THE WATER CONTENT OF THE SOIL AND THE COMPOSITION AND CONCENTRATION OF THE SOIL SOLUTION AS INDICATED BY THE FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS¹

ABSTRACT

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INTRODUCTION

Although certain lines of work presented in the following abstract are not complete it seems advisable at this time to offer the following report on some of the work now in progress.

There are on record results of many studies of the concentration of the cell sap of aerial portions of plants, that is, the leaves and stems, but there is available much less information with respect to the concentration of the root sap of plants. Moreover, certain investigators have contributed valuable data bearing upon the reaction of the plant to the water content of soils and the concentration of the soil solution. Regardless of the numerous researches, there is much to be accomplished, especially by improving methods and studying the concentration and composition of the soil solution, and the water content of soils as measured by the lowering of the freezing-point of the roots and aerial portions of plants.

This paper presents the principal results obtained from studies of the concentration, and composition of the soil solution, and the water content of soils and the rate of water movement in soils as measured by the freezing-point lowerings of the roots and leaves of plants. A number of soils and plants have been utilized in these researches. Moreover, the results presented include studies under both greenhouse and field conditions.

We previously reported (2) that the freezing-point method can be used to determine the concentration of the cell sap directly in the plant tissue. Inasmuch as the details of the method were not presented, it is well to consider them at this time. About 12 gm. of the material to be studied is placed in a freezing tube and slightly macerated by means of a

¹ Received for publication November 18, 1916.

stiff wire flattened and sharpened at one end. The Beckmann thermometer is inserted into the mass, care being taken to have the mercury bulb covered and the tissue gently pressed about it; the tube is then placed directly into the ice and salt mixture (the temperature of which should be -2° to -3° C.) and kept there until the contents have supercooled about one degree. Solidification is brought about by gently agitating the thermometer. Immediately after the mercury begins to rise the tube is removed from the ice and salt mixture and placed in the air bath, and the reading taken when the end point is reached. Usually more time is required for the mercury to become stationary in the case of plant tissues than it is with soils. The work may be greatly facilitated by having one or two tubes of material in ice water while the freezing point of another sample is being ascertained.

After one has acquired skill in making the determinations, concordant results may be obtained with very little difficulty. As a general rule, different samples of the same material do not vary in their freezing-point lowerings more than $.01^{\circ}$ C. and in many cases they are identical.

Before taking up the studies of relations between the water content of soils, the composition and concentration of the soil solution, and the concentration of the cell sap of roots and tops of plants and plant growth, it is logical to present certain data we have obtained which we consider to have justified our methods of procedure throughout our researches herein reported.

Previous to our work above referred to two methods were widely employed to determine the concentration of cell sap of plants, namely, the plasmolytic method (3, 7, 13, 16, 25, 27) and the freezing point of extracted liquid method (2, 5, 6, 8, 9, 10, 14, 18, 22, 26). The conductivity method has been used to some extent but since it has but little bearing upon the work presented we have omitted any discussion of it. Although a brief review of the literature bearing directly on this problem—that we have been able to obtain—is presented later on, it is well to consider at this time the work of Müller-Thurgau (22), and Cavara (5).

Müller-Thurgau, before the advent of the Beckmann thermometer, made rather extensive studies of the freezing-point lowerings of the fruits of apple, pear and grape, as well as potato tubers and leaves of the bean. He determined the depression of the extract of some, of the triturated tissue of others, and the undisturbed tissue in certain cases, by simply inserting the bulb of the thermometer into it.

Cavara made determinations of the freezing-point lowerings of the expressed sap of many species of plants. In some cases, where it was difficult to obtain the cell sap the bulb of the Beckmann thermometer was inserted into the tissue and in others the freezing-point lowerings of the macerated tissue were determined.

The Effect of Different Methods of Treatment of Plant Tissue upon the Freezing-Point Lowerings

In order to determine whether or not the results obtained from the direct freezing method are similar, or strikingly different from those derived after having subjected the mass of plant tissue to repeated freezing, to low temperatures, to low temperatures and trituration, and to low temperatures and extraction under great pressure, respectively, the experiments reported in the immediately following paragraphs were performed.

TABLE I
THE EFFECT OF REPEATED FREEZING UPON THE CONCENTRATION

Times frozen	Corn Roots		Corn Tops			
	Freezing-point depression	Osmotic pressure atmospheres	A		B	
			Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.430	5.182	.640	7.710	.625	7.520
2	.425	5.122	.600	7.229	.624	7.510
3	.435	5.242	.635	7.650	.625	7.520
4	.430	5.182	.633	7.630		

Times frozen	Pea Roots				Pea Tops			
	A		B		A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.865	10.420	.885	10.660	1.250	15.04	1.251	15.05
2	.835	10.055	.890	10.720	1.275	13.34	1.252	15.06
3	.850	10.240	.865	10.420	1.250	15.04	1.270	15.28
4	.842	10.144	.870	10.480	1.268	15.26	1.264	15.20

Times frozen	Clover Roots				Clover Tops			
	A		B		A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.425	5.122	.445	5.362	.825	9.935	.795	9.574
2	.420	5.062	.435	5.242	.830	9.995	.780	9.394
3	.425	5.122	.440	5.302	.815	9.815	.805	9.695
4	.430	5.182	.425	5.122	.820	9.875	.785	9.454

Repeated Freezings. Roots and leaves of corn, Canada field pea, rye, and clover, respectively, were prepared and placed in freezing tubes in the usual manner, supercooled one degree, and the freezing point repeatedly determined without removing the Beckmann thermometer from the mass during the procedure. By so doing, changes in the water content of the material in the tube were prevented. The results obtained are to be found in Table I.

The variations in the above results lie practically within the experimental error and, therefore, we are justified in concluding that repeated supercooling and freezing did not alter the lowering of the freezing points of the different substances studied.

