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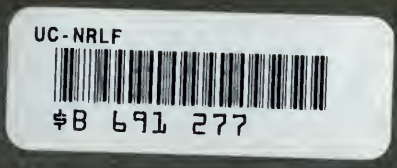

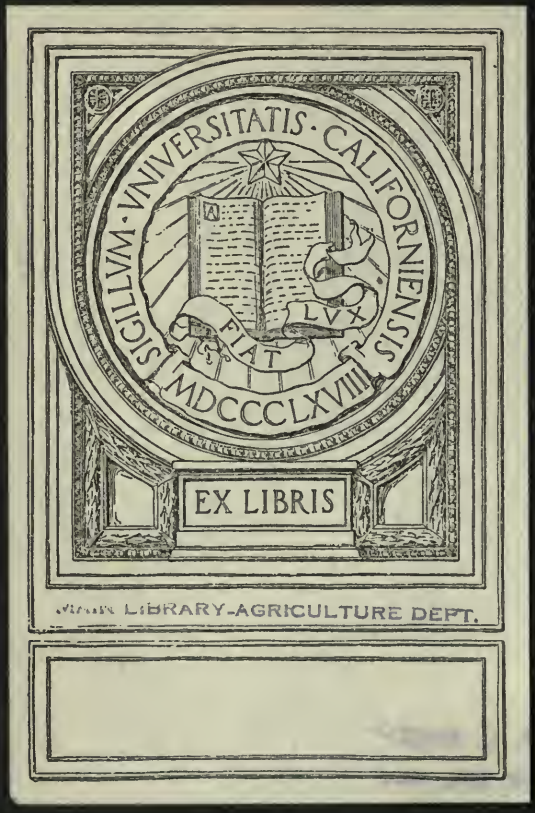

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A PLAN FOR COOPERATIVE RESEARCH ON THE SALT REQUIREMENTS OF REPRESENTATIVE AGRICULTURAL PLANTS

Prepared for a Special Committee

OF THE

DIVISION OF BIOLOGY AND AGRICULTURE

OF THE

NATIONAL RESEARCH COUNCIL

EDITED BY

BURTON E. LIVINGSTON

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SECOND EDITION
BALTIMORE
1919

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THE FIRST EDITION OF THIS PLAN WAS MIMEOGRAPHED, AND A HUNDRED COPIES WERE CIRCULATED. THIS SECOND EDITION IS ESSENTIALLY THE SAME IN CONTENT AS THE FIRST.

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UNIV. OF CALIFORNIA

PREFACE

During the war period the Division of Agriculture, Botany and Zoology of the National Research Council established a special committee to attempt the organization of a nation-wide cooperation among the research scientists interested in plant nutrition. The project is of fundamental importance, and is to be continued under the new Council. The purpose of this cooperation is to hasten the acquisition of definite knowledge regarding the salt requirements of a few representative agricultural plants, and it is hoped thus to accomplish in a small number of years what would usually require many decades. Experimenters are earnestly requested to further this work in every way possible, and it is hoped that at least part of the time of one research worker in each laboratory where this kind of work is carried on may be devoted to this project. If all of the time of one or more persons can be devoted to the work, of course, that would be still better for the progress of knowledge in this field.

The need for some well-established, correlated, quantitative knowledge of the salt nutrition of plants is clearly appreciated by all students of this important subject, as is also the present almost total lack of such knowledge. Our theories are incomplete and vague, and the experimentation on which they rest has not generally been such as to allow correlation between the different pieces of work. It is planned that the present cooperation by a large number of experimenters will soon furnish a great body of correlated information regarding the salt requirements of the plants studied; all workers in the project are urged to follow the methods described on the pages following this preface, to the end that all results obtained may be as truly comparable as possible. While each cooperator will of course be perfectly free to interpret and publish his results as he may see fit, the committee hopes to be able to bring all the contributions together from time to time as the work progresses, so as to build up rapidly a rather complete statement of the salt requirements of each plant that is included in the scheme.

It is clear that this project is a physiological one, and that the results obtained cannot be expected to furnish direct and immediate information as to the fertilizer requirements of these plants when grown on any agricultural soil; each soil offers its own set

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of problems to the agronomist, as does also each climate and each plant form. But it seems safe to predict that the correlated system of physiological knowledge that is to result from this cooperation will place in the hands of agronomists and agricultural chemists many valuable facts and principles. Upon these, with further experimentation in the field, may be built up a greatly improved system of fertilizer practice and crop rotation. The present project is therefore fundamental to the rational advance of agricultural science and practice.

The shortage of potassium in this country during war time emphasized the need that our knowledge of the best ways of using fertilizer salts in general should be increased and put on a definite basis as rapidly as possible. This, together with the high price of nitrogen-bearing fertilizer material, gave to this project some of the characteristics of a war-emergency problem, but the problem is exceedingly important and fundamental to agricultural development in general. A concentrated effort toward the building up of a reliable body of American scientific results in this field may be regarded as highly desirable from the standpoint of national welfare. Also, the fact of cooperation itself should benefit American science very greatly, and it may be hoped that this general method of advancing knowledge may eventually become much more common among democratic peoples than has been the case in the past. Aside from war-emergency matters, this is one of the aims set forth by the President of the United States in his executive order establishing the National Research Council on a permanent basis.

This project itself contemplates only physiological studies, carried on with water and sand cultures, thus avoiding many of the complications introduced when agricultural soils are involved. Besides determining as precisely as possible what are the most favorable total concentrations and sets of salt proportions for the various developmental phases of the plants studied, it is planned to include experimental studies of the relative degrees of susceptibility to fungus attack exhibited by the cultures in different solutions. It is also planned to obtain chemical analyses of the plants grown and to correlate these results with the characteristics of the nutrient media used.

The problem for any single plant is so complicated in itself, and the amount of logically planned and carefully carried-out experimentation required (before even tentative conclusions may be attained) is so great, that it seemed absolutely necessary at the start and for the present to restrict attention to a very few

forms of plants. The work has been begun with the *"Marquis"* variety of *spring wheat,* and an attempt will be made to advance our knowldege of the salt requirements of this plant as rapidly as possible. As a second plant, *soy bean* is to be employed, and work upon it may be begun immediately if workers prefer to deal with this plant rather than with wheat. In the beginning the cooperation is to be limited to these two plants. Other plants may be taken up when plans and methods have been perfected and when the work may be well enough in hand so that the co-operators may afford to leave the two plants just mentioned. All of the wheat and soy bean seed used will be supplied by this committee, so that all experimenters may be considered as dealing with the same complexes of internal conditions as these are presented in the resting seed. Arrangements have been made by which the salts employed by all cooperators may also be of the same lots. The standardized methods to be employed in the beginning, as outlined below, are based on those of Schreiner and Skinner, Tottingham, Shive, McCall, and Hibbard. For some references to the literature in this connection, see the list of citations following this preface.

Cooperators are asked to furnish the special committee with data, as the work progresses, and generally to keep the committee in touch with their work. It is of course understood that the committee will give proper publicity to the work, with due credit to all cooperators.

It is estimated that the materials and apparatus required by one worker for a year will not cost more than $300.00, supposing that some greenhouse space is available for winter work. Much of the needed apparatus is generally at hand in laboratories where studies in plant physiology are carried on.

Those who receive copies of this Plan are urged to look over the outline of the project, and let the chairman of the committee have their decisions at an early date, as to what they may be able to do in this cooperation.* It is desirable to have as large a representation as possible and it is hoped that many workers may feel that this is their project, and that they will do all they can to further it. The work falls readily into numerous sections of different magnitudes, so that a cooperator may devote only a small portion of his time to it and still obtain valuable results toward the general solution of the problem. For example, if a

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worker might devote only an hour or two each day to these experiments, the committee would be able to aid him in selecting a small number of solutions for comparison. Every little piece of careful experimentation (provided only that it fits in as a part of the general plan) will count in the general summation.

K. F. KELLERMAN,
WM. CROCKER,
B. E. LIVINGSTON (Chairman),

Special Committee on Salt Requirements of Representative Agricultural Plants, National Research Council, Division of Biology and Agriculture.

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REFERENCES TO SOME PAPERS DESCRIBING METHODS ON WHICH THE PLAN FOR THIS PROJECT IS BASED.*

1910. Schreiner, O., and J. J. Skinner.

Ratio of phosphate, nitrate and potassium on absorption and growth. Bot. Gaz. **50**: 1-30. 1910. **Idem.** Some effects of a harmful organic soil constituent. U. S. Dept. Agric. Bur. Soils Bull. 70. 1910.

1914. Tottingham, W. E.

A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133-245. 1914.

1915. Shive, J. W.

A study of physiological balance in nutrient media. Physiol. Res. **1**: 327-397. 1915.

1916. McCall, A. G.

The physiological balance of nutrient solutions for plants in sand cultures. Soil Sci. **2**: 207-253. 1916. **Idem.** The physiological requirements of wheat and soy beans growing in sand media. Proc. Soc. Prom. Agric. Sci. **1916**: 46-59. 1916.

1917. Hibbard, R. P.

Physiological balance in the soil solution. Michigan Agric. Exp. Sta. Techn. Bull. 40. 1917.

Shive, J. W.

A study of physiological balance for buckwheat grown in three-salt solutions. New Jersey Agric. Exp. Sta. Bull. 319. 1917.

