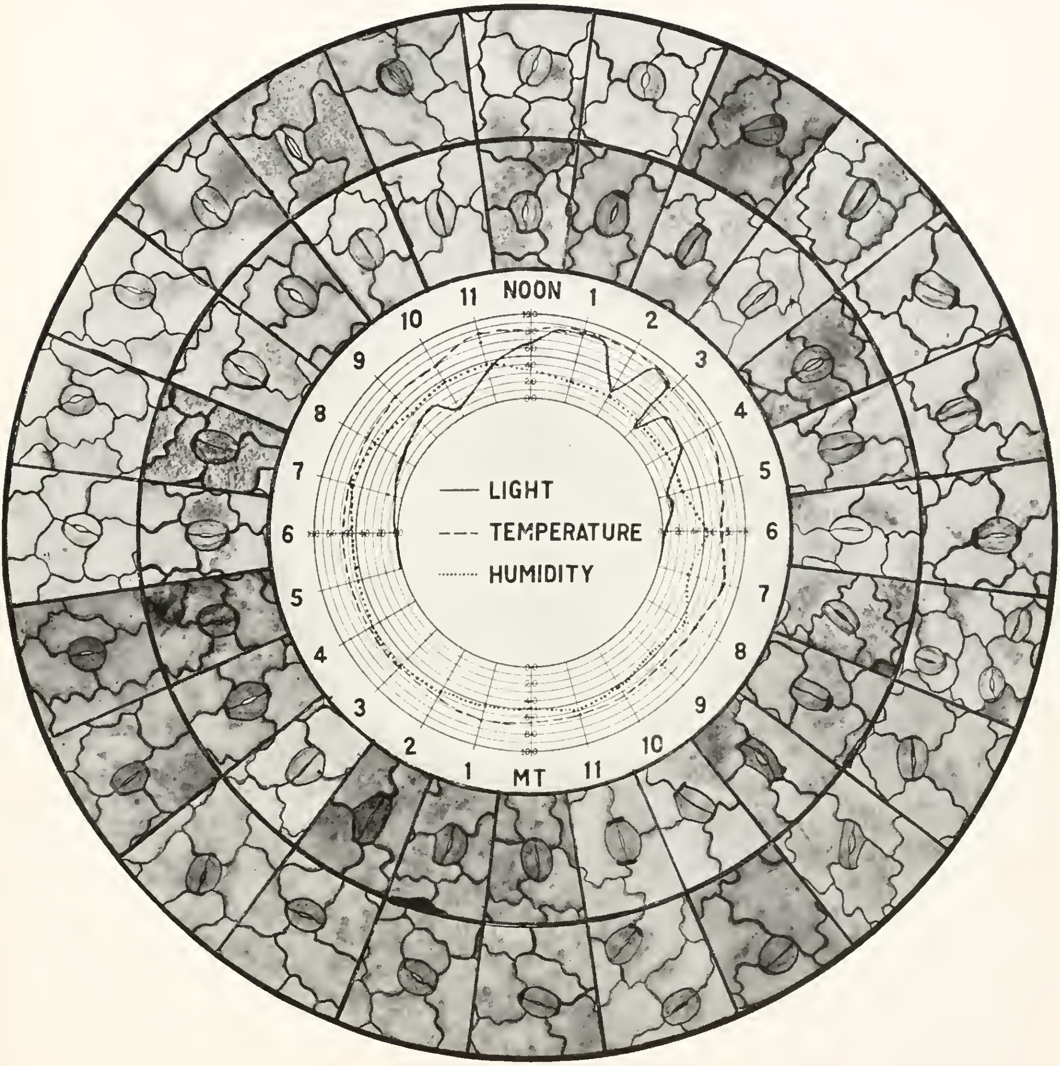
Type of potometer used for stomata experiments.