Repeated Exposure to Low Temperatures. We next conducted experiments to determine if the exposure of different plants to low temperatures appreciably affect their freezing points as determined by the direct method. The plants, or parts of plants, utilized in these studies were placed in tubes which were securely corked, and inserted into the ice and salt mixture, the temperature of which was 10 degrees below zero. The tubes were removed after the contents had solidified (which usually re-

TABLE II
THE EFFECT OF REPEATEDLY SUBJECTING PLANT TISSUES TO LOW TEMPERATURES UPON THE FREEZING-POINT LOWERINGS

Times frozen	Corn Leaves				Pea Leaves			
	A		B		A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
0	.532	6.406	.585	7.048	.580	6.988	.585	7.048
1	.538	6.486	.587	7.078	.585	7.048	.590	7.108
2	.562	6.767	.578	6.978	.590	7.108	.600	7.229
3	.565	6.847	.590	7.108	.620	7.469		
4	.575	6.928						
	Corn Roots				Pea Roots			
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.465	5.603	.470	5.664	.248	2.985	.255	3.074
2	.470	5.664	.485	5.844	.255	3.074	.265	3.194
3	.475	5.724	.490	5.904	.265	3.194	.280	3.375
4	.485	5.844	.496	5.976	.270	3.255	.285	3.435

quired about one hour), the mass thawed and the freezing point determined in the usual manner. It was necessary to remove the thermometer each time the tubes were placed into the cooling mixture in order to prevent it from being broken by the solidification of the mass. The data obtained are given in Table II.

As in the previous series the differences in the results obtained seem to be the result of errors that are introduced. One error, that is impossible to prevent, is the removal of an appreciable amount of liquid on the surface of the thermometer each time it is taken from the freezing tube. Moreover, it is possible that enzymotic activities bring about some changes in the cell contents.

Low Temperature and Trituration. Comparisons of the freezing points of tissue determined directly and subjected to low temperatures

and to trituration were made. Usually aluminum test tubes were employed as the freezing tubes. Unless the material was put through a food grinder while solidified, it was thoroughly macerated by means of a sharpened steel rod while in the aluminum tubes. Great care was taken to prevent changes in the water content of the material, the containers being tightly stoppered at all times, except when it was necessary to insert the thermometer, or the steel rod. The data resulting from these experiments are given in Table III.

TABLE III
THE EFFECT OF LOW TEMPERATURES AND TRITURATION UPON THE
FREEZING-POINT LOWERINGS OF DIFFERENT MATERIALS

Material	Treatment	Freezing-point depression	Osmotic pressure atmospheres
Potato tubers.....	Direct freezing	0.550	6.628
		0.555	6.688
	Frozen solid and macerated to a cream.	0.572	6.887
		0.552	6.648
	Frozen solid and ground	0.565	6.807
		0.570	6.867
Potato tubers.....	Direct freezing	0.518	6.245
		0.565	6.807
	Frozen solid and macerated to a cream.	0.570	6.867
		0.552	6.648
	Frozen solid and ground	0.577	6.938
		0.552	6.648
Clover Leaves.....	Direct freezing	1.018	12.26
		1.045	12.58
	Frozen solid and thoroughly trituated.	1.045	12.58
		1.070	12.88
	Frozen solid and ground	1.117	13.44
		1.102	13.27
Canada Field Pea Leaves	Direct freezing	0.552	6.648
		0.550	6.628
	Frozen solid and thoroughly trituated.	0.565	6.807
		0.558	6.728
Canada Field Pea Roots.	Direct freezing	0.351	4.229
		0.355	4.279
	Frozen solid and trituated	0.372	4.479
		0.360	4.339

An examination of the above data reveals that there is nothing to be gained by subjecting the material to low temperatures and to trituration, inasmuch as the results obtained practically agree. It is noticeable that the results obtained by grinding the frozen material are slightly higher than those obtained by trituration directly in the freezing tube. This is doubtless due to unavoidable loss of moisture during the process of grinding. It is well to mention that the plants used in the above studies, with the exception of clover, were quite succulent.

Low Temperature, Trituration and Extraction. Results reported by Dixon (10) show conclusively that the freezing point of pressed vegetable juices varies with the treatment of the tissue before being pressed, as well as with the degree of extraction. (We have verified the latter point.) Exposure of the tissue to chloroform, to toluene, to heat, to intense cold, and to desiccation and remoistening, respec-

TABLE IV
COMPARISONS OF THE FREEZING-POINT LOWERINGS OF PLANT TISSUE
DETERMINED DIRECTLY AND OF THE EXTRACTED LIQUID

Crop	Direct Freezing		Expressed Liquid	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Oats	1.620	19.480	1.690	20.320
	1.640	19.720	1.690	20.320
Rye	0.890	10.720	0.825	9.935
	0.900	10.840	0.830	9.995
	0.690	8.312	0.740	8.913
	0.700	8.432	0.720	8.672
	0.853	10.280	0.835	10.050
	0.830	9.995	0.825	9.935
ROOTS				
Oats	1.110	13.360	0.895	10.780
	1.120	13.480	0.895	10.780
	1.110	13.360	0.890	10.720
	1.090	13.120	0.880	10.600
	0.380	4.580	0.320	3.857
	0.390	4.700	0.330	3.978
	0.180	2.171	0.190	2.291
	0.180	2.170	0.200	2.412
Barley	0.975	11.740	0.955	11.500
	0.970	11.680	0.950	11.440
Corn	0.412	4.965	0.355	4.279
	0.440	5.302	0.350	4.219

tively, before being pressed, materially increases the lowering of the freezing point of the extracted sap over that obtained from untreated tissue. The author suggests that the liquid pressed from untreated vegetable tissues is not an average sample of the sap contained in the cells of those tissues, but the exposure to intense cold renders the cells permeable and the liquid is not altered in concentration by extraction. It was considered advisable to compare the freezing-point lowerings

of liquid that had been extracted from tissue subjected to intense cold with that of the freezing-point lowerings of the tissue. The mass to be subjected to low temperature was placed in pint mason jars, which were carefully sealed and buried in a freezing mixture and left for 3 hours. Following this exposure the material was at once placed in the press and subjected to a pressure of 300 kg. per square centimeter until all liquid was removed under these conditions. The freezing point of the extract was determined immediately. Both roots and leaves of different plants of high and of low water content and depression have been used in making comparisons. The data obtained are given in Table IV.

As indicated by the data presented in Table IV the freezing-point lowerings of the leaves of the plants studied are practically the same as those of the extracted liquid. We found that precautions must be taken to reduce to the minimum the change in water content of the tissue while being prepared for and during the process of extraction.

