THE EFFECT OF NITRATE FERTILIZER ON STOMATAL BEHAVIOR

By Anne L. Pleasants

Introduction

Of all the elements commonly considered as essential plant food, nitrogen, according to Allison (1), stands out in many ways as the most important. Its effect upon the growth of plants is perhaps greater than that of any of the other elements (18). Not only is it deficient in many soils, but it plays an exceedingly important part in plant metabolism, including both constructive changes and also the respiratory reactions resulting in the destruction of carbohydrates and proteins (1).

That there is a relation between the nitrogen supply and the water requirement of a plant is shown by the review of literature on the water requirement of plants by Briggs and Shantz (2). In this review the following investigators are cited as having found either a slight or a marked reduction in the water requirement of plants according to the use of nitrogen: Von Seelhorst found a marked reduction accompanied the use of nitrogen both alone and in combination. Wilfarth and Wimmer found that as a rule nitrogen lowered the water requirement of each crop considered, and Ohmer, working with squarehead wheat, also found a reduction in the water requirement with the use of nitrogen. Kiesselback (8) gives evidence that the water requirement is lessened in soils to which nitrogen fertilizers are added.

In regard to the effect of stomatal movement on the rate of water loss by transpiration, Loftfield (11) with potted plants in heavily watered and dry soils shows that the stomata regulated the water loss from plants. The same investigator (11) working out of doors found that when the environmental factors did not change too rapidly through the day, transpiration and stomatal apertures varied alike (15), and if water is deficient the stomata close much earlier in the day than if it is plentiful. Sayre (14) points out that the exchange of gases and loss of water from leaves occur mainly through the stomata. Lloyd (9), with the use of potometers, found that the stomata might continue to open while the rate of transpiration was decreasing or the opposite

might be true. Scarth (15) in a review of stomatal movement says, the general conclusion is, therefore, that the stomata must regulate the rate of transpiration at all apertures if there be a slight wind and only at narrow apertures in most indoor experiments.

Since the water requirement of a plant seems to be decreased by the use of nitrogenous fertilizers (2) and the stomata are supposed to regulate the loss of water by transpiration (11), (15) this work was undertaken to determine the effect of nitrogen in the nutrient medium on stomatal behavior.

METHOD AND MATERIALS

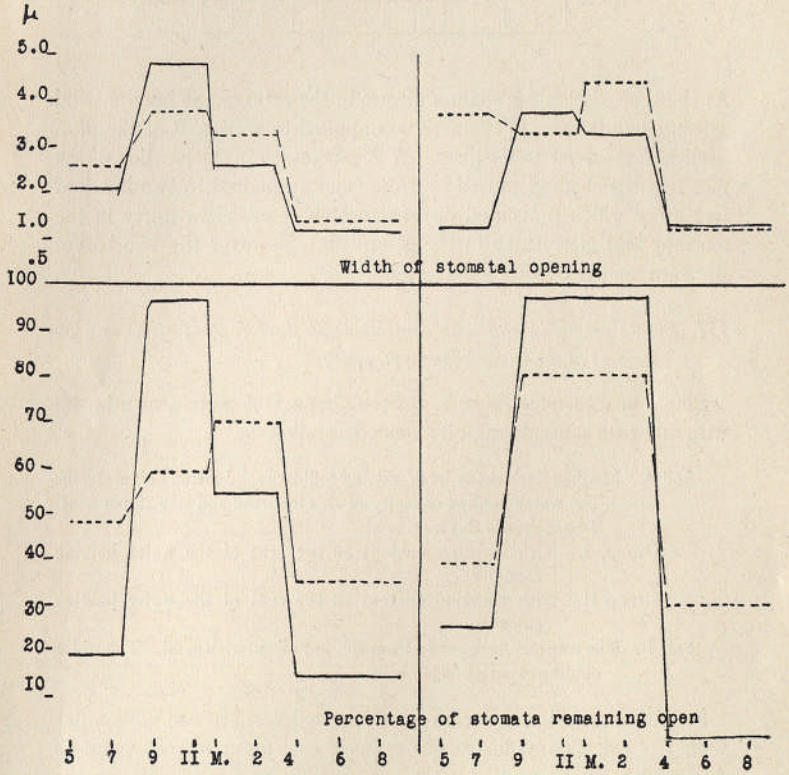
Two species of legumes, *Pisum sativum* and *Phaseolus vulgaris*, vars. Burpee Green Pod and Red Valentine, and *Raphanus sativus* have been used for this work. *Phaseolus vulgaris*, var. Burpee Green Pod, however is the only one which has been used in all of the experiments.

The conditions and nutrient media in which the plants have been grown for examination are as follows:

I. Plants grown indoors in Knop's nutrient solution.

Phaseolus vulgaris, Burpee Green Pod and Red Valentine, and *Pisum sativum* were grown in the laboratory, with south and east exposure, from Feb. to June, 1929. Two nutrient media were used: (a) water cultures contained in pint fruit jars covered with black paper and (b) builders sand, previously leeched and autoclaved at fifteen pound pressure for one and a half hours, and contained in white clay flower pots of approximately four kilogram capacity. Duplicate series of each were supplied with a slightly modified Knop's nutrient solution containing nitrogen: 1.00 gram $Ca(No_3)_2 \cdot 4H_2O$; 0.25 grams $MgSo_4 . 7H_2O$; 0.25 gram KH_2Po_4; and 0.04 $FeCl_3$ in one liter of distilled water. The other series was supplied with the same nutrient solution modified without the nitrogen. $CaCl_2 \cdot 6H_2O$ in the proportion of 0.9 grams per 250 c.c. of water was substituted for the $Ca(No_3)_2$ (4). Changes in the water cultures were made every two to three days and distilled water added between changes to keep the amount constant. The sand cultures were watered with the nutrient solution every two to three days and with distilled water on days when this was not used. One half of each group of plants was kept on tables facing east windows and the other facing south windows. Those facing the south received direct illumination, during clear weather, from ten-thirty or eleven until three

in the afternoon. Those facing the east were illuminated from approximately seven to eleven thirty A.M.

