Transpiration of Wheat Seedlings as Affected by Different Densities of a Complete Nutrient Solution in Water, Sand, and Soil Cultures.[1]

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With 3 figures in the text.

Introduction.

In a series of studies undertaken to ascertain the effect of various chemical agents and of different densities of solution upon the transpiration of plants, the following principal topics were investigated:

1) Transpiration of wheat seedlings as affected by different densities of a complete nutrient solution in water, sand, and soil cultures.

2) Transpiration of corn and bean seedlings as affected by different densities of a complete nutrient solution, in water and sand cultures.

3) Transpiration of wheat seedlings as affected by different densities of non-nutrient salt solutions, in water and sand cultures.

4) Transpiration of wheat seedlings as affected by different quantities of various chemical salts, in water and sand cultures.

5) Transpiration of wheat seedlings as affected by different chemical salts, and by the ions of these salts, in water and sand cultures.

6) Transpiration of wheat seedlings as affected by different densities of an organic substance, in water cultures.

7) Transpiration of wheat seedlings as affected by different densities of solution in different sized particles of sand and soil.

In the present paper, however, only the first topic will be considered.

[1]) Contribution from the Laboratory of Soil Technology at Cornell.

Review of Literature.

The literature bearing directly upon this part of the investigation is rather limited, considering the importance of the subject. Of the most important works reported on the subject may be mentioned the following:

Burgerstein[1]) in his investigation upon the transpiration of plants, studied also the effect of different densities of solutions of $Ca(NO_3)_2$, KNO_3, K_2HPO_4, K_2CO_3, NH_4 NO_3, $(MH_4)_2$ SO_4, and other chemicals. He found that transpiration increased with the increase in density of all these single salts up to a certain point, above which it began to decrease.

Schroeder[2]) found that the amount of water transpired, per unit of dry matter produced increased with the concentration of the nutritive material present, up to a certain density, beyond which it fell.

Sorauer[3]) grew plants for eight weeks in a nutrient solution of different concentrations. He found that the relative transpiration decreased with the increase in concentration.

Heinrich[4]) studied the amount of water transpired by oats in a complete nutrient solution of different strengths. He found that transpiration decreased per unit of dry matter, with the increase in concentration.

Maercker[5]) grew white mustard in an artificial soil, contained in zinc pots. He added to the soil different amounts of moisture and various quantities of Kainit and Carnallite, and studied the effect of these salts upon the amount of water required to produce a given quantity of dry substance. He failed to seal the pots to prevent the direct evaporation from the surface of the soil, but the results he obtained go to indicate that the application of the potash salts produced a distinct economy in the absorption of water, and this was more marked in the lower than in the higher moisture contents.

Kiesselbach[6]) grew corn for two and a half months in pure river sand watered with a complete nutrient solution. His results show that the amount of water transpired per gram of dry matter, tends to increase with the decrease in density.

General Methods of Investigation.

In the present work the effect of the different densities of solution upon the transpiration of plants, was studied in water, sand and soil cultures, at the same time and under the same conditions.

The water cultures were prepared and conducted according to the methods recommended by the Bureau of Soils[7]), with a few modifications.

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[1]) Sitzungsber. Akad. Wiss. Wien. Math.-Nat. Cl. Bd. 83. 1876. p. 191.
[2]) Anw. Inst. Agr. Moscow. 2. 1896. p. 188—226.
[3]) Allgem. Brauer- u. Hopfenzeitg. 23. 1882. p. 15—19.
[4]) 2. Ber. Landw. Vers.-Stat. Rostock. 1894. p. 170 – 174.
[5]) Arbeit. Landw. Ges. H. 20. 1896.
[6]) Nebraska Agr. Expt. Sta. 23rd. Ann. Rept. 1910. p. 138.
[7]) Bull. 23, Bureau of Soils, U. S. Dept. of Agr. 1904.

The method consisted of cutting out four triangular shaped pieces from the circumference of corks which fitted the mouths of glass bottles black painted, of a capacity of 120 cc. The apex of each cork piece was then truncated by the removal of enough cork to allow the fitting of seedlings between the inner angle of the slit and the cork piece. Four equally sized and vigorous wheat seedlings were then fitted into the slits of each stopper with their seeds just below the latter, and a rubber band was placed around the cork to hold the pieces in place. The pieces exerted enough pressure on the seedlings to hold them in their respective positions, but not too great to interfere with their growth. The stopper containing the plants was then pressed firmly into the neck of the bottle which was filled with solution nearly up to the seeds of the plants. The seedlings employed were first germinated in moist sand contained in pans, and allowed to reach the height of about one inch before they were removed from the sand and transferred into the solution.

In pulling out the seedlings from the quartz there was some danger of damaging their tender and delicate roots if the quartz was too dry, but if it was very wet there was no such danger.

The culture solution prepared as described above allowed practically no direct evaporation from the solution but only through the exposed surfaces of the plants. Occasionally, when the cork stoppers became moistened by the layer of concentrated solution held around the wall of the mouth of the bottle by surface tension, a rapid evaporation would take place through the corks by capillarity. The loss of water in this manner was easily prevented by dipping the stoppers, after the slits were cut around them, into melted paraffine, and the holes under the pieces were then opened by a thin wire, just large enough to fit the seedlings. The thin layer of paraffine formed around the stopper not only closed the pores of the latter but also prevented the direct communication between the stopper and the solution when they were in direct contact with each other.