The results obtained from the studies of the roots are not so consistent, in some cases the extracted liquid affording less depression than the tissue frozen directly, and in others practically the same, but the differences are greatest in cases where the depressions are great. At present we are unable to account for such variations unless they are due to adsorption in some cases by the solid mass.

The Rate of Water Translocation in Soils as Measured by the Freezing-Point Lowerings of Roots and Tops of Plants

Early in our work it was found difficult to obtain concordant results with samples of tops of plants taken from the soil at various intervals during the day. Dixon (8) noted that illumination increased the osmotic pressure in the cells of leaves, and conversely, he found that the pressure gradually fell when the leaves were cut off from the light. The difference thus produced amounted to more than 14 atmospheres in some instances. He attributes such differences chiefly to the formation of soluble carbohydrates in the light, thus increasing the concentration of the cell sap. Later on Chandler (6) established that when plants are shaded 24 hours the sap density materially decreases. It is well to call attention to the fact that neither of these investigators presents data to show whether or not the change in concentration is due mainly to the formation of carbohydrates, or to some other condition, such as the rate of water supply by the soil and consequently a change in the water content of the tissue.

Inasmuch as such changes have a very important bearing upon the methods of procedure in our later work, and in view of the fact that they throw light upon the moisture relations of soils and plants, numerous determinations of the concentration of the cell contents of the roots and tops of certain plants were made. In all cases reported, the samples were

placed in the freezing tubes and the freezing point determined immediately. Inasmuch as changes in the water content of plant tissues may alter the concentration of the cell contents markedly; the total loss from the leaves upon drying in an oven heated to 105 °C. was determined. The writers fully appreciate that the total loss in weight so determined may not truly represent the amount of active water present. It may be cited, for example, that Müller-Thurgau (22), many years ago showed that much of the total plant water as determined by loss of weight upon drying may not be frozen and the amount varies with the intensity of the cold to which plants are exposed.

The first series of experiments conducted were under field conditions and no precaution was taken to alter the light intensities. The results of these studies appear in Table V.

TABLE V
VARIATIONS IN THE CONCENTRATION OF THE CELL SAP OF THE LEAVES OF
PLANTS AT DIFFERENT INTERVALS DURING THE DAY

CORN LEAVES			
Hour of Sampling	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
6.00 a. m.	83.56	.544	6.566
12.00 noon	80.47	.778	9.370
6.00 p. m.	82.56	.739	8.903
12.00 midnight	83.23	.607	7.309
CANADA FIELD PEA LEAVES			
6.00 a. m.	90.72	.585	7.048
12.00 noon	87.75	.760	9.154
6.00 p. m.	87.78	.630	7.590
12.00 midnight	88.90	.570	6.867

The results of these studies show very strikingly that the freezing point of the tissues was greatest about noon, appreciably lower at 6.00 p. m., and still lower at midnight, and usually lowest early in the morning, or about sunrise. Moreover, the loss upon drying was found to be greatest when the concentration of the cell sap was lowest. There seems to be, then, a close correlation between our results and the rate of water movement from the soil to the plant at different hours of the day as determined by Briggs and Shantz (4), as well as the results of Lloyd's (21) studies of the leaf water of cotton.

It is assumed that photosynthesis also played some rôle in the change in the concentration of the cell contents. Yunker (28) recently reported that the rate of photosynthesis of the corn plant grown in the greenhouse is greatest at noon and gradually decreases from this time to sunset.

In order to throw additional light upon this question, comparative studies were made with plants that were shaded by means of an inverted

box placed over the plants when the first samples were taken in the morning, and those exposed. These experiments were conducted under field conditions. The data obtained appear in Table VI.

TABLE VI
VARIATIONS IN THE CONCENTRATION OF THE CELL SAP OF THE LEAVES OF PLANTS AT DIFFERENT INTERVALS (FIELD STUDIES)

OATS, UNSHADED				
Time of Sampling and Weather Conditions	Air Temperature ° C.	Freezing point depression	Osmotic pressure atmospheres	Water content per cent
5.30 a. m. Partly cloudy	12.25	0.681	8.201	86.28
9.15 a. m. Completely cloudy ..	17.50	0.755	9.093	86.18
1.00 p. m. Hazy sunshine	20.25	0.889	10.710	83.78
5.00 p. m. Cloudy	18.25	0.878	10.580	84.77
5.30 a. m. Hazy sunshine	12.00	0.650	7.830	87.46
9.15 a. m. Partly cloudy	14.25	0.758	9.134	86.57
1.15 p. m. Cloudy	16.00	0.757	9.124	86.00
5.00 p. m. Misting rain	13.00	0.758	9.134	85.68
OATS, SHADED				
5.30 a. m. Partly cloudy	12.25	0.681	8.201	86.28
9.15 a. m. Completely cloudy ..				
1.00 p. m. Hazy sunshine	20.00	0.638	7.690	87.37
5.00 p. m. Cloudy	17.50	0.623	7.509	88.19
5.30 a. m. Hazy sunshine	12.00	0.650	7.830	87.46
9.15 a. m. Partly cloudy				
1.15 p. m. Cloudy	15.00	0.604	7.279	87.95
5.00 p. m. Misting rain	13.00	0.597	7.208	88.42
RYE, UNSHADED				
5.30 a. m. Fair	13.00	0.790	9.514	78.99
9.30 a. m. Fair	24.00	0.942	11.350	76.61
1.15 p. m. Fair	25.00	1.014	12.200	76.23
5.00 p. m. Hazy	22.00	1.032	12.420	76.62
5.30 a. m. Cloudy	18.50	0.768	9.244	79.87
9.15 a. m. Hazy sunshine	22.75	0.955	11.500	75.74
1.00 p. m. Hazy sunshine	25.00	1.012	12.190	75.79
5.00 p. m. Hazy sunshine	22.50	0.788	9.504	76.55
RYE, SHADED				
5.30 a. m. Fair	13.00	0.790	9.514	78.99
9.30 a. m. Fair				
1.15 p. m. Fair	31.00	0.711	8.562	79.25
5.00 p. m. Hazy	21.00	0.706	8.502	80.09
5.30 a. m. Cloudy	18.50	0.768	9.244	79.87
9.15 a. m. Hazy sunshine				
1.00 p. m. Hazy sunshine	26.50	0.755	9.093	80.68
5.00 p. m. Hazy sunshine	22.50	0.784	9.444	80.97

The results obtained show that the moisture content of the leaves of the plants in the shade remains quite constant throughout the day, but that

the concentration of the cell sap showed a tendency to become more dilute from 5.30 a. m. to 5.00 p. m. Moreover, the decrease in the water content of the exposed plants apparently does not account *in toto* for the increase in concentration of the cell sap or, in other words, the products resulting from photosynthesis probably play their part.

