T H E S I S

on

BEHAVIOR OF STOMATA

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INTRODUCTION

This study was undertaken at Corvallis, Oregon, in 1932 in order to obtain information as to the behavior of stomata under western Oregon conditions where the distinctly wet season of winter and spring is followed by a summer season of practically no rain fall. As the spring rains cease the plant root systems are dependent as a rule entirely upon the stored soil moisture, and their tops are subjected to a continuously arid atmosphere. The question naturally arises whether under these conditions, as the surplus soil moisture is used up, stomata which at first remain open all day will begin to close earlier than usual when the dry air causes water loss to proceed faster than water intake. According to Loftfield's study (12) and the oral report of C. E. Schuster (Horticulturist, Bureau of Plant Industry, U. S. D. A., located at Corvallis) this closing occurs earlier and earlier in the day as the dry season progresses, so that the stomata would finally be found closed during the major part of the daylight hours. This decrease in the normal open period of the stomata would interfere with the gas exchanges that take place through them, including intake of carbon dioxide, and therefore would affect the photosynthetic or food manufacturing processes of the

plant. It would be expected that this reduction of photosynthesis would decrease the storage of manufactured food in and near the forming fruit buds, which might easily affect the next year's crop of fruit and account in part for the presence of devitalized buds and weak bloom in many western Oregon orchards.

The study of stomatal behavior is also of value because of its connection with the penetration into the leaves of germ tubes of many disease producing fungi. Since it has been found that the germination tubes sometimes enter through the stomata if they are open while the leaves are still wet with dew (Hart, 6) or while growing conditions are suitable for the fungus (Pool & McKay, 17), the time of opening of the stomata is important in connection with fungus infection--possibly the critical factor in some cases.

As far as the writer knows, except for preliminary studies by C. E. Schuster with walnuts chiefly, no previous study has been made to ascertain the responses of stomata to the dry season conditions of western Oregon. However, many investigations of stomatal behavior have been made in other regions, including a few studies involving arid conditions or a continuously decreasing water deficiency in the soil. (Loftfield, 12), (Shreve, 18)

The first phase of this study consisted of a review

of the current literature on the subject undertaken in order to learn of the technique used in observing stomata and the conclusions reached in regard to factors that influence the daily cycle of stomatal action.

REVIEW OF LITERATURE

TECHNIQUE USED IN OBSERVING STOMATA

The method devised by Lloyd (10) for observing whether the stomata are open or closed consists of stripping off pieces of the epidermis and plunging them into absolute alcohol, which extracts the water and hardens the cells so quickly that it fixes the stomata as they were when the epidermis was stripped. The epidermis should be plunged into alcohol quickly, so that the guard cells will not have time to change; and pieces of the leaf tissue should not be included, as the water it contains will dilute the alcohol.

Lloyd (11) also observed stomata directly in living attached leaves by focusing direct sunlight or strong artificial light through the leaf, the microscope being placed on a suitable support and provided with a cooling chamber attached under the substage condenser.

Loftfield (12) used Lloyd's two methods, using the absolute alcohol method extensively and checking it by means of the direct observation. He reported that these methods were "reliable in a high degree". For rapid stripping of the epidermis he used scalpel and forceps bound together with rubber bands with the end of the forceps projecting about an inch. Loftfield gives these precautions: epidermis of cereals and grasses is very

sensitive to air exposure and should be plunged into alcohol in less than a second; corn is less sensitive, but should be immersed in less than two seconds; avoid stripping a wet leaf, as the water will dilute the alcohol.

Darwin and Pertz (4) devised the vertical tube porometer, by means of which air is drawn through the stomata, the rate at which it moves through them being an indication of the degree of stomatal opening. A leaf chamber was cemented to the lower leaf surface with glue and connected with a vertical tube containing a water column; the rate of flow of air through the stomata was measured by noting the rate of fall of the water column. The leaf was turned upside down and supported by a glass plate.

In Knight's modification of the porometer (8) a large bottle with tight two hole stopper takes the place of the vertical tube; the tube from the leaf chamber leads into the bottle and a siphon leads out; as water runs from the siphon, air is drawn through the leaf chamber tube and bubbles up through the water; the time elapsing between successive bubbles indicates the degree of opening of the stomata. Knight states that this apparatus has the following advantages: (a) it maintains a constant pressure; (b) it is not necessary to readjust the water level after each

observation; (c) it is applicable to a wide range of stomatal aperature; if bubbles come rapidly the average time of ten or more may be taken. Knight (9) checked on errors due to: (a) quantity of air in the air bubble at different rates of air flow; (b) variation of depth at which the bubble is discharged; (c) change of temperature. He found these errors to be negligible. He found that some stomata tend to close when air is drawn through them, therefore the air current should be stopped when readings are not being taken; also that handling the leaf causes some stomata to close, and that they recover in about two hours.

Eric Ashby (1) compared Lloyd's alcohol method and the porometer. As the porometer reading was taken, epidermis was stripped from a similar leaf and placed in absolute alcohol. The two methods gave results which did not differ significantly except at very small aperatures. At 7:00 P.M. the stomata appeared closed when examined by Lloyd's method, but the porometer still gave readings. The porometer has the advantage that it averages the openings of some thousands of stomata, and the disadvantage that its results are relative, i.e., they do not give an absolute measure of the stomatal opening.

The potometer method of studying stomatal behavior may be disposed of with the statement that when the twig

or leaf is severed the behavior of the stomata is not normal. Loftfield (12) found this to be the case with alfalfa, and Bartholomew (2) tested the transpiration of citrus in a severed branch and arrived at the same conclusion.

Hans Molisch (15) introduced the infiltration method. If absolute alcohol is dropped upon the lower leaf surface it will enter open stomata, and xylol will enter when the pore is too small to permit the entrance of alcohol: these spread in the mesophyll, forming a spot which looks dark by reflected light and watery by transmitted light. This merely tells whether the stomata are open or closed and therefore is not used by investigators who desire quantitative results.

Stalfelt (19) reports that as a result of an investigation made by Deitrich in 1925 a series of ten fluids was so arranged that each would infiltrate a slightly smaller stomatal aperature than the preceding member of the series. The series is as follows: liquid paraffin; castor oil and turpentine mixed in the ratio of 2 to 1; alcohol; castor oil and turpentine mixed in the ratio of 1 to 2; castor oil and turpentine mixed in the ratio of 1 to 3; petroleum; turpentine; benzol; xylol; petroleum ether.

Büsgen and Münch (3) state that the usual infiltration with alcohol or ether will not work with conifers, and

that Stalfelt dips needles into a narrow glass tube nearly filled with ether so slowly that the intercellular air can be displaced by the entering ether. They give another method based on "permeability" to gasses: needles are placed in vapor of ammonia or sulphurous acid; if the stomata are closed the needles remain unchanged, if they are open the cells blacken and die.

Ray R. Hirt (7) used Lloyd's alcohol method and a method which he refers to as 'the ether infiltration method described by Stalfelt'. He used the latter mostly and stated that it is easier, quicker, and just as reliable as Lloyd's method.

Magness and Furr (13) set up a microscope in the shade of the apple trees, mounted strips of epidermis dry under a cover slip, and examined it under the high power. This is very similar to the method used by C. E. Schuster in Oregon in 1931 on walnut and demonstrated to the writer by his assistant, Mr. Voigtel. However, the walnut epidermis cannot be stripped, so thin slices of the lower leaf surface were shaved off with a sharp safety razor blade.

Loftfield (12) stained the epidermis with a saturated solution of congo red in absolute alcohol in order to obtain a good differentiation of tissues for photographing.

The following technique, useful in counting stomata,

is given by Frank A. Patty (16): cut pieces of the leaf one inch square; place them in a mixture consisting of eighty parts of water and twenty parts of clorox; stopper the bottle tightly and place in the sun all day. The pieces are usually clear the next morning. Do not let the clorox act long enough to macerate the leaf; wash in a large volume of water; place on a large glass slide and keep moist while examining.

FACTORS INFLUENCING THE DAILY CYCLE OF STOMATAL ACTION

(A) TYPICAL DAILY MARCH OF STOMATAL MOVEMENT.

Loftfield (12) made twenty-four hour series of observations on over sixty varieties of plants and classified the plants into three groups typified by barley, alfalfa and potato. The cereal type typically has no night opening and rarely shows a maximum opening of all the stomata. Under favorable conditions, alfalfa and most thin leaved mesophytes have the stomata open all day and closed all night. Alfalfa stomata open two to six hours after daylight; remain open three to six hours; and gradually close during a period about twice as long as required for opening. As conditions become unfavorable the stomata close during the middle of the day and open at night: this progresses until under extreme conditions the

stomata are closed all day and open all night. The degree of opening depends upon the water content of the plant. In the third group, illustrated by potato, the stomata open throughout the day and night under optimum conditions--especially of water content. If evaporation becomes critical, the stomata close for a time during the day when the evaporation is greatest.

(B) THE LIGHT FACTOR.

Lloyd (10) found that the stomata were affected by light. He found that in Verbena ciliata and other plants the starch content of the guard cells almost disappeared during the early forenoon, when stomata are wide open, and increased toward evening with the closing of the stomata. Experimenting with the effect of red light on attached leaves of Verbena ciliata, when the experiment was begun early in the morning, with starch at a maximum, the starch in the guard cells was reduced; starch was formed in the chlorenchyma; the stomata opened. Beginning the experiment at the time of the starch minimum, the starch in the guard cells increased; the starch content of the chlorenchyma continued to increase; the stomata gradually closed. Similar experiments were conducted with blue light. When begun in the early morning, the starch in the guard cells was reduced; starch was not formed in the chlorenchyma; the stomata opened, but not as much as with red light. When begun at the time of the normal

starch minimum, the starch increased in the guard cells; the starch content of the chlorenchyma decreased; the stomata gradually closed. Lloyd also tried the effect of darkness on Verbena cuttings with the following results: when the trial was begun in the early morning, with starch at the maximum, the starch content was not reduced; starch was not formed in the chlorenchyma; the stomata opened little if at all. When darkness was begun late in the forenoon, with the starch at a minimum, starch reformed rapidly in the guard cells; it disappeared from the chlorenchyma; the stomata gradually closed. Lloyd observed some opening of the stomata in prolonged darkness.