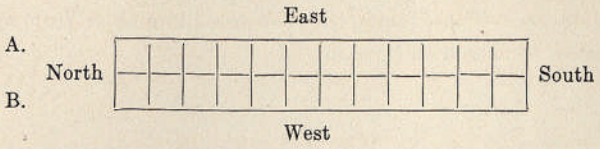


GRAPH 1

——— Grown with fertilizer.
--------- Grown without fertilizer.

II. Plants grown out of doors.

On June 25, 1929, seeds of *Phaseolus vulgaris* var. Burpee Green Pod and *Raphanus sativus* were planted in light clay loam soil in Cincinnati, Ohio. In preparing the soil for planting a three inch sod was removed from a plot 6′ by 8′. After thoroughly pulverizing the soil a 10″ board was sunk through the median length of the plot dividing it into halves;



At the time of planting sodium nitrate in the ratio of one hundred and seventy five pounds to the acre was applied to section B of the plot. Section A received no fertilizer. A disadvantage in the location of the plot was the shading caused by trees from a hundred to two hundred feet away which prevented direct sunshine before eight-thirty in the morning and after three in the afternoon. However the two halves were apparently equally illuminated.

III. Plants grown in soil with controlled moisture content of 20 and 50 per cent of the water holding capacity.

Plants of *Phaseolus vulgaris*, Burpee Green Pod, were grown in soil with moisture content and soil modified as follows:

Soil A. Medium dark sandy loam soil and builders sand mixed half and half. The water holding capacity of this modified soil was 25 per cent. Two groups of this were used:
Group I. With moisture content 50 per cent of the water holding capacity.
Group II. With moisture content 20 per cent of the water holding capacity.
Soil B. The medium dark sandy loam soil not mixed with sand. The water holding capacity 30 per cent.

Sodium nitrate in the ratio of one hundred parts to one million dry weight of soil was applied to one series of each group of soil A and B. A similar series of each group, except soil B, was used without the nitrate.

The soil for that part of the experiment to which no nitrate was added was previously autoclaved for two hours at fifteen pounds pressure in order to destroy nitrogen fixing bacteria. The pH value as determined at the beginning of the experiment was 7.2.

The method used for setting up the experiment was that used and described by Reed (14). This consisted of placing the soil previously mixed with water and the solution of sodium nitrate, in the desired proportions, in wire baskets and sealing with paraffin. The advantage of this method is that the roots of the plants grow more equally dis-

tributed through the soil and not massed at the bottom of the container as they often do when grown in pots.

Seeds of *Phaseolus vulgaris*, Burpee Green Pod, were germinated in moist chambers until the radicles were approximately 2 to 3 centimeters long. The seedlings were then placed with the radicles in holes made through the paraffin equal distances from the center of the basket. Moist cotton was kept over the seedlings until they took root. The water content was kept constant by the daily addition of sufficient tap water through a glass tube, in the center of the top of the basket, to restore the original weight.

In this part of the experiment the plants were grown in the laboratory, as already described, from February to the last of April 1930. The plants were placed on tables which were moved from south to east windows so that all of the plants were illuminated so far as possible for the same length of time each day. During clear weather they were in the sunshine from seven-thirty in the morning to three in the afternoon.

The method used for the study of the size of the stomata of the leaf was the absolute alcohol method introduced by Lloyd (9) and used by Loftfield (11) and Eckerson (7). Stomata examined by this method and checked by direct observations of stomata in position on the uninjured leaf were found by Loftfield (11) to agree very closely. Sayre (15) indicates that caution must be used in the alcohol method as removing the stomata may change the size of the pores. However, because of the size of the openings of the stomata of the plants studied, and the greater convenience offered for the examination of material the alcohol method seemed to offer advantages over the direct method for this work.

Material collected for examination was stripped from the same position, so far as possible, from mature leaves of three different plants at stated intervals of time. The epidermis was removed from near the base of the leaf between the midrib and the first large vein.

In studying this material, examination was made from two fields of the microscope, of the width of the aperture, the percentage of stomata closed and the number per unit area, for each plant stripped, at the hourly interval each day when collections were made. Averages were then obtained for the hourly intervals for all dates given. Since the collections were begun shortly after the leaves matured and continued until after fruiting these data represent the condition of the stomata, more or less, throughout the life of the plant.

Since the measurements made of the length of the stomatal pores did not indicate that those grown with and without the addition of

nitrogen differed greatly, the width of the opening has been given as representing the difference in the two series, for Sayre (15) has pointed out that this is the best means of expressing the degree of opening of the stomata. Also because of the scarcity of the stomata on the upper leaf surface and because of the apparent correlation of those present with those of the lower surface, all data given are representative of the lower surface.

RESULTS

No consistent difference has been noted in the number of stomata per unit area for those grown with and without the addition of the nitrogen to the nutrient media.

While the stomata of the plants grown in the Knop's nutrient solution without the nitrogen were slightly shorter than those grown in the same nutrient solution with the nitrogen, no consistent difference has been noted in the length of those grown in the soil to which the nitrate was or was not added.

Because of the presence of very few nodules, the nitrogen supply has, apparently, been modified very little by the action of nitrogen fixing bacteria, for any of the plants studied.

