THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE MOVEMENTS OF GUARD CELLS

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(WITH TWELVE FIGURES)

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The movements of the guard cells have been shown by many studies to be affected by light, temperature and relative humidity. Most of these studies have been concerned with the separate effects of each of these factors rather than their interrelationship. Since the movements of the guard cells are affected by the total environment rather than any one single factor, it was considered that a study relating the movements of the guard cells to all of the factors should be undertaken. In addition, it was thought that since most of the theories which attempted to explain the movements of the guard cells were related to their photosynthetic activity, a study of the photosynthetic activity of the plants whose stomatal aperture was being measured should also be undertaken. These studies were conducted under both natural and controlled conditions.

Investigation of stomatal aperture under natural conditions

PART I. THE EFFECT OF LIGHT, TEMPERATURE AND VAPOR PRESSURE DEFICIT ON STOMATAL APERTURE

Few investigations on the effect of the aerial factors of the environment on stomatal aperture have been carried out under natural conditions and these were of restricted duration. To obtain the effect of the full range of the environmental factors on stomatal aperture an experiment extending through at least several seasons of the year was considered necessary. The writer is unaware of any such published record. Employing the resistance porometer, WILSON (**33**), records of the stomatal aperture of two species of broad leaf evergreens were obtained from November 1941 through April 1942. Measurements of the light, temperature, and vapor pressure deficit of the atmosphere were also obtained during this same period.

METHODS.—The nature and duration of the experiment determined to a large extent the type of plant which could be employed. Since the experiment was planned to extend from the fall of one year to the spring of the next year, only evergreen species were suitable. It was first thought that conifers would serve as suitable material, but mechanical difficulties in the fixing of the leaf cups, prevented their use. *Camellia japonica* L. and *Ligustrum japonicum* Thunb. were the species used.

A favorable location for the experiment was found on the roof of the Biology building of Duke University. A wooden platform, on which the plants could be placed, was constructed on that part of the roof directly

above an office. Three plants of each species, in fourteen-inch pots, were placed on the platform so that none of the plants shaded each other. The recorder for the resistance porometer was located in the office, and figure 1 indicates how the connections between the leaf cups and the recorder were constructed and how they were protected from temperature fluctuations. All metal connections were soldered and tested under pressure for leaks. When the water was turned on, all of the copper tubing was surrounded by running water and thereby protected from rapid temperature fluctuations. Three water-jacketed, leaf-cup supporters were constructed.

The leaf cups were fastened to two leaves of each of the three plants of one species. The diameter of each leaf cup was nine millimeters and the

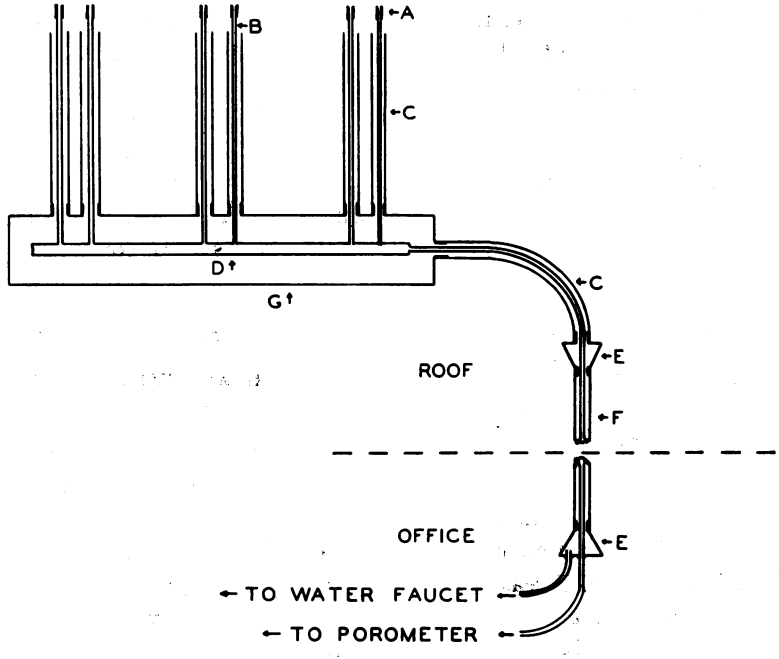

FIG. 1. Diagram to show construction of water jacket for line connecting leaf cups to recorder of resistance porometer. A, leaf cups; B, small bore copper tubing; C, rubber jacket; D, large bore copper tubing; E, copper connecting cups; F, thick wall rubber tubing; G, metal jacket.

total area of the leaf surface within the six cups was 1.22 square centimeters. For Camellia, this area usually contained 26,000 stomates and for privet 31,500 stomates. The leaves were sealed to the leaf cups by means of 40% latex. Card-board squares were fastened to the leaf cups of the third line, which was employed as a control. Since the records obtained from this line indicated no movements of the manometer, it is assumed that the system had been effectively protected from temperature effects.

For comparative purposes, the areas of the curves of successive days, recorded by the resistance porometer, were measured with a planimeter.

The greatest area for any day during the period under investigation was used as a basis for calculating the other areas. Since the swing of the writing arm was kept between the two extremes of pressure for the entire duration of the experiment, the maximum swing was considered to be the maximum stomatal aperture.

The temperature and the relative humidity records were obtained by use of a Friez hygrothermograph located in a standard weather bureau type instrument shelter on the roof adjacent to the platform on which the plants were kept. The thermograph was checked before and at intervals during the experiment with an accurate mercury thermometer, and found to be substantially correct. Periodically, the recording hygrometer was

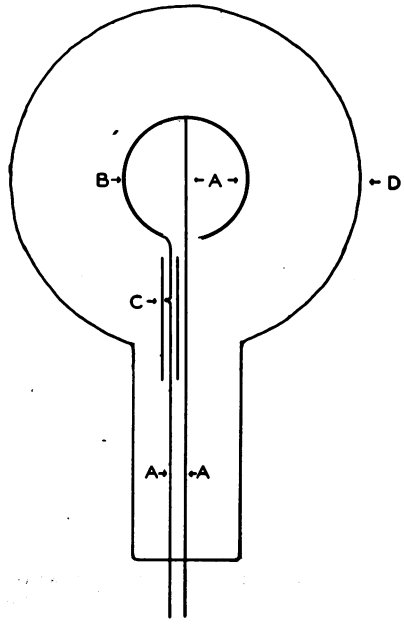

FIG. 2. Diagram of spherical thermocouple. AA, copper lead wires; A, thin copper sphere; B, thin nickel plating and junction wire; C, inside silvered glass tube; D, evacuated glass bulb.

checked for accuracy against readings obtained from a sling psychrometer. The vapor pressure deficit was calculated from the data obtained from the hygrothermograph.

Radiation was measured by means of a radiometer devised by the writer. For the purpose of this investigation, a device which responded to radiation similar to that of a three-dimensional object such as a plant was desirable. It was considered that such a device should be non-selective in its response to the various wave lengths. The device used in this study consisted of a spherical thermocouple with one junction blackened and the other junction silvered, the whole mounted in an evacuated glass bulb (fig. 2). Two radiometers were placed on the platform near the plants and were connected in series to a Leeds and Northrup "Micromax" recording potentiometer.

A record secured on March 25, 1941 was compared with a record cited by KIMBALL (9) of radiation received by a radiometer maintained normal to the radiation on a clear day on the 21st of March near Washington, D. C. Figure 3 shows both of the curves drawn to the same scale. It would appear that the response of this radiometer remains nearly the same as one placed normal to the incident radiation.

If the meteorological conditions at Washington, D. C., can be considered similar to those at Durham, N. C., then a 100% light value of the writer is equivalent to 1.33 gm. cal./min./cm.^{2} The greatest daily intensity for the entire year, which was equal to approximately 797 calories, was recorded on June 22, 1942, while the greatest daily intensity measured during the course of the experiment was on April 20, 1942 and was equal to 753 calories. In

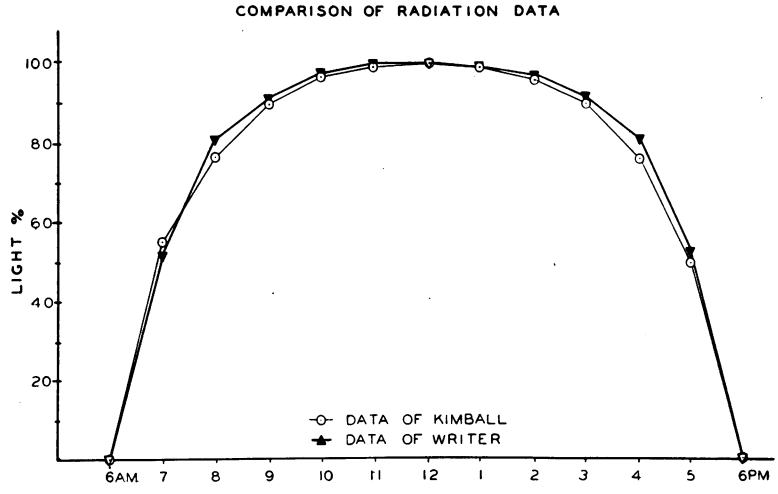

FIG. 3. Comparison of radiation data obtained by writer from spherical thermocouple with data obtained by Kimball from pyrheliometer maintained normal to incident radiation.

computing the average light value for each day, the total number of calories received was calculated and expressed as percentage of 797.

RESULTS.—Data were obtained of the stomatal aperture for both species of plants for approximately sixty-five days distributed between November 1941 and May 1942. These data together with the corresponding values for the light, temperature and vapor pressure deficits are presented in table I. To determine the independent effect of both temperature and light upon stomatal aperture graphs were constructed in which the relation between mean stomatal aperture and light under the same average temperature (fig. 4a) and the relation between mean stomatal aperture and temperature under the same average light (fig. 4b) was expressed.