The cultures were weighed at intervals of three to four days and the loss in weight was taken as a measure of transpiration. The decrease was replaced at each time with the solution, and about once every week the entire solution was changed. In this manner the entire root system was constantly kept covered with the solution, and the stagnation or any inequality in concentration in the latter was avoided by its frequent renewal.

The tests for the soil or sand cultures were conducted in the paraffined wire baskets, also recommended by the Bureau of Soils.[1])

The baskets used for this work were about three inches deep and three inches wide. Those in which soil was added were prepared by repeated dipping the rim about one inch in height in melted paraffine, with the intervening cooling in water until a firm wall was formed. Then exactly the same amount of soil was added to

[1]) Loc. cit.

each basket, and pressed firmly at the bottom and at their sides so it would form a very close contact with the walls. The baskets were next dipped several times in melted paraffine slightly above the lower edge of the paraffined rim, until a thick coating was formed which cemented the outer surface of the mass of soil to the walls of the pots. The soil was next brought up to the desired moisture content by the addition of a certain amount of the solution and then an equal number of well selected seeds were planted in each pot, the weight of the pot being then recorded. When the seeds were germinated and the seedlings were about one inch in height, each basket was reweighed and the loss in weight was replenished by the addition of water. Then an equal number of the healthiest and most vigorous seedlings of as near as possible the same size, were selected and the remainder pulled out. The baskets were next sealed and their final weight recorded.

The sealing of the baskets was done somewhat differently from that recommended by the Bureau of Soils. The new method was devised in the course of the investigation and consisted of placing over the selected seedlings small glass tubes with a diameter sufficiently large to permit the fitting of the seedlings through them, and then melted paraffine was poured over the surface of the soil. The glass tubes, being pushed a little below the surface of the soil, prevented the hot paraffine from coming in contact with the seedlings and thus injuring them.

This method has given very satisfactory results and is probably the better of the two, for the following reasons: 1) it takes less time to seal each basket; 2) it reduces the evaporation from the surface of the soil more, because it forms a better uniform cover; it gives less amount of exposed surface of the soil through the holes; it creates a closer contact between the surface of the soil and the cover; and 3) it reduces to the minimum the possibility of injury to the plants during manipulation.

The only serious objection to this method is that if the paraffine is too warm it may heat the glass tubes, and if the seedlings are in close contact with them, they may probably be scalded. This danger can be easily avoided, however, by placing over the glass tubes very thin rubber tubing before they are placed over the plants, and also by not using too warm paraffine.

Just as in the case of the water cultures, the baskets were weighed at intervals of from three to four days and the loss in weight was attributed to transpiration. The loss was replenished in this case not by the solution as in the case of the water cultures, but by distilled water only.

The sand cultures were prepared and conducted exactly like the above, excepting that the sand was added in the baskets after they were entirely paraffined.

The seed employed throughout the work was Pringels Champion. In order to eliminate errors which are due to inherent differences in the individuality of the plants, a large number of seedlings of the same variety was used for the different kinds of cultures within the same experiment. Special care was also taken to select seedlings of nearly uniform size and of the same vigor and health, as possible.

Experimental Results.

As already stated the object of this line of investigation was to determine the effect of different densities of a complete nutrient solution on the amount of water transpired by wheat seedlings per unit of dry matter produced. For this purpose the wheat plants were grown in solution, sand and soil cultures, containing various densities of a full nutrient solution. The concentrations of this solution were seven in number, and ranged in strength from 0.0 to 4500 parts per million. The sand and soil cultures were used first to duplicate the water cultures, and secondly, to see whether the different densities of the solution would affect the transpiration in these cultures, in the same order or in the same magnitude as in the water cultures. For the determination of these two points, all the three different kinds of cultures were conducted at the same time and under the same conditions, in order that their results might be comparable. The sand and soil employed were of various sized particles. The sand consisted of three different sizes, namely, numbers, 1, 2 and $3^1/_2$. In other words, those which could pass through a mesh of 1, 2 and $3^1/_2$ millimeters respectively, in diameter. The soil consisted of a Dunkirk clay loam and of a Dunkirk sandy loam, the first being assumed to be the finer of the two. The object of employing the sand and soil of different textures, was to ascertain whether the size of particles exerted any influence upon the density of the solution in affecting the transpiration. To investigate this point, the cultures of the various sizes of sand, and of the two soils, were carried on at the same time, and under the same conditions. This part of the work, however, will be reported elsewhere.

In order to obtain definite and conclusive evidence on this part of the investigation, the three different kinds of cultures were repeated a large number of times. The solution and sand cultures were made five times each, and the soil cultures two times, Inasmuch, however, as there were two different sizes of sand in four out of the five cases, and two different kinds of soil in both instances, and since all received the same treatment, there were really nine sand and four soil cultures, respectively. The solution cultures conducted at the different times, contained in each density, from three to five bottles with four plants in each receptacle. The sand and soil cultures, also carried on at different periods, contained in each density, from three to six baskets, with five to six plants in each. The total number of plants in each concentration in the different cultures was, therefore, 96 in the five solution cultures, 204 in the nine sand cultures and 80 in the four soil cultures.