Darwin and Pertz (4) using the porometer on a potted Tropaeolum in a dark room got a prompt partial closure of stomata with darkness. With a cut leaf of Helianthus annus in water, fifty minutes of darkness started at 11:25 A.M. brought about partial closure; thirty minutes of darkness started at 1:00 P.M. brought about more complete closure. They report that marsh plants do not close their stomata in darkness: also that in laurel (P. Laurocerasus) and Nicotiana the stomata begin to open about an hour before sunrise.

Loftfield (12) studied the effect of light on the starch relation by means of four cabinets which reduced the light in different degrees, and by means of mazda lights. He experimented on alfalfa, cow beet, and potted wheat and

corn, and arrived at the following conclusions:

(1) Light induces the opening of stomata after daybreak by initiating conversion of starch in the guard cells into sugar. The starch content of the guard cells does not wholly disappear, but is lowest about 10:00 A.M.

(2) During the middle of the day, until shortly before the stomata start to close, the rise in starch content is very slow, but rapid during closure, then slow again after closure, and further retarded during the night.

(3) "Changes of opening caused by factors other than light are not necessarily accompanied by corresponding changes in the starch content of the guard cells."

(4) Reduction of light to less than half of normal is usually necessary to affect the stomata of plants growing in the open.

(5) Decreasing the light has more affect when the stomata are closing than when they are opening.

(6) Stomata open at night due to moonlight or strong artificial light of much less intensity than 1% of the sunlight maximum. They open more readily toward morning than before midnight.

Helen Hart (6) found that wheat stomata open gradually after sunrise, and states that sunlight is the most important stimulus for the opening of stomata. In her study, artificial light did not prolong the period of open-ness for stomata of cereals in the greenhouse.

Büsgen and Münch (3) state that wave length of light influences stomatal behavior and transpiration; that the stomata close rapidly at night or in artificial darkness; and that in case of prolonged darkness reopening may occur.

Magness and Furr (13) found, in their studies of stomatal activity in apple leaves, that opening began soon after daylight; that the stomata were almost fully open half an hour later; and that the stomata of leaves in direct sunlight open somewhat earlier than those in the shade.

Bartholomew (2) tried the effect of two hours of artificial darkness on the rate of transpiration of citrus leaves and found that it reduced the moisture loss of the lower leaf surface to about one third of normal.

Miller (14) gives the following two viewpoints as to the cause of opening and closing of stomata:

(a) In the morning, light initiates the action of diastase, probably by decreasing the acidity of the cell sap of the guard cells; diastase changes the starch to sugar, which increases the osmotic value of the cell sap and causes water to enter the guard cells; the osmotic value of the cell sap of the epidermal cells remains constant; the guard cells swell and open the stomata.

(b) Illuminated and open guard cells are alkaline in reaction, probably from utilization of carbon dioxide in photosynthesis, and certain colloidal contents of these

cells become much swollen as the pH value increases. The high turgidity of the guard cells is mainly caused by colloidal imbibition. Closure is accompanied by increased H ion concentration due to accumulation of carbon dioxide of respiration when photosynthesis ceases on account of low light intensity. The colloids are dehydrated as they approach their isoelectric point; the bound water is freed; the guard cells lose their surplus water to the surrounding cells and the stomata close. If hyperacidity develops due to prolonged darkness, the colloids of the guard cells may become more acid than their isoelectric point, produce an acid swelling of the colloids, and bring about night opening.

(C) THE WATER CONTENT FACTOR.

The water content factor seems to be next to light as to importance in determining behavior of stomata. Either withering of a detached leaf or reduction of water content in rooted plants below a certain critical point results in closure of stomata.

Darwin and Pertz (4) observed the closing in withering leaves. They also observed that closing was often preceded by a period of increased transpiration which they ascribed to temporary opening of stomata. For example, in the experiment with Nicotiana glauca the stomata began to open rapidly within five minutes of the severance of the leaf; the opening continued for twenty-eight minutes, then

rapid closure occurred which gradually became slower.

We have previously noted Loftfield's observations that, as water deficiency increases, the stomata close earlier in the day and finally in case of extreme water deficiency reverse their normal behavior and are closed all day and open all night.

Thus the water factor may modify the response to the light factor and finally completely nullify it. Moderate decrease of water content results in the stomata becoming more responsive to evaporation, and to light as well. Unfavorable conditions result in a more irregular behavior of the stomata, which may go to the extreme of alternate opening and closing. When leaves of alfalfa, sweet clover, and nasturtium were wetted, in Loftfield's experiments, the stomata opened. He states than when leaves were wet by dew or rain the stomata usually opened if closed or opened more widely if partly open. When the water dried, partial or complete closure occurred. Water content of the soil is one of the chief factors determining the rate of supply of water to the leaves: if the soil is dry the rate of supply is slow, turgor is lost early in the day and the stomata close. Water logging of the soil causes closing of stomata and wilting due to the failure of the roots to function.

The succulence of the leaves and growth habit of the plant affects the "working margin" of water stored in the tissues, and therefore affects stomatal behavior.

Loftfield (12) stresses this "working margin." He expresses the opinion that it offers an explanation of mid-day closing and night opening of stomata. He says, "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 percent of water rises once more above this point and the leaf again has a margin with which to safely operate."

Magness and Furr (13) found that the open period for apple stomata depended largely upon moisture conditions. In the case of their irrigated plot, on a warm day ninety-five percent of the stomata were open at 7:00 A.M., practically one hundred percent from 8:30 to 9:30, and about forty percent still open at noon: on the dry plot not over five percent were open at the peak of opening. Furr and Magness (5) state that 'the length of time the stomata remain open is apparently determined by the amount of soil moisture and the evaporating power of the air'; and that, 'the tree adjusts itself to reduced water supply through earlier closing of the stomata each day'. The writer has read of but one case running contrary to the above; Ray R. Hirt (7) in his report of 1931 work on the white pine blister rust states that stomatal movements were not noticeably affected by weather conditions such as rain, fog, dew, sunshine, and moonlight. He says nothing about soil moisture.

(D) THE TEMPERATURE FACTOR.

Loftfield (12) conducted twenty-three experiments on the relation of temperature to stomatal behavior, discarded results from six, and from the remaining seventeen reports that:

(a) The temperature of the air affects the rate of opening in the morning. The time required for opening is reduced about one-half for every 10° C. rise in temperature.

(b) When the temperature of the soil rises too much the stomata close and in extreme cases the plant wilts.

Miller (14) reports that Zalenski (1921) found that when the temperature rises to 35° to 50° C. starch in the guard cells is transformed to maltose, the turgor rises, and the stomata open widely. This also occurs in the wilting of plants. Fifty species of plants were observed and all but millet and succulent plants showed opening of stomata at high temperatures.

(E) THE HUMIDITY FACTOR.

Loftfield (12) states that high humidity of the air permits the stomata to open wider and remain open longer than low humidity under most conditions, especially if the plant has difficulty in obtaining enough water to offset the daily evaporation.

Pool and McKay (17) found that high humidity favored stomatal opening in sugar beet leaves and that low

humidity was associated with closure. The stomata were likely to be found open if the humidity remained above 60 percent during daylight hours, and closed if it fell below 50 percent.

(F) THE AGE FACTOR.

The age of the leaf affects stomatal action. According to Büsgen and Münch (3) the stomata are fully mobile only on a mature leaf; leaves lowest on the shoot are the first to have adjustable stomata; and mobility decreases with age, especially in several-year old needles. Bartholomew (2) mentions the differences due to age of the leaf in his study of transpiration of citrus leaves. The extreme difference occurred in lemon in which the transpirational loss of young leaves compared to old leaves was in the ratio of 3½ to 1: the age difference was not over four to six months. He found that orange leaves varying from two to twenty months in age were fairly similar in transpiration. Pool and McKay (17) found that in the case of sugar beet leaves stomatal activity was greater in mature than in young leaves, and slight in old leaves.

(G) MISCELLANEOUS FACTORS.

Loftfield (12) reports that usually the plant shows less response to wind than does the atmometer, but with a sudden high wind the plant shows greater response. Wind coats the leaves with dust and often wedges particles into

the open stomata, wedging them open.

The removal of hairs from a leaf causes the stomata to open earlier in the morning and close very much sooner than normally according to Loftfield's observations.

Büsgen and Münch (3) state that withholding carbon dioxide leads, in either light or darkness, to opening of stomata. Lloyd (10) conducted experiments with carbon dioxide free air but does not give conclusions as to any effect on behavior of stomata.

Magness and Furr (13) report some indication that the presence of fruit (apples) results in the stomata remaining open longer than in its absence.

THE PRESENT INVESTIGATIONS

The studies reported here were undertaken in the spring and early summer. Several different methods of determining the relative aperature of stomata were tested out. In connection with this work a short series of experiments was conducted to learn how the stomata of certain test plants would respond to changes in the moisture content of the soil, to variations in such factors as light, atmospheric humidity and temperature, and to a reduction of total transpiration rate for the plant by foliage removal. The experiments were not intended to develop detailed and complete information regarding stomatal behavior in the test plants nor to learn the effect on such behavior of the entire series of environmental changes which take place in the course of the growing season. They were undertaken rather to determine the most satisfactory types of stomatal tests to use under different conditions, to shed some light in a preliminary way on the sort of stomatal response which might be expected under various conditions and to learn something of the degree of variability or extent of experimental error which might be expected in the data arrived at by different means. The studies were intended to clear the path and point out the way for the more detailed and more prolonged studies which must be conducted in the future

before the full influence of external factors on stomatal activity in various crops can be determined for Oregon conditions.