From a consideration of the graphic presentation of the data, it would appear that at a temperature between minus three and four degrees centi-

TABLE I

AVERAGE DAILY LIGHT, TEMPERATURE, VAPOR PRESSURE DEFICIT AND STOMATAL APERTURE FROM NOVEMBER 1941 TO APRIL 1942

STOMATAL APERTURE		LIGHT	TEMP.	V.P.D.
PRIVET	CAMELLIA			
		%	0° C.	mm. of Hg
69.0	75.0	57.6	16.3	5.54
54.0	49.6	27.4	17.0	3.73
84.0	77.5	68.0	15.0	8.49
57.5	53.3	55.0	7.8	4.37
28.0	23.0	9.0	10.0	0.11
47.0	51.6	52.8	9.1	3.86
58.3	52.4	74.0	6.0	4.08
61.4	51.3	71.2	8.4	4.84
77.7	70.0	60.5	16.5	6.93
45.0	41.9	31.5	14.3	4.98
52.5	66.7	39.7	15.8	6.22
17.5	23.2	7.4	17.4	0.60
97.1	85.5	61.4	20.5	6.76
50.0	54.1	32.7	14.1	5.70
47.0	56.3	69.5	10.4	5.86
38.5	51.8	39.3	11.3	5.11
65.3	66.8	59.4	12.8	6.69
58.7	64.4	58.7	15.1	7.94
28.3	39.6	48.2	4.2	2.89
7.0	20.6	9.7	2.5	0.84
13.7	22.2	10.2	4.1	0.14
37.2	47.5	53.4	7.1	3.06
27.0	36.3	70.7	5.2	3.78
23.8	26.5	57.4	7.1	4.01
50.1	34.1	54.1	9.9	4.62
49.0	49.1	61.4	9.3	3.56
34.7	38.1	38.5	12.1	5.36
67.5	67.2	65.2	13.6	7.28
34.5	31.1	59.3	5.6	3.32
27.5	26.1	37.5	6.6	3.17
16.0	15.7	6.5	13.0	0.59
62.5	69.0	56.8	15.0	4.79
44.5	51.7	66.8	10.4	3.82
24.0	15.3	15.0	11.3	0.61
47.7	36.2	39.1	10.0	3.69
50.0	49.5	58.6	7.7	2.81
32.0	34.5	61.5	4.5	2.95
19.5	16.5	49.3	2.7	2.06
37.0	43.7	59.7	7.1	3.92
8.5	.....	8.4	6.0	0.58
67.5	59.0	70.3	15.0	7.29
10.0	13.0	13.1	5.6	2.57
13.0	16.0	34.4	4.1	0.57
15.0	11.5	20.4	4.3	2.02
6.0	6.0	61.8	−1.2	3.00
4.0	6.5	18.4	−2.0	2.36
0.0	0.0	56.0	−4.0	1.20
0.0	0.0	58.8	−3.8	3.60
0.0	0.0	77.7	−5.2	1.20
0.0	0.0	67.2	−4.0	0.90
0.0	0.0	15.5	−3.5	1.70
26.0	.....	20.0	14.6	1.11
40.0	.....	75.0	8.1	3.90
100.0	100.0	88.2	24.5	14.49
100.0	93.5	84.7	23.0	11.21
95.0	9.0	73.7	26.5	14.46

TABLE I (*cont'd*)

Stomatal aperture		Light	Temp.	V.P.D.
Privet	Camellia			
		%	0° C.	mm. of Hg
52.5	42.5	46.1	16.7	3.44
47.5	37.5	47.5	12.3	1.58
91.0	86.7	90.7	18.5	11.50
77.5	59.5	88.8	12.5	6.79
90.0	82.5	84.7	23.7	15.91
81.0	71.5	88.1	21.4	11.31
88.0	76.2	76.8	23.1	11.90
97.0	90.4	83.1	24.0	13.38
90.0	81.0	89.9	24.2	14.01
85.0	85.0	93.3	16.3	8.67
81.3	75.0	93.4	14.7	6.97
83.4	80.7	79.2	18.5	9.76
77.5	75.0	79.3	18.2	9.03
82.5	100.0	90.9	21.8	13.15
100.0	100.0	88.9	24.0	15.68
100.0	100.0	83.1	26.2	18.65

grade, the stomates remained closed regardless of any other factors. As the temperature increased, the reactivity of the guard cells also appeared to increase. This fact made it likely that there would be greater variability of stomatal aperture under conditions of high temperature than under low temperature. It thus appeared that the variability of the stomatal aperture

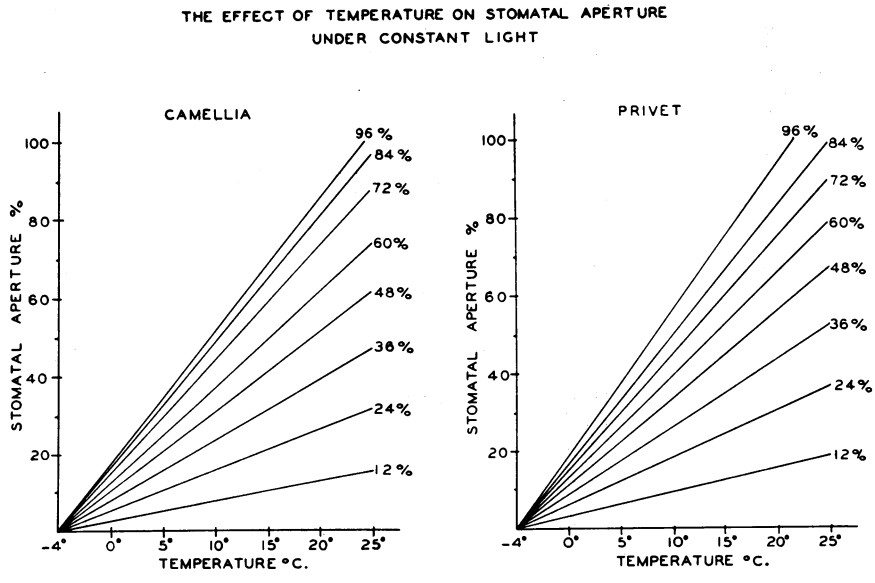

Fig. 4a. The effect of temperature on stomatal aperture under the same average light. Light indicated as percentage in columns at the right; each percentage marks the upper limit of the points used. Thus 24% marks the average stomatal aperture for all light values lying between 12 and 24%.

was proportional to the temperature. The weight of each observation was inversely proportional to the temperature. Certain limitations are thus imposed upon the form of an equation which would present the relation between stomatal aperture and the environmental factors. At a temperature of between minus three and four degrees, the equation must pass through zero stomatal aperture, and the entire equation should be weighted inversely proportional to the temperature.

From the graphic presentations, it appears that if the origin of the temperature scale t, be placed at $-4°$ C., then for constant light intensity l,

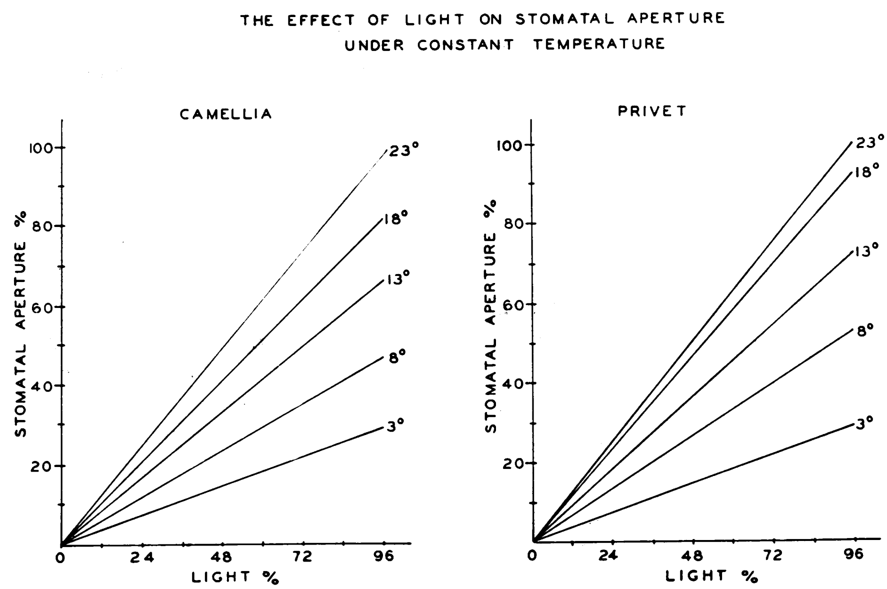

FIG. 4b. The effect of light on stomatal aperture under the same average temperature. Each temperature marks the upper limit of the points used. Thus 8° C. marks the average stomatal aperture for all temperatures between 3° and 8° C.

and vapor pressure deficit v, the calculated stomatal aperture Y, varies directly with $(t+4)$ such that

$$Y = p(t+4)$$

in which p is the proportionality factor. But this factor p itself varies directly with l and with v, and may even accelerate with increase in temperature such that

$$p = b_1 l + b_2 lv + b_3 lv(t+4)$$

upon substituting this expression for p, the equation takes the form

$$Y = b_1 x_1 + b_2 x_2 + b_3 x_3 \qquad (1)$$

in which

$$x_1 = l(t+4)\ ;\ x_2 = lv(t+4)\ ;\ x_3 = lv(t+4)^2.$$

As remarked above, the weight of each observation should be taken as inversely proportional to $(t+4)$; hence, if y be an observed aperture corresponding to the calculated Y of equation (1), the coefficients b_1, b_2, and

b_3 may be evaluated according to the method of least squares, such that the sum of the weighted squares of residuals between observation and calculation, that is

$$\int\left[\frac{1}{t+4}\{y - b_1x_1 - b_2x_2 - b_3x_3\}^2\right]$$

will be minimum.

Numerical values were calculated from the data, and the contents of each of the variables was calculated. The significance of each of the variables was tested and it was found that for both species, x_1 and x_2 were significant, while x_3 made no contribution to the equation. The failure of x_3 to be significant indicates that no confidence can be placed in a curvilinear relation between the independent variables. The final equation for Camellia was as follows:

$$Y = 0.0702\ l(t+4) - 0.0023\ lv(t+4)$$

and for privet

$$Y = 0.0635\ l(t+4) - 0.0017\ lv(t+4)$$

The standard error of the mean for both species was approximately ten %.