Each basket within the same density, and each group of baskets representing the various concentrations, both in the sand and soil cultures, contained at each time, exactly the same amount of sand or soil, and exactly the same percentage of moisture content. The moisture content was brought up to the desired point by the addition of the nutrient solution, and then kept at that point throughout the duration of the experiment, by the addition of distilled water.

All the experiments conducted at the different periods always received the same density of the same complete nutrient solution. The composition and preparation of the latter are shown below.

Calcium Nitrate	27.0	grams
Magnesium Sulphate	6.0	„
Potassium Phosphate (Monobasic)	15.0	„
Ferric Sulphate	0.5	„
Potassium Chloride	7.5	„
Total	56.0	grams

These salts were dissolved in 10 liters of distilled water. In order to avoid as much precipitation as possible, they were dissolved in the following manner and then mixed together.

$Ca(No_3)_2$ plus KCL dissolved in 3 liters of water.
$MgSO_4$ plus $Fe_2(SO_4)$ 3 dissolved in 3 liters of water.
K_2HPO_4 dissolved in 3 liters of water.
They were then brought together and the volume made up to 10 liters.

Even when the above precautions were taken, some precipitation did take place and the solution had to be filtered through ordinary filter paper. The density of the filtrate was then determined by evaporating 50 cc. of the solution and weighing the residue. This gave 4500 parts per million. From this stock solution, the different densities needed for the experiments were prepared. These various concentrations with their strength in P. p. m., and in electrical resistance as determined by the Wheatstone bridge, are shown herewith.

Density in P. p. m.	Density in Ohms
4500.0	66.0
2250.0	121.1
750.0	327.4
375.0	588.3
187.5	1115.5
93.5	2775.0
0.0	10000.0

Each experiment was continued for three weeks. During this period, the daily development and general appearance of the plants, were carefully noted. It was observed that, as a general rule, the seedlings grown in the higher densities always made, at the beginning, a much slower growth than those in the lowr concentrations. Indeed, it always appeared that the rate of growth increased as the density decreased. After the first four or five days, however, this order was reversed; the plants in the higher densities began to grow very rapidly and the rate or magnitude of growth became decidedly proportional to the concentration.

In connection with the above observation, it might also be mentioned, that the germination of the seeds was also retarded by the higher densities. The time required to sprout was proportional to the concentration, and the difference in time between the two extremes, was from one to two days.

At the termination of each experiment a careful examination was always made of the total growth and of the general appearance of the plants, including both the tops and the roots, in all three kinds of cultures. In the case of the tops, it was observed that they were healthy, vigorous, and their total growth always tended to increase with the increase in density. The roots also always appeared to be healthy, vigorous and turgid, and their total weight to be proportional to the actual weight of the tops. As to the latter relation, several attempts were made to confirm it quantitively by determining the dry weight of the roots and then comparing this with the dry weight of the tops. It was found, however, that this comparison was not reliable, because the roots in the different densities contained various amounts of fine sand particles which were practically impossible to wash out. In view of this difficulty, the determination of the dry weight of the roots had to be abandoned, and therefore, the relation of the roots to tops, roots to transpiration, and roots to density, all of which would have been of great interest, could not be ascertained.

The dry matter of the tops was determined by drying them in a drying oven at the temperature of boiling water for about 10 hours and then after cooling, they were weighed on a chemical balance. The total dry matter was then compared with the total transpiration, and the amount of water required to produce one gram of dry substance, calculated.

Mention has already been made that this line of the research involved five series of solution cultures, nine series of sand cultures, and four series of soil cultures. Inasmuch as all the different series within the same culture received practically the same treatment, and show almost the same order of results, it warrants us in arranging their individual results, in averages. The main conclusions will, therefore, be drawn from these averages. These data are given in the following tables. Each table is accompanied by a diagram to illustrate its salient facts in a graphical form.

It is evident from the foregoing talbes and diagrams that the different densities of the complete nutrient solution have a very significant effect upon the transpiration of the wheat seedlings, and that this effect manifests itself in all three of the different

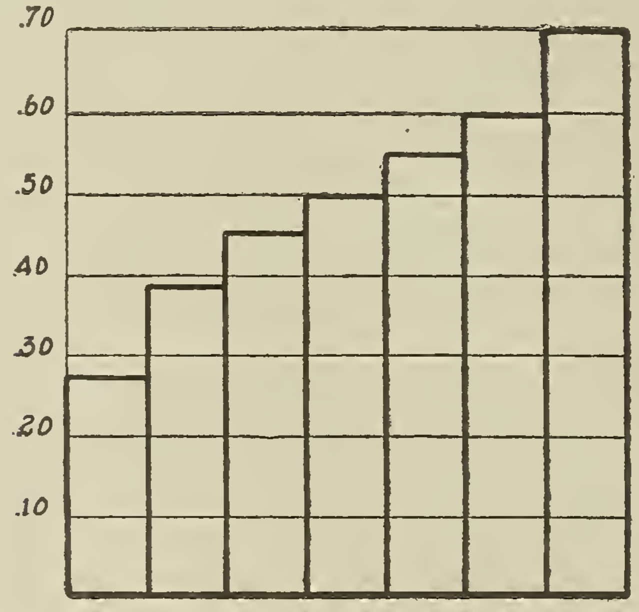
Table I.