As a preliminary step in this investigation, in order to acquire some facility in examining plants for stomatal behavior, the writer made observations by the following methods:

(a) Lloyd's method (10) plunging stripped epidermis into absolute alcohol;

(b) The immediate microscopic examination of stripped or shaved epidermis;

(c) The porometer method of Darwin and Pertz (4);

(d) The infiltration method devised by Molisch (15), using absolute alcohol and xylol;

(e) Stalfelt's method (19) of dipping pine needles into a narrow tube filled with ether.

Knight's modification (8) of the porometer was used: the aspirator bottle was of gallon size; the long arm of the siphon was 14 cm. longer than the short arm and the water was allowed to drip directly from the tube without the use of an overflow vessel. The short arm ended within the bottle in a capillary tip about 1 mm. in internal diameter. The leaf chamber used in experiment 1 had a cross section area of 1.33 square centimeters: the leaf chambers used in all the later porometer experiments were approximately 1 square centimeter in cross section. The

leaf chamber was connected, by means of rubber and glass tubing, through a two-way stopcock to the short arm of the siphon. A piece of rubber tubing about 9 centimeters long was slipped upon the stem of the leaf chamber and wired securely, then a piece of glass tube was inserted into the line of tubing leading to the short arm of the siphon so that the tubing could be easily disconnected at this point and shifted from one leaf chamber to another. When readings were not being taken either the tube was disconnected from the leaf chamber or the stopper was removed from the stopcock. A pinchcock on the long arm of the siphon prevented the emptying of the aspirator bottle.

The leaf chambers were cemented to the under surface of the leaves with "Tree Seal", an emulsion of asphalt in water. The leaf remained in its natural position, the leaf chamber being supported by a ring-stand clamp in experiment 1, and in other experiments by a piece of number 6 wire with one end wound into a spiral to receive the leaf chamber; the distal end of the wire was thrust into the soil in the case of potted plants, or bound to a twig with friction tape in the case of shrubs or trees.

In taking readings the short arm of the siphon was put into communication with the leaf chamber as previously described, the pinchcock removed from the long arm, and as bubbles of air rose within the bottle they were counted for a period of 15, 30, or 60 seconds. If the bubbling

was very slow, intervals between successive bubbles were noted, an average obtained, and the result recorded in terms of number of bubbles per 30 or 60 seconds.

Some difficulty was experienced in keeping the leaf chambers sealed to the very pubescent surfaces of geranium leaves so that air leakage would not occur. Some of the leaks were especially insidious, as they usually occurred in the morning when the turgidity of leaf and trichomes was at the maximum, then were spontaneously sealed by the somewhat plastic "Tree Seal" as the day advanced. Even if positively identified at once, the presence of a leak necessitated the loss of the reading for the time period involved, since if fresh "Tree Seal" was applied, it had to be allowed to dry out and set for a time in order to be sure of a tight joint. The seal gave no trouble on the smooth leaves of lilac and fuchsia.

The porometer method provides a quantitative measure of stomatal aperature changes at convenient intervals over considerable periods of time but changes in temperature affecting the air volumes in the apparatus introduce a source of error difficult to evaluate and hard to overcome.

The following studies were conducted by means of the porometer and infiltration methods, supplemented by some use of Lloyd's method:

(A) Comparative effectiveness of absolute alcohol and

and formalin-acetic-alcohol fixing fluid in fixing open stomata.

(B) The response of stomata to continuous drying out of the soil in the case of herbaceous potted plants.

(C) The response of stomata upon watering potted plants after drying out of the soil.

(D) The effect of pruning upon stomatal behavior in potted plants subjected to drought.

(E) The daily cycle of stomatal behavior in lilac, and the accompanying weather and soil moisture conditions.

(F) The daily cycle of stomatal behavior in the Royal Anne cherry and accompanying soil moisture conditions.

(A) COMPARATIVE EFFECTIVENESS OF ABSOLUTE ALCOHOL AND FORMALIN-ACETIC-ALCOHOL FIXING FLUID IN FIXING OPEN STOMATA.

Lloyd's method of stripping epidermis and plunging into absolute alcohol was tested and for comparison a single trial of formalin-acetic-alcohol fixing fluid was made with each of three kinds of leaves in order to find out whether this material would fix them equally well with whatever degree of opening they happened to possess at the time. The composition of the fluid was: formaldehyde, 5 cc; glacial acetic acid, 5 cc; 70% alcohol, 90 cc. Leaves of cherry, nasturtium, and geranium were used; a piece of epidermis was stripped and quickly plunged into absolute alcohol, then a second strip of epidermis was at once

removed from the same leaf and plunged into formalin-acetic-alcohol fixing fluid. Later the strips of epidermis were mounted on a slide in the fluid in which they were fixed and a count made of the degree of opening of thirty stomata. The results are recorded in Table I.

Table I. Fixation of stomata by absolute alcohol and by formalin-acetic-alcohol.

Degree of stomatal opening	Absolute Alcohol			Formalin-acetic-alcohol		
	Cherry	Nastur-tium	Geran-ium	Cherry	Nastur-tium	Geran-ium
0	0	0	1	Mostly	0	15
Slit	0	0	2	slits;	1	15
1/4	15	4	3	nothing	27	0
1/2	11	7	21	one	2	0
3/4	4	11	3	half	Many	0
Fully open	0	8	0	open	scarcely more than slits	0

The results of these three trials were so uniformly unfavorable to the use of formalin-acetic-alcohol as an agent for fixing open stomata that it was not considered necessary to test it further.

(B) THE RESPONSE OF STOMATA TO CONTINUOUS DRYING OUT OF THE SOIL IN THE CASE OF HERBACEOUS POTTED PLANTS.

In these experiments the effect of continuous drying out of the soil on stomatal aperature was studied. A single potted geranium plant was used in the first test, two geraniums in one pot in the second test, and two

fuchsias in a single pot in a third test. The porometer method was used.

In each of the three experiments leaf chambers were sealed to a leaf of a plant in normal growing condition, the soil was saturated with water, and porometer readings were taken at sufficiently frequent intervals to establish a daily curve of stomatal behavior. When the stomata showed an unmistakeable response to the drying out of the soil, a soil sample was taken from the pot with a cork borer and the percent of moisture determined. Porometer readings were recorded in terms of the number of bubbles in thirty seconds. In Experiment 1 the number of bubbles was usually counted for two sixty-second periods or four thirty-second periods; in Experiments 2 and 3, for two or three thirty-second periods. The plants were kept on a north window ledge where they were subject to natural outdoor conditions except that they did not receive direct sunlight and were quite fully sheltered from winds.

In Experiment 1 the plant was thoroughly watered at 6:00 P.M. May 17 and allowed to dry out continuously until immediately after the taking of the soil sample at 5:30 P.M. May 24, when the soil was again saturated with water seven days after the first watering.

The results for Experiment 1 are presented in Tables II A and II B and in the graphs in Figure 1.

Table II A, Experiment 1, May 18-21. Porometer readings in bubbles per minute, and accompanying conditions of weather and temperature.

Date	8 A.M.	10	12	2	4	6	8	10P.M.
18	--	--	66	77	52½(1)	-	24½	20
	Cl	Cl	Cl	C	C			
	Temperatures, Max. 24, Min. 9½ Centigrade.							
19	60	54	62½	78	53	36	21	19
	C	R	R	Cl	C	Cl		R
	Temperatures, Max. 27, Min. 10.							
20	64	43(2)	34	32	32	22	9	7
	C	C	R	Cl R	C	Cl	Cl	Cl
	Temperatures, Max. 32, Min. 11.							
21	63	42½	37	64	27	12	8	--
	PC	R	PC	R	PC	Cl	Cl	
	Temperatures, Max. 28, Min. 13							

Symbols: Cl, clear; C, cloudy; PC, partly cloudy; R, rain. Notes: (1) taken at 5:00 P.M.; (2) at 10:45 A.M.

Table II B, Experiment 1, May 22-28 (Continuation from Table II A) Porometer readings in bubbles per minute and accompanying conditions of weather and temperature.

Date	8 A.M.	10	12	2	4	6	8	10 P.M.
22	50	30	7	34	12½(3)	--	8(4)	6(3)
	C	C	C	C	R			
	14	15	16	17	15		14	13
23	36	8	6	8	16	23	10	10
	R	PC	Cl	Cl	Cl	Cl	Cl	Cl
	15	16	18	18	17	15	11	10
24	23	6	1½	--	8½	5½(W)	5½	4
	Cl	C	C		Cl	Cl	Cl	Cl
	12	14	14		16	16	12	11
25	38	25½	21	42	33(5)	17	6½	3½
	C	PC	Cl	C	C			
	11	13	14	15	16	15	14	13
26	--	--	39	23	32	14½	5	3½
	C	C	C		PC	Cl	Cl	
	12	15	17	18	17	17	15	13
27	42	46	17	7	14½	3½(5)	1½	1½(6)
	C	C	C	C	R	R	R	
	13	15	17	17½	15	14	15	16
28	48½	28	12	8(7)	6			
	C	C	C	Cl	C			
	16	18	19	21½	22			

Symbols: (W), plant watered; other symbols as in Table II A. Notes: The third line under each date in Centigrade temperature. (3) taken 20 minutes after the hour; (4) at 7:20 P.M.; (5) taken 30 minutes after the hour; (6) at 9:00 P.M.; (7) at 3:00 P.M.

Explanation of Figure I

Graphs of porometer and temperature readings obtained in Experiment 1.

Upper nine graphs--porometer readings.

Lower three graphs--temperatures in Centigrade degrees to accompany the porometer reading graphs just above them.

"W", May 24 Graph--time of watering.