The equations derived for both species of plants were tested by an analysis of variance to determine if there was a significant difference between the two equations. The test indicated that there was no significant difference, which indicates that the guard cells of both species behave in a similar manner under similar environmental conditions. An equation was calculated from the combined data of both species. This new equation was as follows:

$$Y = 0.067\ l(t+4) - 0.002\ lv(t+4)$$

The similarity of behavior of both species suggested the possibility that the equation derived from the two species studied might also describe the response of the guard cells of other species. Unfortunately, time did not permit an extension of the experiment to check this hypothesis. There is available in the literature, however, a small amount of data on the activity of the guard cells together with observations of the necessary environmental factors. GRAY and PIERCE (**4**), working with various cereal crops, recorded for several days the stomatal aperture, approximate light, temperature, and relative humidity. From their data stomatal apertures were calculated by means of the combined equation. The results are presented below:

LIGHT	TEMPERATURE IN 0° C.	V.P.D. MM. OF HG	CALCULATED S. A.	ACTUAL S. A.
80	27.5	13	94	94
70	12.5	3.5	72	68

POOL and MCKAY (**20**) working with *Beta vulgaris* presented data which, although not completely quantitative, were thought to be usable in this connection. Their values for the diameter of the stomates were plotted in terms of what they considered a standard day, and a diameter of

six microns determined as equal to 100% opening. The results of these calculations are presented in the following table:

LIGHT	TEMPERATURE IN 0° C.	V.P.D. MM. OF HG	CALCULATED S. A.	ACTUAL S. A.
80	30	26	24	5
70	20	8.6	71	71
50	24	8.0	65	70

Using the combined equations, three-dimensional graphs were constructed in which the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture could be expressed (fig. 5a, b, c, d, e). The vapor pressure deficit was calculated at the indicated

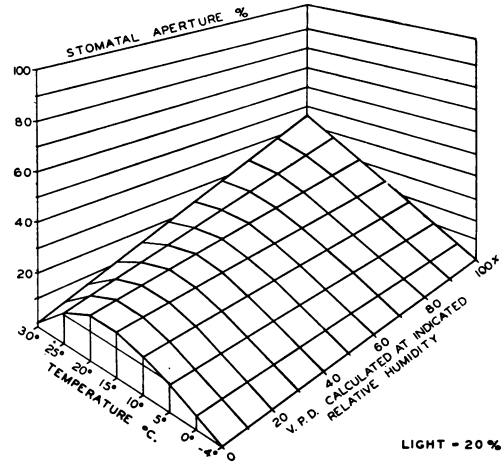

FIG. 5a. Graph showing the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture.

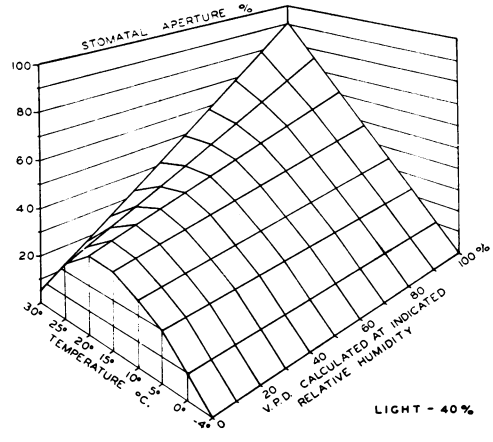

FIG. 5b. Graph showing the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture.

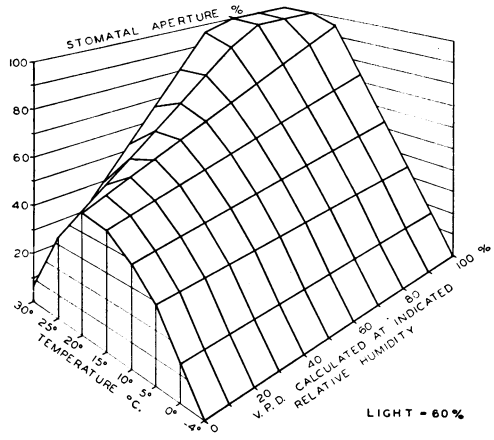

FIG. 5c. Graph showing the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture.

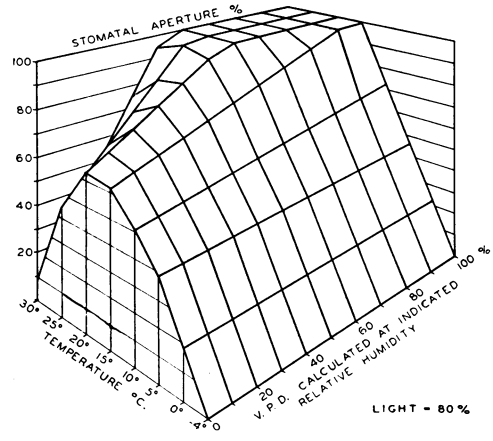

FIG. 5d. Graph showing the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture.

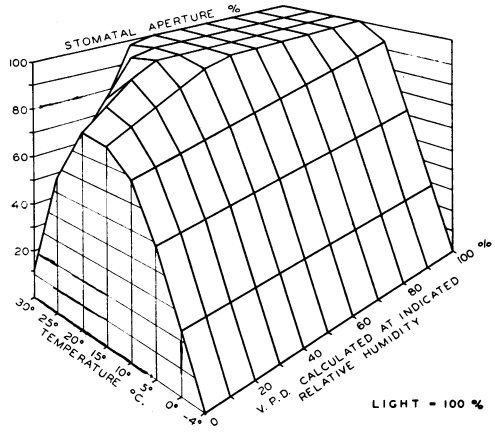

FIG. 5e. Graph showing the effect of temperature and vapor pressure deficit under different light intensities on the stomatal aperture.

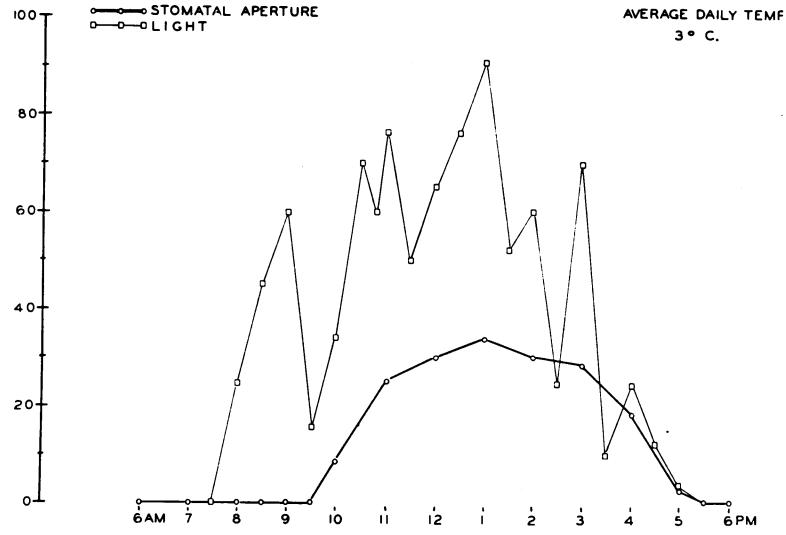

FIG. 6a. Graph showing the effect of light on stomatal aperture of Ligustrum at various average daily temperatures.

relative humidity for each temperature. As indicated by figure 5a, b, c, d, e, the effect of low relative humidities on stomatal aperture is comparatively slight at medium and low temperatures and only becomes important at high temperatures.

The relation of vapor pressure deficit to the moisture content of the leaf is undoubtedly very close. Since the movements of the guard cells

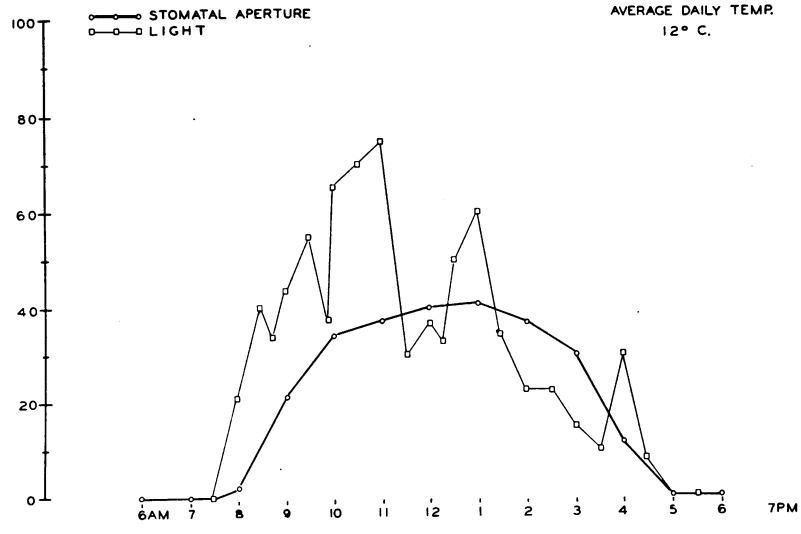

FIG. 6b. Graph showing the effect of light on stomatal aperture of Ligustrum at various average daily temperatures.

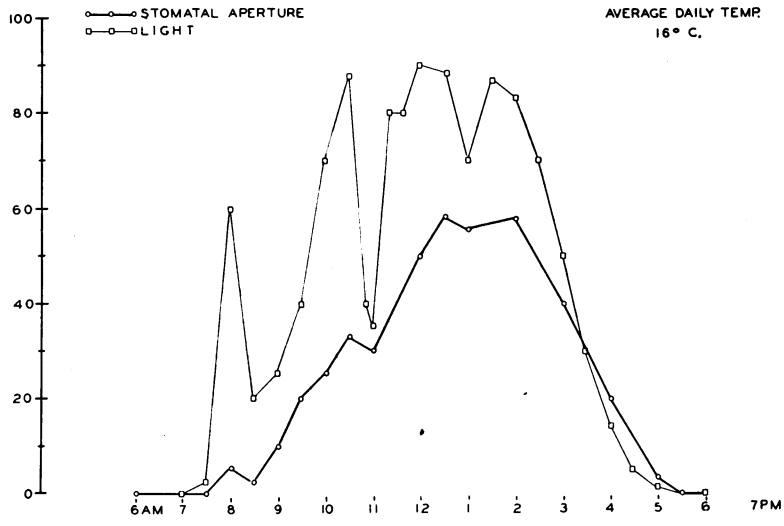

FIG. 6c. Graph showing the effect of light on stomatal aperture of Ligustrum at various average daily temperatures.

have been assumed to be associated with turgor changes, they would be affected by the water balance of the leaf as a whole. It would appear that the stomatal aperture increases with an increase in the moisture content of the leaf.