Effect of Different Densities of a Complete Nutrient Solution upon the Transpiration Per Gram of Dry Matter of Wheat Seedlings Grown in Solution Cultures.

Average of Five Series.

Density of Solution in P. p. m.	Grams of Dry Matter Produced	Grams of Water Transpired	Grams of Transpiration Per Gram of Dry Matter
4500	.7071	298.67	360.0
2250	.6017	290.99	415.0
750	.5530	272.98	426.7
375	.4995	266.52	454.8
187.5	.4494	243.25	471.4
93.5	.3808	196.13	463.7
0.0	.2766	83.63	256.8

Grams of Dry Matter Produced.



Grams of Transpiration Per Gram of Dry Matter.

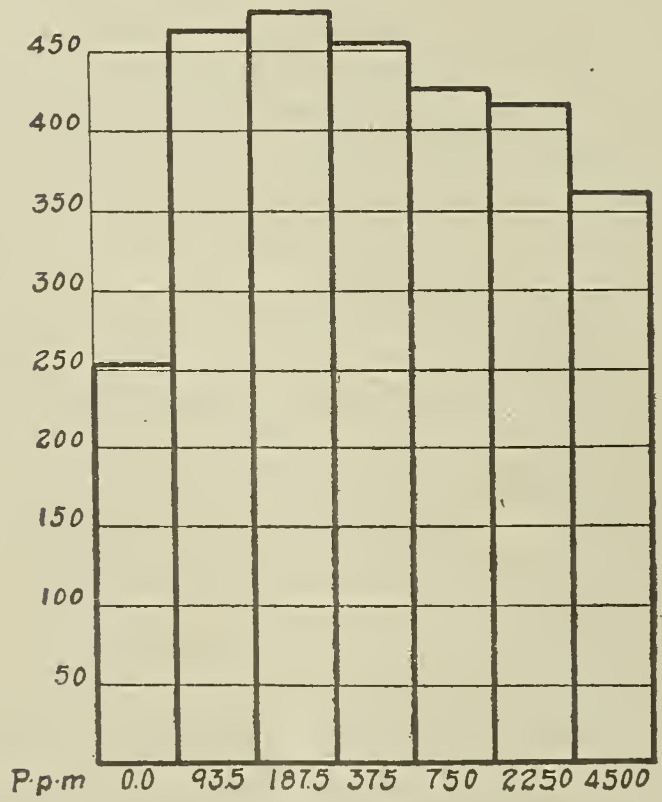


Fig. I.

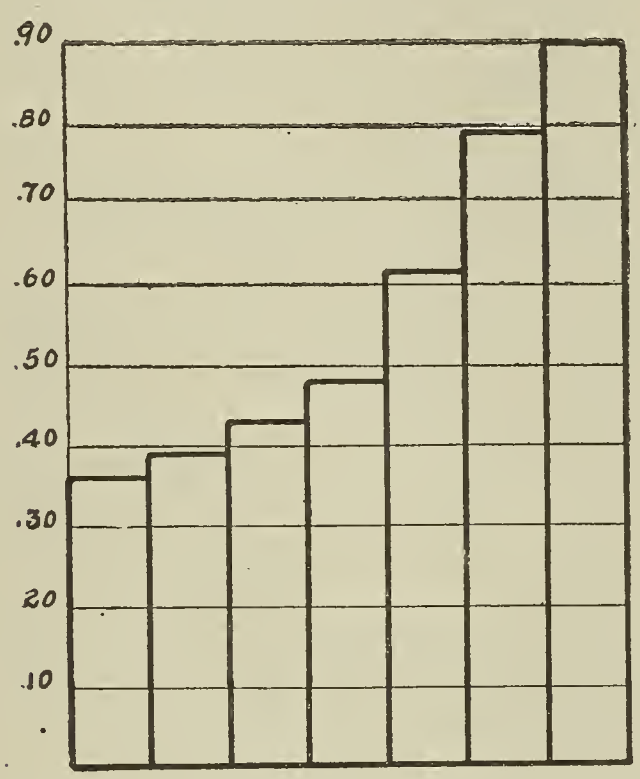
Table II.

Effect of Different Densities of a Complete Nutrient Solution upon the Transpiration Per Gram of Dry Matter of Wheat Seedlings Grown in Sand Cultures.

Averages of Nine Series.

Density of Solution in P. p. m.	Grams of Dry Matter Produced	Grams of Water Transpired	Grams of Transpiration Per Gram of Dry Matter
4500	.9077	373.64	374.6
2250	.7965	370.21	425.7
750	.6179	275.71	446.7
375	.4862	232.95	463.7
187.5	.4307	193.56	460.6
93.5	.3951	173.71	442.6
0.0	.3601	151.19	429.4

Grams of Dry Matter Produced.



Grams of Transpiration Per Gram of Dry Matter.