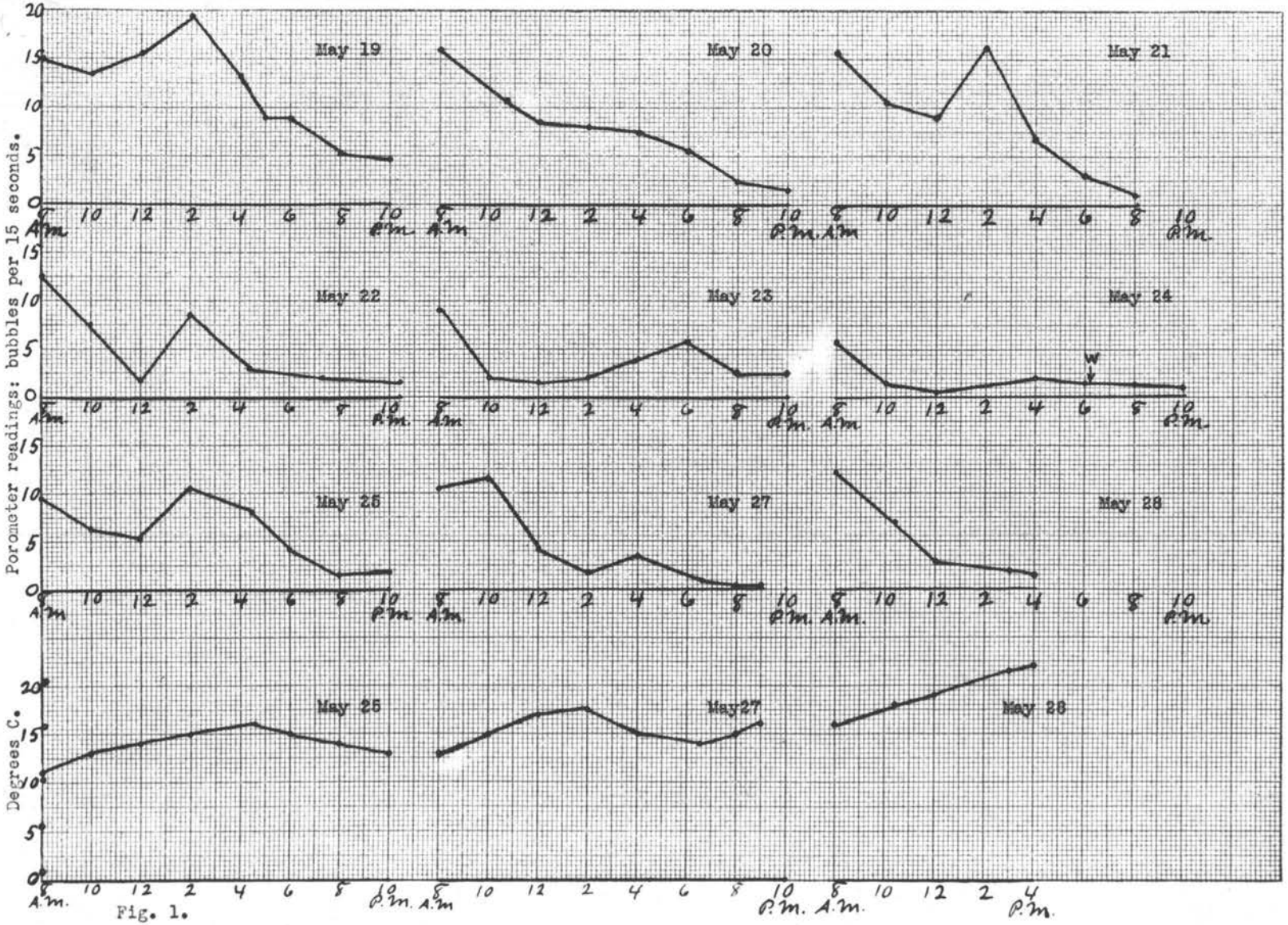


Fig. 1.

It will be noted from the tabulated results of Experiment 1 (Table II) and from the accompanying graphs (Figure 1) that the open period of the stomata had decreased so that they were only moderately open at 8:00 A.M. and were nearly closed from 10:00 A.M. to 10:00 P.M. on May 24. On May 23 the 8:00 A.M. opening was only 58% of the average 8:00 A.M. opening for May 19, 20, and 21, and throughout the rest of the day the stomata were nearly closed except for a moderate opening from 4:00 to 6:00 P.M. May 23 and 24 were the sixth and seventh days of drying out. A soil sample taken at 5:30 P.M. May 24 showed 12.1% of moisture.

The results could not be considered as proving that this decrease in degree and duration of opening was due entirely to soil moisture deficiency, since there was considerable variation in the daily cycle of stomatal behavior; but that much of the decrease in stomatal activity was due to lack of moisture supply is indicated by the fact that considerable recovery occurred after the plant was watered at 6:00 P.M. May 24. This recovery is shown in the data and curves for May 25-28.

The recovery was far from complete and seemed to be of only about three days duration; therefore the soil was again thoroughly saturated with water at 4:30 P.M. May 28 and porometer readings were taken on May 31 and 31.

These readings showed still less recovery than those of May 25-27. Upon examining the leaf to which the leaf chamber was attached it was found to be showing signs of age and deterioration. A little yellowing was beginning to show at the edge of the leaf to one side of the leaf chamber. The leaf was mature when the experiment was started, and the leaf chamber had been attached to it eleven days at the end of May 28.

In this experiment there does not appear to be any close correlation between porometer readings and temperatures. To illustrate this inference, there are shown in Figure 1 three temperature curves which accompany the three porometer reading curves of similar date.

Experiments 2 and 3 constituted the second attempt to test the effect of drought on behavior of stomata of potted plants. These two experiments were carried on simultaneously; Experiment 2 with two geranium plants growing in one pot, and Experiment 3 with two fuchsias of the Phenomenal variety in one pot. The plants were potted together in each case in order to be sure that the two specimens of each plant were growing under the same conditions and in soil of the same moisture content. A leaf chamber, held by one of the wire supports previously described, was attached to a leaf of each plant, using care to select healthy leaves that were neither old nor young.

The leaf chambers were attached to the two geranium plants June 10, but other experiments took the writer's time so that regular observations on these plants were not taken until June 18. The two plants, which had been growing in separate pots, were placed in a large pot and watered on May 18. Since the leaf used for the porometer readings in Experiment 1 had deteriorated before the experiment was fully finished, successive porometer attachments were made in Experiment 2, even though readings were continued with the old attachment. Thus on June 21 a second leaf chamber, and on June 25, a third, was attached to each plant. The first attachment was removed shortly before the third was made. The geranium plants were designated as No. 1 and No. 2; the numbers 1, 1A, and 1B were assigned to the leaf chambers successively attached to geranium No. 1; and 2, 2A, and 2B, to those attached to geranium No. 2.

The porometer readings resulting from this experiment are given in Table III and graphically represented in Figure 2.

Humidity observations taken in the immediate locality of the experiment were not available; the humidity figures used in Figure 2 were obtained at the East Farm, about 2 miles away, and were not corrected for lag of the hydrothermograph pen. Therefore, no particular accuracy can be claimed for the humidity curves; they are introduced merely to show the general trend of humidity on the

Table III. Porometer readings in bubbles per minute and accompanying temperatures in degrees Centigrade. Experiment 2; June 20-29. Symbols: L. Leak in leaf chamber; Po.1, Po.2, etc. Readings of porometer leaf chamber No.1, No.2, etc.

6/20	8:30A.M.	10:30	2:00	4:00	5:30	8:00	10:00P.M.
Temp.	20	24	27	27	--	23	21
Po. 1	L	18	14	17	15	14	14
Po. 2	L	168	14	16	43	22½	14½

6/21	6:25A.M.	7:50	10:00	12:00	2:00	5:00	8:25	9:25P.M.
Temp.	19	20	26	--	26	24½	20	19
Po. 1	28	37	22	15	96	22	15	14
Po. 1A	65	38	31	19	125	32	17	13
Po. 2	--	47	22	53	35	59	12	11½
Po. 2A	L	112	41	72	126	40	12	10

6/22	9:00A.M.	10:00	12:00	1:50	4:00	5:30	8:00	9:30P.M.
Temp.	--	20	23	24	25	--	18½	17
Po. 1	23	35½	27	5¼	10	9¼	13	10
Po. 1A	40	115	89	4	3¼	45	9½	8
Po. 2	82	44	34	9½	14	19	5¼	4½
Po. 2A	129	121	51	8	6½	28	13	12

6/23	8:30A.M.	10:45	1:30	3:00	5:00	7:45	9:00P.M.
Temp.	15½	18	21	22	22½	21	18
Po. 1A	29	14½	19	23½	10	6¼	4¼
Po. 2A	24	22½	16	12	15½	12	8½

6/24	8:30A.M.	10:00	12:00	2:30	4:00	9:00	10:00P.M.
Temp.	18	19½	22	24	23	16	16
Po. 1A	25	12	3½	2½	2	14	12
Po. 2A	17	14	9	11½	13	21	16½

6/25	8:15A.M.	10:00	12:00	2:00	4:00	6:00	8:00	10:00P.M.
Temp.	14	17	20½	23	24	24	22	20
Po. 1A	46	23	9	6	4½	6	5	3½
Po. 1B	19	9½	6½	2	0	3½	1	.8
Po. 2A	31	15½	10	9¼	9	11½	9½	7½
Po. 2B	13	1½	6	1	.8	3½	1¼	0

6/28	8:20A.M.	10:00	12:00	2:00	4:00	7:30P.M.
Temp.	20	24	27	29	29	24
Po. 1A	20½	6½	3	4	4	1
Po. 1B	14½	8	5	4	5	4¼
Po. 2A	31½	21	19	21	24	17
Po. 2B	11½	7¼	6½	8½	10	5

(continued on following page)

(Table III continued)

6/29	8:20A.M.	10:00	12:00	2:00	5:00	8:15P.M.
Temp.	18	20	22½	25	--	21
Po. 1A	34	25½	11	6½	3	2½
Po. 1B	24	24½	19	16	11½	7½
Po. 2A	141	122	78	61	51	12
Po. 2B	28	30½	27	25	22	8½

Explanation of Figure 2.