I. Plants grown indoors in Knop's nutrient solution.

In this somewhat preliminary part of the work no data were obtained when the plants were not directly illuminated. Also it may be suggested that since the data from nine until eleven are from plants illuminated from the east windows from seven to eleven-thirty, and that given from twelve to two-thirty from the plants grown in front of the south windows and illuminated from eleven to about three, the data from twelve to three might be modified if the plants had been in the sunshine during the forenoon. However, it agrees with that of the forenoon in indicating that the nitrogen has influenced the stomata to open wider and for fewer of them to remain closed when the plants are in the sunshine.

The material from which the data for these plants were obtained was collected on March 21, 27, 28, April 2, 1929. Camera lucida drawings are given for those made on April 2. Averages of the data are given in table I.

Pots of both varieties of *Phaseolus vulgaris* were sealed on April 21, 1929, with warm paraffin. The weight at the beginning and at the

TABLE I

The data represent the percentage of stomata closed and the average width in microns, at the stated intervals for the plants grown indoors in Knop's nutrient solution, from the time of mature leaf formation until after fruiting. Material for examination was collected March 21, 27, 28 and April 2, 1929.

TIME	PHASEOLUS VULGARIS, VAR. RED VALENTINE—GROWN IN KNOPS' NUTRIENT SOLUTION							
	Sand cultures				Water cultures			
	Nitrogen		No nitrogen		Nitrogen		No nitrogen	
	Per cent closed	Width	Per cent closed	Width	Per cent closed	Width	Per cent closed	Width
Material from plants at east windows:								
9:00 a.m.	14	1.9	46	1.6	23	1.8	67	1.8
9:30 a.m.					15	2.5	40	1.4
10:00 a.m.	13	2.2	33	1.8	52	2.2	56	1.8
11:00 a.m.	18	1.7	61	1.3	21	2.0	62	1.2
11:30 a.m.	61	1.1	80	1.6	11	3.3	30	1.9
Material from plants at south windows:								
12:00 m.	69	1.2	46	1.6	37	2.0	63	1.3
12:30 p.m.	30	1.8	43	2.0	8	2.8	13	2.1
1:30 p.m.	6	2.0	39	1.4	16	1.3	63	1.1
2:30 p.m.	8	1.5	78	1.6	60	1.6	78	2.0
Averages of the above data 9:00 a.m. to 2:30 p.m.	38	1.8	53	1.4	11	2.2	55	1.5
	PISUM SATIVUM—GROWN IN KNOP'S NUTRIENT SOLUTION; WATER CULTURES				PHASEOLUS VULGARIS-BURPEE—KNOP'S NUTRIENT SOLUTION; SAND CULTURES			
Material from plants at east windows:								
9:00 a.m.	16	1.5	30	1.2	23	2.0	67	1.3
9:30 a.m.					40	1.9	90	1.3
10:00 a.m.	1	2.8	23	1.4	21	1.5	51	1.1
11:00 a.m.	9	1.8	71	0.9	0	2.4	0	1.4
11:30 a.m.					30	2.0	53	1.4
Material from plants at south windows:								
12:00 m.	0	2.8	76	1.0	30	2.2	65	1.2
12:30 p.m.					0	2.4	50	1.7
1:30 p.m.					9	2.3	15	2.1
2:30 p.m.					18	2.2	60	1.2
Averages—9:00 to 2:30	8	2.2	47	1.1	19	2.1	50	1.4

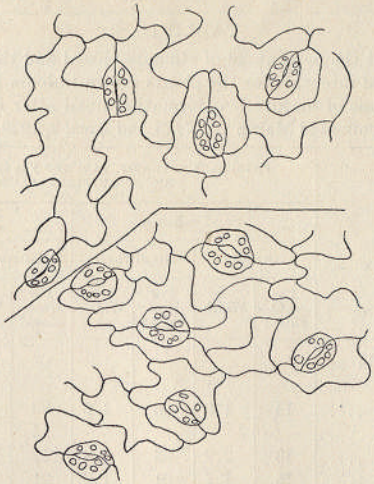


FIG. 1. CAMERA LUCIDA DRAWINGS OF STOMATA OF PHASEOLUS VULGARIS-BURPEE, AT 10 A.M.

Those above the line were from plants grown with Knop's nutrient solution modified without nitrogen and only 5 per cent were open. Below the line are these grown with nitrogen and 92 per cent were open.

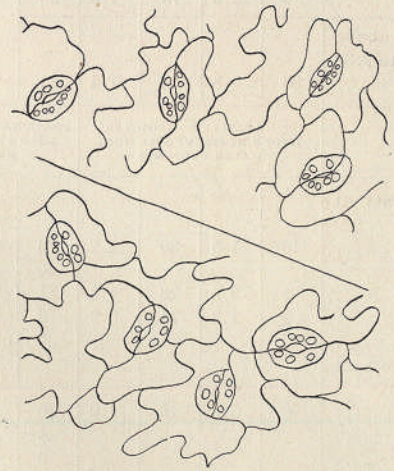


FIG. 2. CAMERA LUCIDA DRAWINGS OF STOMATA OF PHASEOLUS VULGARIS-BURPEE AT 11 A.M. WHEN GROWN IN SAND WITH KNOP'S NUTRIENT SOLUTION MODIFIED WITH (BELOW) AND WITHOUT (ABOVE) NITROGEN

At this time 90 per cent of those grown without this element were closed, while 33 per cent of those grown with it were not functional.

end of the experiment was obtained and the water loss computed. After obtaining the leaf surface and dry weight of the plants, the water loss per gram of dry weight and per square centimeter of leaf surface was obtained. The results showed that both species of plants which had been grown in the nutrient solutions with the nitrogen lost less water both per square centimeter of leaf surface and also per gram of dry weight than did those grown without this material. Since similar