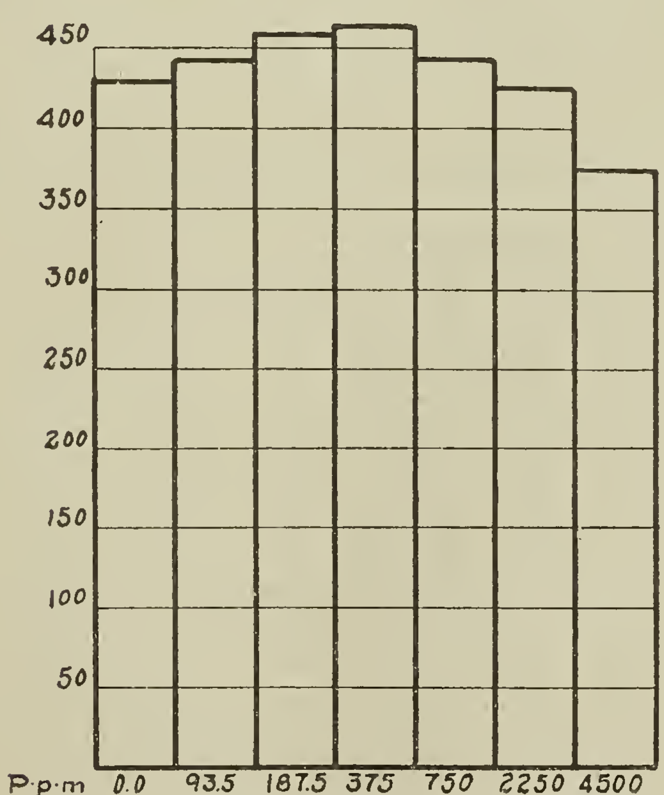


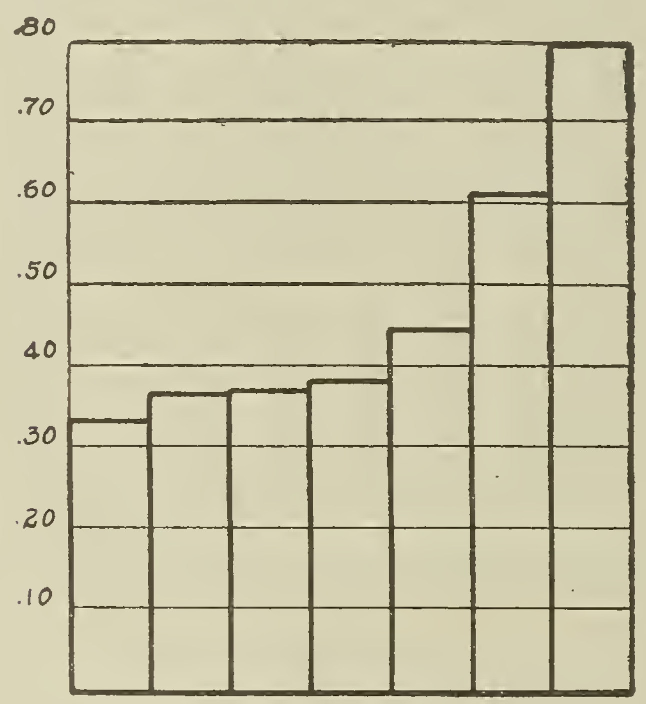
Fig. II.

Table III.

Effect of Different Densities of a Complete Nutrient Solution upon the Transpiration Per Gram of Dry Matter of Wheat Seedlings Grown in Soil Cultures. Averages of Four Series.

Density of Solution in P. p. m.	Grams of Dry Matter Produced	Grams of Water Transpired	Grams of Transpiration per Gram of Dry Matter
4500	.8077	407.9	501.1
2250	.6127	359.3	584.2
750	.4444	251.5	570.4
375	.3838	232.9	584.0
187.5	.3718	235.7	618.0
93.5	.3709	239.1	645.4
0.0	.3392	204.1	588.9

Grams of Dry Matter Produced.



Grams of Transpiration Per Gram of Dry Matter.

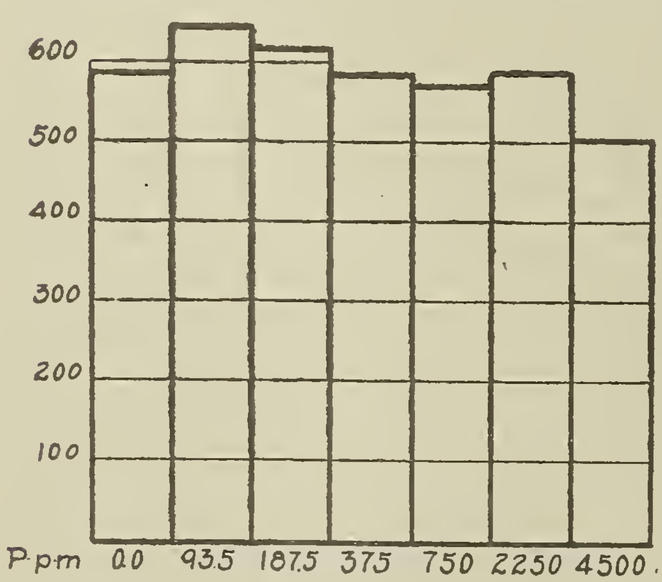


Fig. III.

cultures. It will be seen that the transpiration per gram of dry matter increases in every one of the three cultures with the decrease in density, until a certain dilution is reached and then decreases with a further decrease in concentration. To state the same thing in the converse form, the relative transpiration rises with the concentration up to a certain point beyond which it declines with a further increase in density. The concentration at which the change occurs is different for the various cultures, but it lies between 93.5 to 375 parts per million. For the solution culture, it is at 187.5 P. p. m.; for the sand culture, it is 375 P. p. m.; and for the soil culture, it is at 93.5 P. p. m. The regularity with which the relative transpiration increases with the diminution in density and then declines with the further reduction in concentration, is very remarkable for the sand and solution cultures, but in the soil culture, there is one exception. It will be observed that in both the water and sand cultures, there is not the slightest break in the curve from the highest density to that where the change takes place, nor from there to the lowest concentration. In the soil culture, however, there is one irregularity in the curve before the density where the change commences, is reached. Undoubtedly this discrepancy would probably not be noticed had there been as many trials of this culture as there were of the other two.