1918. Livingston, B. E., and W. E. Tottingham.

A new three-salt nutrient solution for plant cultures. Amer. Jour. Bot. **5**: 337-346. 1918.

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*Of course a large number of papers might be mentioned in this list, as bearing in one way or another upon this project. From the point of view of the logical analysis of the problem the papers cited here will be specially valuable, and numerous other literature references may be obtained from them. Tottingham's bibliography will be found very useful in connection with the general proposition of controlled chemical environment as far as the root system of the plant is concerned.

McCall, A. G., and P. E. Richards.

Mineral food requirements of the wheat plant at different stages of its development. Jour. Amer. Soc. Agron. **10:** 127-134. 1918.

Shive, J. W., and W. H. Martin.

A comparison of salt requirements for young and for mature buckwheat plants in water cultures and sand cultures. Amer. Jour. Bot. **5:** 186-191. 1918. **Idem.** A comparative study of salt requirements for young and for mature buckwheat plants in solution cultures. Jour. Agric. Res. **14:** 115-175. 1918.

Schreiner, O., and J. J. Skinner.

The triangle system for fertilizer experiments. Jour. Amer. Soc. Agron. **10:** 225-246. 1918.

INTRODUCTION.

This project aims to test a large number of combinations of the necessary chemical elements, to find out what combinations give the most satisfactory growth of the plants considered. Different phases of the development of the plants are to be treated separately, to bring out any changes in the nutritional requirements that may supervene as growth proceeds. As many different complexes of climatic conditions as are practicable are to be tested, to find out in how far the salt requirements may depend upon climatic conditions. It is planned to find out just what sets of salt conditions give the best growth for each type of climatic complex.

The project thus contemplates a very thorough experimental study of the physiological possibilities of the plants dealt with. Just as the atomic weights of the chemical elements need to be known with considerable precision before their relations to their surroundings may be satisfactorily studied, so the physiological characteristics of agricultural plants need to be known before scientific agriculture may progress very far.

The cooperative feature of the project aims to secure a large body of comparable data as rapidly as possible. A large number of research workers acting simultaneously will be able to test the numerous possibilities in a comparatively short time, and the fact that all employ the same standard methods should make all of the results fit into one general whole.

The following outline, which is planned to be extended and improved later, has been elaborated through consultation with a large number of specialists in this sort of work. It is hoped that the essentials of the method here described will be followed, since likeness of method is the prime consideration in work of this sort. When alterations of the plan seem necessary the other cooperators should be informed and an agreement reached through the committee. Correspondence regarding the work should be addressed to Dr. B. E. Livingston, Laboratory of Plant Physiology, the Johns Hopkins University, Baltimore, Md.

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THE PLANTS TO BE USED.

The first and most important plant for the present purpose is wheat, and a supply of seed of the "Marquis" variety of spring wheat was obtained by purchase from Professor Leith, of the Wisconsin Agricultural Experiment Station. Cooperators will

receive seed from Baltimore, upon request. A new supply will be available in the fall of 1919.

The second plant to be studied is soy bean, and a supply of seed has been made available through the kindness of Mr. J. E. Metzger of the Maryland Agricultural Experiment Station. This seed may also be obtained from Baltimore, on request.

TWO METHODS OF CULTURE.

It is planned to carry out this elaborate series of physiological tests with both water and sand cultures, but it seems desirable to press forward somewhat more rapidly with the former; most workers may prefer to attack the water cultures first, since the operations are simpler here. Both methods are set forth below. Special details for soy bean and for the later phases of wheat are still to be elaborated and modifications of the outline here given may be required as the work progresses.

WATER CULTURE OF WHEAT.

Four Developmental Phases.

The development of the plant will be considered in four separate stages or phases, three of these being defined by the degree of development attained and the other by time duration. These phases are as follows:

1. *Germination Phase,* from beginning of soaking till the shoot is four centimeters high, measured from the seed to the tip of the shoot.

2. *Seedling Phase,* from the end of phase 1 *for a period of five weeks,* without regard to the size of the plant.

3. *Vegetative Phase,* from the end of phase 2 until the first appearance of flowering in the *controls.* [Controls are always in Shive's best solution for wheat seedlings, IR5C2, by 1/10-increments (1.75 atm.) ; see below.]

4. *Reproductive Phase,* from the end of phase 3 until maturity is reached by the best five cultures of the group of 20 or 21.

These phases should be adhered to with care, in order that all the parts of the work may be comparable. It may be that their definition will require alteration after the work is well in hand, but the definitions here given will serve for the beginning.

It is aimed first to find out what four solutions should be consecutively used to produce plants that are the best at the ends of all four stages of development. Thus, after the plan has been carried out we should be able to say that phase 1 should have

solution *a;* phase 2, solution *b;* phase 3, solution *c;* and phase 4, solution *d.* Many other possibilities are clearly in the prospect, but this plan appears to be best for the first study. It is not at all well known how the solution requirements for best growth may alter from one developmental phase to the next succeeding one, nor is it known what may be the most satisfactory method of dividing the growth period into partial periods to represent the phases. The scheme of employing these four phases and attempting to find the best solutions for each phase (the preceding phase or phases having had their own best solutions) is simple and readily practicable for making a start.

1. Germination Phase.

General Treatment of Seed. The supply of seed should be stored in a loosely closed container, with some access of air, and in a dry, cool place, free from laboratory gases, etc. The seed to be used in any test should be inspected, and obviously imperfect or otherwise apparently undesirable seeds should be discarded. Soak ten times as many seeds as the number of seedlings needed, for five or six hours in a glass vessel, with a volume of germination solution equal to twice their apparent volume. Then place them on the germination net.

The seeds should be uniformly distributed over the germination net. Since excretion and absorption by the seeds on the germinating net tend to alter the germinating solution, care should be taken that the seeds are not too crowded on the net. Germination is to be continued until the shoots reach a height of four centimeters, measured from the seed. A uniform series of seedlings is then to be selected and placed in the culture jars. The treatments for the four phases for wheat will now be presented. Later modifications may be required, especially for the last two phases.

Germination Apparatus and Method. For germinating the seeds the following scheme is to be followed, at least in its essentials. This is not necessarily the best plan conceivable, but it is *one* fairly good way, and it is thought to be well adapted to the various exigencies of a large number of laboratories and workers. Uniformity of method among the various cooperators is again to be emphasized here as absolutely requsite. Failure to adhere to the standard method would effectually prevent the different sets of results from being comparable. If modifications are planned, the committee should be informed in this regard.

The germination net consists of ordinary mosquito netting, thoroughly paraffined by dipping in melted "Parawax" (or other paraffin of equally high melting point). The net is to be tied as tightly as possible over the top of the germination jar. A new net should be used for each germination.

The germination jar is an ordinary 5-gallon stoneware jar, approximately 28 cm. in inside diameter and 34 cm. high, glazed inside and out. The germination net should be tied tightly over the opening of the jar, so that when the jar is filled with solution the water surface practically coincides with the plane of the net.

Insert in the jar a vertical glass tube, which will lie close to the jar wall and project a few centimeters above the net, through which it passes. This tube should be broken obliquely at its lower end, where it rests on bottom of jar. The upper end should be cut squarely off. The bore of the tube should be about a centimeter, or more. New solution is added through this tube, so as to be introduced at bottom of jar.

From a suitable support above, a glass thermometer is to be suspended vertically, so that its bulb lies just entirely below the net, extending through the latter at its center. This shows the temperature of the solution that lies about the seeds.

It is aimed to maintain the temperature for germination within about 2° C., and it will therefore be necessary generally to place the whole jar in a larger water-bath, to which cold or warm water may be added two or three times a day, according to the needs of the temperature control. A better form of control may of course be used; this method "by hand" is taken as the simplest and least expensive. An ordinary galvanized iron, wooden or fiber wash-tub is suitable for the bath in which the germination jar stands. The bath water should not reach as high as the edge of the netting where the latter projects over the edge of the jar.

The apparatus should stand in a lighted place, subject to the ordinary fluctuation of day and night, free from poison gases and laboratory fumes, but should not receive direct sunshine at any time. It should be so placed that the overflow will be drained away. Ordinary water is used for the bath; nutrient solution for the jar. Solution is added to the jar once a day during germination, and water is added to the bath as frequently as may be necessary to obtain the needed temperature control. In both cases there will of course be an overflow, the water solution from the jar rising through the net and passing over into the tub.

The jar is filled at the start with the solution to be used and two liters of new solution is added once each day, by means of a siphon or funnel, the new solution entering through the glass tube above mentioned. The same kind of solution is to be used throughout the germination period.

Tests on the relation of the nature of the solution and its temperature to rapidity of germination in the wheat to be employed have been carried out at Baltimore, and one of the best temperatures and sets of salt proportions for this wheat has proved to be 25° C. and Shive's solution R5C2 (0.175 atm.). The germination temperature is to be maintained at from 24° to 26° C., and the solution to be used is Shive's R5C2, in 1/10 "optimal" total concentration (osmotic value about 0.175 atm.). This solution has the following volume-molecular partial concentrations of the three salts; KH_2PO_4, 0.0018 mol.; $Ca(NO_3)_2$, 0.00052 mol.; $MgSO_4$, 0.0015 mol. No ferric phosphate is to be used in the solution for the germination phase of growth. This solution may safely be prepared, for stock, thirty times as concentrated as is required, so that 67 cc. of the stock solution *plus* two liters of water will give a rather close approximation to the needed germination solution.