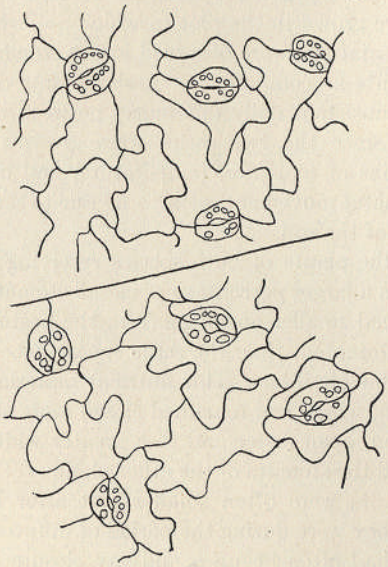


FIG. 3. CAMERA LUCIDA DRAWINGS OF STOMATA OF PHASOLUS VULGARIS-BURPEE, AT 12 M.

Those above the line were grown without nitrogen, and 62 per cent of the stomata were closed at this time. Below the line shows the stomata of the plants grown with nitrogen, and only 30 per cent were closed.

results are shown by the data given for the plants grown in the sealed wire baskets in part III of the experiment the data for this part of the work have not been given.

II. Plants grown out of doors in soil with and without the addition of sodium nitrate.

The conditions under which the plants were grown for this part of the work have already been described.

As for the differences in the plants grown in the fertilized and non-fertilized plots, the fertilized plants of *Phaseolus vulgaris* were considerably larger than those not so treated. But while the fertilized plants of *Raphanus sativus* were larger the difference in size was not so noticeable. Less difference in the size of the leaves of both species was apparent. However the fertilized one usually produced more of them.

There was a very evident difference observed in the stomatal movement of both species of plants receiving the sodium nitrate, in contrast to those that were grown in the plot to which no fertilizer was added. Although daily variations were observed in the stomatal movement of the fertilized plants as compared to similar plants on different days, there were also consistent daily differences between them and the unfertilized ones. Since the two series were grown under apparently identical conditions of moisture, temperature, and light, these differences in the stomatal movement seem to be due to the difference in the nitrogen content of the soil.

The leaves of the plants of both species receiving the nitrate were found to maintain a larger percentage of closed stomata, and those that were open averaged smaller openings, from the beginning of day light until directly illuminated, than the same species when grown without the addition of this fertilizer. This nutrient material, also seemed to make the stomata, which had remained closed more successfully before direct illumination, open faster and to a greater width when in direct sunshine, than did the stomata of the other plants. The stomata of the non-fertilized plants were often smaller just after being directly illuminated than they were during the period of diffused light. Also the fertilized plants had often begun a mid-day closure before the others had reached their maximum size. And although the plants from the fertilized plot averaged more stomata open and with wider openings for the entire period of sunshine, the openings were usually smaller and more of them were closed in the early afternoon. Just as the stomata of the plants grown on the fertilized plot, seemed to open more suddenly in direct light, so they also seemed to close more rapidly after the sun had left the plants in the afternoon.

Another difference noted between the two series of plants was that, although the stomata of those not fertilized were larger in the early afternoon during continuous clear weather, on clear days following cloudy weather, they maintained smaller stomata during both afternoon and forenoon than did the fertilized plants.

The data were obtained from the material collected at hourly inter-

TABLE II

DATA GIVING THE PERCENTAGE OF STOMATA REMAINING CLOSED AND THE AVERAGE WIDTH IN MICRONS OF THOSE OPEN FROM THE TIME OF MATURE LEAF FORMATION UNTIL AFTER FRUITING, OF THE PLANTS GROWN OUT OF DOORS

These data indicate that both species of plants when grown with the addition of nitrogen to the soil maintain a greater number of stomata open and with larger openings, when in the sunshine than did those not fertilized. In diffused light this was reversed. Collection of material was made July 5, 8, 23, 25 and August 13, 1929.

TIME	PHASEOLUS VULGARIS-BURPEE GREEN POD				RAPHANUS SATIVUS			
	Nitrate added to the soil		Nitrate not added		Nitrate added to soil		Nitrate not added	
	Per cent closed	Width	Per cent closed	Width	Per cent closed	Width	Per cent closed	Width
Diffused light:								
5:00 a.m.	90	2.3	48	2.7	90	2.0	88	1.3
5:30 a.m.	96	1.7	88	1.7				
6:00 a.m.	73	2.0	44	1.1	100	0.0	88	1.5
7:00 a.m.	78	2.7	42	3.5	34	1.6	6	1.6
7:30 a.m.	66	1.6	37	1.5				
8:00 a.m.	10	2.9	58	4.4				
Direct:								
9:00 a.m.	12	4.5	36	2.8	34	2.0	00	0.0
10:00 a.m.	7	4.4	57	2.8	4	5.6	27	3.1
11:00 a.m.	0	5.4	25	3.9	0	3.5	33	2.8
12:00 m.	52	3.0	32	4.0	0	3.0	17	3.0
12:30 p.m.	25	4.1	15	2.9				
1:00 p.m.	28	1.8	9	3.9	5	4.3	00	5.6
1:30 p.m.	21	2.0	33	4.6				
2:00 p.m.	58	2.6	52	3.0	6	2.8	23	3.3
3:00 p.m.	59	2.8	40	2.6	0	3.2	00	6.0
Diffused light:								
4:00 p.m.	78	2.0	75	1.3	64	1.7	50	2.3
5:00 p.m.	88	1.7	59	1.5	98	0.8	89	0.8
6:00 p.m.	77	1.1	45	2.3	93	1.6	65	1.5
6:30 p.m.	91	1.5	84	1.5				
7:00 p.m.	78	1.4	65	1.5	93	1.3	83	1.0
8:00 p.m.	95	0.9	85	1.1	85	0.9	80	1.1
9:00 p.m.	99	0.4	88	0.9	98	0.3	96	0.3
Averages:								
5:00– 7:30 a.m.	81	2.0	52	2.5	75	1.2	39	3.7
9:00–11:30 a.m.	4	4.8	41	3.8	03	3.7	20	3.2
12:00– 3:00 p.m.	46	2.6	30	3.3	03	3.3	20	4.4
9:00 a.m.–3:00 p.m.	25	3.7	55	3.5	08	3.8	20	3.8
4:00– 9:00 p.m.	86	1.1	65	1.3	99	1.4	71	1.4
5:00–7:00 a.m.–4:00–9:00 p.m.	87	1.6	60	1.9	83	1.3	55	2.5.