At very high relative humidities there appears to be a direct relation between temperature and stomatal aperture. At approximately $-4°$ C. light is ineffective in causing stomatal opening. As the temperature in-

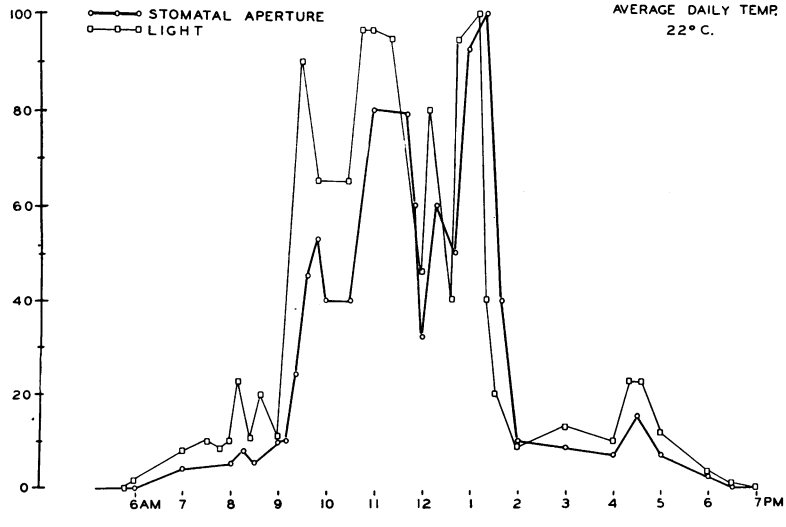

FIG. 6d. Graph showing the effect of light on stomatal aperture of Ligustrum at various average daily temperatures.

creases, the stomates become more responsive and more sensitive to the effects of light. Above approximately 20° C., there appears to be a direct relation between light and stomatal aperture. This situation is shown in figure 6a, b, c, d, e, in which graphs of stomatal aperture of Ligustrum are shown for different days during the experiment. When the average daily temperature was below 12° C., fluctuations in the light intensity caused no appreciable movements of the guard cells. When the temperature was high, there was a practically instantaneous response of the guard cells to fluctuation in light.

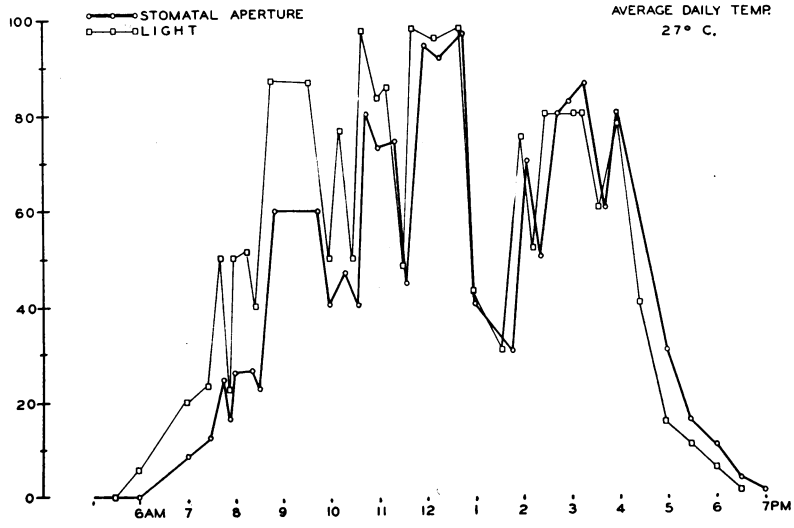

FIG. 6e. Graph showing the effect of light on stomatal aperture of Ligustrum at various average daily temperatures.

PART II. THE RELATION OF PHOTOSYNTHESIS TO STOMATAL APERTURE

The relation between photosynthesis and stomatal aperture may be considered from two distinct viewpoints. One view is that the stomates by controlling the diffusive capacity of the leaf might control the rate of photosynthesis; the other view maintains that photosynthesis, along with its effects, determines the opening of the guard cells. If the first view is correct, it would appear that the movements of the guard cells are independent of the photosynthetic process and could control its rate. If the second view is correct, the movements of the guard cells and the rate of photosynthesis should be essentially parallel. Photosynthetic measurements were made on the plants at the same time that stomatal aperture was being measured.

METHODS.—The apparent rate of photosynthesis was determined by measuring the amount of carbon dioxide present in the air before and after it had passed over the leaves of the experimental plants. The difference between the two readings was taken as a measure of the carbon dioxide used

by the plants. The carbon dioxide content of the air was determined by passing the air stream through absorption towers and measuring the change in conductivity of the absorbing solutions (**29**).

Celluloid envelopes, into which the leaves could be placed, were constructed as shown in figure 7. These chambers were tested carefully for eddy effects with smoke before being employed. These tests indicated no apparent dead spaces so that it might be assumed that the air stream passed continuously over the entire leaf surface. During the photosynthetic measurements, the air within each cup was renewed in approximately 0.8 of a second at a rate of 2.5 liters per cm.^{2} of leaf surface per hour. The celluloid was sufficiently thin so that no appreciable reduction of light intensity was apparent. The temperature of leaves within these cups was compared with that of normal leaves by means of a thermocouple. When

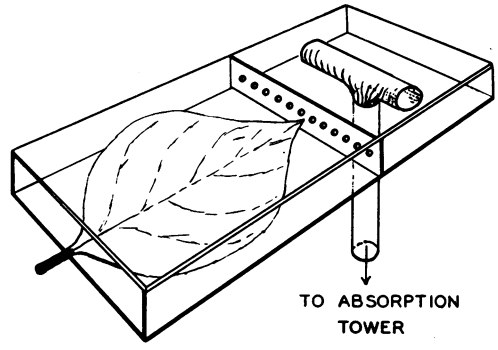

FIG. 7. Diagram of leaf chambers for determination of apparent rate of photosynthesis.

air was moving through the cups, as in photosynthetic measurements, the temperature of the leaves within the cups was approximately one degree higher than that of normal leaves. These determinations were made on a day of high light intensity. When the light intensity was low, there was no measurable difference in temperature between the leaves.

Five leaves from three plants of Ligustrum and four leaves from three plants of Camellia were used. These leaves were chosen carefully so that the total area for each species was approximately fifty square centimeters. Since it has been shown that paired opposite leaves exhibit morphological and physiological similarity, the leaves of Ligustrum employed for photosynthetic measurements were paired with the leaves whose stomatal activity was being recorded. Since Camellia has an alternate arrangement of the leaves, such a pairing could not be employed. The leaves whose photosynthetic activity was to be determined were chosen alternately from the node above and below the leaves whose stomatal activity was being recorded.

Photosynthetic determinations were made on both species of plants on approximately forty days of the experimental period. Determinations

were of ten-minute duration, each half hour of the day, from approximately one hour before sunrise to approximately one hour after sunset.

RESULTS.—The relationship between stomatal aperture and apparent photosynthesis in Ligustrum are shown in figures 8a, b, c, d, and 9a, b, c, d, e. Data from Camellia were similar. These graphs were constructed from data obtained on nine different days throughout the experimental period. At temperatures from zero to five degrees centigrade, the stomates require from two to four hours to open even under high light intensity. As the graphs in figure 8 indicate, there is no appreciable photosynthesis evident until the stomates have opened. Photosynthesis does occur before

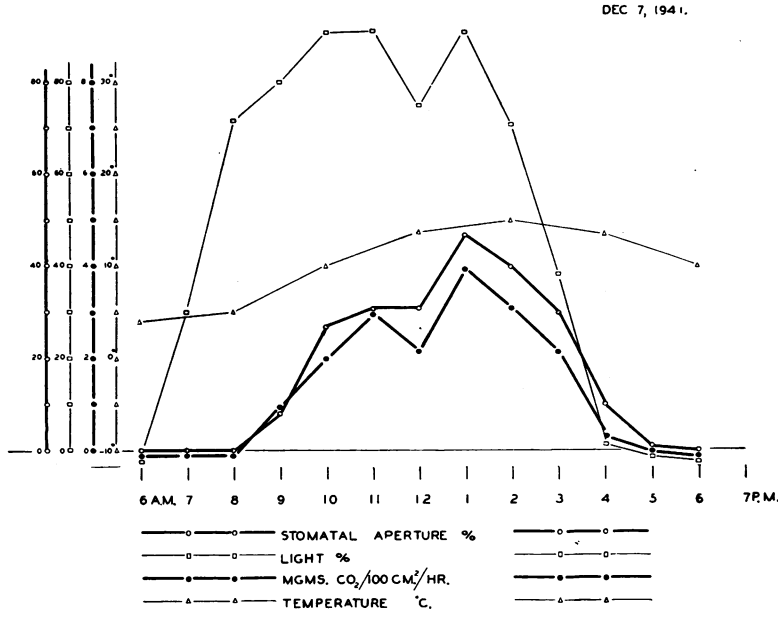

FIG. 8a. Graph showing relation between stomatal aperture and photosynthesis on first year leaves of Ligustrum.

the opening of the guard cells but only at a rate similar to the normal respiration rate. At no time was a photosynthetic rate of a greater magnitude than the respiration rate observed while the guard cells were apparently closed. On one day in which the average temperature was $-3°$ C., no stomatal aperture was apparent although the plants carried on slight photosynthesis for five hours on that day.

The graphs shown in figure 9 were constructed from data obtained during the following spring when the leaves were beginning their second season. In practically all instances, there was a mid-day drop in the rate of apparent photosynthesis. On some days this reduction in the apparent photosynthetic rate was so great that the plants released carbon dioxide. The only exceptions were on days in which the vapor pressure deficit was low. Although respiration exceeded photosynthesis occasionally on days

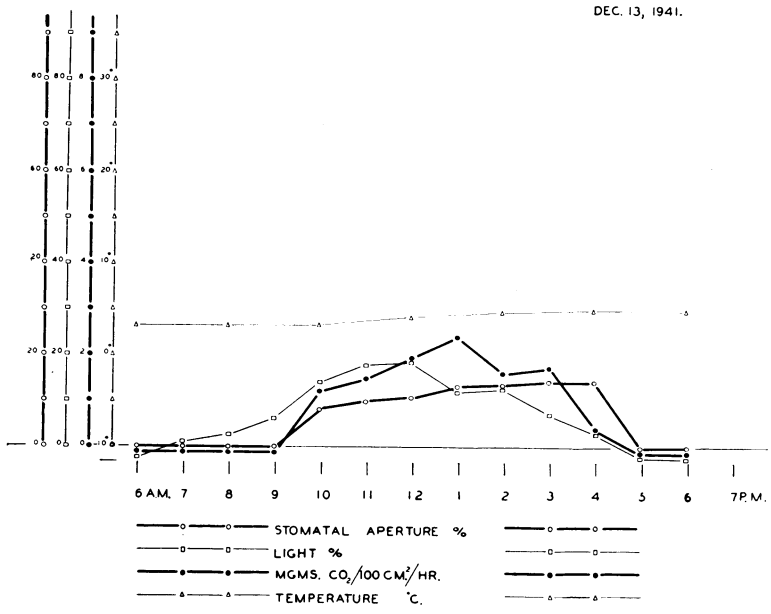

FIG. 8b. Graph showing relation between stomatal aperture and photosynthesis on first year leaves of Ligustrum.