This stock solution from which the germination solution is to be prepared by dilution (and from which the control solution for later phases of growth is also to be prepared, with the addition of $FePO_4$ in that case) thus has the following volume-molecular partial concentrations of the three salts (these values being thirty times the corresponding values given above);— KH_2PO_4, 0.054 mol.; $Ca(NO_3)_2$, 0.0156 mol.; $MgSO_4$, 0.045 mol. To prepare the two liters of germination solution required daily, add 67 cc. of this stock solution to two liters of water. The "error" is negligible and this quick method conserves time.

The seedlings to be placed in the culture jars for the seedling phase are to be selected for uniformity as to height (4 cm.) and general appearance. Since ten times as many seeds are to be placed on the net as are to be needed after germination, this selection should be as satisfactory as is possible with present knowledge.

It is convenient to select at once all the seedlings to be used (and some extra ones), lifting them from the germination net with care not to injure the roots nor to bring them into even momentary contact with the hands, table, etc. The seedlings thus selected are placed in one or more glass pans with germination solution 2 cm. deep, so that the roots are in the solution and

the shoots project above its surface. Then they are taken from these pans, one by one, and fixed in the prepared cork stoppers. They should not be placed in distilled water or tap water at all, but are thus kept in the germination solution until they are actually placed in the culture jars. Have the hands clean; paraffined forceps are useful.

2. Seedling Phase.

Culture Jars and Corks, and the Setting Up of Cultures. The culture jars used for the seedling phase are to be ordinarily glass fruit jars, of the "Mason" type, the quart size. It is essential only that the capacity be approximately correct and that the opening be suitable for the corks carrying the seedlings. One distinct advantage of the "Mason" jar is that it is supplied in three sizes (pint, quart and two-quart), all with the same size of opening; another advantage is that this jar is procurable practically everywhere in the United States, and at a low price. The tops supplied with the jars are not needed in this work.

Each jar is to be covered by a cylindrical jacket of opaque paper, white on the outside, to keep strong light from reaching the roots of the plants. It is desirable that the jacket be so arranged that it can be readily removed without disturbing the plants (for examination of the root systems, etc.). A good method is that employed by Shive (1915). A jacket that is dark-colored on the *outside* is not suitable, since absorption of radiant energy is undesirable.

The corks used for closing the jars and for supporting the seedlings should be of good quality, of the flat form, one-half inch thick and of a diameter to fit the opening of the jar used. They are to be thoroughly impregnated and thinly coated with paraffin, such as "Parawax." A very excellent arrangement for mounting the seedlings is that adopted by Tottingham (1914). Only five seedlings per jar will be used in the present cooperation. It is strongly urged that this arrangement be employed, for the sake of uniformity, but if another arrangement is used it should conform in the essentials. Those essentials are: that the five seedlings must be firmly held just above the seed in each case, with but slight pressure opposed to subsequent increase in the stem diameter in this region; that the jar be practically closed; that the stopper be protected from fungi; and that the technique of placing the seedlings be simple (so as to be rapid and to avoid undue disturbance of the delicate plantlets during the process of placing). Ready-prepared corks may be obtained

from the committee at cost,—perforated ones $4.00 per hundred, plain ones $3.50 per hundred. Corks require reparaffining from time to time if they show fungus growth. The same corks are to be used for later growth phases.

A good quality of ordinary cotton (batting) is best for fixing the seedlings in the corks; absorbent cotton is not desirable (it absorbs water much more readily than ordinary cotton). Use just enough cotton to hold the seedling securely in position, so that it will not slip downward. The cotton will become compressed as the stem enlarges. The cotton is to be kept dry.

Jars should be thoroughly washed, rinsed in distilled water, and dried (or each finally rinsed with the solution to be used therein) before using. Each is filled to a point 1 cm. below the lower surface of the cork. A non-perforated, paraffined cork is inserted (to reduce evaporation and other changes during the interim) and the jar is clearly marked with its solution number (wax pencil). When all the jars of a series are thus filled and marked, the work of placing the seedlings is taken up, the cork bearing the five seedlings now replacing the non-perforated cork that previously stoppered the jar. After each jar is supplied with seedlings its jacket is put in place. The jacket should also bear the number of the solution contained in its jar (marked in pencil).

The seedlings should be kept from direct sunlight and from laboratory gases or other disturbing influences throughout the process of changing from germination apparatus to jars, and the series of jars is to be placed in the experiment location as soon as all are ready. It is desirable that the placing of all the seedlings for any comparable series be completed as rapidly as possible, on the same day; the jars may be supplied with their solutions on the preceding day, but are then to be kept stoppered, as described above.

Any series may be set up either as a simple series or in duplicate, triplicate, etc., according to the number of cultures included, the time at the disposal of the experimenter, etc. In all cases the control culture should be in triplicate; i. e., three cultures in solution IR5C2 (1/10-increments, 1.75 atm.) are to be included in every series.

Renewal of Solutions, and Observations During the Five-Week Period of the Seedling Phase. The solution of each culture is to be renewed every 3½ days (nine renewals in the five-week period). Details regarding the renewals are given below, also

regarding the preparation of the solutions, the experiment location and exposure, and the records of aerial conditions.

Records are to be kept of visual observations made at the times the solutions are renewed, regarding differences that may be manifest among the various cultures. Attention should be given to the root systems as well as to the tops of the plants. It is possible that a culture showing the apparently most vigorous plants of a series at the end of the third week, for example, may not show the most vigorous plants at the end of the fifth week, etc., and these records of visual observation are planned to bring out such occurrences. Without such records important features may escape notice entirely. (On comparing cultures, etc., when measurements cannot be employed, see:—Free, E. E. Plant World **18**:249-256. 1915.

Plant Measurements at End of Five-Week Period. At the end of the seedling phase the following plant measurements are to be made:—

(1) Length of longest and shortest top in each culture, measured from the seed (or the position where it was attached) to the extreme tip of the plant.

(2) Fresh weight of tops for each culture as a whole. Cut off each main root at its junction with the stem and consider all that remains as the top of the plant. Cut tops into pieces as much as is necessary, and immediately place all tops from each single culture in a weighed test-tube, stoppering with rubber or paraffined cork stopper. Stopper is removed when weighing occurs. Weigh as soon as possible, and record fresh weight of tops for each culture as a whole. Calculate this value to represent 5 plants in every case where the entire 5 plants are not available.

(3) Dry weight of tops for each culture as a whole. After fresh weight has been obtained, dry the tops, at a lower temperature first and then at 102° C., and determine dry weight. It is well to place tubes in a desiccator on removal from drying oven and to stopper each tube as it is weighed, using the same weighed rubber or paraffined cork stopper for all tubes; this avoids having a large number of weighed stoppers. Of course other weighing vessels, etc., may be used; the method above suggested seems adequate and is simple and inexpensive.

(4) Dry weight of roots for each culture as a whole. Place all root systems from single culture together and press gently between sheets of blotting paper to remove most of the liquid.

Then dry in weighed test-tube, as for tops, and obtain the dry weight of roots. Other suitable methods may of course be used.

(5) Dry weight of entire plants for each culture as a whole. This datum is simply the sum of the corresponding dry weights of tops and roots.

(6) Record any differences that may be manifest, but not measurable in the above terms. Of course these observations are to be made before plants are removed from the jar and stopper, and the whole manipulation of getting the above measurements is to be carried out so as to prevent appreciable changes in fresh weight before this is measured. Treat each culture separately till its tops are in the stoppered tube, then proceed to next culture. Preserve the dry material in envelopes, properly marked, so that a reweighing or other future examination may be made if desirable.

3. Vegetative Phase.

Preparation of the Plants. Germination is to be carried out as for the seedling phase, and twice as many jars are to be set up for the seedling phase as will be needed for the vegetative phase. These cultures are to be carried through the seedling phase in the manner described for that phase, but ALL CULTURES ARE TO BE SUPPLIED WITH THE SAME SOLUTION, which is the one previously found to be best for the seedling phase. At the end of the five-week period there should be twice as many plants, all nearly alike, as are to be needed for the vegetative phase. Select five or ten plants from this lot to represent the average, and record for them the final measurements of the seedling phase, as stated above. From the remaining plants select a uniform lot for the vegetative phase. It will generally not be necessary to remove plants from their corks; the cork and its plants will simply continue in one of the cultures of the vegetative phase. All cultures are of course left in the solution used for the seedling phase until transferred to the particular solution to be used for that culture in the vegetative phase. Plants that are not needed are to be discarded after the series for the vegetative phase is entirely set up. Of course, duplicate, triplicate, etc., cultures may be employed; and triplicate control is to be included, as in the seedling phase.

Treatment During the Vegetative Phase. All the different solutions (see below) are to be tested for the vegetative phase, to find out what solution is best for this phase, as will have been done for the seedling phase. The procedure is the same as for

the seedling phase, with solutions renewed twice per week. It may be necessary to furnish the plants with mechanical support. The basis of the support should be a cylindrical wooden stake (¼-inch dowel is good) and care should be exercised that the leaves are not seriously crowded. The support described by Hibbard (1917) is one satisfactory form. The stake is set in the center of the cork in which the plants are arranged. This phase ends when flowers appear in the controls, but this statement may require modification as the work progresses.