As far, therefore, as these results may be considered conclusive, they go to show two facts; first, that the relative transpiration increases with the decrease in density, down to a certain point; and secondly, after this point, it declines with the further diminution in concentration. Evidently, the highest and lowest densities diminish it the most, and the intermediate ones the least.

These results are significant not only on account of the relation they indicate between the density and the transpiration, but also because they throw some doubt upon the statement made by certain investigators[1]) that the total transpiration is as good a criterion for measuring plant growth as the weight of the plants. Indeed, the foregoing data tend to disprove this view most decidedly. They show that the media or density of solution in which the plants are grown, have a most significant effect upon the relative transpiration, and that the latter varies in some cases, between the different densities within the same culture, from 360.0 to 471.4 grams, or a difference of 11.4 grams, in view of these facts, therefore, it can hardly be considered, from these results at least, that the total transpiration is an accurate and true criterion of measuring plant growth, even though the total transpiration may be to a certain extent a function of plant growth.

The next question to decide is, what forces cause these phenomena. It must be stated in advance that no definite and proved explanation can be offered; the following suggestions, however, may be worth considering.

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[1]) Bot. Gaz. 40. 1905. p. 178—195.

The increase in the relative transpiration with the decrease in density down to the point where the change takes place, may be attributed to three factors; namely, 1) to the different modification of the structure of the plants, brought about by the different densities; 2) to the different osmotic pressure of the various concentrations; and 3) to the different densities of the cell sap of the plants grown in the various densities of solution.

As to the first factor, it was realized at the beginning of the investigation that if too high densities were used, they would not only prove harmful to the plants, but they would also modify their structure or anatomy, i. e., cause them to assume the xerophytic form, which would tend to reduce transpiration, and thus destroy the central object of the reserach. The concentrations employed, therefore, were of a strength below that which would bring about the above conditions. Furthermore, from a large number of examinations, there were never observed any noticeable differences in the external structure of the plants grown in the different solutions. Under these conditions, therefore, it seems that the increase of the relative transpiration with the diminution in density, is not due to any difference in the outer anatomy of the plants grown in the various concentrations.

In the second factor it is conceived that in the highest concentrated solution there is a high osmotic pressure but not so high as to cause plasmolysis. This high osmotic pressure has a limiting influence in the amount of water the plants can absorb. As this high osmotic pressure decreases, however, by the decrease in the density of the solution, the amount of water the plants can take up, increases. The ultimate result of this would be what the results already indicate.

In the third factor, it is assumed that the density of the cell sap of the plants increases with the concentration of the solution in which they are grown. That is to say, the density of the cell sap of the plants grown in the highest concentrated solution is greater than that of the plants grown in the less dense solution, and that it decreases in direct ratio with the diminution in density of the solution. In this condition, the high concentration of the cell sap in the high density of solution, would possess very high osmotic pressure, viscosity, and low vapor tension, all of which tend to produce the same effect namely, to retain water. As the density of the cell sap decreases, however, the degree of magnitude of transpiration which these physical properties can diminish decreases proportionally, and consequently, the evaporation of the water from the surface of the plants increases, and therefore, results in what has already been mentioned.

It must have already been seen that both factors may and can act together at the same time, for both tend to produce the same effect. This is easy to see. As the density of the solution decreases, its osmotic pressure diminishes almost proportionally, and therefore, everything else being equal, the ease or facility with which the plants can absorb water increases in the same ratio. Also as the density of the cell sap decreases, the degree or power of its physical properties to diminish evaporation decreases, and therefore, the rapidity with which the water tends to go off rises proportionally. Thus, in either case, the amount of water lost increases directly with the reduction in concentration, and since the density of the cell sap diminishes with the increase in dilution of the outside solution, the increased transpiration per unit of growth as already noted, is probably the result of the reduced intensity of the physical properties of both factors.

The assumption made under the third factor, was tested experimentally, and from all evidence it appears to be correct. This point was investigated by extracting the cell sap of the plants grown in the different concentrations of solution and determining its density. The extraction of the plant juice was performed by placing the green plants in a mortar, crushing them with a pestle till the sap was pressed out, and washing out the latter with distilled water. The pestling and washing were continued till the plant juice had been taken out, and only the white fiber was left behind. The later was then put in a piece of cheesecloth and rinsed several times in order to wash out every trace of the cell sap, and then pressed very firmly inside of the cloth to strain out all the solution. The extract was then filtered through ordinary filter paper, its volume brought up to a definite point, and its density determined. For the latter, two methods were used; 1) the freezing point; and 2) the electrical resistance, as determined by the Wheatstone bridge. A good deal of preliminary work was done with both methods, and it was found that both gave exactly the same results, namely, that the density of the cell sap decreased with the diminution in concentration of the outside solution. Since the resistance method was the quicker and the simpler of the two, the majority of the final determinations were done by this method. While the resistance may not be as accurate or give as definite quantitative results as the freezing point, it shows, however, the comparative relation sought for.