vals, on clear days of bright sunshine, July 5, 8, 23, 25, and August 13, 1929. Measurements were made and recorded for each of these dates, and averages computed for all. Also averages of the width of the stomata and the percentage remaining closed have been given for the time before the plants were directly illuminated; while they were in direct sunshine; and after the sun had left them. These results are given in table II.

III. Plants grown indoors in soil having a controlled water content of 20 and 50 per cent of the water holding capacity.

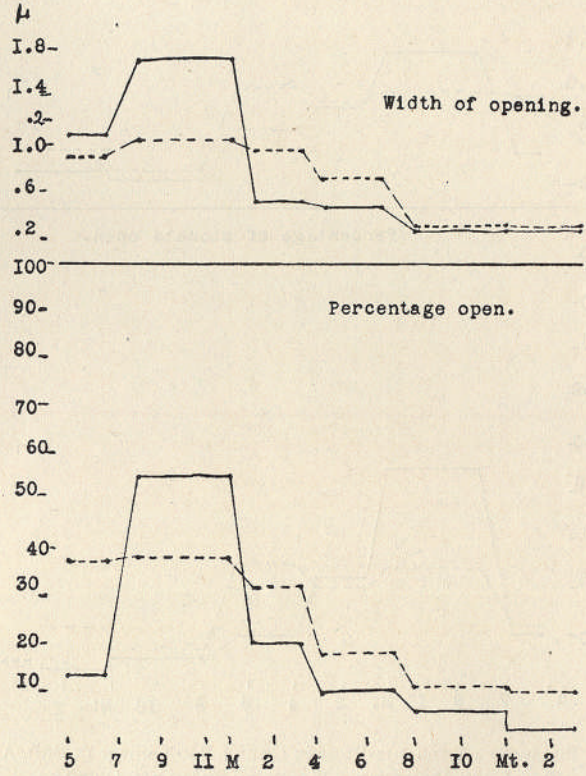
The plants grown in the wire baskets in this part of the experiment remained in very good condition, apparently, throughout the period of examination. The largest were those grown in the 50 per cent moisture and nitrogen. There was, however, not a very noticeable differance between them and those of the same series grown without the nitrogen. The fertilized plants had more leaves than those not fertilized. The greatest contrast in size was found between those grown with 20 per cent moisture content. Those of this series without the nitrate were the smallest. Both the plants and the leaves of these were small.

The observations of this part of the work as contrasted with the other two experiments, gave evidence of considerable variations in the stomata of the same species of plant when grown under different environmental conditions. While the stomata of Burpee Green Pod were largest when grown out of doors, and fewer of them remained closed during the period of sunshine, this species when grown in the wire baskets had the smallest stomata and more remained closed than they did in any other part of the work. These differences may in part be due to differences in temperature, humidity, and degree of illumination. The more poorly developed root system of those in the sealed baskets may also be responsible for some of the variations. The pH value of the soil as tested at the end of the experiment was approximately the same as found at the beginning.

However, practically the same results, so far as what seemed to be the effect of nitrogen on the stomata is concerned, were observed from this study as in the first two series of experiments.

The stomata of all of the plants grown with the nitrogen added to the soil, as compared to the duplicate series without the nitrogen, were found to be larger and more of them were open during forenoon when in direct sunlight. Also they were usually smaller during the early after-

noon. When not directly illuminated they seemed to close more quickly and to remain closed more completely than the other series did. This difference was not as evident during early morning as it seemed to be when the plants were grown out of doors. One reason might be because fewer collections of material were made just before sunrise.

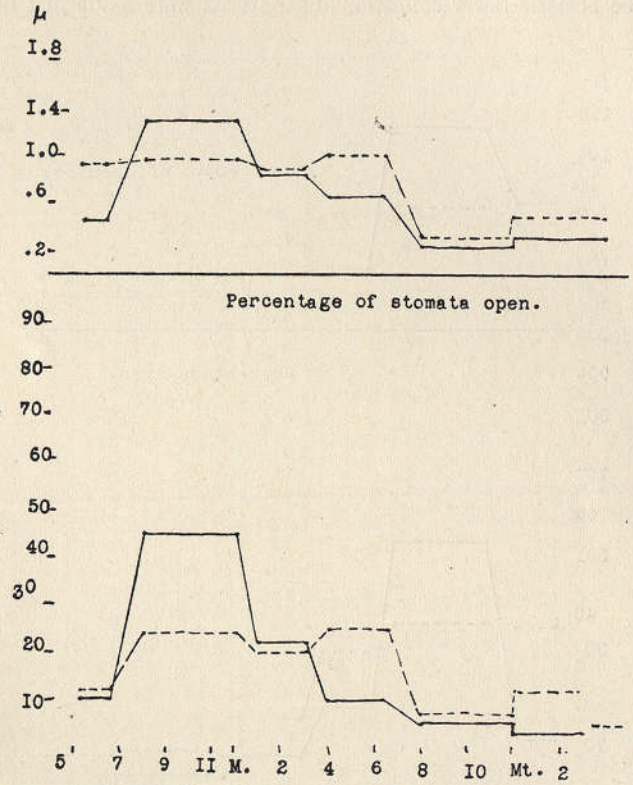


GRAPH 2. PHASEOLUS VULGARIS-BURPEE. GROWN IN SOIL A WITH 50 PER CENT MOISTURE

——— Grown with fertilizer.
--------- Grown without fertilizer.