Although this question is quite incidental to the problem we had under investigation, a series of experiments with potted plants was run in order to obtain additional information concerning it.

In these studies, the plants were kept in a saturated atmosphere by inverting a large bell jar over them. The results presented in Table VI show that the concentration of the cell sap of the leaves was lowest at sunrise and greatest at 1.00 p. m., and, furthermore, indicate that these differences are not due wholly to the water content of the tissue. Moreover, the concentration of the roots varied, but less strikingly than that of the tops, and in a reverse order with respect to time of sampling. Similar results were obtained with peas.

TABLE VII
FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT AT
DIFFERENT INTERVALS DURING THE DAY

Time	Tops			Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
Sunrise	.503	6.061	93.4	.375	4.519
9.00 a. m.	.570	6.867	91.8	.388	4.676
1.00 p. m.	.625	7.529	91.5	.336	6.050
5.00 p. m.	.570	6.867	92.7	.450	5.423
10.00 p. m.	.558	6.724	92.8	.359	4.327

EXPERIMENTAL

The freezing-point lowerings of the roots and tops of plants as a measurement of the composition and concentration of the soil solution, as well as the different water contents and rate of water movement in soils, have been studied. Several of the experiments herein reported were conducted in a greenhouse but many were carried on under field conditions.

The Freezing-Point Lowerings of Plants as Indicators of the Concentration of the Solution in Which They Grow

Certain investigators have established that there is some relationship between the concentration of the media in which plants grow and their cell sap. Weiler (27) early determined the concentration of the cell sap of *Helianthus annuus*, *Vicia faba* and *Phaseolus multiflorus* by the plasmolytic method and reported that the roots and tops were the same in concentration, and that the strength of sugar required to cause plasmoly-

sis lies between 6 and 7 per cent. He also showed that stronger solutions were required to bring about plasmolysis after the plants had been in contact with sugar solutions of higher concentrations several hours.

Copeland (3) employing solution cultures presented data which show that the cell sap of the roots and leaves of *Phaseolus multiflorus*, *Phaseolus vulgaris*, *Pisum sativum*, *Sinapsis alba*, *Fagopyrum* and *Zea Mays* is altered by the composition and the concentration of the solutions in which they grew. He also found that the cell sap of the roots in some instances is slightly less concentrated than that of the aerial portions of the plants.

Drabble and Drábble (11) report that the osmotic pressure of the sap in turgid cells in the plants studied (total number being 48), ranges from 3386.6 to 1495.13 mm. of mercury. In addition they found the osmotic strength of the sap of plants growing in fresh water marshes to be lower than that of those growing in salt water marshes. Moreover, the concentration increases with the degree of physiological drouth, and in any area it varies with the physiological scarcity of water, but plants growing under the same conditions generally have the same concentration of cell sap. Moreover, "the depression of the freezing point of the sap within the range of strengths found will be small, but may be effective in the case of plants with the highest osmotic strength of sap."

Hill (19) in studies with salt marsh plants found that the root hairs vary their concentration with the strength of the soil water and the concentration of the different root hairs, varies on the same plant. Moreover, different individuals of the same species likewise vary in this respect.

Bouyoucos (1) noted a close relation between the density of the cell sap of wheat and bean seedlings, and the outside solution in which they were grown.

The senior author made many determinations of the concentration of the cell sap of *Tradescantia Zebrina* that had grown 30 to 60 days in distilled water, full nutrient solution, and single and mixed salt solutions, respectively. Unpublished data show very strikingly that the density of the solutions in which the cuttings grow appreciably influences the osmotic pressure of the cell sap and indicate that the composition also influences it.

In view of the fact that the direct freezing point method has never been used, to our knowledge, to determine whether or not the concentration of the cell sap of roots and leaves of plants is indicative of the density of the solutions in which they grow, several experiments were conducted in the greenhouse with Canada field pea and wheat seedlings.

The three-salt solution of Shive (24) was employed. The concentration ranged from one-tenth to four times that suggested by this investigator. The experiments were continued 17 and 24 days in the case of

peas, and 14 and 30 days, respectively, with wheat seedlings. Several hours previous to the freezing-point determinations, the tops were rinsed in distilled water and allowed to dry, the roots remaining in contact with the solution. The roots of the plants were likewise rinsed and carefully dried between sheets of filter paper before freezing. In all cases the loss upon drying at 105° C. was recorded. The results obtained from these studies are presented in Table VIII.

TABLE VIII
THE FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF PLANTS, AS A MEASUREMENT OF THE DENSITY OF THE CULTURAL SOLUTIONS

CANADA PEA SEEDLINGS, 17 DAYS OLD						
Tops			Roots			
4 x Nutrient Strength of solution	.673 Freezing-point depression	8.101 Osmotic pressure atmospheres	91.97 Water content per cent	.424 Freezing-point depression	5.102 Osmotic pressure atmospheres	95.23 Water content per cent
1/10 Nutrient	.543	6.536	93.38	.371	4.469	93.64
Nutrient	.549	6.616	93.42	.369	4.449	95.47
2 x Nutrient	.608	7.319	93.47	.405	4.881	95.67
CANADA PEA SEEDLINGS, 24 DAYS OLD						
1/10 Nutrient	.613	7.379	92.24	.328	3.947	93.11
Nutrient	.650	7.830	92.53	.353	4.249	95.24
2 x Nutrient	.712	8.572	94.38	.404	4.861	95.34
4 x Nutrient	.790	9.514	92.09	.424	5.102	95.35
WHEAT SEEDLINGS, 14 DAYS OLD						
1/10 Nutrient	.641	7.720	90.53	.181	2.181	94.48
Nutrient	.780	9.394	93.05	.360	4.339	94.82
2 x Nutrient	.861	10.370	93.07	.391	4.710	94.53
4 x Nutrient	.887	10.680	92.37	.374	4.499	94.89
WHEAT SEEDLINGS, 30 DAYS OLD						
1/10 Nutrient	.748	9.003	90.71	.402	4.841	92.44
Nutrient	.835	10.050	91.94	.592	7.128	92.63
4 x Nutrient	.995	11.980	91.37			

The data show conclusively that the freezing point of the roots and tops of Canada field pea and wheat seedlings is indicative of but not directly proportional to the density of the nutrient solution in which they are grown. Although the conditions for growth were identical, the concentration of the cell sap of the two plants, as measured by the direct freezing point method, differed appreciably. The tops of the wheat seedlings gave greater depressions than those of the pea seedlings at the close of each period. Moreover, the roots of the former showed greater difference in concentration at the close of the second period than the roots of the latter. It is well to note that in all cases the roots were lower in concentration than the tops, undoubtedly due in part at least to a higher water content.