The plants used in this work were wheat. Beans were also used, but only in one case. Both kinds of plants were grown in solution cultures from about three to six weeks, and one kind also in sand cultures.

In order that the density of the cell sap may be comparable among the different densities of solution, exactly the same weight of green plants grown in the various concentrations, was taken.

Experiment I. This experiment consisted of growing wheat seedlings for 16 days in two different densities of solution, namely, 4500 and 93.5 P. p. m. At the end of the above period they were harvested and exactly 3.9610 grams of green weight was taken, the cell sap was extracted by the method already described, diluted to 200 cc. with distilled water, and the density determined by both methods. The data obtained are given in the following tables.

Table IV.

Density of Cell Sap as Determined by the Freezing Point Method.

P. p. m. of Solution in which Plants Grew	Freezing point of the Cell Sap.
0.0	4.200° C
93.5	4.190° C
4500.0	4.175° C

Table V.

Density of Cell Sap as Determined by the Electrical Resistance Method.

P. p. m. of Solution in which Plants Grew	Resistance of Cell Sap in Ohms
93.5	592.5
4500.0	411.9

It is evident from both tables that the density of the cell sap diminished with the decrease in density of the solution. It will be seen that in the first table the cell sap of the plants grown in the highest density of solution lowered the freezing point .015° C more than that in the lower concentration, and .025° C more than that in the check. In the second table, the electrical resistance was 180.6 ohms less in the plant juice derived from the plants grown in the highest concentrated solution than in the other.

Experiment II. This experiment was the same as the preceding one except that in this case only the electrical resistance method was used to measure the density of the cell sap. The results obtained are given in the following table.

Table VI.

Density of Cell Sap as Determined by the Electrical Resistance Method.

P. p. m. of Solution in Which Plants Grew	Resistance of Cell Sap in Ohms
93.5	422.0
4500.0	210.9

It will be observed that as in the foregoing experiment the density of the cell sap was decreased with the reduction in concentration of the solution.

These experiments were repeated also in sand cultures, and the data obtained were identical as the above.

Experiment III. In this experiment beans were used instead of wheat, the object being to see whether the above relation would hold true with other species. These plants were allowed to grow for 45 days in complete nutrient solutions of 4500 and 750 P. p. m. Exactly 5.6900 grams of green weight were taken, the cell sap extracted as usual, diluted to 500 cc., and the density determined by the electrical resistance method. The table below contains the results secured in this case.

Table VII.

Density of Cell Sap of Bean Seedlings as Determined by the Electrical Resistance Method.

P. p. m. of Solution in Which Plants Grew	Resistance of Cell Sap in Ohms
750	1479.6
4500	1158.1

These figures show most strikingly that the foregoing relation holds true also with beans. It will be noticed that the 4500 P. p. m. solution gave cell sap with 321.5 ohms more concentration than the 750 P. p. m. solution.

Experiment IV. This experiment consisted of growing wheat seedlings for 15 days in solutions of $\frac{N}{50}$ and $\frac{N}{250}$ of $CaCl_2$ to each one of which was added exactly the same amount of a complete nutrient solution in order that the plants may make a normal growth. The object of this series was to see whether the density of the cell sap of the plants grown in different densities of the non-nutrient solution, would run in the same order as that of the different concentrations of the complete nutrient solution already noted. The cell sap was extracted in this case, from 1.6330 grams of green weight, diluted to 200 cc., and the density determined by the electrical resistance method. Following are the results obtained.

Table VIII.

Density of Cell. Sap of Wheat Seedlings as Determined by the Electrical Resistance Method.

P. p. m. of Solution in Which Plants Grew	Resistance of Cell Sap in Ohms
$CaCl_2$ $\frac{N}{250}$	1007.2
$CaCl_2$ $\frac{N}{50}$	952.8

It is evident that even with the non-nutrient salt solutions, the concentrations of the cell sap decreased with the decline in density of the solution. It will be apparent that in the case of the $\frac{N}{50}$ solution, the resistance of the cell sap is 54.4 ohms less than that of the $\frac{N}{250}$ solution.

It seems then from all the preceding experiments, that the assumption originally made, namely, that the density of the cell sap may be proportional to the concentration of the solution in which the plants are growing, is correct, or at least the foregoing data have confirmed it in every case without a single exception.

If it is true that there is a close relation between the density of the cell sap and that of the outside solution, this would at once suggest the method already described of determining the concentration of the soil solution. Whether it would be a practical way, however, is another question.