The plants grown with the limited water supply and the addition of nitrogen differed from those of the same series grown without the fertilizer in keeping more of the stomata closed, especially during the

afternoon. The stomata of the former during forenoon were only slightly smaller than those of the plants grown with the maximum water supply. Those grown in the soil B-dark sandy loam—to which was added nitrogen, showed the widest opening in the morning with the



GRAPH 3. PHASEOLUS VULGARIS-BURPEE GREEN POD GROWN IN SOIL A WITH 20 PER CENT MOISTURE. WIDTH OF STOMATAL OPENING

---------- From plants not fertilized.
———— From plants with fertilizer.

smallest in the afternoon of any of the plants grown in the 20 per cent moisture content. They also had more stomata closed, especially in the afternoon.

During cloudy weather the plants grown without the addition of the sodium nitrate maintained the widest stomatal aperture with more

TABLE III

The table gives averages of data, obtained during clear weather, from the plants grown in soil with controlled moisture content. During the forenoon the nitrated plants had more stomata open and the apertures were wider. The same plants had fewer functional stomata in the afternoon, especially in the soil with low moisture content. Material collected for examination February 27, March 2, 12, 20, and April 5, 1930.

TIME	PHASEOLUS VULGARIS-BURPEE GREEN POD									
	Grown in soil A								Grown in soil B	
	With 50 per cent moisture				With 20 per cent moisture				With 20 per cent moisture	
	Nitrate added		Nitrate not added		Nitrate added		Nitrate not added		Nitrate added	
	Closed	Width	Closed	Width	Closed	Width	Closed	Width	Closed	Width
5:30 a.m.	97	1.0	54	0.5	95	0.4	88	0.4	92	0.3
6:30 a.m.	78	1.3	72	1.3	86	0.6	86	0.6	84	0.6
7:30 a.m.	80	1.6	86	0.6	63	1.6	73	1.6	60	1.6
8:00 a.m.	49	1.5	67	1.1	55	1.2	60	0.8	78	1.4
8:30 a.m.	44	1.7	61	1.2	55	1.1	74	1.0	41	1.5
9:00 a.m.	52	1.7	65	1.3	53	1.4	75	0.7	77	1.4
9:30 a.m.	27	2.1	56	1.0	35	1.0	58	0.8	69	1.2
10:00 a.m.	47	1.6	59	1.1	60	1.2	70	0.8	46	1.4
11:00 a.m.	42	1.6	48	1.0	52	1.6	60	1.0	62	1.3
12:00 m	55	1.5	81	0.9	79	1.2	55	1.8	68	1.7
1:00 p.m.	87	0.6	68	0.9	79	0.7	72	0.6	89	0.7
2:00 p.m.	90	0.4	62	1.1	78	0.8	86	1.2	99	0.3
3:00 p.m.	72	1.1	75	1.0	77	1.0	74	1.0	85	0.5
4:00 p.m.	85	1.0	79	1.2	92	0.9	67	0.9	90	0.4
4:30 p.m.	60	0.7	75	0.5	95	0.5	63	0.8	95	0.5
5:00 p.m.	93	0.2	76	1.0	92	0.4	91	0.4	90	0.6
6:00 p.m.	100	0.0	94	1.0	71	1.0	98	1.0	98	0.6
6:30 p.m.	99	0.5	98	0.3	96	0.4	85	0.9	88	0.6
8:15 p.m.	90	0.3	89	0.4	91	0.3	91	0.3	94	0.3
9:15 p.m.	93	0.4	88	0.2	90	0.3	90	0.3	98	0.3
10:00 p.m.	90	0.2	95	0.5	100	0.0	98	0.3	99	0.2
12:00 midnight	90	0.3	85	0.4	99	0.3	97	0.3	99	0.2
3:30 a.m.	98	0.3	90	0.4	97	0.3	88	0.5	99	0.3
Averages										
5:30–6:30	87	1.1	63	0.5	90	0.4	88	0.9	88	0.4
8:00 a.m.–12:00 m.	45	1.7	62	1.0	55	1.3	76	1.0	63	1.4
1:00–3:00 p.m.	80	0.5	68	1.0	78	0.8	80	0.9	91	0.5
4:00–6:30 p.m.	90	0.5	82	0.7	88	0.6	75	1.0	90	0.5
8:15–12:00 midnight	94	0.3	89	0.3	95	0.3	93	0.4	98	0.3

of them open than did the fertilized plants. This difference was more evident for those grown with the low water content. On the other hand

TABLE IV

DATA GIVING THE CONDITION OF THE STOMATA OF THE PLANTS GROWN WITH 20 AND 50 PER CENT MOISTURE, AND WITH AND WITHOUT THE ADDITION OF NITRATE, DURING CLOUDY WEATHER, FROM THE TIME OF MATURE LEAF FORMATION UNTIL AFTER FRUITING

Material collected for examination February 28, March 7, 18, 27, and April 8, 1930.