Graphs of porometer and temperature readings obtained in Experiment 2: humidity records obtained two miles away on the same dates.

Porometers 1, 1A, and 1B were attached to geranium No. 1 on June 10, 21, and 26 respectively. Porometers 2, 2A, and 2B were attached to geranium No. 2 on the same dates.

Humidity figures were divided by two in graphing; i.e. on the humidity and temperature graph for June 21, the 6:00 A.M. humidity stands at 44, which means that the humidity was 88%.

Temperatures are in degrees Centigrade.

Plants were watered at noon, June 18.

Plant No. 1 was pruned just before 6:00 P.M. June 24 (See "Pruned" on the June 24 graph).

Plants watered again at noon June 27.

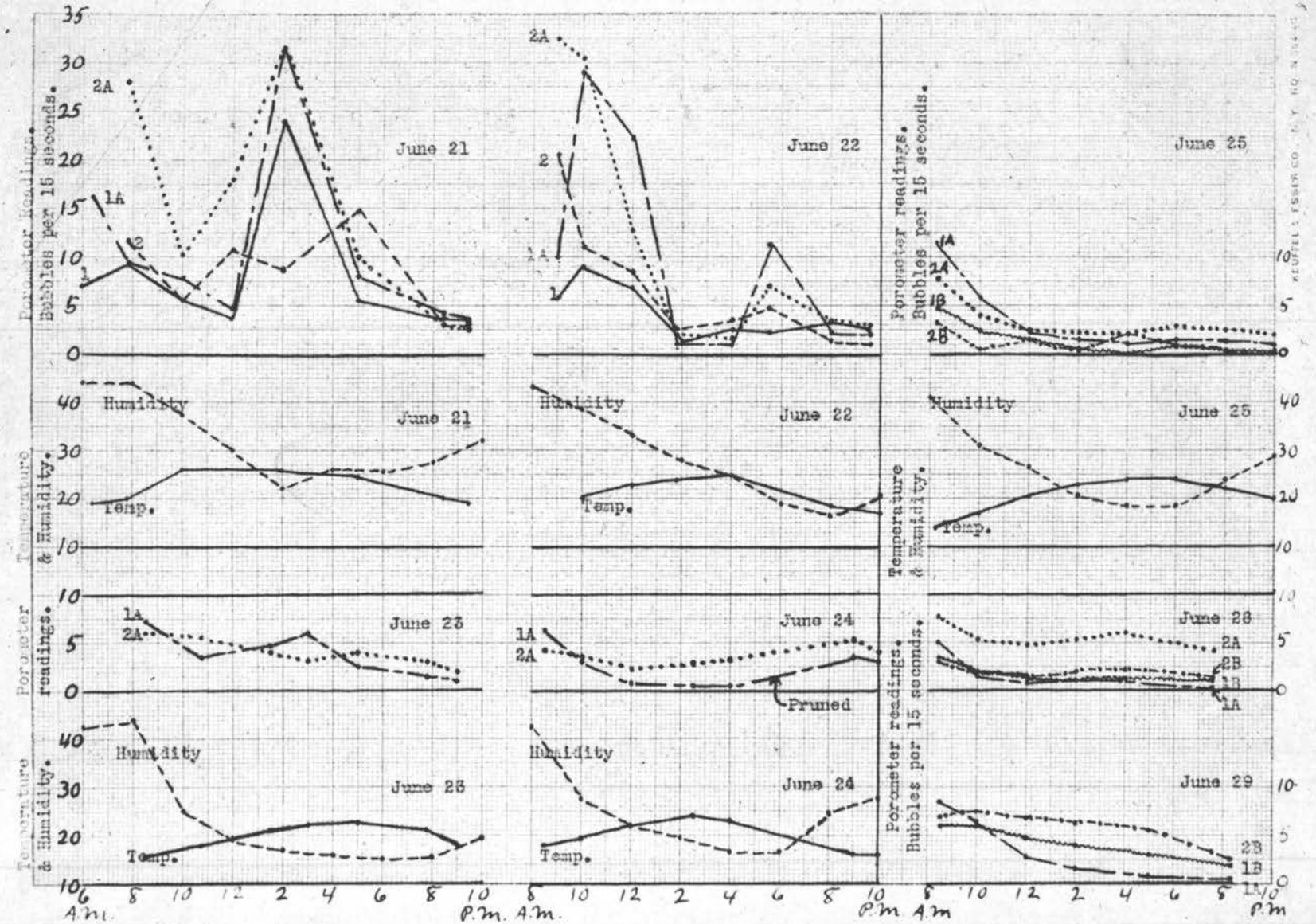

Porometer readings. Bubbles per 15 seconds.
Temperature & Humidity.
June 21
June 22
June 25
June 23
June 24
June 26
June 27
June 28
June 29
Humidity
Temp.
1A
2A
1B
2B
Pruned
A.M.
P.M.

Fig. 2.

days concerned. The data on which they are based are given in Table IV.

Table IV. Humidity observations for the period June 20-25 and 28-29, 1932, from hydrothermograph record at the East Farm.

Date	6 A.M.	8	10	12	2	4	6	8	10 P.M.
20	89%	88%	78%	57%	49%	38%	34%	43%	53%
21	88	88	77	60	44	50	51	57	64
22	84	86	77	66	56	50	38	33	41
23	85	88	50	39	34	32	30	31	39
24	76	85	55	44	39	37	37	49	55
25	85	82	62	53	41	37	37	48	58
28	65	55	45	38	30	31	47	68	75
29	75	58	45	35	33	28	38	73	79

A soil sample taken from the pot with a cork borer at 8:00 P.M. June 24 showed 22.28% of moisture.

An examination of the porometer readings in Table III and graphs of Figure 2 shows that there was, as in Experiment 1, a distinct decrease in duration of daily stomatal opening as drying out of the soil proceeded. This is graphically illustrated in the curves for June 21 and 22 as contrasted with those for June 23 and 24.

On account of the difficulty experienced in maintaining an air-tight seal between leaf and leaf chambers with geranium, fuchsias were selected as a subject in a duplication of the drying out experiment. These plants had the apparent advantage of possessing smooth glazed

leaves, and, like geranium, thrive well as potted plants. They were quite satisfactory so far as the leakage trouble was concerned, but proved to be poor subjects with regard to stomatal activity. The stomata did not show a clear cut daily cycle of behavior: the porometer readings were low during most of the day so that there was no marked closing of stomata as evening approached, and small temperature changes affecting the porometer made such readings less reliable than would be the case with readings of greater magnitude. Such conclusions as could be drawn from the porometer readings with the fuchsias supported the conclusions from the experiment with geraniums; i.e. there was a tendency toward minimum opening of the stomata as drying out of the soil progressed: this tendency was less clearly shown than in geranium, presumably for the reasons above stated.

(C) THE RESPONSE OF STOMATA UPON WATERING POTTED PLANTS AFTER DRYING OUT OF THE SOIL.

In Experiment 1 the geranium plant was watered May 24 at 6:00 P.M. after the soil had been drying out seven days and stomatal opening had greatly decreased. There was a distinct though incomplete recovery in degree of stomatal opening which is shown in Figure 1 by the curves for May 25 and 27 as contrasted with those for May 23 and 24. The incompleteness of this recovery and a probable cause for it were mentioned in the dis-

cussion in Experiment 1. (See pages 30-31.) The plant as a whole showed no visible bad effects from the drying out. It was watered occasionally, and was put into use June 18 as geranium No. 1 in Experiment 2, giving normal porometer readings.

In Experiment 2 the two geranium plants were watered at noon June 27, after nine days of drying out. Recovery could not be claimed with any assurance. The curves for June 29 appear to show some recovery as compared with those for June 25, but if the increase in the June 29 porometer readings were due to recovery then the June 28 readings should show some increase: porometer reading 2A does show an increase, but the other three readings are fully as low as those of June 25. Though recovery did not occur within a day or two, with occasional watering the plants proceeded to make a healthy growth, as did the single plant after Experiment 1. It is therefore evident that the plants were not carried to the point of permanent injury.

(D) THE EFFECT OF PRUNING UPON STOMATAL BEHAVIOR IN POTTED PLANTS SUBJECTED TO DROUGHT.

We have previously noted that as the soil becomes depleted of its moisture the stomata close early in the day thus reducing carbon dioxide intake and storage of manufactured food. As this is due to the rate of water

loss exceeding the rate of water intake, it is logical to suppose that pruning the plant would reduce the water loss, enable the root system to maintain the water content of the leaf tissue with resulting opening of stomata and normal rate of photosynthesis.

With this point of view in mind, before the moisture was restored to the potted plants in Experiments 2 and 3, plant No. 1, in each case, was pruned to the extent of removing every other leaf; plant No. 2 was left untouched. This operation was performed on geranium No. 1 June 24 at 6:00 P.M. The result--or rather lack of result--is shown by the curves for June 24 and 25 in Figure 2. The porometer readings were higher on June 25 than on June 24, but they were higher in the case of both pruned and unpruned plants and bore about the same relation to each other during both days: i.e. the 1A reading (pruned plant) dropped below the 2A reading (unpruned plant) before 11:00 A.M. on both days and remained below and approximately parallel to 2A throughout both days. A few readings taken June 27 verify those of June 25, 1A reading lower than 2A. The few readings taken after pruning one of the fuchsias likewise showed no recovery.