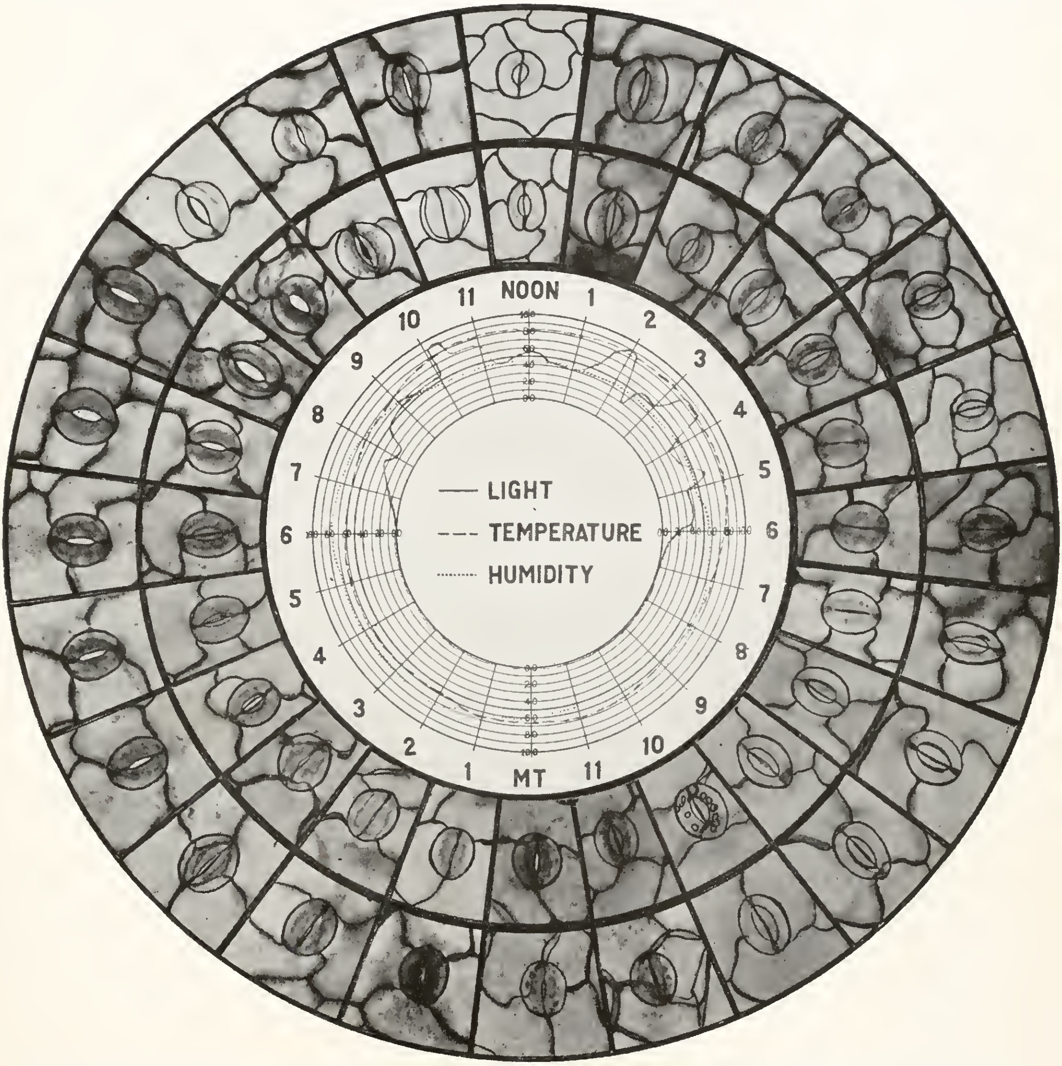
Series 28, showing upper and lower stomata of *Fouquiera splendens*, × 240, and factors during a 24-hour day.