TIME	PHASEOLUS VULGARIS-BURPEE GREEN POD									
	Soil A								Soil B	
	With 50 per cent moisture				With 20 per cent moisture				With 20 per cent moisture	
	Nitrate added		Nitrate not added		Nitrate added		Nitrate not added		Nitrate added	
	Closed	Width	Closed	Width	Closed	Width	Closed	Width	Closed	Width
5:00 a.m.	92	0.3	95	0.7	96	0.7	82	0.4	90	0.4
6:30 a.m.	97	0.5	98	0.3	88	0.3	90	0.3	90	0.4
7:00 a.m.	90	0.8	80	0.4	80	0.3	92	0.3	91	0.4
8:00 a.m.	78	0.6	65	1.0	84	0.6	61	0.9	80	0.8
9:30 a.m.	80	0.5	74	1.8	85	0.4	52	0.3	90	0.4
10:00 a.m.	90	0.8	56	0.3	72	0.6	56	0.6	87	0.7
10:30 a.m.	94	0.5	96	0.4	95	0.4	64	0.2	97	0.5
12:00 m.	75	1.7	86	1.0	79	0.9	88	0.7	80	0.9
1:30 p.m.	81	0.9	69	0.9	85	0.6	45	0.8	80	0.5
3:00 p.m.	77	0.6	74	0.6	89	0.4	32	1.6	75	0.8
4:15 p.m.	76	0.6	87	0.7	90	0.8	91	1.0	94	0.7
6:15 p.m.	95	0.3	89	0.5	97	0.7	93	0.3	95	0.3
7:15 p.m.	92	0.4	96	0.5	98	0.6	98	0.6	96	0.3
8:15 p.m.	90	0.3	89	0.4	91	0.3	93	0.3	94	0.3
9:15 p.m.	93	0.4	88	0.2	90	0.3	90	0.3	92	0.3
10:00 p.m.	90	0.2	95	0.5	100	0.0	98	0.3	99	0.3
12:00 midnight	99	0.3	85	0.4	99	0.3	97	0.3	99	0.2
Averages:										
5:00 a.m.–12:00 midnight...	88	0.6	84	0.6	85	0.5	78	0.7	89	0.5
8:00 a.m.–6:00 p.m.....	83	0.7	76	0.8	89	0.6	65	0.7	85	0.6

both groups of plants grown with the limited water supply and with nitrogen added kept more stomata closed and those that opened had smaller openings than those grown with the nitrate but with the higher

percentage of moisture. Table III gives the data obtained during the period of sunshine, and table IV during cloudy weather.

The water loss from the plants grown in the sealed wire baskets was recorded daily from February 26 to March 28, 1930, just before the addition of water necessary to maintain a constant weight. A record was made of the loss during certain clear and cloudy days. The loss has

TABLE V

DATA GIVING THE AMOUNT OF WATER LOST PER GRAM DRY WEIGHT, AND PER SQUARE CENTIMETER OF LEAF SURFACE, BY THE PLANTS GROWN IN THE WIRE BASKETS SEALED WITH PARAFFIN

	PHASEOLUS VULGARIS-BURPEE GREEN POD				
	Grown in soil A				Grown in soil B
	50 per cent moisture		20 per cent moisture		20 per cent moisture
	Nitrate added	No nitrate added	Nitrate added	No nitrate added	Nitrate added
Total water loss 2/26 to 3/28	3947.2	3920.0	2387.0	1596.0	2130.9
Water loss for five 24-hour periods—clear days	726.5	680.6	497.5	341.8	425.5
Water loss for two 24-hour periods—cloudy days	134.1	138.5	101.1	94.4	117.0
Dry weight of the plants	20.6	17.24	13.35	7.33	14.95
Leaf surface in square cm	9026.0	3678.0	5038.0	1198.0	6012.0
Total water loss for 1 sq. cm. of leaf surface	00.44	1.06	00.47	1.33	00.34
Total water loss 1 gram dry weight	191.60	227.37	178.82	217.20	143.70
Water loss per sq. cm. leaf surface during clear period	0.08	0.18	0.09	0.29	0.07
Water loss per sq. cm. of leaf surface during the cloudy period	0.015	0.038	0.020	0.079	0.019

been computed for the square centimeter of leaf surface and per gram of dry weight. The results confirm those of Experiment I. The fertilized plants lost less water per gram of dry weight and also per centimeter of leaf surface than did those not fertilized. The plants which lost less per gram of leaf surface were those grown in the better grade of soil (B) with 20 per cent moisture and nitrate. The plants grown in soil A with 20 per cent moisture and no nitrate apparently required three times as much water as the duplicate series with the nitrate, and

only slightly less than four times as much as those grown with a similar moisture content in soil B with the nitrate added. During cloudy weather approximately twice as much water was lost by the plants grown without the addition of nitrogen as with it.

Comparisons of the leaf surface produced by these plants give interesting differences. All of the fertilized plants produced more than those not fertilized. More than one and a half times as much was produced by those grown in the better grade soil B, fertilized and grown with only 20 per cent moisture, as was produced by those grown in soil A with 50 per cent moisture without the nitrate. Those in soil A grown with 50 per cent moisture and with fertilizer, produced more than two and a half times as much as those grown under similar conditions but without the nitrate. The fertilized plants in soil A and 20 per cent moisture developed a leaf surface more than four times as extensive as those in the same soil and moisture content but with no nitrate added.