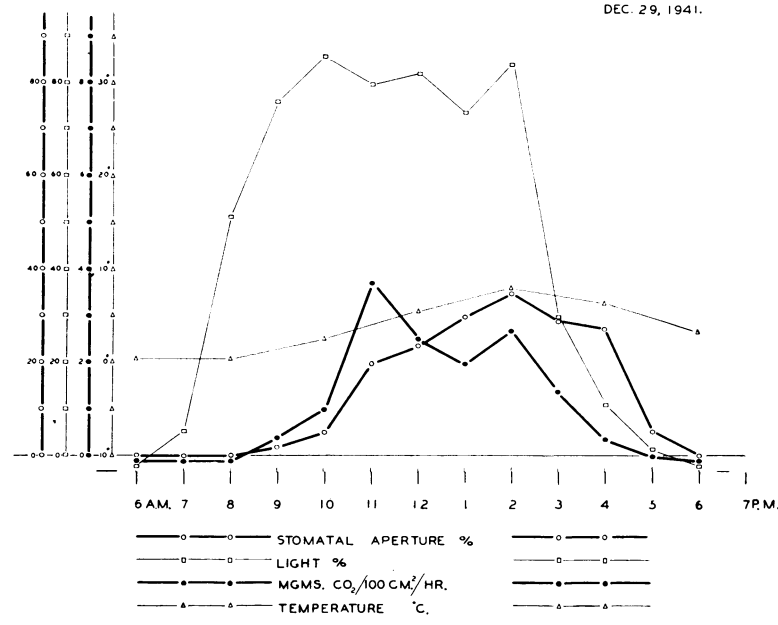

FIG. 8c. Graph showing relation between stomatal aperture and photosynthesis on first year leaves of Ligustrum.

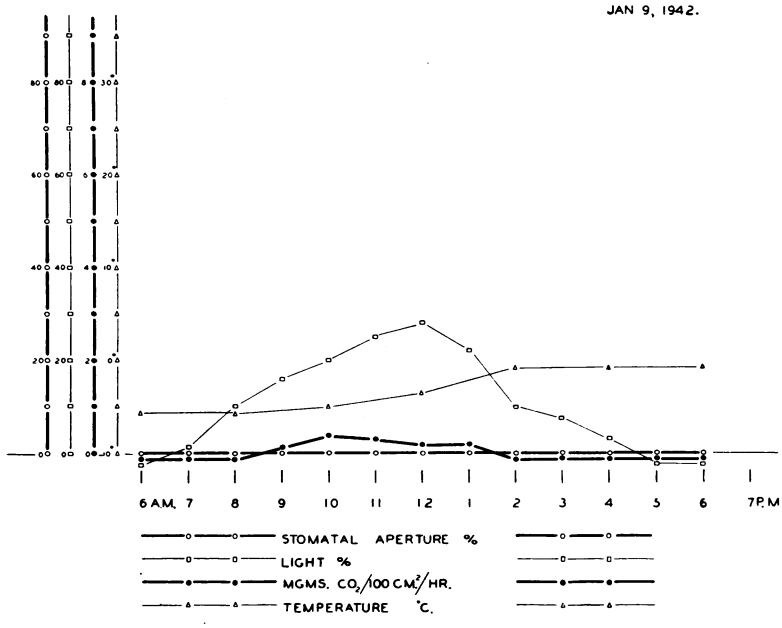

FIG. 8d. Graph showing relation between stomatal aperture and photosynthesis on first year leaves of Ligustrum.

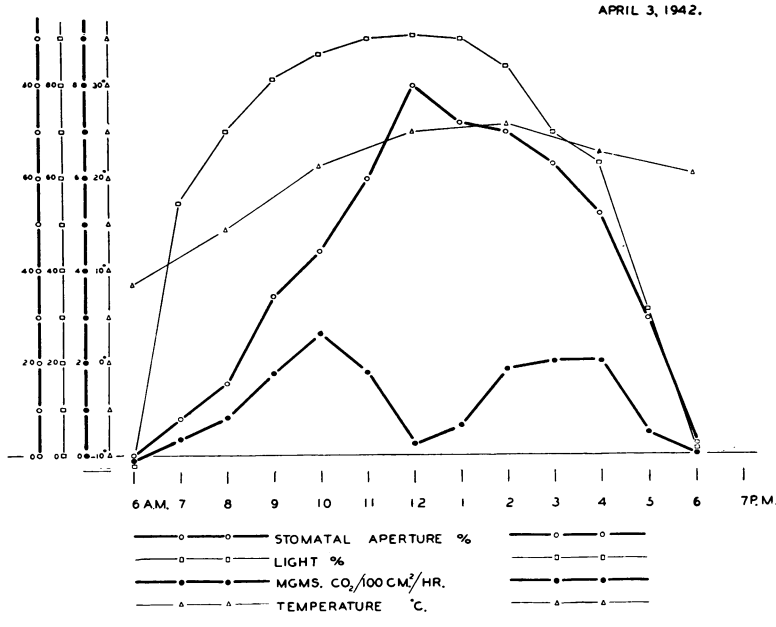

FIG. 9a. Graph showing relation between stomatal aperature and photosynthesis of second year leaves of Ligustrum.

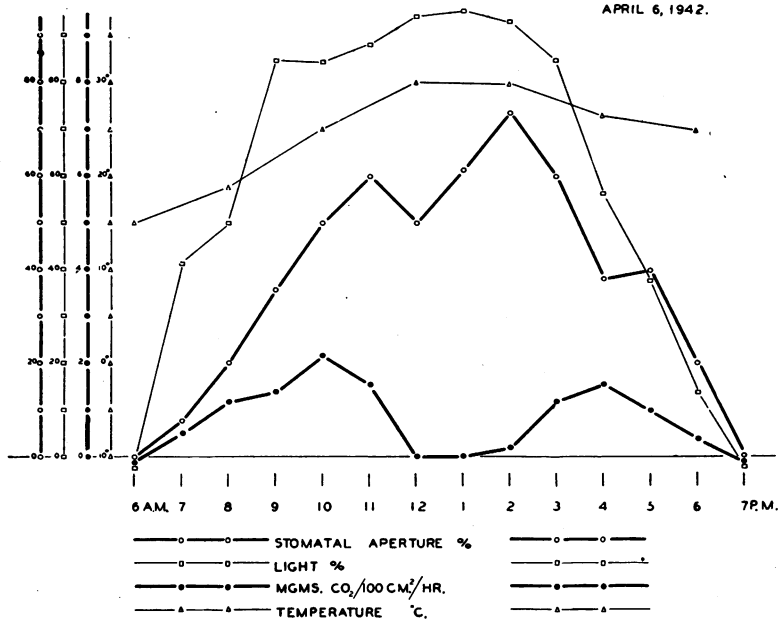

FIG. 9b. Graph showing relation between stomatal aperature and photosynthesis of second year leaves of Ligustrum.

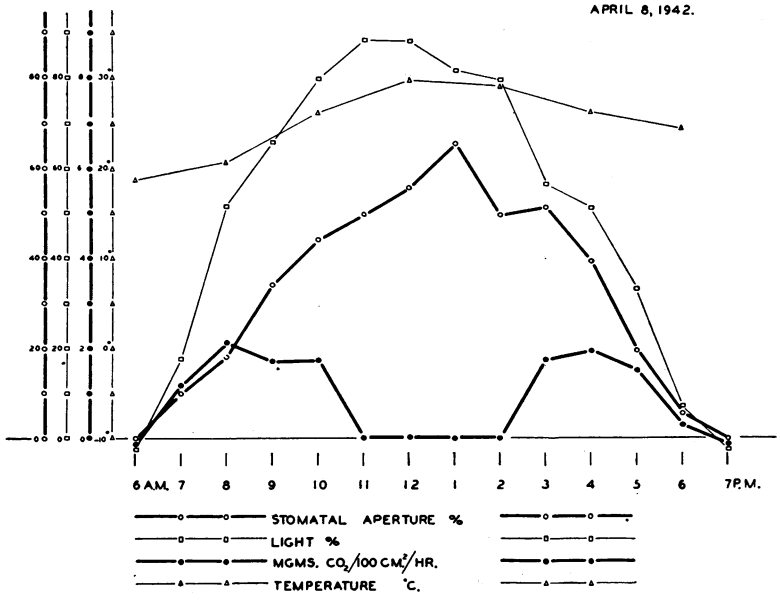

FIG. 9c. Graph showing relation between stomatal aperature and photosynthesis of second year leaves of Ligustrum.

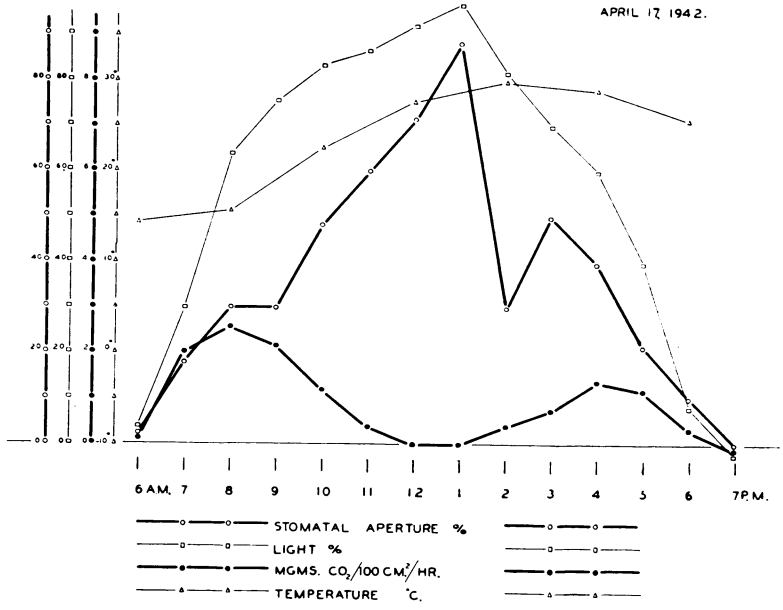

FIG. 9d. Graph showing relation between stomatal aperature and photosynthesis of second year leaves of Ligustrum.