This, in short, is the manner in which the osmotic pressure of the solution and the density of the cell sap may cause the relative transpiration to increase with the decrease in density of the solution down to a certain point. The decrease of the correlative transpiration from this point down, i. e., with the further decrease in density of solution, may be attributed to another factor, namely, to the decreased power of certain densities of solution to stimulate transpiration. It is assumed here that the lowest density of the nutrient solution has a slight stimulative action upon transpiration, and that it increases with the increase in concentration up to a certain point, beyond which it ceases, or is overcome by the osmotic pressure; or decreases from this point with the diminution in density, just as has already been noted. In other words, the decreased transpiration per unit of growth from the intermediate to the most dilute solution, is ascribed to the reduced stimulative action of the decreased density of solution. To state the same thing in the converse form, the rise in the relative transpiration from the most dilute to the intermediate densities, is due to the greater stimulative action of the increased concentration. The stimulative action cannot continue much further upward than where the change in the curve occurs, because it either reaches its maximum, or it is overcome by other factors, namely, the osmotic pressure of the solution, and the different physical properties of the cell sap.

In what manner the transpiration is stimulated or accelerated by the dilute solutions is not known, it may be chemical, physiological or both.

The whole problem may now be stated in either of the following ways: 1) the transpiration per unit of growth increases with the increase in density of solution, due to the stimulative action of the latter, until a certain concentration is reached and then begins to decrease with a further rise in density, due to the increased osmotic pressure of the latter and to the increased density of the cell sap; or 2) the relative transpiration increases with the decrease in concentration down to a certain point, due to the reduced osmotic pressure of the dimished densities of solution and to the decreased power of the cell sap to reduce evaporation, and then it begins to decrease with a further decline in density, due to the reduced stimulative action of the latter upon it.

Going back to the original tables (I—III) of solution, sand, and soil cultures, it is very significant to note that while the relative transpiration increases down to a certain point and then decreases, the total dry matter produced increases in all three cultures with the rise in density. In other words, the actual plant growth is proportional to the concentration of the solution. This ratio is certainly remarkable for its regularity, being perfectly constant without any interruption in the curve from the lowest to the highest density in all three cultures.

Mention has been made that the sand and soil cultures were employed, first, to duplicate the solution cultures; and secondly, to see whether the different densities of solution would affect the transpiration of the wheat seedlings in these media in the same order or in the same magnitude as in the water cultures. The table below gives these data.

Table IX.

Difference in the Transpiration Per Gram of Dry Matter Among Solution, Sand and Soil Cultures.

Density of Solution in P. p. m.	Greater Transpiration per Gram of Dry Matter in Sand Cultures over Water Cultures	Greater Transpiration per Gram of Dry Matter in Soil Cultures over Water Cultures
4500	14.6	141.1
2250	10.7	169.2
750	20.0	143.7
376	8.9	129.2
187.5	—10.8	146.6
93.5	—21.1	181.7
0.0	172.6	332.1
Average	27.8	177.7

This table shows the difference in the transpiration per gram of dry matter between solution and sand cultures and between solution and soil cultures, solution cultures being taken as a standard in both cases. It will be seen that there is a significant difference among these comparisons. Both the sand and soil cultures transpired more per gram of dry matter than the solution culture, and the soil culture transpired the most. The sand and the soil cultures gave off 27.8 and 177.7 grams of water per gram of dry matter respectively, more than the solution culture. Evidently, the different densities in the culture solution diminished the magnitude of the transpiration per unit of growth, more than in the sand and soil cultures.

One other interesting feature that may be worked out from tables I, II and III, is the difference in the dry matter produced between solution and sand cultures, solution and soil cultures, and sand and soil cultures. The table below gives these data.

Table X.

Difference in the Dry Matter Produced Among Solution, Sand And Soil Cultures

Density of Solution in P. p. m.	Greater Growth in Solution Cultures Over Sand Cultures Grams	Greater Growth in Solution Cultures Over Soil Cultures Grams	Greater Growth in Sand Cultures Over Soil Cultures Grams
4500	—.00331	—.00355	—.00024
2250	—.00390	.00071	.00461
750	.00146	.00658	.00512
375	.00451	.00683	.00232
187.5	.00435	.00482	.00047
93.5	.00235	.00129	—.00106
0.0	—.00152	—.00255	—.00103
Average	.00056	.00206	.00145

These figures show the difference in weight per single plant among the three different cultures. It is very evident that the average dry matter of the solution culture is greater than that of the sand or soil, receiving the same densities of solution, and that of the sand cultures, is larger than that of the soil cultures.

Summary.

In the foregoing investigation an attempt was made to study the effect of different densities of solutions upon the relative transpiration of wheat seedlings. The results obtained may be summarized as follows.

The transpiration per gram of dry matter of wheat seedlings grown in solution, sand and soil cultures containing 4500, 2250, 750, 375, 187.5, 93.5 and 0.0 P. p. m. respectively of a complete nutrient solution, increased in every case with the decrease in density down to a certain point and then decreased with the further diminution in concentration, while the actual dry matter produced increased with the rise in density.

This general run of the relative transpiration was explained thus: The increase from the highest concentration to that where the change occurs, was thought might be due to the decreased osmotic pressure of the solution and to the decreased density of the cell sap; while the decrease from where the change occurs to the lowest density, was believed might be due tho the decreased stimulative action of the dilute solutions.

The relation of the density of the solution to that of the plant cell sap was tested experimentally and it was found that the density of the latter increased with the concentration of the former.

The relative transpiration was greater in the sand and soil cultures than in the solution cultures receiving the same densities of solution, and greater in the soil than in the sand cultures; while the actual dry matter produced was larger in the solution than in the sand or soil cultures and larger in the sand than in the soil cultures.

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