These negative results undoubtedly mean, not that the pruning idea is incorrect, but that the experiment needs to be carried on under more carefully controlled conditions. In the geranium experiment (Experiment 2) for example, the

rate of drying out was very rapid. Weighings of the two potted geraniums covering the period from 7:00 A.M., June 21, to 11:00 A.M., June 23, show that moisture was being lost at the average rate of 126.4 grams per day during those two days from a soil and plant mass of about 1600 grams. It seems entirely probable that the rapid loss of moisture caused the plant to pass at once beyond the point of recovery before the pruning could take effect; or that the saving of moisture due to pruning was inadequate to offset the rapid loss due to transpiration and evaporation. The necessity of bringing this study to an end did not permit a repetition of this experiment with pot and soil sealed to retard the rate of water loss.

(E) THE DAILY CYCLE OF STOMATAL BEHAVIOR IN LILAC, AND ACCOMPANYING WEATHER AND SOIL MOISTURE CONDITIONS.

Observations were made upon the two clumps of lilac standing just west of the south entrance to Agricultural Hall (Experiment 4). Two porometer leaf chambers, designated as Porometer 3 and Porometer 4 were attached to two leaves of the purple lilac, Syringa vulgaris, standing nearest the entrance, and one leaf chamber, Porometer 5, to the white lilac, Syringa vulgaris alba, just west of the purple clump. The leaf chambers were cemented to the under side of the leaves with "Tree Seal" and supported by a wire with one end coiled to receive the leaf chamber and the other end bound to the twig with

friction tape.

The leaf chambers were attached June 10, and a few readings taken in order to find out during what parts of the day frequent readings would be desirable; then readings were taken from all three attachments throughout the days of June 11 and 13, and from Porometer 3 on June 15. The results are tabulated in Table V. Temperatures were taken at the same time as the porometer readings. June 11 soil samples were taken with a soil auger at the five locations A, B, C, D, and E shown in Figure 5. The depths at which these samples were taken, and their moisture content, are given in Table VI. Humidity observations were obtained from the same source as in Experiment 2. In the making of the hydrothermograph record covering this period the pen was set so high that it ran off the scale: a correction was made by using the humidity observations taken by the Soils Department at 5:00 P.M. each day. The average difference between the 5:00 o'clock hydrothermograph readings and the Soils Department humidity observations was subtracted from the hydrothermograph readings for June 11, 13, and 15. The corrected figures are given in Table VII. Here, as in Experiment 2, the figures should not be interpreted as showing any more than the general trend of humidity.

Table V. Porometer readings in bubbles per minute and accompanying temperatures in degrees Centigrade. Experiment 4; June 11, 13, 15.

Porometer leaf chamber No. 3.

June 11			June 13			June 15		
Time	Por.	Temp.	Time	Por.	Temp.	Time	Por.	Temp.
7:00A.M.	2.0	18	8:20A.M.	31.3	21	7:15A.M.	--	13.5
7:30 "	3.8	18	9:05 "	20.6	23.5	9:00 "	30.0	17
7:36 "	5.4	18	9:30 "	4.6	24	9:45 "	40.6	17
7:45 "	5.6	20	10:00 "	3.4	26.5	10:00 "	44.0	--
8:20 "	8.0	20	10:50 "	2.4	27	11:00 "	51.5	18
9:00 "	15.0	22	11:50 "	3.8	28	12:00 "	52.5	--
9:30 "	15.0	23	1:15P.M.	5.4	30	2:00P.M.	60.0	19
10:15"	4.6	24	2:40 "	3.8	31	3:00 "	35.5	--
11:45"	3.4	--	3:30 "	2.2	30	4:00 "	8.6	--
2:30P.M.	1.4	31	4:35 "	1.0	28.5	5:40 "	14.0	--
4:45 "	.8	28	5:30 "	1.0	26	8:15 "	--	13
			7:30 "	.6	21			
			9:15 "	.6	19			

Porometer leaf chamber No. 4.

June 11			June 13		
Time	Por.	Temp.	Time	Por.	Temp.
8:35A.M.	1.4	20.5	8:10A.M.	0.0	21
9:05 "	2.0	22	9:15 "	.8	23
9:45 "	2.4	24	9:45 "	20.0	25
10:20"	3.4	26	11:00 "	5.6	28
11:55"	1.0	27.5	12:00 "	7.0	30
2:35P.M.	0.0	31	1:20P.M.	2.4	32
4:40 "	9.5	28	2:30 "	8.3	31
			3:30 "	14.3	30
			4:30 "	7.0	28.5
			5:35 "	1.2	26
			7:30 "	0.0	21
			9:15 "	.6	19

Porometer leaf chamber No. 5.

June 11			June 13		
Time	Por.	Temp.	Time	Por.	Temp.
7:55A.M.	1.6	19	8:05A.M.	16.6	21
8:05 "	2.6	19	8:25 "	18.3	23
8:15 "	5.2	20	9:00 "	20.6	23.5
8:55 "	14.0	22	9:40 "	12.6	25
9:40 "	14.5	24	11:00 "	11.6	28

(continued on following page)

(Table V. Continued)

June 11			June 13		
Time	Por.	Temp.	Time	Por.	Temp.
10:15A.M.	9.3	26	12:00A.M.	17.0	30
11:50 "	7.0	27	1:30P.M.	9.3	30.5
2:45 P.M.	5.4	30	2:30 "	11.6	30
4:40 "	4.5	28	3:40 "	9.7	30
			4:30 "	9.6	28.5
			5:45 "	11.0	26
			7:40 "	8.5	20.5
			8:30 "	5.3	19
			9:05 "	5.4	19
			9:25 "	3.4	--

Table VI. Soil Moisture Determinations. Experiment 4.

Location	Depth	Per cent of moisture
A	1 Ft.	21
B	1 "	21
B	1½"	23
C	1 "	20
C	1½"	21
D	1 "	18
D	1½"	20
E	1 "	23
E	1½"	25
E	2 "	26
E	3 "	21

Table VII. Humudity observations for June 11, 13, and 15, from hydrothermograph record at the East Farm: corrected for displacement of the pen.

Date	6 A.M.	8	10	12	2	4	6	8	10P.M.
11	--	49	50	39	32	65	63	68	79
13	64	50	48	39	41	47	56	60	76
15	73	53	52	52	49	48	55	72	76

Both lilac bushes were shaded from the earliest direct rays of the sun by the building, and the purple lilac shaded the lower part of the white one for a short time: thus the sunlight first struck the region of Porometer 3 at 7:25 A.M. and at 7:50 the white lilac branch bearing Porometer 5 was in direct sunlight. The ascending curves of June 11 for these two series of porometer readings (Figure 3) appear to reflect quite clearly this difference in timing of the illumination. The ascending curve of June 13 for Porometer 3 was lost because of the only leak that occurred after this experiment was once under way. However, this curve reached its peak at or before 8:20 A.M., whereas the curve for Porometer 5 continued to ascend until 9:00 o'clock. The branch of the purple lilac bearing Porometer 4 was shaded until between 9:00 and 10:00 A.M., and after that the leaf to which the leaf chamber was attached was more or less shaded by surrounding leaves. This may partly account for its erratic behavior. Giving our attention to the curves for the other two leaves--on June 11 the stomata opened from the time the sun's rays touched the leaves until 9:00 o'clock, remained moderately open until 9:30 and 9:40, then proceeded to close rapidly until 10:15, they closed gradually through the remainder of the day. It is noteworthy that the stomata of the white lilac leaf (Porometer 4) remained much more widely open throughout both June 11 and June 13

than did those of the leaf to which Porometer 4 was attached. Attention sould be called to the shallowness of the soil at location A and greater moisture supply at location E. Brick and gravel filling was encountered at a depth of a little over one foot at location A. However, the difference in stomatal behavior of these two leaves may also be due to difference in variety of plant, variation of individual leaves, and to an unidentified malady which affects this particular purple lilac bush and causes some of its leaves to wither and change color before the middle of the summer.

The influence of the weather is apparent in the behavior of the leaf bearing Porometer 3. Its stomata closed rapidly and fairly completely by 10:15 and 9:30 A.M. June 11 and 13 respectively, but on June 15 they opened much more, did not begin to close until 2:00 P.M., and were wider open at 4:00 o'clock than at any time after 10:00 A.M. June 11 and 13. June 11 and 13 were hot clear days, while the 15th was cool and cloudy with a little sunshine at intervals. The evaporation observations furnished by the Soils Department of the College for June 11, 13, and 15 were .254, .250, and .205.

Explanation of Figures 3, 4, and 5.

Figure 3.

Graphs of porometer and temperature readings obtained in Experiment 4: humidity records were obtained from the hydrothermograph record taken at East Farm, and corrected by means of wet and dry bulb readings taken at 5:00 P.M. each day by the Soils Department of the College.

Por. 3, Por. 4, Por. 5--location of porometer leaf chambers numbers 3, 4, and 5.

Figure 4.

Daily cycle of stomatal behavior of Torgerson's Royal Anne cherry tree.

The infiltration index figures for the east, south, and west sides, and top were averaged together: the result was multiplied by five to increase the amplitude of the curve.

Figure 5.

Location of soil samples and porometer leaf chamber attachments in Experiment 4. (See explanation of Figure 3.)

I. Clump of purple lilac, Syringa vulgaris.

II. Clump of white lilac, Syringa vulgaris alba.

A, B, C, D, E. Location of soil samples.