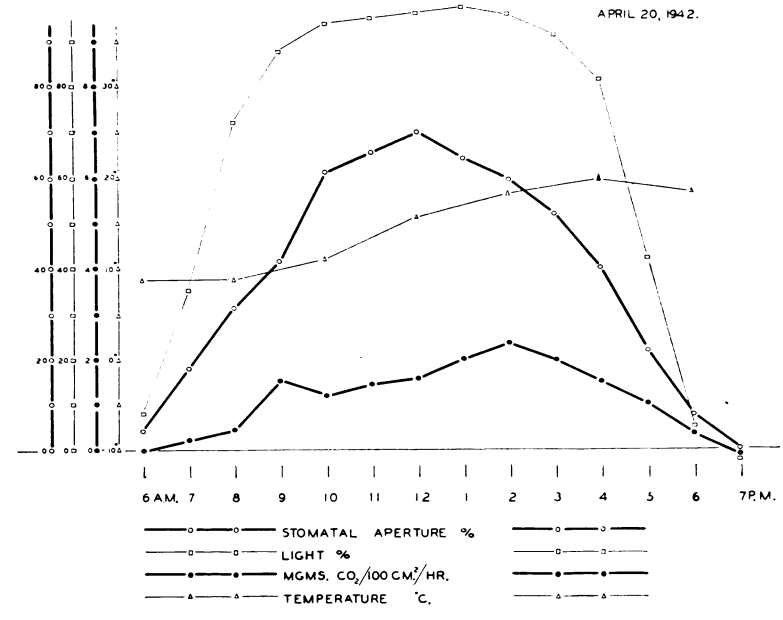

FIG. 9e. Graph showing relation between stomatal aperature and photosynthesis of second year leaves of Ligustrum.

in which the average daily temperature was high there was no corresponding fluctuation in the stomatal aperture. The only appreciable mid-day closure of the stomates observed occurred at the time in which the plants were resuming their apparent photosynthetic activities. Although no data were obtained on the moisture content of the leaves, it is not improbable that the stomatal closure was associated with a low leaf moisture content rather than with the mid-day drop in the apparent photosynthetic rate.

The relation between stomatal aperture and photosynthesis of Ligustrum during different months of the experimental period is shown in figure 10.

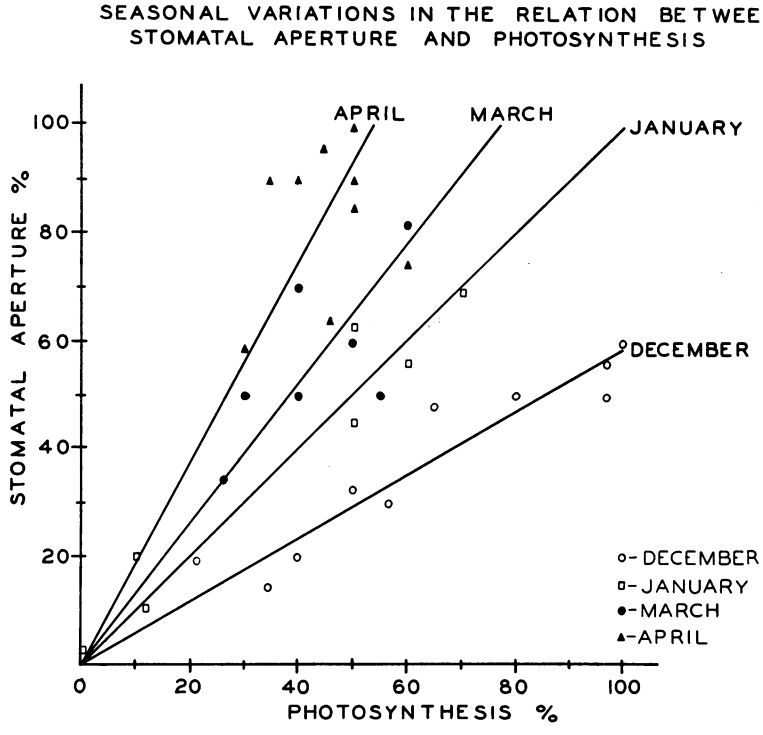

FIG. 10. Graph showing seasonal variations in the relation between stomatal aperture and photosynthesis in Ligustrum.

The highest apparent photosynthetic rate for the entire period was used as a base for calculating all the other photosynthetic rates. As the graphs indicate, the average daily apparent rate decreased from December to May while the average daily stomatal aperture increased during the same period. It would appear that the age of the leaf affects its apparent photosynthetic activity more than it does the activity of its guard cells.

Investigation of stomatal aperture under controlled conditions

PART I. THE EFFECT OF TEMPERATURE UNDER CONSTANT LIGHT INTENSITY

The effect of temperature on the stomatal aperture has been the subject of several investigations (**13, 14, 32**). These investigations have resulted

in the formulation of divergent opinions concerning the effects of temperature. Since a temperature effect was clearly indicated on the plants growing under natural conditions, an investigation under controlled conditions was undertaken to verify this effect and to aid in the clarification of the reported contradictions.

METHODS.—Two plant chambers were constructed in which it was possible to control light, air temperatures, and soil temperatures independently of one another. No attempt was made to control the relative humidity beyond keeping the air as saturated with water vapor as possible. The independent air and soil temperatures obtained by the use of this chamber were considered desirable in a study of the effect of temperature on stomatal aperture. Unpublished data of the writer indicate that if soil and air

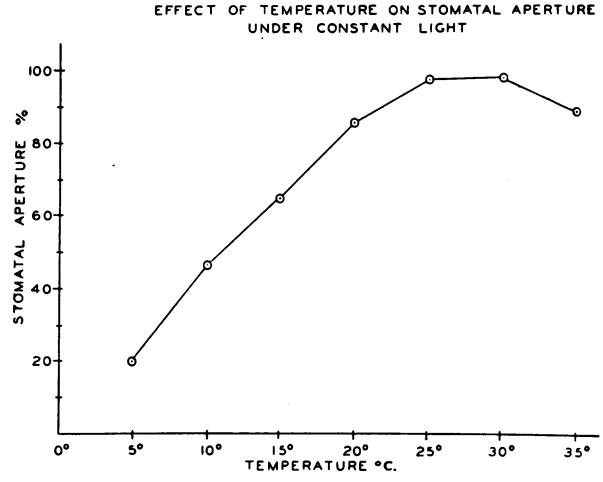

FIG. 11. Effect of temperature on stomatal aperture of cotton under constant light.

temperatures are both low, a lack of water associated with low soil temperatures may affect stomatal aperture.

Since time did not permit an extended investigation of the effect of temperature upon stomatal aperture of many species of plants, it was considered desirable to choose a species which does not normally undergo a wide range of temperature during its normal growing season. *Gossypium hirsutum* L. and *Nicotiana tabacum* L. were the species employed.

Plants were grown out-of-doors in large metal containers and were approximately two and a half months old when they were used in this study. The stomatal aperture was determined by means of the resistance porometer in the manner previously described. The plants were tested at various temperatures from 5° to 35° C. under a light intensity of ten thousand foot candles as measured by a Weston light meter. Four plants of each species were placed in one chamber and four independent records of the stomatal aperture were obtained for each plant at each temperature.

The light was automatically controlled by a time switch to give four hours of light alternating with four hours of dark. The four-hour period was used to eliminate any time effects. Plants were maintained at each air temperature for two days.

RESULTS.—The effect of temperature under constant light and humidity on the stomatal aperture of cotton and tobacco was very similar to that observed for Camellia and privet. Both the period of opening and the rate of movement of the guard cells decrease with decreasing temperature. At 35° C., the stomatal aperture was less than at either 25° or 30° C. The experiment was not extended beyond 35° C. but it seems reasonable to conclude from the form of the curve shown in figure 11 that beyond 30° increasing temperature may be associated with decreasing stomatal aperture. It would appear that temperature exerts a direct influence upon stomatal aperture.

PART II. THE EFFECT OF CHLOROFORM UPON STOMATAL APERTURE

It has been shown by IRVING (8) that chloroform in small concentrations inhibits photosynthesis without appreciably affecting respiration. PAWLENKA (18) has shown that this gas has no pronounced effect upon stomatal aperture other than to accelerate its rate of movement. The writer is unaware of any study in which the effect of chloroform was determined on stomatal aperture and photosynthesis simultaneously. Such a study was undertaken so that the relation between stomatal aperture and photosynthesis might be further clarified.

METHODS.—The plants to be investigated were placed in the plant chambers previously described. The chambers were maintained at 25° C. and the light at ten thousand foot candles. The rate of apparent photosynthesis and the stomatal aperture of the plants were determined by the methods previously described. The concentration of chloroform was approximately 0.02 cc. per liter of air. The species employed were cotton and tobacco.

RESULTS.—Small concentrations of chloroform appeared to inhibit the photosynthetic activity of both cotton and tobacco. Chloroform, also stimulated an increase in the rate of respiration which, however, was not sufficient to account for the lack of apparent photosynthesis. The effect of chloroform on the stomatal aperture was somewhat irregular. When the chloroform was added while the stomates were open and the plants exposed to light, a slight stomatal closure was observed. The stomatal aperture soon regained its normal position although no apparent photosynthesis could be detected at the same time. When chloroform was added to plants in the dark whose stomates were closed, there was no apparent movement of the guard cells. When the light was turned on soon after, the plants continued to emit carbon dioxide while the stomatal aperture appeared to follow its normal light-induced opening. The plants did not appear to be affected by the treatment and one day after treatment the photosynthetic rate was

normal. The guard cells of the plants whose photosynthetic activity was inhibited by small concentrations of chloroform, appear to respond in a fairly normal fashion to the effects of light.