The early work of Copeland (3) with solution cultures point to similar relations between concentration of the sap of the roots and tops of seedlings. On the other hand, Stange (25) did not report difference in the osmotic pressure of the stems and of the roots of *phanerogamia*. Dixon (10) by means of the thermo-electrical method found sap expressed from certain plants to range in pressure from 11.6 to 16.9 atmospheres, but that taken from roots of the same plants gave pressures of 4.3 to 5.9 atmospheres. At about the same time, Nathanson (23) discussed this relationship. More recently, Hanning (16) employing the plasmolytic method determined the concentration of the cell sap of the roots and leaves of a large number of plants, the total number being 62. With few exceptions the sap of the leaves showed greater concentrations than that of the roots.

In connection with the difference in depression exhibited by the cell sap of the aerial portion of the plants studied and root juice, the question suggests itself, whether there is an actual difference in the amount of soluble material present, or is it possible that there is a difference in the amount of water that freezes?

TABLE IX

A COMPARISON OF THE LOSSES ON DRYING THE ROOTS AND TOPS OF PLANTS AT 105° C. AND OF THE AMOUNTS OF WATER IN THE MATERIAL THAT FREEZES

Plant: Barley	Tops		Roots	
	Percentage loss at 105° C.	Percentage water that froze	Percentage loss at 105° C.	Percentage water that froze
Morning	80.32	55.06	88.33	61.51
	80.43	54.63	86.84	62.91
Noon	80.47	42.22	82.09	57.08
	81.63	44.46	85.89	60.78

To throw some light on this point the amount of water that freezes in the plant tissue was measured in the following manner. A weighed sample of the material, about 10 gm., was thoroughly triturated in a dilatometer¹ by means of a sharpened steel plunger, such as was used for crushing similar material in the freezing point tubes. The bowl of the dilatometer was then filled with ligroin. It required considerable time to expel air from the interstices of the tissue but with thorough trituration and due care replacement ceased. The instrument was placed in a bath about —3° to —4° C. and allowed to remain undisturbed until the contents reached the same temperature as the bath, as shown by no further shrinkage of the ligroin in the stem of the dilatometer. By agitating the instrument solidification was now induced and the expansion measured.

¹ Dr. Geo. J. Bouyoucos is directly responsible for the development of dilatometer method. His work along these lines is now in the process of publication.

The percentage of water that freezes and the percentage of loss on drying the material at 105° C. is shown in Table IX.

The data presented indicate a striking difference in the per cent of water freezing in the roots and tops of barley plants, and also a marked difference in the per cent of water freezing in the tops in the morning and at noon. The plants were kept under such conditions that transpiration was retarded while photosynthesis could proceed. It seems probable that the physiological activities of the cells play an important rôle in the amount of free water being decreased.

An additional series was conducted with Canada pea seedlings. In these experiments soil extracts were used which were obtained from both virgin and cropped sandy loam and silt loam soils in the usual manner, that is by shaking with five parts of distilled water, allowing to settle and then filtering through Chamberland-Pasteur filters. Three-gallon jars were used as containers, the seedlings being inserted through holes in heavily paraffined paper, which covered the jars, into the solution. At the close of 14 and 25-day periods the freezing-point lowerings of the roots and tops of the plants were determined. The data obtained are presented in Table X.

The data presented show no apparent relation between the type of soil from which the extract was obtained and the freezing-point depression of the roots and tops of the plants grown therein. In the case of the roots there seems a tendency for those growing in the extract from the cropped soil to have a slightly higher freezing-point depression than those growing in the extract from the virgin soil of the same type.

Since the freezing-point depressions of the various extracts are practically the same, it seems that any variation in the freezing-point depression of the plants must be due to the composition of the extracts, or to some other uncontrolled factor.

The Concentration of the Soil Solution as Measured by the Freezing-Point Lowerings of the Roots and Tops of Plants

Since the results derived from solution cultures show clearly that the density of the same is indicated by the freezing-point lowerings of roots and tops of certain plants grown therein, the question at once arises as to whether or not similar conditions obtain with respect to the soil and plants. In order to obtain further evidence a series of soil cultures was prepared.

Sandy loam was placed in 1-gallon pots, the moisture maintained uniform and constant, throughout the experiment, while the concentration of the soil solution was varied by the addition of soluble salts. The experiments were run in triplicate, but the results obtained are reported in duplicate. Previous to freezing, the roots and tops were rinsed with distilled water and carefully dried between layers of filter paper. Samples of each were brought to constant weight in an oven heated to 70° C. and

TABLE X
THE FREEZING-POINT LOWERINGS OF PLANTS GROWN IN SOIL EXTRACTS

ROOTS

Soil	Period of Growth 14 Days				Period of Growth 25 Days		
	Freezing-point depression of the extract	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent
Sand virgin		.310	3.737				
	.009	.312	3.757	95.08	Not	run	
Sand cropped		.375	4.519		.300	3.616	
	.015	.358	4.319	94.18	.305	3.676	93.66
Sandy loam virgin..		.430	5.182		.330	3.978	
	.009	.395	4.760	94.20	.335	4.038	93.34
Sandy loam cropped		.430	5.182		.365	4.399	
	.011	.419	5.052	94.58	.365	4.399	94.49
Silt loam virgin....		.300	3.616		.320	3.857	
	.011	.305	3.676	94.56	.310	3.737	95.88
Silt loam cropped...		.335	4.038		.390	4.700	
	.010	.325	3.917	94.04	.395	4.760	91.86

TOPS

Sand virgin		.575	6.927				
	.009	.572	6.897	89.26	Not	run	
Sand cropped		.555	6.788		.605	7.289	
	.015	.550	6.628	90.14	.615	7.409	87.16
Sandy loam virgin..		.625	7.529		.715	8.612	
	.009	.620	7.469	88.84	.720	8.672	87.98
Sandy loam cropped		.600	7.229		.590	7.108	
	.011	.617	7.439	89.64	.605	7.289	93.58 ?
Silt loam virgin....		.535	6.446		.710	8.552	
	.011	.542	6.526	89.30	.700	8.432	86.20
Silt loam cropped...		.560	6.747		.725	8.732	
	.010	.550	6.628	88.78	.738	8.893	86.30

the total loss in weight recorded. For 24 hours previous to freezing the plants were not exposed to light.