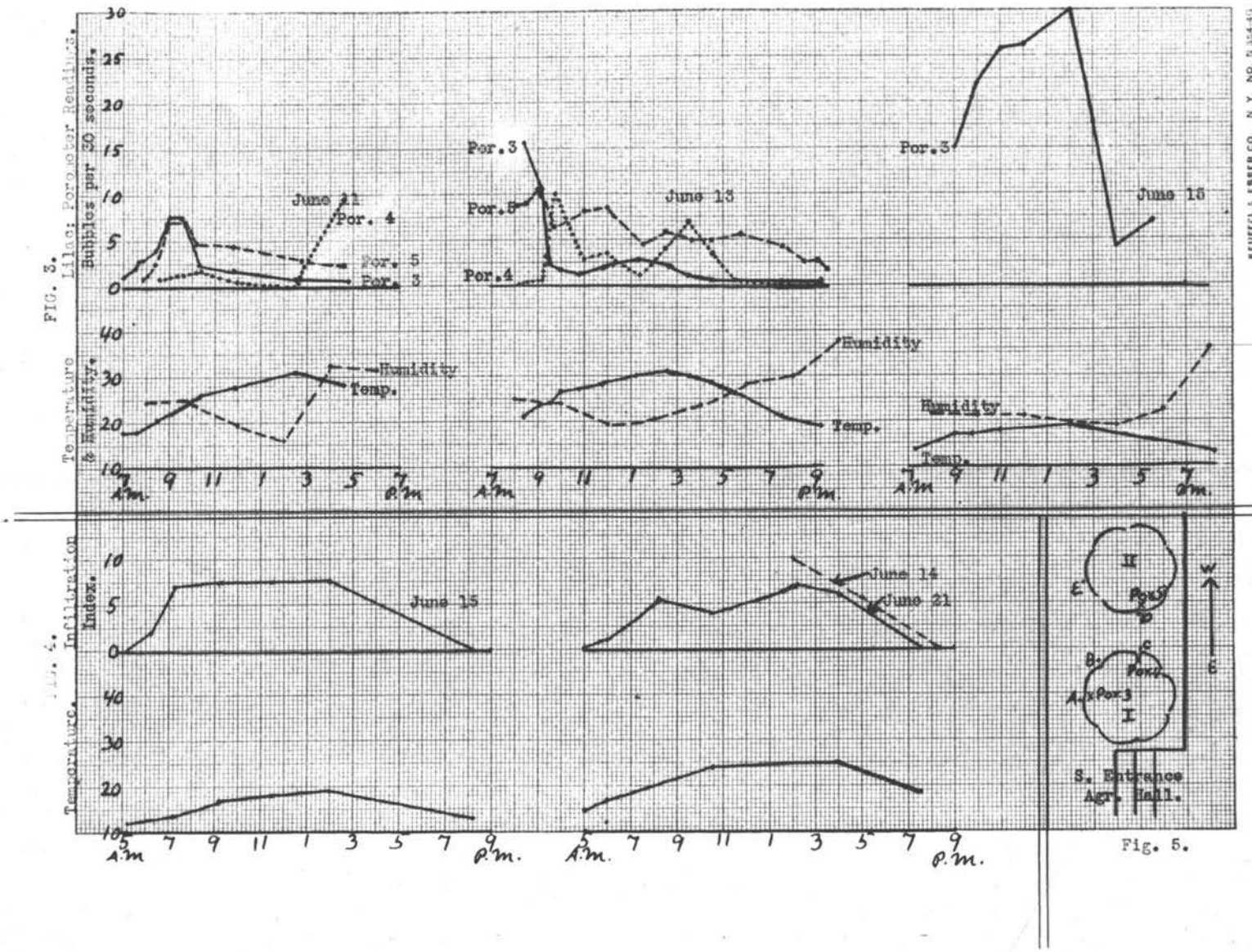

Lilac: Porometer Readings.
Bubbles per 30 seconds.
Por.3
Por.5
Por.4
June 11
Por. 4
Por. 5
Por. 3
June 13
Por.3
June 15
Temperature & Humidity.
Humidity
Temp.
Infiltration Index.
Temperature.
June 15
June 14
June 21
A.M.
P.M.
II
I
Por.4
Por.5
Por.2
Por.3
S. Entrance Agr. Hall.
W
E

FIG. 3.

Fig. 5.

(F) THE DAILY CYCLE OF STOMATAL BEHAVIOR IN THE ROYAL ANNE CHERRY, AND ACCOMPANYING SOIL MOISTURE CONDITIONS.

The stomatal behavior of the Royal Anne cherry was studied by means of the infiltration method devised by Molisch (15), using absolute alcohol. The tree at the residence of E. F. Torgerson, 15 Park Terrace, was the principal subject for this study. Some observations were also made on the Royal Anne tree at 8 Park Terrace and, for the sake of comparison, on a seedling cherry tree (in the row of trees extending northward from the road north of the Horticultural Building, the third tree in the row, going from south to north). Observations were made on the east, south, west, and north sides, and the top of the Torgerson tree in the order named. No tests were made on the tops of the other two trees. In making the observations at each location, from three to five leaves were picked and absolute alcohol dropped upon the under side of each leaf, covering the area between two branch veins from mid rib to margin. The alcohol filtered through the stomata, if they were open, causing small scattered darkened areas when viewed by reflected light. The results were represented by the figures 1, 2, 3, etc. The intention was to record the result as "1" if the darkened areas covered about one-tenth of the area treated with alcohol; "5" would indicate that the infiltration darkened

one half the leaf area, etc. Since the dark spots were very irregular in shape, area and distribution, it was impossible to judge accurately as to just what percent of the leaf area was affected, especially when there was little infiltration. In the case of the cherry leaves the infiltration never affected as much as one-third of the leaf area, consequently the recorded figures should not be considered as measures of area, but rather as index figures, the accuracy of which depends upon the judgment and memory of the observer: at the best, they would involve a large personal error. On account of the small proportion of the leaf area darkened by the infiltration, the writer used fractions in expressing the results, rather than change to a new basis of judgment after the observations were under way. Observations were made on the Torgerson tree during the afternoon of June 14 and the entire days of June 15 and 21. The figures recorded at a given time from the several leaves at each location were averaged together to obtain the results tabulated in Table VIII. The following is a typical observation: June 15, 7:15 A.M., south side; 1½, 1½, 1, 2, 0; Av., 1.2.

If the data from any one location were used to construct a graph, the result would be a very irregular curve owing to the personal error in making observations and to the error introduced by individual variation of the

leaves. Therefore, since the stomata of the east, south, and west sides, and the top of the tree behaved essentially alike, the figures for these four locations were averaged together for each time of observation and curves drawn from this average to represent the daily cycle for the stomata of Torgerson's tree June 15, 21, and the afternoon of June 14. (See Figure 4.)

A few soil moisture determinations were made: borings were made at two opposite points four feet from the trunk of the tree, a sample was taken from the two-foot depth at each point, and the two samples mixed together. The locations, dates, and results were as follows:

Location	Date	Per cent of moisture
Torgerson's tree	June 13	23.0
Torgerson's tree	June 21	22.2
Tree at 8 Park Terrace	June 21	21.1
Seedling tree	June 21	17.0

Table VIII. Infiltration Observations on Cherry trees.

Torgerson's Royal Anne Cherry Tree.

June 14 Time	Temp.	East	South	West	North	Top
2:00 P.M.	--	2.0	2.0	2.0	.6	2.0
5:00 "	--	.8	1.4	1.0	.6	1.2
8:15 "	--	0 0	0 0	0 0	0	0 0
June 15						
5:15 A.M.	12	0*	0 0	0 0	.2	0 0
6:15 "	--	.5	.35	.3	.35	.5
7:15 "	13.5	1.8	1.2	1.4	.8	1.2
9:15 "	17	1.75	1.5	1.6	.9	1.3
11:30 "	18	2.0	1.6	1.4	.75	.9
2:00 P.M.	19	2.0	1.6	1.2	.5	1.4
8:15 "	13	0 0	0 0	0 0	0	0 0
June 21						
5:00 A.M.	14.5	0 0	0 0	0 0	0	0*
5:30 "	--	.75	0*	0*	0*	.5
6:00 "	17	.2	0*	.17	.2	.6
8:10 "	--	1.25	1.4	1.17	.4	1.4
10:30 "	24	1.0	1.4	.3	.25	.5
2:10 P.M.	--	1.75	1.5	1.75	.6	.7
4:00 "	25	1.8	.6	1.3	.37	1.0
7:30 "	18.5	0 0	--	0*	--	0 0

Royal Anne at No. 8 Park Terrace

June 21 Time	East	South	West	North
5:05 A.M.	0 0	0 0	0*	--
5:50 "	0*	.5	0*	--
8:30 "	1.16	2.5	1.66	1.5
10:50 "	.75	1.0	.5	.6
2:30 P.M.	1.5	1.0	2.25	1.5
4:00 "	.5	.6	1.0	1.75
7:40 "	0 0	0 0	0*	--

Seedling Cherry Tree

June 21 Time	East	South	West	North
5:15	.66	--	0 0	--
11:00	1.8	.8	.6	.9
2:30	1.0	1.5	1.5	1.4
4:00	.9	1.4	1.6	.8
7:40	.2	0 0	--	0*
8:00	--	--	0**	

Symbols: "0*" indicates that the infiltration index was practically zero but with a very few infiltered spots occurring: two stars indicate several spots. Numerals represent the infiltration index. Temperatures in degrees Centigrade. The portion of the tree where leaves were tested is indicated at the top of each column.

The graphs show a rapidly progressing opening of the stomata for a period of two to three hours after sunrise. While the sun was above the horizon at 5:15, June 21, the Torgerson tree was shaded by a row of maples half a block east of it so that it was not in full sunlight until about 6:00 o'clock. The graph for June 15 indicates a gradual closing of stomata throughout most of the afternoon: however, no observations were made between 2:00 and 8:15 P.M. on that day, therefore the afternoon curve for June 14 is introduced next to the June 21st curve to throw additional light on the rate of closing. June 21 the sun set at 7:20 to 7:25 and the stomata of the Torgerson tree were closed at 7:30. Some of the stomata on the west side of the seedling tree were still open at 8:00 P.M. (See Table VIII, Seedling Cherry Tree.) This tree was not shaded on the west, while Torgerson's tree is shaded by the house, and the tree at 8 Park Terrace is shaded on the west by trees half a block west of it. The seedling tree showed a little earlier opening of stomata on the east side than did the other two trees. The writer attributed this to the fact that the seedling tree is not shaded on the east: the tree at 8 Park Terrace stands just west of a house: the Torgerson tree, west of a row of maples.