PART III. THE EFFECT OF THE ABSENCE OF CHLOROPHYLL ON STOMATAL APERTURE

The behavior of guard cells with chlorophyll-free plastids has been variously reported. LLOYD (**13**) considered such cells capable of movement. PAETZ (**17**) concluded that such cells responded to changes of humidity but not of light. SCARTH (**25**) noted that guard cells located above chlorophyll-free tissue responded gradually to the effect of light and

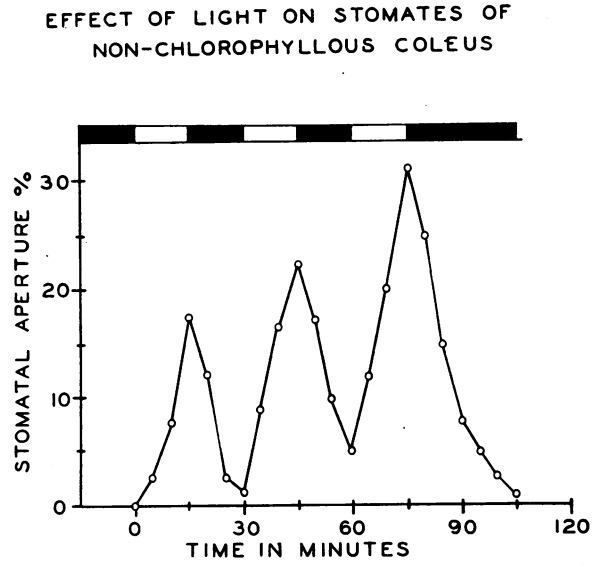

FIG. 12. Effect of light on stomatal aperture of non-chlorophyllous Coleus.

thought the response spread from the cells located next to the chlorenchyma. HARMS (**6**) reported that the light induced opening of guard cells situated over chlorophyll-free tissue was proportional to the intensity of light absorbed by the tissue. A further investigation of this problem was thought desirable.

METHODS.—The guard cells of several species of plants were observed through a fluorescent microscope to determine whether or not they contained chlorophyll. Although the guard cells located above chlorophyll-free tissue of several species of plants were examined, only one species was found to contain non-fluorescent plastids. These guard cells, of a variegated species of *Coleus blumei* were studied by means of the resistance porometer.

Sweet potato plants were grown in total darkness. Guard cells from leaves of these plants were observed through the fluorescent microscope.

No fluorescence was observed in any of the plastids of any of the cells of the leaves. Several of these plants were removed from the dark and placed under a bright light. The movements of the guard cells were observed through a microscope at ten-minute intervals and the plastids examined for fluorescence.

RESULTS.—The effects of light on the guard cells of *Coleus blumei* are shown in figure 12. Although the amplitude of their response is less than that of varieties containing fluorescent plastids, the rate of response appears to be the same. The guard cells exhibited a response to changes in light within one minute.

The guard cells of the sweet potato which had been growing in the dark were closed. When the plant was placed under a light of approximately one thousand foot candles for ten minutes, a slight opening of the guard cells was observed. No fluorescence was observed during the same time. After one half-hour many guard cells were quite widely open and still no fluorescence was observed in the plastids of any of the cells of the leaf. It would appear that guard cells containing non-fluorescing plastids and located over chlorophyll-free tissue are capable of exhibiting light-induced movements.

Discussion

Although it has been established by many workers that the movements of the guard cells are effected by changes in the environment, it is difficult to formulate a satisfactory explanation of these effects. It has been assumed that the movements of the guard cells are associated with turgor changes which result from the action of the external environmental factors. Several theories have been proposed to explain the effects of these factors upon the movements of the guard cells. In general, these theories can be grouped into three classes: those concerned with the effect of the factors upon the osmotic and enzymatic relations of the guard cells; those concerned with the effect upon the permeability of the guard cells; and those concerned with the effect upon the colloidal material in the guard cells.

The theory that the external environmental factors cause reversible changes of the osmotically active constituents of the guard cells, has been widely held. VON MOHL (**28**) was the first to propose that the plastids of the guard cells produced an osmotically active substance which resulted in an increase of the turgor of the cells and thereby promoted opening. An increase in the osmotic pressure of the guard cells in the day-time has been reported by many workers: ILJIN (**7**), WIGGANS (**32**), STEINBERGER (**27**), LLOYD (**13**), LOFTFIELD (**14**), and SAYRE (**22**). Most of these workers assumed that starch was changed to sugar as the guard cells open and that the sugar was converted to starch as they close. Sayre considered that there was approximately two and a half times as much sugar present in the guard cells when they were open as when they were closed. He further considered that there was a reciprocal relation between starch and sugar. LLOYD reported that in *Verbena ciliata,* as well as in certain other plants, starch

almost wholly disappeared during the early afternoon, when the stomates were at their widest, and increased toward evening with the closing of the stomates. LOFTFIELD studied "the starch content of the guard cells . . . in order to determine its relationship to light intensity and stomatal movement." He reported that "the starch index did not show the close correlation to either light changes or stomatal movement that ILJIN and LLOYD have found. 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. 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 have supposed, and must be the subject of renewed investigation." Although LOFTFIELD wrote the above in the body of his study, in his summary he wrote the following: "light induces opening of the stomata after day-break by initiating conversion of the starch within the guard cells into sugar."

The results of some other workers indicate an apparent disparity between their facts and their conclusions. MILLER (**16**) states that "the results of WIGGANS show that the osmotic value of the guard cells averaged about thirteen atmospheres higher than that of the epidermal cells." It is not clear what the effect of an "average osmotic" value would have on the guard cells if the fluctuations in the osmotic value were not associated with changes in the movements of the guard cells. A reexamination of WIGGANS, data did little to clarify this point. There were no data upon the stomatal aperture except the statement that the stomates were usually at their maximum aperture on bright days at 10 A.M. He states that on November 29th, it was dark until 9:30 A.M. and then bright throughout the day. It is assumed from his data that the stomates did not open until there was light, which would be at approximately 9:30 A.M. and yet the greatest difference between the osmotic value of the guard cells and the other epidermal cells was recorded at 8 A.M. The differences in the osmotic values decreased from that time until 2 P.M. when there was no appreciable difference between them. On December 6th, when he reported the sun as bright throughout the day, he recorded no appreciable difference between the osmotic pressure of the guard cells and that of the cells of the epidermis at any time. WIGGANS also found that in *Zebrina pendula* the osmotic pressure of the guard cells was always distinctly higher than that of the epidermal cells. In considering this, LOFTFIELD states that "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."

SAYRE (**22**) determined the osmotic values of the guard cells and epidermal cells of *Rumex patientia* by using sucrose, glucose, and calcium chloride as plasmolyzing agents. He found that there was a wide variation in the osmotic pressures of the cells so determined. In tabulating his values,

he gave maximum and average values but not minimum values. It seems fair to conclude that the minimum values would deviate from the average as much as the reported maximum values. If his values, using cane sugar as the plasmolyzing agent, are inspected, it can be seen that there is no difference between the average osmotic pressure of the guard cells and the subsidiary cells until 1:30 P.M. at which time the stomates were at their maximum aperture. The guard cells, however, were reported 25% open at 8:30 A.M. and 50% open by 10:30 A.M. The minimum values for the osmotic pressure of the guard cells, calculated as described above, were less than those found for the epidermal cells, making it difficult to understand the relation between stomatal aperture and osmotic pressure of the guard cells from the above data. The values obtained using glucose and calcium chloride as plasmolyzing agents were even more unsatisfactory.

In addition to the above problems of evaluation, there exist in this field some directly contradictory data. Although most workers report a decrease in the starch content of the guard cells during the day and a maximum at night, the opposite was reported by ALEXANDROV (1). It would appear that the experimental evidence is not so conclusive as many have assumed and that a re-investigation of the problem might well be undertaken.

If the results of the investigation on the osmotic relations of the guard cells are provisionally accepted, the results lead to the further problem of the cause of the changes in the osmotically active substances. Since it has been assumed that during the day starch in the guard cells would change to sugar, the existence of an enzyme to promote this change was postulated. KOHL (11) considered that light activated an enzyme which promoted the change of starch to sugar. He considered that photosynthesis occurred in the plastids of the guard cells and that in some way this activity promoted the production of the enzyme. This theory with various modifications has been held by many modern workers including LOFTFIELD (14), SAYRE (22), and WEBER (30). SAYRE associated the activity of the enzyme with a change in the pH of the guard cells. He considered that . . . "in the morning, light initiates the action of diastase, probably by decreasing the acidity of the cells sap of the guard cells. The diastase changes the starch to sugar, which results in an increase of the osmotic value of their cell sap. This causes water to enter the guard cells, since the osmotic value of the cells sap of the epidermal cells remains constant. A swelling of the guard cells results, and this causes the stoma to open. The procedure during the closing of the stomata is perhaps the reverse of that during the opening."

The presence of diastase has not been demonstrated in the guard cells. HAGEN (5) caused the guard cells to open by placing them in a solution of diastase. He considered that the starch in the guard cells had been hydrolyzed to sugar and the turgor of the cells thereby increased. The fluctuations in the starch content of the guard cells which have been observed by many workers have been considered the best indirect evidence for the presence of diastase. LLOYD (13) and ARENDS (2) could not confirm the

presence of sugar in open guard cells as reported by HAGEN (**5**) and by SAYRE (**22**). If diastase was present, it appeared to act in a manner contrary to that found in the cells of the mesophyll. In the latter cells, starch accumulates during the day and is hydrolyzed at night, whereas in the guard cells the contrary has been reported.

It has been reported by some workers, and confirmed by the writer, that the guard cells of some species react to a light stimulus in less than one minute. It has been considered that the speed of reaction of the guard cells is so rapid that turgor changes caused by enzyme reactions are too slow to account for the changes (**12, 25, 31**).

This hypothesis appears inadequate in explaining the effects of fluctuations of light upon the movements of the guard cells after the stomates are open. If the action of light, presumably by its photosynthetic effect, produces a shift in the pH in a direction favorable to the hydrolytic activity of the enzyme, it would appear likely that as long as photosynthesis was occurring in the leaf, the pH would remain relatively constant. On the basis of this theory, we would then expect a gradual opening of the guard cells when photosynthesis begins, followed by a gradual closing after photosynthesis ceased. It has been shown by the writer that there were marked fluctuations in the movements of the guard cells which were closely correlated with fluctuations in light intensity while the photosynthetic activity of the leaf remained fairly constant. The enzyme theory apparently provides no explanation of these fluctuations. The writer observed that the guard cells remained open in the day-time during periods when the photosynthetic activity of the leaf had ceased. If the pH change is associated with photosynthetic activity, the stomates should close when photosynthesis ceases, but they actually remain open.