Plant Measurements at End of Vegetative Period. These are to be the same as those made at end of seedling period. Of course visual observations on the plants are to be made from time to time through the vegetative period, just as in the case of the seedling period. Especially should the final records show observations on differences in inflorescence, for those cultures that show flowers.

4. *Reproductive Phase.*

Preparation of the Plants. Germination is to be carried out as for earlier phases and twice as many jars are to be set up for the seedling phase as will be needed for the reproductive phase. These cultures are all to be carried through the seedling phase (with the best solution for that phase) just as in preparation for the vegetative phase. Then a selection of one or two representative cultures is made, for records of plant measurements of the seedling phase, and the remainder are carried through the vegetative phase, but ALL WITH THE SAME SOLUTION, which has been found to be best for that phase. At the end of the vegetative phase one or two cultures are selected to represent the average, records are made of plant measurements from these for the vegetative phase, and the remainder furnish the selection for the reproductive phase. This selection is made just as in the case of the vegetative phase described above. The series may of course be in duplicate, etc.; and triplicate control is introduced as in earlier phases.

Treatment During the Reproductive Phase. All of the solutions (see below) are to be tested for the reproductive phase. The procedure is the same as for the two preceding phases, with renewal of solutions twice per week. The plants will probably need mechanical support. This phase continues till maturity is reached by the best plants of the series. The exact criteria to be used may receive more attention as the work proceeds; it is of course aimed to find out what set of salt proportions is best for

the reproductive phase, when the plants have previously been supplied with the respectively best solutions for the germination, seedling and vegetative phases.

Plant Measurements at End of Reproductive Period. These are to be generally the same as those made at the ends of the preceding periods, but it will be desirable to separate the grain produced from the rest of the tops in the present case. The dry weight of grain is to be determined, also the number of grains. Of course visual observations are to be made from time to time during the reproductive phase, with special attention given to flowering and fruiting.

Renewal of Solutions.

The solution placed in any culture jar as the beginning of an experiment is to be completely discarded and replaced by a fresh one of the same composition, after 3½ days, and this renewal of every solution is to occur at 3½-day intervals (twice per week, thus avoiding Sunday renewals) throughout the course of the experiment. The following points apply to all growth phases alike.

Before the renewals are to be made, a new set of jars are prepared, as at the beginning, each filled with its proper solution and properly marked and stoppered with non-perforated cork. Then these jars are placed near the series of cultures and each stopper, bearing its plants, is removed from its original jar and placed in the new jar of corresponding number. In making the change, care is of course to be taken not to injure the roots nor to disturb them more than is unavoidable. Finally, the opaque jackets are transferred to the new jars, and the old jars are emptied, the volume of the contents is recorded, and they are washed, to be used again at the next renewal.

The purpose of determining the volume of solution remaining in each jar at each renewal is to furnish data on the relative amounts of water absorbed by the various cultures. The volume of solution originally placed in each jar will of course be known, and this value *minus* the volume left after 3½ days will represent the volume absorbed in that period. The rate of absorption is approximately the same as the rate of transpiration for such plants as we deal with. Of course this volume measurement need not be of great precision; a plus or minus error of as much as 3 or 4 per cent. is probably allowable until results indicate necessity for greater precision. It should be added that a better method of dealing with this feature is to weigh each culture be-

fore and after each filling of the jar with fresh solution. It is desirable to employ the weighing method for such work as this, but experience suggests that many experimenters prefer the volumetric method. Either will serve our purpose, however. In either case the desideratum is to have a record of the approximate amount of solution removed from each jar during each three and one-half day period.

Experiment Location and Exposure.

The cultures are to be exposed generally in a greenhouse, but at some stations they may be out of doors. In any event, they are to receive the climatic light (with shade, as of painted glass, etc., only when this seems necessary in order that the plants may thrive), but they must not receive any rain. For the colder months at most stations the temperature will be artificially much above the climatic temperature. When artificial heat is not considered as necessary it will of course be omitted; in general, any greenhouse in which other plants are kept growing should be suitable for these cultures. It is planned to carry out these tests with a large number of climatic complexes, such as will be obtained by employing a large number of geographical locations and various seasons of the year at each station.

The special problem of uniform exposure to the aerial complex of conditions (uniform for all cultures of any comparable series) is of considerable importance, and it is hoped that all experimenters will use rotating tables. This point has been discussed by Shive (1915), who describes one way to build a rotating table for this sort of work. It is desirable that all cultures that are to be comparable should stand on the table in a *single* circle; two circles of them form two series with slightly different aerial surroundings, although the different cultures of any one circle are themselves comparable. (If a rotating table cannot be employed, the cultures should be shifted on the bench so as to pass through each position of exposure every few days; it is well to shift them daily according to a definite plan.)

Rotating tables built on motor-cycle wheels can be supplied by the Plant World, but it is probably best for each cooperator to superintend the building of his own. Tripod bases can be supplied at $15.00 apiece, just the casting. Five-ply wood circles, painted, four feet in diameter, can be supplied at $12.00 apiece, packing included. The bearing portion may be built of a bicycle hub, etc., in various ways that will suggest themselves, and second-hand wheels may perhaps be obtained in some places.

A satisfactory rotating bearing (with an insufficient reducing gear) is offered by Winfield H. Smith, Buffalo, N. Y., and the table top may be attached to this, which itself may be attached to a greenhouse bench or other suitable support. The same firm offers an excellent reducing gear (which is needed besides the one coming with the bearing, and which is of course needed with any table built on a bicycle wheel). A finer type of gear was recently supplied by the Eberbach Company, Ann Arbor, Mich. The electric motor for this work should be of a rating of about ¼ horse-power. It is to be remembered that the motor and table operate continuously, night and day, for many weeks or even months at a time, and proper lubrication is of course essential.

Records of Aerial Conditions.

The non-solution conditions will not be controlled in these tests; the complex of these will vary from hour to hour and from day to day for any series, and it will differ from season to season at the same station and from station to station at the same season. In order to secure a rough description of this complex for each experiment, four kinds of records are to be obtained. These have to do with (1) air temperature, (2) reading of white spherical porous-cup atmometer, (3) reading of black spherical porous-cup atmometer, and (4) duration of sunshine. Methods for these records are set forth below.

(1) The records are to show the maximum and minimum air temperature in shade at the experiment location for every day of each experiment. These records may be obtained by the use of a max.-min. thermometer (read daily after the occurrence of the maximum for the day), or they may be taken off from a thermograph record sheet. The data are to appear in terms of the centigrade scale.

(2), (3) Standardized black and white spherical atmometer cups, with simple mountings, will be furnished by the committee at cost ($10.00 for two whites and two blacks, with two simple mountings). Orders should be sent to Baltimore. The two instruments should stand on the rotating table, within the circular area left free by the peripheral row, or rows, of cultures. They should be operated with distilled water, the porous surfaces should be scrubbed with distilled water and a tooth-brush once a week, and they should be read weekly, always at the same hour of the day. Since water is apt to enter the instrument during the operation of scrubbing, this operation should take place *after*

the reading and *before* the final setting of the instrument for the next week's run. The best way to obtain readings is to *weigh* the entire instrument at the beginning and end of each weekly run. The weekly procedure is consequently as follows: weigh the instrument, scrub the sphere, wipe off what water clings to tube, stoppers and bottle, add water to bottle more than sufficient for the next run, and reweigh. The loss in weight by evaporation for each week constitutes the weekly reading, and five of these data for each type of instrument are needed for the five-week period of the seedling phase. These data will give evidence of the kind of evaporation and sunshine conditions to which the plants have been subjected.

At the end of a given series the spheres are to be dismounted and dried, wrapped in paper, and sent (in a *strong* container and by parcel post) to the Laboratory of Plant Physiology, Johns Hopkins University, Homewood, Baltimore, Md. They will be re-standardized, and the new coefficients will be sent to cooperators. The spheres will be returned, or other ones will be sent in their place (in case they seem injured). (On operation of porous-cup atmometers see:—Livingston, B. E., Atmometry and the porous-cup atmometer. Plant World **18:** 21-30, 51-74, 95-111, 143-149. 1915. Reprints may be procured from the Plant World, Tucson, Ariz. The second pair of spheres is to be used during the time required for restandardization.

(4) Sunshine records are to be obtained for the period of each experiment series, from the nearest U. S. Weather Bureau Station operating a Marvin sunshine-recorder, these records being in the form of the daily duration (hours) of sunshine, as shown by that instrument.

THE CULTURE SOLUTIONS FOR ALL PHASES.

Introduction.

It is obvious that there are actually an infinite number of different solutions that must needs be tested if we are to find out just what solution is the very best for a given plant and for a given growth phase. Of course our experimentation must merely approach finding this best solution and it must proceed by *sampling* the range of solution possibilities (or promising portions of that range), as it were. It is highly desirable that the sample solutions be selected, for the beginning, at uniform intervals throughout any promising range of possibilities, and that the intervals be not so broad as to let the best solution in the region (for which we are looking) escape being fairly represented by

one of the samples tested. On the other hand, the intervals between the solutions selected for test—out of the infinite number of possible ones— might be made so narrow that the large number of tests required would render the whole project hopeless. We cannot hope to test millions of different solutions, nor should we hope for much real progress if we were to test only a dozen, for instance. In arranging the scheme of solution to be first tested, as presented below, the judgments of a number of specialists in this general field have been combined. It must be remembered that the present plan does not pretend to lay out the experimentation excepting for the beginning of our work. We begin by sampling *a certain region only* of the whole infinite field of solution possibilities. Other regions may be attacked later.