The depression in the curve about midday of June 21 is evidently not due entirely to personal error nor variation in individual leaves since it also occurred in the

other trees. (See 10:50 A.M. observation at 8 Park Terrace and 11:00 o'clock observation of the seedling.) The day was warm and clear, but light clouds came up about 2:00 P.M. The hydrothermograph record at East Farm showed a temporary 2½ degree drop (Fahrenheit) at that time, though the temperature commenced to rise again in about half an hour and at 6:00 o'clock was about 3½ degrees F. higher than just before the 2:00 o'clock drop.

June 28 being an excessively hot, dry day, an observation was made at 4:00 P.M. on the two Royal Anne trees used in this investigation. The temperature was 30° C. yet the average infiltration index given by the Torgerson tree was .8 and by the tree at 8 Park Terrace, .88, indicating that the stomata of the Torgerson tree were about two-thirds as widely open as on the warm day of June 21 and the cool day of June 15, and those of the tree at 8 Park Terrace, as widely open as on June 21.

In the case of the seedling tree the spotting produced by the infiltration was finer than that on the Royal Anne leaves. Judging from the observations of June 21 and a few other scattering observations, the behavior of the stomata was essentially similar in seedling and Royal Anne cherry trees.

DISCUSSION

In these investigations several methods of stomatal examination were studied, of which one proved valueless. This involved the use of formalin-acetic acid-alcohol as a fixing solution. It did not preserve the stomata at their natural aperature but resulted in their closure.

The desire was to find methods which would indicate the variations in diurnal periodicity of stomatal opening and closure under changing conditions, and do so with as little difficulty as possible. It was not the intention to arrive at quantitative estimates of stomatal aperature. Therefore, the method of direct observation, which involved the setting up of a microscope in the field, and also the method of fixation in absolute alcohol, which involved carrying many sample bottles into the field and keeping of detailed records on each, were discarded as too cumbersome.

The porometer method was used extensively for certain of the studies conducted and has the advantage of averaging the degree of opening of hundreds of stomata, and of giving definite and detailed information in regard to the stomata of one leaf. It has the disadvantage of giving results which are merely relative; i.e., it does not tell the actual degree of opening of the stomata. It has the further disadvantage of taking no account of the wide variation in stomatal behavior of individual leaves. The

porometer method is too cumbersome and time consuming to be used on many leaves of a plant in order to secure a reliable average.

Of all the methods studied, the infiltration method of Molisch involved the least trouble and gave the most rapid readings. It is well adapted to outdoor use and requires the least apparatus. It is satisfactory where the need is merely to find out whether the stomata are open, nearly closed, or closed, at the time of examination. It can be applied to a large number of leaves in a few minutes. It has the disadvantage of involving a large personal error in estimating the percent of infiltered leaf area. For any great accuracy or differentiation of results the reagent used would need to be adapted to the kind of leaf under observation and also to the condition of the stomata at different times of the day. Absolute alcohol filters through lilac stomata so readily that it will darken the whole area covered by the liquid when the stomata are only moderately open; on the other hand, it filters into Royal Ann cherry leaves only in rather widely scattered spots when the stomata are open at their widest. The use of the series of ten liquids arranged by Dietrich and reported by Stalfelt--or which ever one of the ten proved best adapted to the given leaf--would undoubtedly help to overcome this difficulty.

The experiments on geraniums conducted on the labora-

tory window ledge by the porometer method showed clearly the effect of gradual drying out of the soil in the closing down of the stomatal aperature earlier and earlier each day as the drying out of the soil progressed, until leaves that originally did not begin to show stomatal closure until about 2:00 P.M. finally showed considerable closing down of stomata as early as 10:00 A.M. thus greatly curtailing the daily period of gas exchange.

The first plants tested showed some recovery from this tendency to early closing, upon restoration of soil moisture, although the recovery was never quite complete. The other plants showed no recovery, indicating that perhaps fundamental irreversible harmful changes in stomatal behavior had occurred during the preceding drought period.

The early closing of stomata noted above may be induced without carrying the plant to the point of permanent wilting.

The outdoor tests on lilac by the porometer method could not be continued long enough to show the effect of gradual soil moisture depletion, especially as unseasonable rains and lawn watering counterbalanced the usual drying out. The tests, however, showed clearly the effect of high transpiration rate on hot drying days in producing stomatal closure by mid-forenoon whereas the stomata remained open much longer on a cool cloudy day with no

essential change in the soil moisture, the maximum aperature being recorded at 2:00 P.M. on this day.

The outdoor tests on sweet cherries were conducted by the infiltration method. Here again the tests could not be carried on long enough to permit the dessication of the soil to proceed to the point where the behavior of the stomata would be affected. The Royal Ann trees studied showed apparently normal stomatal behavior throughout this period. The stomata were closed at night, opened rapidly after sunrise, remained open--with some fluctuations--through the day, gradually closing from the middle of the afternoon on, and becoming fully closed to infiltration with alcohol within a few minutes after sunset. No essential difference from this stomatal behavior was noted in the seedling cherry tree studied.

SUMMARY

1. A review of the literature on stomatal behavior and its observation is given.

2. Various methods of estimating the degree of opening of the stomata and the daily duration of such opening were studied.

3. The porometer method of Darwin and Pertz as modified by Knight was found to be a very sensitive method capable of use for over a week on a single leaf, but it involves rather cumbersome apparatus, and is somewhat time-consuming and too limited for extensive field use.

4. The infiltration method of Molisch, using alcohol and xylol to test the aperature of the stomata, was found most rapid and satisfactory for field use where many leaves are to be tested within a short time.

5. That plants respond to excessive drying out of soil by closing the stomata and reducing gas exchange early in the day was indicated by the porometer tests on geraniums. Stomata show this effect long before any serious injury to the plant has been sustained, as shown by final recovery on restoration of soil moisture.

6. That excessive transpiration during hot and dry air conditions in absence of soil moisture deficiency can result in premature stomatal closure was brought out by the porometer tests with lilac.

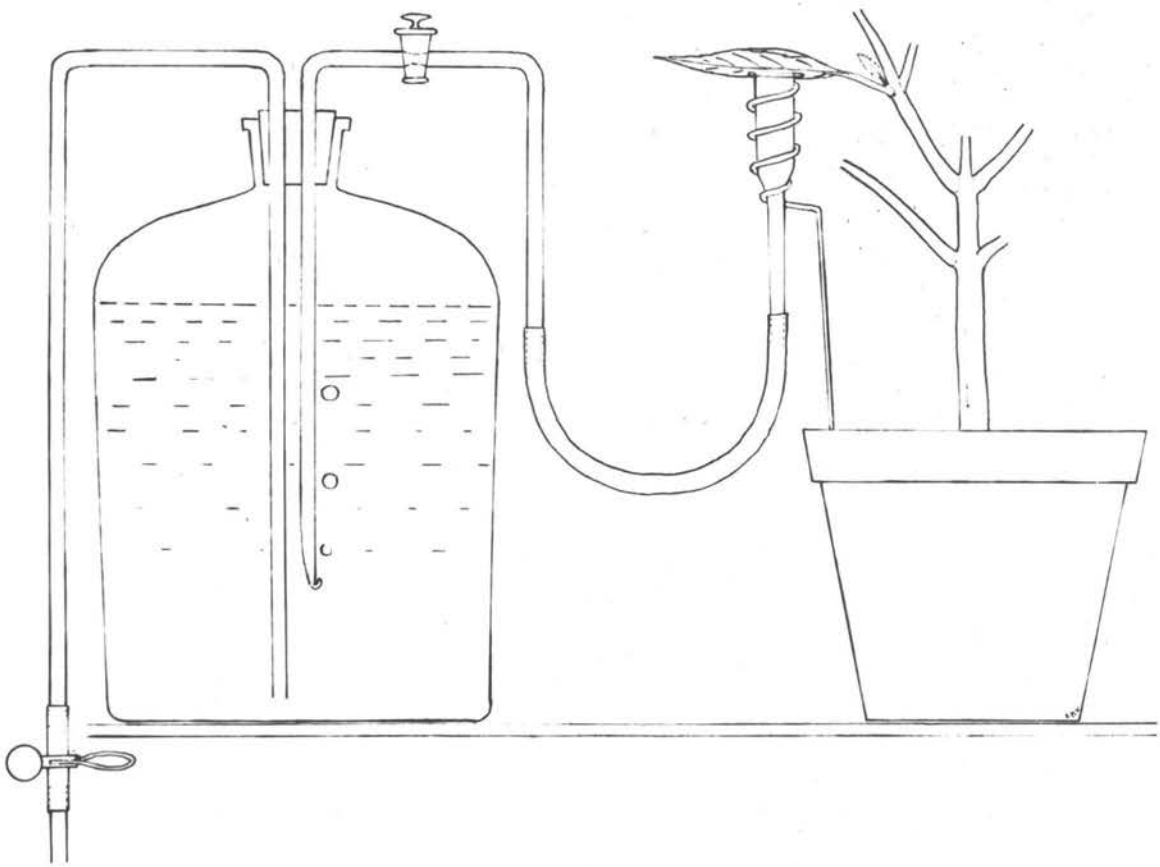
7. That sweet cherry trees behave normally even on hot dry days when provided with sufficient soil moisture was disclosed in the infiltration tests here reported.

Explanation of Porometer Figure

The leaf chamber was sealed to the lower leaf surface and supported by a wire thrust into the soil.

The stopcock stopper was removed or the tube disconnected from the leaf chamber when readings were not being taken.

The long arm of the siphon was 14 centimeters larger than the short arm.



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