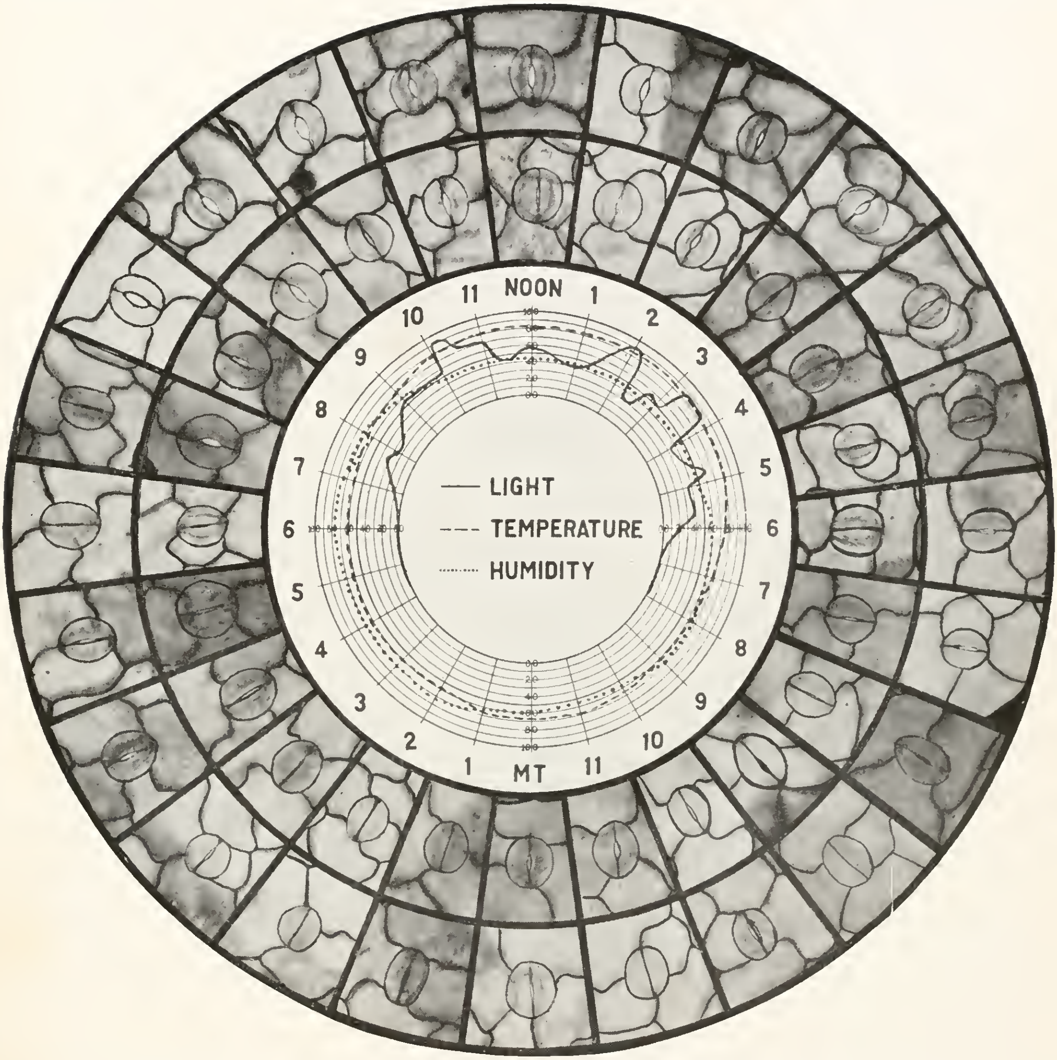
Series 32, showing upper and lower stomata of alfalfa, × 240, from a heavily irrigated plot, and factors during a 24-hour day.



Series 32, showing upper and lower stomata of cut stems of alfalfa, × 240, and factors during a 24-hour day.



Series 33, showing upper and lower stomata of cow-beet in dry pots, and factors during a 24-hour day.



Series 33, showing upper and lower stomata of cow-beet in moist soil, and factors during a 24-hour day.

THE BEHAVIOR OF STOMATA

BY

J. V. G. LOFTFIELD

PUBLISHED BY THE CARNEGIE INSTITUTION OF WASHINGTON
WASHINGTON, 1921