We proceed on the general physiological principle that the best solutions for plant growth must always contain at least *some* of each of the seven chemical elements known to be essential for all plant activity: K, Ca, Mg, Fe, N, P. and S. It appears safe to say that it would be useless to test any solution not containing all of these elements, in a search for the best solution for any plant and growth phase. Consequently, under the guidance of our present knowledge, we do not need to deal with any solution that does not contain all of the seven recognized essential elements for higher plants in general.

Furthermore, experience has already shown that no solution may be expected to support good plant growth if it contains more than a small trace of *iron*. On this account, and because agriculture seldom meets with either a deficiency or an excess of iron as a source of trouble, we shall at first make no study of different partial concentrations of that element. We shall follow Tottingham in supplying iron as $FePO_4$ in the same, very small, amount to every solution employed after the germination phase. (For that phase the suuply of iron in the seed may safely be considered as sufficient.)

It is of course logically possible (perhaps even probable) that the very best solution for any plant and phase may eventually be found to contain still other elements besides the seven recognized essential ones. We shall ignore this proposition at the beginning of our work, however, and shall study only solutions containing just the six elements K, Ca, Mg, N, P, and S, in various proportions and in various total concentrations, besides a trace of Fe. Later plans will perhaps introduce some of the apparently most promising non-essential elements, such as Na, Cl, Si, Mn, etc.,

but it would be quite hopeless to attempt to bring these into consideration at the start, especially considering the limited number of cooperators available. It should be remarked that there will surely be a considerable amount of silicon in all the solutions tested (since uncoated glass containers are to be employed), but the amount of this element present may be considered as practically the same in all solutions. Like iron, it will be uniformly present in small amount. The same is true, to a degree, of sodium; and to a smaller degree of chlorine. But the traces of these three elements that may be present are ignored for the part of the project now being planned.

From these considerations it emerges that our present plans involve simply the testing of solutions containing the six elements that are essential in considerable quantities, with addition of a trace of the seventh essential element, Fe. As Shive (1915) has pointed out, the simplest way to get these six elements into solution is to prepare the solution from *three* salts; since three of the elements occur as kations and the other three occur in anions. It appears best, at the start, to employ N as the nitrate ion (NO_3), P as the di-hydrogen phosphate ion (H_2PO_4), and S as the sulphate ion (SO_4). Other carriers of these three elements may be studied later, such as nitrates, ammonium salts, mono-hydrogen phosphates, sulphites, etc. It is even probable that ammonium may need to be introduced for later growth phases of soy bean to get even presentable growth, but this matter is not now before us.

Of course it may be that no possible three-salt solution is best suited to the growth of a given plant in a given developmental phase; it is clear that there are many sets of element or ion proportions that cannot be obtained in a three-salt solution at all, and four-, five-, six-, etc., salt solutions must logically be brought into the experimental comparison before the very best possible six-ion solution may be established. It seems desirable, however, to make a very thorough study of the three-salt possibilities before moving forward to attack the much more complex types of solution with more than three salts. One type of such solution has been studied by Tottingham (1914) for young wheat plants. As our project goes forward, plans for later campaigns may be formulated, but an attempt at their consideration would be bootless at present.

The Three-Salt Solutions Characterized.

As Livingston and Tottingham have pointed out (Amer. Jour. Bot. **5**: 337-346. 1918. A reprint may be obtained from either

author), there are exactly six possible types of three-salt solutions that can be made to contain just these six ions, K, Ca, Mg, H_2PO_4, NO_3, and SO_4, and it is a rather thorough test of these six types that is contemplated for the first stage of the present project.

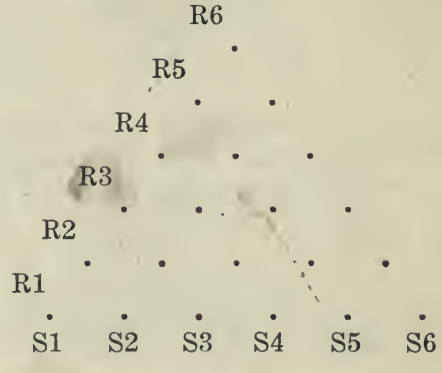
It should be added that there are three other essential elements, besides the ones mentioned above, that are always present in any of these solutions; namely, C, H, and O. The solution is mostly water, oxygen occurs as a solute and also in every one of the three anions employed, hydrogen occurs in the di-hydrogen phosphate ion, and CO_2 occurs as a solute (thus adding the element C). It may be that the oxygen atom in an ion will prove to be negligible in such work as this (it seems not to have been given serious consideration in any of the published discussions of nutrient solutions and fertilizers), but it is certain that the H-ion, as such, is of great importance in determining the physiological properties of nutrient solutions for plants. (See, for example:—Sörenson, S. P. L. Ueber die Messung und Bedeutung der Wasserstoffionenkonzentration bei biologischen Prozessen. Ergeb. Physiol. **12:** 393-532. 1912. Sharp, L. T., and D. R. Hoagland. Acidity and absorption in soils as measured by the hydrogen electrode. Jour. Agric. Res. **7:** 123-145. 1918. Plummer, J. K. Studies on soil reaction as indicated by the hydrogen electrode. Jour. Agric. Res. **12:** 19-31. 1918.)

The six possible types of three-salt solution are represented below, the arrangement being the same as that employed by Livingston and Tottingham (1918). On the basis of present knowledge the presence of carbon dioxide in these solutions may be ignored as without significant influence upon the plants.

I.	II.	III.	IV.	V.	VI.
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

Our first problem is to find (by actual test with each growth phase) the best set of volume-molecular proportions and the best total concentration for each type of solution. Now, there is clearly an infinite number of possible sets of proportions of any three things, and for each set of salt proportions of each of the six types there is an infinite number of total concentrations. We choose, from the very large series of different sets of salt

proportions possible for each solution type, just twenty-one; and we select them so as to be representative of the mathematically possible range of sets of proportions. This is accomplished by letting the volume-molecular partial concentration of each salt differ from solution to solution (in a series of twenty-one solutions, all with the same total concentration, measured osmotically) by increments of *one-eighth* of the total volume-molecular concentration. Previous workers in this field have mainly used increments of one-tenth, and have employed *osmotic* proportions instead of volume-molecular proportions. For each of the six types of solution (numbered in Roman numerals above) all the possible sets of salt proportions, with increments of one-eighth, are represented by the points in a triangular diagram, as first used in this sort of work by Schreiner and Skinner (U. S. Bur. Soils, Bull. 70, 1910. Also Bot. Gaz. **50**: 1-30. 1910.) and later by Shive, McCall, Hibbard. (See Shive—1915—for the general plan of such a diagram, but it is to be remembered that he used increments of one-tenth and thirty-six sets of proportions, while we employ increments of one-eighth and have only twenty-one sets.) In diagraming the solutions for the present project it is earnestly requested that all diagrams follow the same system. Let the base line for the potassium salt be the *base* of the triangle, and let that for the calcium salt be the left side. The right side will then be the base line for the magnesium salt. Each of these three base lines represents a row of solutions each having one-eighth of its total volume-molecular concentration due to the salt for which the line is named. The apex opposite this line represents a solution in which six-eighths are due to that salt. The volume-molecular proportions of all three salts are quickly determined for any solution represented on the diagram. The diagram is given herewith. To designate the solutions, the rows on the diagram are numbered from below upward, and the solutions are numbered in each row from left to right. To refer to a solution we first write the Roman numeral denoting the type (what three salts are used), then we write the row number (preceded by the letter R), then we write the solution number



in the row, preceded by S), and finally we state (in parentheses) the total concentration, in terms of atmospheres of osmotic pressure representing the calculated osmotic value of the solution in question. Examples are:—IR2S3 (1.00 atm.), IIIR1S2 (1.50 atm.), VR6S1 (1.65 atm.), etc. This form of notation is employed in the tables given below, and it is hoped that all cooperators will adhere strictly, in order to facilitate comparisons. (Previous writers have employed C in place of S, but it seems a little better to use S as standing for *solution* rather than C, denoting culture.)

Blank triangular diagrams, printed on sheets 8½x11 in., may be obtained from Baltimore at a price of $1.00 per hundred.