The concentration of the tops of the plants that grew in the soil to which the full treatment was afforded, was slightly greater than that of those grown in the soil which received only one-half the ration of soluble salts, as well as those in the untreated soil. On the other hand, by the freezing point of the tops the concentration of the soil solution found to exist in the two latter soils was not indicated. In other words, all other conditions being maintained as nearly the same as possible, only rather large variations with respect to the soil solution are indicated by the freezing-point lowerings of the tops of plants. The results also show that the roots of the plants grown under the above conditions are better indicators of the concentration of the soil solution than the tops, the freezing-point lowerings decreasing with, but not directly proportional to the decrease in density of the soil solution with which they come in contact.

TABLE XI
THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS A MEASUREMENT OF THE CONCENTRATION OF THE SOIL SOLUTION

Treatment per Acre	Tops			Soil	Roots		
	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent	Moisture per cent	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent
MgSO ₄ 300 lbs. . . .	.537	6.466	89.32	10.08	.434	5.232	90.53
NaNO ₃ 300 lbs. . . .	.535	6.446	88.83	8.82	.483	5.814	89.62
K ₂ HPO ₄ 500 lbs. . . .	.562	6.767	89.42	10.25	.445	5.362	89.70
Average	.545	6.566	89.19	9.72	.454	5.473	89.95
1/2 Treatment	.504	6.075	89.78	10.85	.428	5.162	87.82
	.509	6.135	90.00	9.28	.421	5.072	89.83
	.533	6.426	89.37	10.11	.431	5.192	89.24
Average	.515	6.205	89.72	10.08	.427	5.152	88.96
Check	.540	6.506	87.83	11.25	.365	4.399	88.64
	.519	6.245	88.89	10.53	.410	4.941	88.67
	.519	6.245	88.79	12.26	.357	4.299	89.45
Average	.526	6.336	88.50	11.35	.377	4.539	88.92

An additional series of experiments was conducted. Jars of 3-gallon capacity were filled with silt loam soil, the same amount of water was added to each, and maintained constant by means of an inverted funnel that extended to within about 6 inches of the bottom of the jar and glass tubes that penetrated through the wax seal. The concentration of the soil solution was varied markedly by additions of different amounts of a strong solution of calcium nitrate, magnesium sulfate, and potassium phosphate. In this series the roots of the plants were freed of as much soil as possible by vigorous shaking before they were frozen. The

error so introduced, due to the presence of the soil particles on the roots, is probably very slight, as many determinations seem to indicate.

The concentration of the tops of the plants proved to be less valuable indicators of the concentration of the soil solution than the roots. Consequently, there was less difference between the concentration of the roots and tops of plants grown in the soils possessing high concentration; indeed, the roots of the plants removed from cultures 4 and 5 afforded greater depressions than the tops. The data obtained appear in the table below.

TABLE XII
THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS INDICATORS OF THE CONCENTRATION OF THE SOIL SOLUTION

Culture No.	Soil			Corn Tops			Corn Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
1	.101	1.216	16.18	.511	6.155	89.89	.381	4.590
2	.165	1.990	16.56	.585	7.048	89.50	.455	5.483
3	.281	3.385	16.99	.577	6.957	90.00	.549	6.618
4	.412	4.961	17.20	.601	7.239	89.46	.624	7.509
5	.600	7.229	17.22	.605	7.289	90.16	.680	8.191

In order to throw additional light upon this question, nutrient salts were added to pots of sandy soil in which were growing rye plants about one foot in height, in sufficient amounts to increase markedly the concentration of the soil solution. The jars were sealed and remained outside the laboratory, and the soil permitted to become dry, without further addition of water. In the meantime the concentration of the soil solution, the water content of the soil as well as the freezing-point lowerings of the roots and tops of the plants were determined. The data presented in Table XIII show very strikingly that the concentration of the soil solution is rather closely correlated with the freezing-point lowerings of the roots of the plants in contact with it, but the tops of the plants are less sensitive to changes in the concentration of the soil solution.

Moisture Relations of Soils and Plants

According to Drabble and Lake (10) a greater concentration of cell sap occurs in those plants which are most strongly subjected to factors tending to promote loss of water by transpiration. Livingston (20) concludes, from certain of his studies, that the concentration of the cell sap of desert plants is not appreciably greater than that of ordinary land plants, while Fitting (13) maintains, as a result of his investigations, that the concentration of the sap of epidermal leaves taken from desert plants is very great, ranging from 30 to 100 atmospheres, and that the root sap of the same must likewise be very highly concentrated. Hibbard and

Harrington (18) report, as a result of a single series of experiments, that as the water content of a soil decreases the depression of the freezing point of both tops and roots of plants increases.

TABLE XIII
THE CONCENTRATION OF THE SOIL SOLUTION AS INDICATED BY THE FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS

Soil			Tops			Roots	
Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
.285	3.435		1.240	14.92		0.555	6.688
.285	3.435	9.60	1.240	14.92		0.555	6.688
.500	6.025		1.420	17.08		1.250	15.04
.495	5.904	4.10	1.440	17.32	78.66	1.240	14.92
.890	10.72		1.420	17.08		0.870	10.48
.900	10.84	3.25	1.405	16.90	80.70	0.850	10.24
*			1.660	19.96		1.450	17.44
*		2.18	1.670	20.08	79.92	1.470	17.68
*			1.400	16.84		2.060	24.75
*		2.06	1.420	17.08	74.53	2.050	24.63

* Did not freeze.