DISCUSSION

Published information concerning the influence of nitrogenous fertilizers on stomatal activity seems to be limited. Thus far no reference in literature has been found on the subject. This study indicates that nitrogen does influence these structures physiologically. But the way in which this is brought about is not so clear. If the plastids of the guard cells were similar to those of the leaf mesophyll the interpretation would be more evident. But it has been pointed out that these bodies differ structurally, physiologically, and genetically from the plastids of the mesophyll, and the green color contained by them does not give the micro-chemical test for chlorophyll (15). Also while starch occurs in the guard cells under any conditions, even in plants grown from the seed in complete darkness, starch is found in the chloroplasts only in light and soon disappears in continued darkness (15).

That the characteristic changes in the guard cells are due to fluctuations of the H-ion concentration, has been demonstrated by both Sayre (15) and Scarath (16). Both investigators found the pH value more alkaline when the guard cells were fully open but Scarath indicates that there may also be an "acid" value for the opening. Starch was found to appear in the pH zone of closure and to disappear in that of the opening. The causes suggested for this were the changes in sap colloid produced in pH condition which would initiate the opening, and the activity of enzymes in the acid free medium which would cause changes of starch to sugar and thus change the osmotic value of the cells.

Sayre (15) gives as one of the theories which might be advanced to account for the decrease in acidity of the guard cells (which would lead to their opening) as being the accumulation of CO_2 from respiration which would be used in photosynthesis in sunlight but not in darkness.

That nitrogen may play a part in respiratory reactions has been suggested by Allison (1) and Schertz (18) and Ville suggests that it also bears a relation to chlorophyll. Ville, according to Denber (6), while working with hemp plants found that a great decrease in chlorophyll and carotin was brought about by a deficiency in nitrate. Schertz (18) in working with *Coleus* shows that nitrogen is the element lacking which causes the mottling of leaves, and suggested that either chlorophyll would be prevented from forming or if formed would be decomposed, for in those plants having a low concentration of chlorophyll, this material seems to be decomposed in light, while plants with much chlorophyll show little or no breaking up of the chlorophyll (18).

The relation of chlorophyll to photosynthesis is well known. That CO_2 is one of the limiting factors in the same process has been shown by Blackman (16).

It may be suggested that the effect which nitrogen has seemed to produce in the activity of the stomata of the plants used in this study, may be one of interrelation between the part which Allison and Schertz suggested is played by nitrogen in the respiratory reactions, and chlorophyll formation or prevention of its decomposition. If more chlorophyll is present it is supposed that photosynthesis might take place more rapidly, which would lead to CO_2 being taken out of solution, and so changing the pH value, and thus lead to the wider opening of the stomata. If respiration is influenced by nitrogen to cause more carbohydrates and proteins to be destroyed (1) the acid condition found in those plants grown with its use might be such as to lead to the more complete and continued closure of the stomata, when the plants were not in light sufficient to use the excess CO_2 in photosynthesis.

The greater efficiency in the use of water which the plants with the nitrate fertilizers seem to make in comparison with those grown without this element, may be partially accounted for by less water being lost through transpiration due to the stomata closing more completely when photosynthesis is not taking place. Sayre has pointed out that 50 per cent of the maximum amount could diffuse through the pore when it is only 10 per cent of its maximum size. From that it would seem that considerably more water would be lost by those plants which retained a larger number of open stomata during diffused light as has been found

in this work. Also when in the sunlight, since the fertilized plants maintained the widest open stomata, they would, it is supposed, be using more water due to increased photosynthesis.

SUMMARY

I. Plants used for this study of the effect of nitrate fertilizer on stomata have been grown in duplicate series of sand and water cultures; sandy loam soil with moisture content of 20 and 50 per cent of the water holding capacity; and in light clay loam soil out of doors. One series of each was grown with nitrate added at the time of setting up of the experiment. The other series received no fertilizer.

II. Variations have been noted in the daily march of the stomatal movement from the time of the mature leaf formation until after fruiting of each nitrated series as contrasted with the duplicate series of those not fertilized. These differences are as follows:

A. During clear weather—

1. The stomata of all the fertilized plants have been wider and more of them were open during the forenoon period of direct illumination.
2. During the early afternoon the width of the aperture has been smaller, and more have been closed during the same period.
3. However, during the entire period of direct illumination the stomata of the fertilized plants have averaged a wider aperture and the percentage open has been greater than for those not fertilized.
4. During the period of diffused light, both in early morning and afternoon, the plants grown with addition of nitrate have, with few exceptions, maintained stomata with smaller openings and a larger per centage have remained closed.
5. More stomata remained closed during the period of illumination in both nitrated and non-nitrated plants when grown with low moisture content than when water was not deficient.
6. The stomata of those grown with low water content and nitrogen, opened wider and fewer remained closed during the forenoon than the similar series grown without nitrogen.

7. During the afternoon those fertilized had smaller openings and more remained closed when the water was limited.

B. During cloudy weather:

1. The stomatal apertures of the fertilized plants were smaller and more remained closed.
2. The stomata of the fertilized plants grown in the better grade of soil B, with 20 per cent moisture, maintained the lowest percentage of open stomata, and those open had smallest apertures.
3. Those grown in the soil A with low water content and no nitrate kept more stomata open and the aperture was usually larger than any other of the plants.

III. Concerning water loss.

1. The plants grown with addition of nitrogen have been found to lose less water per square centimeter of leaf surface and per gram of dry weight the twenty-four hour period both during clear and cloudy weather than those not fertilized.
2. Those plants apparently having the lowest water requirement per centimeter of leaf surface and per gram of dry weight were those grown in soil B with the limited water supply and addition of sodium nitrate.

Since nitrogen seems to make the stomata more responsive, opening more quickly and more completely during the period of direct sunshine and closing more quickly and more completely when the plant is not illuminated this may partially account for the lower water requirement, as observed in this work and by other investigators (2).

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