Aside from the kinds of salt entering into one of these solutions (solution type) there are four characteristics by which that solution may be distinguished: (1) volume-molecular salt proportions, (2) osmotic salt proportions (see Tottingham, 1914), (3) total volume-molecular concentration (proportional to the number of molecules, of all kinds, per liter), and (4) total osmotic concentration (taken to be proportional to the number of *particles*,—molecular groups, molecules and ions, of all kinds,—per litre; this last characteristic may be expressed in various ways, but the atmosphere will be the unit here used as a measure of the calculated osmotic value, or the calculated potential osmotic pressure (of which the solution is taken to be capable). On account of ionization (and probably hydration), nos. 1 and 2 do not vary proportionally to each other from solution to solution in our series; nor do nos. 3 and 4. It follows that if we plan our series of twenty-one solutions to vary by definite increments on the basis of no. 1 (volume-molecular salt proportions), then they must vary irregularly in respect to no. 2 (osmotic salt proportions). The variation cannot be regular by both criteria at once, and we have chosen the former as the one to be used. This criterion (no. 1) can be definitely stated for any of the solutions without any assumption regarding ionization, osmotic phenomena, etc. On the other hand, since the total concentration of a solution must act upon plants primarily in an osmotic way, the calculated *osmotic value* (no. 3, above) is here used for this measure of the main *physical* character of any solution. This usage involves calculation, and assumptions as to ionization, or direct measurements of the lowering of the freezing-point, but it seems desirable (at the start, and in spite of uncertainties) to compare all the twenty-one selected sets of salt proportions, keeping the calculated total osmotic value constant throughout

each series. The dilutions shown in the tables given below are based on freezing-point determinations made by Dr. Shive, especially for this work. Other plans of dilution (which cannot alter the volume-molecular salt proportions in any given case) may of course be employed. The osmotic values are to be considered as much less precise than the salt proportions.

In this general connection, it may be noted that actual *ionic* partial concentrations of the various salts in any of these three-salt solutions is at present impossible to determine (with the single exception of the hydrogen-ion, for which a method is of course available), and any mental picture of assumed values of the degrees of ionization of the various kinds of salt molecules must depend upon more or less probable assumptions, for the actual testing of which no methods have yet been devised. The physical chemistry of such three-salt solutions as these is still far beyond us, excepting in its most general aspects. But physiology and agriculture need not wait for the advance of physical chemistry in this connection; we aim to determine the *physiological* properties of our solutions (by plant tests) and merely to define our solutions in such a way that they may be reproduced at any time in the future, for advanced physical-chemical study, etc. Any solution is sufficiently defined for exact reproduction when the volume-molecular partial concentration is stated for each of the salts used.

For a beginning, we wish to compare the twenty-one different sets of salt proportions for each of the six types of solution and for the same total osmotic concentration throughout the entire series. The uniform total concentration adopted should be one that promises to be suitable for good growth of the plants, and, at the same time, it must be such that all of the solutions may be possible in this concentration without the formation of precipitate. The osmotic value of *1.00 atmosphere at 25° C.* is chosen as the index of this uniform total concentration. There are thus 126 different solutions (really only 123, see below) to be compared in the beginning, all having the same osmotic value (1.00 atm.), but all differing in other ways (salt proportions or kinds of salts used).

The formulas for the 126 solutions, and for the universal control solution (Shive's IR5C2, increments of 1/10, 1.75 atm.) are given in the accompanying tables, which have been prepared by Dr. Shive for this project. It will be noted that there is a separate table for each of the six types of solution and that each table presents the twenty-one different sets of volume-molecular salt

proportions for its own type. In each table, the first column shows the twenty-one solution numbers, the next three columns present the volume-molecular proportions of the three salts used, and the remaining three columns give the actual volume-molecular partial concentrations of the three salts—the latter always stated for a mixture having a total osmotic value of 1.00 atmosphere. The reader should be warned that the *relative* partial concentrations cannot be read vertically; for example, in table I it cannot be said that the volume-molecular partial concentration of KH_2PO_4 is the same in all solutions of row 1, etc., although the relative *proportional* values for this salt are all set down as unity for these solutions. The data of the last three columns of the table show how this comes about; the unit used in reckoning the relative proportions steadily becomes smaller as we proceed along the row from left to right (on the triangular diagram). This reduction is necessary on account of the phenomena of ionization, etc. (as evidenced when the complete solutions are subjected to the freezing-point determination), and on account of the desideratum that all solutions should have approximately the same osmotic value (1.00 atm.). But the relative salt proportions for each individual solution are stated correctly if simply read from left to right in the table; thus, for IR1S1, KH_2PO_4: $Ca(NO_3)_2$:$MgSO_4$::1:1:6, etc. It will be noted that the proportions of elements and ions may be read in a similar way, note being taken of the number of atoms or ions in each molecule considered. Thus, K:Ca:Mg :: 1:1:6; but PO_4: NO_3: SO_4:: 1:2:6, and H:N:S:: 2:2:6. The proportions of the three kations K, Ca, and Mg are read directly from the position of the solution on the triangular diagram for types I, III, V and VI, since none of the molecules used for these types gives more than a single one of these ions. For the other two types it must be remembered that there are two K ions in each molecule of K_2SO_4. (In this last consideration is will be noted that the term "ionic proportions" refers to atoms or atomic groups—Ca, PO_4, etc.—as *ions*, without regard to the actual degree of dissociation of the corresponding molecules. When we say that the ionic proportions of a solution are:—PO_4: SO_4: NO_3:: 1:2:6, we merely signify that these atomic groups *were placed* in the solution in these proportions, not implying at all that the *ionized* portion alone shows such proportions.)

TABLE I. SOLUTIONS OF TYPE I.

Partial volume-molecular concentrations and molecular proportions of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be used in stock solutions (mixed) without change in the molecular proportions, are obtained, in each case, by multiplying each value given in the table by the factor 3.50. One liter of each of these strongest stock solutions, properly diluted with distilled water, will make 3.50 liters of nutrient solution with an osmotic value of approximately 1.00 atm. at 25°C.

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
IR1S1	1	1	6	.0027	.0027	.0161
S2	1	2	5	.0025	.0049	.0123
S3	1	3	4	.0024	.0071	.0094
S4	1	4	3	.0022	.0089	.0067
S5	1	5	2	.0022	.0108	.0043
S6	1	6	1	.0020	.0122	.0020
R2S1	2	1	5	.0053	.0027	.0132
S2	2	2	4	.0049	.0049	.0099
S3	2	3	3	.0047	.0071	.0071
S4	2	4	2	.0045	.0090	.0045
S5	2	5	1	.0041	.0104	.0021
R3S1	3	1	4	.0076	.0025	.0101
S2	3	2	3	.0072	.0048	.0072
S3	3	3	2	.0068	.0068	.0045
S4	3	4	1	.0065	.0086	.0021
R4S1	4	1	3	.0099	.0025	.0074
S2	4	2	2	.0094	.0047	.0047
S3	4	3	1	.0090	.0068	.0022
R5S1	5	1	2	.0123	.0024	.0049
S2	5	2	1	.0118	.0047	.0023
R6S1	6	1	1	.0145	.0024	.0024
Control, Shive's R5C2-(1.75 atm.) *	3.77	1.09	3.14	.0180	.0052	.0150

* Shive's solution was planned on the basis of osmotic proportions and increments of 1/10 of the total osmotic value, so that the molecular proportions when stated as eighths are not whole numbers. On the present basis Shive's best solution is described as IR3.77 S1.09 (1.75 atm.), and its location is easily found on our triangular diagram.

TABLE II. SOLUTIONS OF TYPE II.

Partial volume-molecular concentrations and molecular proportions of K_2SO_4, $Ca(NO_3)_2$, and $Mg(H_2PO_4)_2$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be used in stock solutions (mixed) without change in the molecular proportions, are obtained, in each case, by multiplying each value given in the table by the factor 3.70. One liter of each of these strongest stock solutions, properly diluted with distilled water, will make 3.70 liters of nutrient solution with an osmotic value of approximately 1.00 atm. at 25°C. Instead of 3.70, the factor 3.00 is used, however (see Table VIII).

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	K_2SO_4	$Ca(NO_3)_2$	$Mg(H_2PO_4)_2$	K_2SO_4	$Ca(NO_3)_2$	$Mg(H_2PO_4)_2$
R1S1	1	1	6	.0019	.0019	.0118
S2	1	2	5	.0019	.0039	.0097
S3	1	3	4	.0019	.0059	.0078
S4	1	4	3	.0019	.0075	.0056
S5	1	5	2	.0019	.0094	.0037
S6	1	6	1	.0019	.0116	.0019
R2S1	2	1	5	.0038	.0019	.0096
S2	2	2	4	.0036	.0036	.0072
S3	2	3	3	.0036	.0054	.0054
S4	2	4	2	.0036	.0072	.0036
S5	2	5	1	.0036	.0091	.0018
R3S1	3	1	4	.0057	.0019	.0076
S2	3	2	3	.0056	.0037	.0056
S3	3	3	2	.0056	.0057	.0038
S4	3	4	1	.0056	.0075	.0019
R4S1	4	1	3	.0074	.0018	.0056
S2	4	2	2	.0074	.0037	.0037
S3	4	3	1	.0075	.0056	.0019
R5S1	5	1	2	.0094	.0019	.0037
S2	5	2	1	.0094	.0037	.0019
R6S1	6	1	1	.0112	.0019	.0019
Control, Shive's R5C2 (1.75 atm.)*	3.77	1.09	3.14	.0180	.0052	.0150

* See footnote to Table I.

TABLE III. SOLUTIONS OF TYPE III.

Partial volume-molecular concentrations and molecular proportions of KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be used in stock solutions (mixed) without change in the molecular proportions, are obtained, in each case, by multiplying each value given in the table by the factor 3.70 . One liter of each of these strongest stock solutions, properly diluted with distilled water, will make 3.70 liters of nutrient solution with an osmotic value of approximately 1.00 atm. at 25°C. Instead of 3.70, the factor 3.50 is used, however (see Table IX).