Many facts concerning the movements of the guard cells are not satisfactorily explained by the theory that the change in the positions of the guard cells is associated with changes in the activity of diastase. The assumptions which have been made to support the hypothesis rest mainly upon inconclusive evidence. There is no evidence for the presence of diastase in guard cells or for the effect of a pH change upon the hydrolytic-synthetic activity of the enzyme. If such a mechanism does exist, it would probably act as a reinforcing agent incidental to some other agent.

The possibility that the movement of the guard cells is associated with light-induced permeability changes has been advanced by several workers. There appears to be a general agreement that light causes an increase in the permeability of most plant cells. KISSELEW (**10**) investigated the permeability of the guard cells under open and closed conditions. He employed several methods in this study and, although his results have been open to question, one of his methods appears to be acceptable. By studying the rate of plasmolysis of closed and open guard cells, he stated that the closed guard cell is more permeable to water than the open guard cell. LVOV (**15**) considered that the permeability of the guard cell as determined by the

method of isotonic coefficients was the opposite to that reported by KISSELEW. ILJIN (7) and STEINBERGER (27) studied the changes in the osmotic pressure of the guard cell using potassium nitrate as the plasmolyzing agent and report that their results indicated that the osmotic pressure of the guard cells was very much higher in open than in closed cells. SAYRE (22) has criticized their work on the ground that potassium nitrate penetrates the cells. It is possible that their results indicated a change in the permeability of the guard cells rather than an osmotic change.

It seems reasonable to assume that there are changes in the permeability of the guard cells. It is not clear whether these changes are the same as those reported for other plant cells or whether the guard cells behave in an anomalous manner.

An hypothesis which has gained wide acceptance in recent years states that "the normal turgor changes in guard cells are, in large part, merely a specialized example of a phenomenon which is very general not only in living cells but in biocolloids apart from life, namely, changes of hydration in relation to H-ion concentration." This view was presented independently by both SCARTH (24) and LINSBAUER (12). SCARTH considered the series of events that leads to opening and closing of the guard cells in the natural state to be the following: "The morning light initiates photosynthesis resulting in a reduction of CO_2 concentration and the development of a more alkaline reaction within the guard cells. In response to the change of reaction the vacuome apparently in virtue of its colloidal content quickly imbibes more water from the adjacent cells and causes distension of the guard cells. More gradually, as a result of the same H-ion change starch is hydrolyzed, and unless the cells are freely permeable to the soluble product—a point still subjudice—turgidity will be further increased thereby.

Cessation of photosynthesis whether from lack of light, accumulation of photosynthetic products (?) is followed by a similar series of opposite changes. Prolonged closure in darkness may possibly result in sufficient acidity, through accumulation of CO_2, to cause temporary opening along with slight hydrolysis of starch."

This theory appears to offer many advantages over the previous theories. Since colloidal hydration and dehydration can occur very rapidly, the turgor changes which they produce in the guard cells are capable of explaining the observed speed of stomatal opening and closing. The work of SCARTH also indicates that starch-sugar changes are subsidiary to the pH changes and require a much longer time for completion. This observation may explain why the starch-sugar changes reported by other workers do not necessarily coincide with movements of the guard cells. Several facts have been reported, however, which cast some doubt upon this hypothesis.

SAYRE (22) was the first to observe movements of the guard cells in solutions of different pH. He noted that the guard cells of *Rumex patientia* opened in a pH of 4.2 to 4.4 and closed in the pH range above and below those figures. He was not able to demonstrate pH changes in the guard

cells of normal leaves under natural conditions. SCARTH (**24**) observed that the guard cells of *Zebrina pendula* closed when placed in solutions of pH 5.0 to 8.0 and opened above and below that range. He stated that: "experiments with non-adsorbed indicators show that the vacuole of the guard cells may range from about pH 4.5 on plants kept in the dark to about pH 7.0 on plants exposed to full sunlight. The experiments do not show whether or not it reaches a point (probably about pH 7.3) at which hydrolysis of starch and opening of stomata can take place without the aid of light."

PEKAREK (**19**) observed that "the cell sap of guard, subsidiary and epidermal cells in the light have a pH of about 6.0. In the dark, the pH of the guard cells falls towards the acid side while that of the subsidiary and epidermal cells increases towards the alkaline side." The results obtained by the above investigators indicate that the effect of pH changes on the movement of the guard cells is somewhat complex.

ZIRKLE (**34**) stated that "the pH of the cell sap can be measured only with great difficulty and the values obtained are generally somewhat inaccurate. Different vacuoles, sometimes within the same cell, may acquire very different colors when stained by such dyes as neutral red." He reported the use of natural indicators as particularily valuable. However, when SCARTH (**25**) used the natural indicators in Zebrina, he noted that alkali-induced opening corresponded to a pH of 7.4 which apparently never occurs under natural conditions.

SCARTH also reported that there was no detectable trace of tannin in the vacuole of the guard cells of Tradescantia. This would place the guard cells of Tradescantia in the B category of ZIRKLE of which he says: "the range indicator method gave very uncertain results with the B type vacuoles. Twelve dyes were found which stained these vacuoles, and the color of the stained vacuole matched different buffer solutions of the dyes ranging from pH 7.2 when they were stained with neutral red, to pH 13 when they were stained with brilliant cresyl blue, depending on the particular dye which was used. Obviously, such results show that the vacuoles contain some substance which markedly alters the normal virage of basic dyes."

It was considered by SCARTH (**24**) that the colloids of the vacuole became dehydrated as they approached their isoelectric point and became hydrated at a pH above and below that point. It is not clear how this concept can be harmonized with the data presented by SAYRE (**22**), who noted that the guard cells opened in an intermediate pH and closed on either side of that pH. Such a mechanism is contrary to that required by the theory of colloidal hydration.

If SCARTH's hypothesis is correct, there should be a direct relation between photosynthesis and the movements of the guard cells. As soon as photosynthesis has started in the plastids, the concentration of carbon dioxide surrounding the plastids should be greatly reduced. If the vacuoles of the guard cells are not well buffered then the diffusion of carbon dioxide

to the plastids should increase the pH of the vacuole. Since carbon dioxide diffuses very rapidly through the cells, an equilibrium between the free carbon dioxide of the vacuole and the plastids should be quickly attained. As long as the plastids continue photosynthesis, the equilibrium should remain fairly constant. Thus, once the guard cells have opened, it is not clear how fluctuations in the movements of the guard cells could arise as long as photosynthesis is maintained. The writer has observed that the guard cells responded to fluctations in the light intensity while the photosynthetic rate remained fairly constant.

Many investigators have attempted to determine the effect of different regions of the spectrum on the movements of the guard cells. In most of the early investigations, no attempt was made to measure the intensity of the light falling upon the guard cells. The result of these investigations indicated that the guard cells were more strongly affected by the red end of the spectrum. They considered that yellow, green, infra-red, and ultraviolet exerted essentially no influence on stomatal opening (**3, 11, 13**). SAYRE (**23**) considered that the guard cells did not open in wave lengths longer than 6900 A and that other regions of the visible transmitted by his filters seemed equally effective.

A very careful investigation of the opening of the guard cells in different regions of the visible spectrum was carried out by SIERP (**26**). Great precautions were taken with the photometric methods and the total energy actually falling on the guard cells under observation was measured by means of a thermopile. The total energy transmitted by each of the filters used was so arranged that the radiation which fell on the guard cells differed in light quality but not in total energy. SIERP stated that: "blue, green, yellow, and orange-yellow light of equal intensity had equal effect on the stomates of Helianthus. Red was only about sixty percent effective while the infra-red had no effect. Since the different wave lengths used were all about equally absorbed by the leaf, it is concluded that if the stomates are affected by the quanta of light absorbed then in some region of the red no quanta were absorbed. This is in contrast to assimilation studies so that reasonable doubt is cast on the stomatal movement being directly linked to assimilation."

HARMS (**6**), using the same careful technique as that employed by SIERP (**26**), conducted an extensive investigation of the effects of quality of light on the movements of the guard cells. He reported that most species were more sensitive to blue than to red by the ratio of 2:1. He also noted that the stomates situated over the colorless mesophyll of variegated species responded according to the formula, intensity $\times$ percentage of wave length absorbed, to open to the same aperture.

It would appear from a consideration of the available evidence that light of a given wave length affects photosynthesis and the guard cells differently. Observations of the writer appear to confirm this conclusion. It was found that guard cells, containing non-fluorescent plastids, located above non-

chlorophyllous tissue exhibited light-induced movement. It was also noted that guard cells of etiolated sweet potato leaves in which no fluorescence could be observed also exhibited light-induced movement. Simultaneous determinations of stomatal aperture and photosynthesis under natural conditions indicated that the stomates could be open at a time when photosynthesis had ceased during the middle of the day. The effect of chloroform upon stomatal aperture and photosynthesis was investigated and it was found that stomatal opening occurred although photosynthesis was inhibited. These observations appear to confirm the independence of photosynthesis and movement of the guard cells.

Summary

1. The effects of light, temperature, and humidity on stomatal aperture were observed on leaves of potted specimens of *Camellia japonica* L. and *Ligustrum japonicum* Thunb. growing out-of-doors. The stomates are affected by variation in temperature, remaining closed at temperatures of $-4°$ C. or less. The guard cells respond slowly to light when the temperature is low. With temperatures of 20° C. or higher the stomatal aperture is directly affected by variations in the light intensity. The effect of low relative humidity on stomatal aperture is comparatively slight at medium and low temperatures, but is more pronounced at high temperatures. An equation was derived from these data which describes the effects of light, temperature and humidity on the stomatal aperture of both species. The effect of temperature on the stomatal aperture of cotton and tobacco under constant light and humidity was also investigated. It was found that the stomatal aperture of both cotton and tobacco decreased with decreasing temperature.

2. Photosynthetic measurements are reported for the plants whose stomatal apertures were also being determined. Fluctuations in the rate of photosynthesis do not appear to affect the stomatal aperture of Camellia and privet. The apparent photosynthetic rates of leaves of both species are affected by age differently from the stomatal aperture. The effect of chloroform is to inhibit apparent photosynthesis but not to affect greatly stomatal aperture. Guard cells containing non-fluorescent plastids on leaves devoid of chlorophyll exhibit light-induced movements. These results indicate that probably there is no causal relation between photosynthesis and stomatal opening.

3. Stomatal behavior has been explained by turgor changes associated with changes in permeability, changes in enzyme activity and variations in colloidal hydration. It has been assumed that these changes are initiated by fluctuations in photosynthesis. It is considered that none of these proposed theories are adequate to account for all of the observed facts concerning the movements of the guard cells.

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