Now the question arises—Is the increase in concentration brought about, mainly, the result of slow movement of water from the soil to the

TABLE XIV
THE FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT AS INDICATORS OF THE WATER CONTENT OF SOILS

Soil Condition	Soil				Leaves			Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent
High water content	.0170	0.201	22.3	.522	6.280	89.4	.285	3.43	86.5
	.0210	0.251		.504	6.130	89.4	.365	4.34	
	.0150	0.181	18.8	.531	6.390	88.7	.305	3.67	89.9
Average	.0177	0.211	20.6	.521	6.267	89.2	.318	3.82	88.2
Low water content	.5140	6.180	8.6	.505	6.080	88.9	.542	6.56	87.8
	.5210	6.270	7.7	.490	5.900	88.4	.483	5.81	87.6
	.4570	5.510	10.1	.503	6.050	87.8	.479	5.77	88.2
Average	.4970	5.980	8.6	.499	6.010	88.4	.501	6.03	87.8

plant, or is it the result of greater density of the soil solution, or of both? It is rather difficult to settle this point, inasmuch as we have shown that the concentration of the soil solution may be altered quite strikingly by

changes in the water content. Many experiments have been conducted to throw light upon this question. The results of certain of these are presented in Table XIV.

Silt loam was placed in 3-gallon jars, the water content was maintained slightly above the critical content in three, and near the so-called optimum content in three others. The corn plant was employed as the indicator, the duration of the experiment being 35 days. The roots of the plants were rinsed and dried between filter paper before the freezing points were determined. The losses in weight of tops and roots upon drying in an oven heated to 105° C. were also ascertained.

The results presented in Table XIV show clearly that the water content of the soil and therefore the concentration of the soil solution is closely correlated with that of the cell sap of the roots of the plants growing therein.

The next series of experiments conducted under greenhouse conditions, although of short duration, affords additional information concerning this question. Sandy loam was placed in pots, the water content being maintained constant at 8, 12, and 16 per cent, respectively.

TABLE XV
THE WATER CONTENT OF SOILS AS INDICATED BY THE FREEZING-POINT
LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT
(Duration of experiment 14 days)

Water content of soil	Soil		Tops		Roots	
	Freezing- point depression	Osmotic pressure atmospheres	Freezing- point depression	Osmotic pressure atmospheres	Freezing- point depression	Osmotic pressure atmospheres
8%	.555	6.506	.530	6.386	.560	6.747
	.540	6.688	.535	6.446	.567	6.837
	.220	2.652	.506	6.085	.435	5.242
12%	.235	2.832	.520	6.266	.445	5.362
	.135	1.518	.520	6.266	.470	5.664
16%	.150	1.809	.515	6.205	.480	5.784

The above results show less striking differences with respect to concentration of the roots of the plants grown in the soils of different water contents. It is a notable fact also that the concentration of the soil solution did not vary so markedly as in the previous series. It should also be noted that the soil was removed, as far as possible, by shaking the roots. The freezing-point lowerings of the leaves are practically the same throughout, those grown in the soil of lowest water content being very slightly higher than the others.

On November 1, rye plants about one foot in height, growing in groups of 50 or more, were transferred with the immediately surrounding soil to glazed jars. The soil was kept moist 10 days by additions of water. At the end of this period the jars were sealed and the soil permitted to

become dry by loss of water through the leaves of the plants. The freezing point of the roots and tops, as well as the water content and the freezing-point lowerings of the soil were determined at successive stages until the plants were completely wilted. In this manner the moisture relations of the soil and plants were determined.

TABLE XVI

CHANGES IN SOIL MOISTURE AS INDICATED BY THE ALTERED FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS

Soil			Tops			Roots	
Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
.06	0.724		1.240	14.92		0.312	3.757
.07	0.844		1.240	14.92		0.317	3.827
.22	2.652		1.170	14.08		0.600	7.229
.22	2.652	4.44	1.150	13.84		0.585	7.048
.22	2.652	4.12	1.190	14.32		0.590	7.108
.23	2.772		1.230	14.80		0.560	6.747
*			1.310	15.76		1.275	15.340
*		2.01	1.360	16.36	79.57	1.280	15.400
*			1.770	21.28		1.300	15.640
*		2.01	1.780	21.40	75.98	1.300	15.640
*			1.670	20.08		1.330	16.000
*		2.21	1.660	19.96	75.88	1.300	15.640

* Did not freeze.

The data in Table XVI indicate that there is no relation between the water content of the soil and the freezing-point lowerings of the leaves of the plants growing in it until the critical water content of the soil is reached. The freezing-point depression of the roots, on the other hand, correlates very closely with the water content of the soil, in which they are growing, and with the concentration of the soil solution. Similar results were obtained when the freezing-point depressions of rye growing in soil of varying water content to which salts had been added, were determined. This is shown in Table XIII.

A similar set of experiments with corn plants, one foot in height, also was run. The results obtained appear in Table XVII.

The above data, as well as many others not presented in this paper, show that the freezing-point lowerings of the roots of plants indicate the water content of the soil in which they are growing, inasmuch as they are altered by changes in the water content of the soil. Moreover, they strongly indicate that the concentration of the soil solution at the wilting

point of the soil under ordinary conditions of temperature, etc., is equal to or greater than that of the roots of the plants. Again, we have failed to obtain evidence in these and many other determinations that the freezing-point lowerings of the leaves of plants are greatly affected by the water content of the soil until the critical point is approached. We should not fail to note that all comparative determinations of the leaves were carried on at approximately the same hour of the day—between 8.00 and 9.00 a. m.

TABLE XVII

THE FREEZING-POINT LOWERINGS OF THE ROOTS AND LEAVES OF THE CORN PLANT AS INDICATORS OF THE WATER CONTENT OF SOILS

Condition of Plants	Soil			Roots		Tops		
	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point-depression	Osmotic pressure atmospheres	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent
Excellent	0.070	0.844	16.22	.393	4.740	.507	6.115	87.90
Good	0.320	3.857	5.21	.470	5.664	.500	6.025	88.10
Signs of wilting	1.000	12.040	3.23	.463	5.603	.473	5.694	88.00
Badly wilted ..	*		2.13	.810	9.755	.635	7.650	86.00

* Did not freeze.