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	KNO_3	$Ca(H_2PO_4)_2$	$MgSO_4$	KNO_3	$Ca(H_2PO_4)_2$	$MgSO_4$
R1S1	1	1	6	.0027	.0027	.0165
S2	1	2	5	.0026	.0053	.0132
S3	1	3	4	.0024	.0073	.0098
S4	1	4	3	.0023	.0093	.0070
S5	1	5	2	.0021	.0106	.0042
S6	1	6	1	.0021	.0125	.0021
R2S1	2	1	5	.0054	.0027	.0135
S2	2	2	4	.0048	.0048	.0096
S3	2	3	3	.0045	.0067	.0067
S4	2	4	2	.0042	.0084	.0042
S5	2	5	1	.0041	.0103	.0020
R3S1	3	1	4	.0075	.0025	.0099
S2	3	2	3	.0070	.0047	.0070
S3	3	3	2	.0067	.0067	.0045
S4	3	4	1	.0064	.0086	.0021
R4S1	4	1	3	.0099	.0025	.0074
S2	4	2	2	.0093	.0047	.0047
S3	4	3	1	.0085	.0064	.0021
R5S1	5	1	2	.0125	.0024	.0048
S2	5	2	1	.0113	.0045	.0023
R6S1	6	1	1	.0139	.0023	.0023
Control, Shive's R5C2 (1.75 atm.)*	3.77	1.09	3.14	.0180	.0052	.0150

* See footnote to Table I.

TABLE IV. SOLUTIONS OF TYPE IV.

Partial volume-molecular concentrations and molecular proportions of K_2SO_4, $Ca(H_2PO_4)_2$, and $Mg(NO_3)_2$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be used in stock solutions (mixed) without change in the molecular proportions, are obtained, in each case, by multiplying each value given in the table by the factor 4.20. One liter of each of these strongest stock solutions, properly diluted with distilled water, will make 4.20 liters of nutrient solution with an osmotic value of approximately 1.00 atm. at 25°C. Instead of 4.20, the factor 3.00 is used, however (see Table X).

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	K_2SO_4	$Ca(H_2PO_4)_2$	$Mg(NO_3)_2$	K_2SO_4	$Ca(H_2PO_4)_2$	$Mg(NO_3)_2$
R1S1	1	1	6	.0018	.0018	.0108
S2	1	2	5	.0018	.0036	.0092
S3	1	3	4	.0019	.0056	.0075
S4	1	4	3	.0019	.0075	.0056
S5	1	5	2	.0019	.0093	.0037
S6	1	6	1	.0019	.0113	.0019
R2S1	2	1	5	.0037	.0018	.0091
S2	2	2	4	0.037	.0037	.0074
S3	2	3	3	.0037	.0056	.0056
S4	2	4	2	.0038	.0077	.0038
S5	2	5	1	.0038	.0097	.0019
R3S1	3	1	4	.0056	.0019	.0075
S2	3	2	3	.0056	.0037	.0056
S3	3	3	2	.0056	.0056	.0038
S4	3	4	1	.0057	.0075	.0019
R4S1	4	1	3	.0076	.0019	.0057
S2	4	2	2	.0077	.0039	.0039
S3	4	3	1	.0078	.0059	.0019
R5S1	5	1	2	.0097	.0019	.0039
S2	5	2	1	.0098	.0039	.0019
R6S1	6	1	1	.0116	.0019	.0019
Control, Shive's R5C2 (1.75 atm.) *	3.77	1.09	3.14	.0180	.0052	.0150

* See footnote to Table I.

TABLE V. SOLUTIONS OF TYPE V.

Partial volume-molecular concentrations and molecular proportions of KNO_3, $CaSO_4$, and $Mg(H_2PO_4)_2$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be employed in the nutrient solutions of this series are those actually given here.

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	KNO_3	$CaSO_4$	$Mg(H_2PO_4)_2$	KNO_3	$CaSO_4$	$Mg(H_2PO_4)_2$
R1S1	1	1	6	.0019	.0019	.0115
S2	1	2	5	.0021	.0041	.0103
S3	1	3	4	.0021	.0061	.0081
S4	1	4	3	.0021	.0088	.0067
S5	1	5	2	.0023	.0104	.0041
[S6*	1	6	1	.0024	.0143	.0024]
R2S1	2	1	5	.0041	.0021	.0104
S2	2	2	4	.0042	.0042	.0083
S3	2	3	3	.0044	.0067	.0067
S4	2	4	2	.0043	.0085	.0043
S5	2	5	1	.0048	.0121	.0025
R3S1	3	1	4	.0065	.0023	.0086
S2	3	2	3	.0066	.0044	.0066
S3	3	2	2	.0072	.0072	.0048
S4	3	4	1	.0074	.0098	.0025
R4S1	4	1	3	.0086	.0022	.0065
S2	4	2	2	.0091	.0046	.0046
S3	4	3	1	.0094	.0071	.0024
R5S1	5	1	2	.0112	.0023	.0045
S2	5	2	1	.0113	.0045	.0023
R6S1	6	1	1	.0139	.0024	.0024
Control, Shive's R5C2 (1.75 atm.)**	3.77	1.09	3.14	.0180	.0052	.0150

* The solubility of $CaSO_4, 2H_2O$ is approximately .0140 m. Solution R1S6 is therefore not possible with an osmotic value of 1.00 atm.

** See footnote to Table I.

TABLE VI. SOLUTIONS OF TYPE VI.

Partial volume-molecular concentrations and molecular proportions of KH_2PO_4, $CaSO_4$ and $Mg(NO_3)_2$ in 21 solutions all having a calculated osmotic value of approximately 1.00 atm. at 25°C., but differing (by increments of ⅛) in salt proportions.

The highest partial volume-molecular concentrations of the salts of this table, that may be employed in the nutrient solutions of this series are those actually given here.

Solution number.	Molecular Proportions.			Partial Volume-molecular Concentrations.		
	KH_2PO_4	$CaSO_4$	$Mg(NO_3)_2$	KH_2PO_4	$CaSO_4$	$Mg(NO_3)_2$
R1S1	1	1	6	.0020	.0020	.0116
S2	1	2	5	.0021	.0041	.0102
S3	1	3	4	.0022	.0065	.0086
S4	1	4	3	.0023	.0091	.0069
S5	1	5	2	.0024	.0120	.0048
[S6*	1	6	1	.0026	.0155	.0026]
R2S1	2	1	5	.0040	.0021	.0101
S2	2	2	4	.0044	.0044	.0087
S3	2	3	3	.0050	.0070	.0070
S4	2	4	2	.0053	.0107	.0053
[S5*	2	5	1	.0054	.0136	.0028]
R3S1	3	1	4	.0062	.0021	.0082
S2	3	2	3	.0068	.0045	.0068
S3	3	3	2	.0073	.0073	.0048
S4	3	4	1	.0079	.0104	.0027
R4S1	4	1	3	.0089	.0023	.0067
S2	4	2	2	.0093	.0047	.0047
S3	4	3	1	.0103	.0078	.0026
R5S1	5	1	2	.0120	.0024	.0048
S2	5	2	1	.0130	.0052	.0027
R6S1	6	1	1	.0146	.0025	.0025
Control, Shive's R5C2 (1.75 atm.)**	3.77	1.09	3.14	.0180	.0052	.0150

*The solubility of $CaSO_4, 2H_2O$ is approximately .0140 m. Solutions R1S6 and R2S5 are therefore not possible with an osmotic value of 1.00 atm.

** See footnote to Table I.

Preparation of the Solutions.

Introduction. The Baker Chemical Company, of Phillipsburg, N. J., has very kindly agreed to prepare the salts needed for this cooperation in special lots, and at cost, so that all of any given salt will be of the same lot. These salts will be put up in sealed bottles with a label to indicate this project of the National Research Council, and the analysis (including the mean water content) will be shown on the label. These salts may be obtained from the above-named manufacturers by specifying that they are needed for the Plant Nutrition Project of the National Research Council. At the outset of our work salts from other lots will have to be used, since it will take some time for the various laboratories in this cooperation to obtain the special salts. Orders should be placed as soon as possible, and it will help the work if the committee is informed by sending to Baltimore a memorandum of the salts ordered when the order is sent to the manufacturer. Cooperators are asked to do this.

The nutrient solutions are to be prepared by adding, in each case, measured amounts of the single-salt stock solutions to a measured amount of distilled water. The first operation is, consequently, to make up the single-salt stock solutions.

The Single-Salt Stock Solutions. These should be made up so as to have the following volume-molecular concentrations. They will keep for a long time and should be tightly stoppered and preserved in darkness.

KNO_3,	... 1.00 vol.-mol.	$CaSO_4 \cdot 2H_2O$,	... 0.013 vol.-mol.
KH_2PO_4,	... 0.10 vol.-mol.	$Mg(NO_3)_2 \cdot 6H_2O$,	... 1.00 vol.-mol.
K_2SO_4,	... 1.00 vol.-mol.	$Mg(H_2PO_4)_2$,	... 0.10 vol.-mol.
$Ca(NO_3)_2 \cdot 4H_2O$,	... 1.00 vol.-mol.	$MgSO_4 \cdot 7H_2O$,	... 1.00 vol.-mol.
$Ca(H_2PO_4)_2$,	... 0.40 vol.-mol.		