Field Studies

Many determinations of the freezing-point lowerings of leaves and roots of different crops growing under field conditions have yielded much

TABLE XVIII

THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS INDICATORS OF THE WATER CONTENT OF SOILS

Soil Class	Per cent of water in soil	Corn Leaves		Corn Roots	
		Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Sand	10.5	0.461	5.553	.254	3.064
Sandy loam	21.1	0.457	5.513	.227	2.742
Loam	22.6	0.482	5.804	.278	3.355
		Rye Leaves		Rye Roots	
		Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Sand	12.2	1.220	14.68	.285	3.435
Sandy loam	20.5	1.235	14.86	.310	3.737
Loam	24.2	1.264	15.20	.315	3.797

information with respect to the moisture relations of soils and the plants growing therein, precautions having been taken to obviate local differences as far as possible. We have shown that the variations in concen-

tration of the solution of all classes of soils are not great at or near the point of saturation. Such being the case, the freezing-point lowerings

TABLE XIX

VARIATIONS IN THE MOISTURE CONTENT OF SOILS AS MEASURED BY THE CHANGE IN THE FREEZING-POINT DEPRESSION OF THE ROOTS AND TOPS OF PLANTS GROWING IN THEM

Soil Class	Per cent of water in soil	Corn Tops		Corn Roots	
		Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Sand	12.50	.475	5.724	.254	3.064
	3.04	.695	8.372	.745	8.973
Sandy loam	20.00	.520	6.266	.325	3.917
	7.50	.785	9.454	.750	9.033
Muck	49.00	.601	7.239	.455	5.302
	40.50	.655	7.890	.610	7.349

of the roots and leaves of a given crop growing under such conditions should not vary greatly, unless the composition of the soil solution is an important factor.

TABLE XX

FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF DIFFERENT CROPS GROWING UNDER SIMILAR FIELD CONDITIONS

MUCK SOIL

Crop	Soil			Tops		Roots		
	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
Canada Pea ..	43.30	.320	3.857	0.670	8.071	89.20	0.475	5.724
Corn	43.30	.320	3.857	0.430	5.182	87.80	0.412	4.961
Oats	43.30	.320	3.857	0.780	9.394	89.20	0.450	5.423
Wheat	43.30	.320	3.857	0.860	10.360	87.00	0.410	4.941
Barley	43.30	.320	3.857	0.800	9.635	87.30	0.390	4.700
Rye	43.30	.320	3.857	0.820	9.875	86.90	0.500	6.025

SANDY LOAM SOIL

Canada Pea ..	6.22	.718	8.648	1.090	13.20	81.50	0.842	10.14
Corn	6.22	.718	8.648	0.965	11.62	82.04	0.932	11.23
Barley	6.22	.718	8.648	1.275	15.34		1.005	12.10

SANDY SOIL

Canada Pea ..	3.04	.290	3.496	0.735	8.85	88.20	0.735	8.850
Corn	3.04	.290	3.496	0.690	8.31	87.70	0.745	8.973

In Table XVIII are presented the results obtained with corn and rye taken from different classes of soil in a certain field on the college farm.

The above determinations show that the freezing-point lowerings of the leaves and roots of the same crop growing on different soil classes of high water content do not vary appreciably.

On the other hand, the concentration of the cell sap of the roots of the same crop—as measured by the freezing-point method—is greatly altered by appreciable changes in the water content of the soil, as the following results obtained from field studies with corn show.

Freezing-point lowerings of different crops seeded the same date on muck, sandy loam, and sandy soils, respectively, were determined. The results as set forth in Table XX bring out that the leaves of crops growing under adjacent field conditions differ markedly in concentration and the roots much less.

SUMMARY

Consistent determinations of the freezing-point depression of plant tissue may be obtained by inserting the thermometer directly into material triturated in the freezing tube.

Results with material repeatedly reduced to the point of solidification, repeatedly frozen at low temperatures, and material frozen at low temperatures and macerated or triturated were essentially the same as those obtained by direct freezing of the triturated material.

The freezing-point depression of sap expressed under great pressure from the aerial portion of the plants studied, previously frozen at low temperatures, was practically the same as that of the material frozen directly, but greater if the pressure is not so great. The freezing-point depressions of the juice expressed from roots frozen at low temperatures were not consistent.

It was found necessary to take samples of vegetable material for freezing-point work at the same time of day or else protect the plants from light and retard transpiration, since the freezing-point depression of the leaves was found to increase from morning till noon and decrease again in the evening. It appeared that this change was due to the products of photosynthesis, as well as to the moisture content of the material which decreased from morning till noon and increased again in the evening.

The concentration of the solution in which roots of Canada field pea and wheat are grown is indicated by the freezing-point lowerings of the root tissues, but on the other hand, only rather wide variations are indicated by the freezing-point lowerings of the tops.

Changes in the concentration of the soil solution induced by the addition of salts may be detected by determining the freezing-point lowerings of the roots of the plants growing therein. It was found that the tops of the plants are far less sensitive to changes in the concentration of the soil solution.

The moisture content of soils is closely correlated with the freezing-point lowerings of the roots of the plants in contact with them, due in part at least to changes in the concentration of the soil solution so induced, but again the tops of the plants studied, under both greenhouse and field conditions, prove to be far less sensitive to soil moisture changes, at least until the critical water content is approached.

The indications are that the soil solution and the root sap of plants approach each other in concentration at or near the critical (low) water content of the soil.

The freezing-point lowering of a given crop growing in widely different soils of high water content were found to differ but slightly. On the other hand, different crops growing on the same soil, under similar conditions vary appreciably in this respect.

The field studies show that crops may be subjected to sudden and very wide variations in the concentration of the soil solution during the growing season.

During the progress of the work herein reported many soil problems have suggested themselves. Some of these being studied are, the optimum concentration of the soil solution for crop production, the effects of different treatments upon the concentration of the soil solution at very low water contents, as measured by the freezing-point lowerings of the roots of plants, reasons for the adaptation of a given soil to the production of certain crops, as well as the changes, if any, induced in the concentration of the soil solution by crop production.

The authors wish to thank Mr. I. C. Wheeting for making many of the determinations and otherwise assisting greatly in carrying on these investigations.

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