The amount of salt to be used is weighed out and placed in distilled water in a volumetric flask or other suitable container, being shaken from time to time until solution is complete after which the solution is made up to required volume by adding water. Heat may be applied to hasten solution in some cases, but it would produce decomposition in others. It is best to dissolve all salts at room temperature, excepting those noted below, for which lower temperature is needed.

The three phosphates should be dissolved at temperatures below 20° C., and calcium sulphate would be dissolved at a temperature below 15° C. Its solubility limit is lower with

higher temperature and the concentration required (0.013 vol.-mol.) is almost at the limit for ordinary temperatures. If the larger crystals that are left when solution is nearly complete are crushed with a glass rod the completion of the operation is hastened.

It is well to have a chemical determination (analysis for one element) made of each single-salt stock solution (or at least of the first lot of solution made from a given bottle of salt) so as to determine whether the concentration called for is actually attained.

The Mixed Stock Solutions and the Nutrient Solutions Themselves. To prepare any mixed stock solution, find the three partial molecular salt concentrations for that particular solution (tables I-VI) and multiply each of these three values by the factor given for this purpose. This factor is *unity* for solution types V and VI; it is 3.50 for types I and III, and 3.0 for types II and IV. From the three values thus obtained (and from the molecular concentration of each of the three corresponding single-salt solutions) calculate the number of cubic centimeters of each single-salt stock solution needed for making a liter of the mixed stock solution in question (or several liters; for simplicity these directions are made to read for a single liter). Add the three numbers thus secured and subtract the sum from 1000, to give the number of cubic centimeters of water required. Place this amount of water in a suitable bottle and proceed to add the proper amount of each of the three single-salt solutions, beginning with the least concentrated of these and ending with the most concentrated, and shake thoroughly to insure mixing as the liquid runs in from the burette. Of course other procedures may be followed; this one is simple and requires no large volumetric flasks, but it does require the careful measuring of the three single-salt solutions and of the water. Throughout all making of solutions it will be well to estimate the range of error introduced and to keep record of this for each step of the proceeding. (Thus, 1 cc. as actually used may be, perhaps, anything between 0.98 and 1.02 cc., etc. This error will of course depend upon the burettes used, the temperature variations, the care exercised by the operator, etc.)

It will be noted that the mixed stock solutions are more concentrated than the corresponding nutrient solutions themselves (as prepared to have osmotic values of 1.00 atm.), excepting for types V and VI. In these two cases the mixed stock solutions *are* the 1.00-atm. nutrient solutions themselves.

All stock solutions are to be preserved, tightly stoppered, in darkness. The nutrient solutions to be actually employed in the cultures are to be prepared, with proper dilution, from these mixed stock solutions. The nutrient solutions may, of course, have any calculated osmostic value below that of the mixed stock solution. It is planned to begin the work using calculated osmotic values of 1.0 atm. for all solutions and the data of tables I-VI all refer to this value. The nutrient solutions themselves may be preserved, tightly stoppered, in darkness (to prevent algal growth, etc.), but it is undesirable to keep them too long, since alterations might possibly occur. It seems safe to preserve them as long as five or six weeks, but a more frequent preparation of new ones is probably desirable. There seems to be no doubt that the stronger mixed stock solutions (types I-IV) will keep indefinitely in darkness, as will also the single-salt stock solutions. If solutions are preserved for very long periods the solubliity of the glass of the containers may become a considerable feature. If the bottles are internally paraffined this possibility is largely removed.

Supplementary Tables. Tables VII-XII give the amounts of water and of each of the requisite single-salt stock solutions that are needed to make a liter of each of the most concentrated nutrient solutions for each of the six different solution types. For example, solution IR1S1 (table VII) is made by placing 924.7 cc. of water in a suitable container and then adding to it, with shaking, first 9.45 cc. of molecular KH_2PO_4, then 9.45 cc. of molecular $Ca(NO_3)_2$, and finally 56.4 cc. of molecular $MgSO_4$. Of course every single-salt stock solution is always employed with the concentration shown in parentheses below the formula for that salt in these tables. (See page 36 of this Plan.) The stock nutrient solutions of types V and VI have an osmotic value of 1.0 atmosphere, while those of types I-IV are more concentrated. The solutions of types V and VI cannot be generally made with higher concentrations than this value of 1.00 atm. From tables VII-X and from page 36 it is clear that the stock nutrient solutions of types I and III are planned to be made 3.5 times—and those of types II and IV 3.0 times—as concentrated as will be needed for solutions having an osmotic value of 1.00 atmosphere. Of course other procedures may be followed in making the nutrient solutions actually used, but this plan supposes that the stock nutrient solutions will be prepared as here set forth, using the factor 1.0 for types V and VI, 3.5 for types I and III, and 3.0 for types II and IV. (See p. 37 of this Plan.)

Solutions VR1S6, VIR1S8, and VIR2S5 are not possible with an osmotic value of 1.00 atm.; they cannot be tested except in series having a lower total concentration than this.

TABLE VII.

Supplementary for Solutions of Type I.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each concentrated stock nutrient solution of type I. The stock nutrient solutions thus made are each 3.5 times as concentrated as the corresponding nutrient solution having an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	KH_2PO_4 (1.0m.)	$Ca(NO_3)_2$ (1.0m.)	$MgSO_4$ (1.0m.)
IR1S1	924.7	9.45	9.45	56.4
S2	931.1	8.75	17.15	43.0
S3	935.85	8.4	24.85	32.9
S4	937.7	7.7	31.15	23.45
S5	939.45	7.7	37.8	15.05
S6	943.3	7.0	42.7	7.0
R2S1	925.8	18.55	9.45	46.2
S2	931.05	17.15	17.15	34.65
S3	933.85	16.45	24.85	24.85
S4	937.0	15.75	31.50	15.75
S5	941.9	14.35	36.4	7.35
R3S1	929.3	26.6	8.75	35.35
S2	932.8	25.2	16.80	25.20
S3	936.55	23.8	23.80	15.75
S4	939.80	22.75	30.10	7.35
R4S1	930.7	34.65	8.75	25.9
S2	934.2	32.9	16.45	16.45
S3	937.0	31.50	23.80	7.7
R5S1	931.4	43.05	8.4	17.15
S2	934.2	41.3	16.45	8.05
R6S1	932.45	50.75	8.4	8.4
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

TYPE VIII.

Supplementary for Solutions of Type II.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each concentrated stock nutrient solution of type II. The stock nutrient solutions thus made are 3.0 times as concentrated as the corresponding nutrient solution having an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	K_2SO_4 (0.4m.)	$Ca(NO_3)_2$ (1.0m.)	$Mg(H_2PO_4)_2$ (0.1m.)
IIR1S1	626.05	14.25	5.7	354.0
S2	683.05	14.25	11.7	291.0
S3	734.05	14.25	17.7	234.0
S4	795.25	14.25	22.5	168.0
S5	846.55	14.25	28.2	111.0
S6	893.95	14.25	34.8	57.0
R2S1	677.8	28.5	5.7	288.0
S2	746.2	27.0	10.8	216.0
S3	794.8	27.0	16.2	162.0
S4	843.4	27.0	21.6	108.0
S5	891.7	27.0	27.3	54.0
R3S1	723.55	42.75	5.7	228.0
S2	778.9	42.0	11.1	168.0
S3	826.9	42.0	17.1	114.0
S4	878.5	42.0	22.5	57.0
R4S1	771.1	55.5	5.4	168.0
S2	822.4	55.5	11.1	111.0
S3	869.95	56.25	16.8	57.0
R5S1	812.8	70.5	5.7	111.0
S2	862.2	70.5	11.1	57.0
R6S1	853.3	84.0	5.7	57.0
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

TABLE IX.

Supplementary for Solutions of Type III.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each concentrated stock nutrient solution of type III. The stock nutrient solutions thus made are each 3.5 times as concentrated as the corresponding nutrient solution having an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	KNO_3 (1.0m.)	$Ca(H_2PO_4)_2$ (0.1m.)	$MgSO_4$ (1.0m.)
IIIR1S1	838.3	9.45	94.5	57.75
S2	759.2	9.1	185.5	46.2
S3	701.8	8.4	255.5	34.3
S4	642.9	8.05	325.5	24.50
S5	606.8	7.35	371.1	14.70
S6	547.8	7.35	437.5	7.35
R2S1	839.3	18.90	94.5	47.25
S2	781.6	16.80	168.0	33.60
S3	726.3	15.75	234.5	23.45
S4	675.6	14.70	294.0	14.70
S5	618.	14.35	360.6	7.00
R3S1	851.5	26.25	87.5	34.7
S2	786.5	24.50	164.5	24.5
S3	726.3	23.45	234.5	15.75
S4	669.2	22.40	301.0	7.35
R4S1	851.9	34.7	87.5	25.9
S2	786.5	32.55	164.5	16.45
S3	738.9	29.75	224.0	7.35
R5S1	855.4	43.75	84.0	16.80
S2	794.9	39.55	157.5	8.05
R6S1	862.8	48.65	80.5	8.05
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

TABLE X.

Supplementary for Solutions of Type IV.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each concentrated stock nutrient solution of type IV. The stock nutrient solutions thus made are each 3.0 times as concentrated as the corresponding nutrient solution having an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	K_2SO_4 (0.4m.)	$Ca(H_2PO_4)_2$ (0.1m.)	$Mg(NO_3)_2$ (1.0m.)
IVR1S1	900.1	13.5	54.0	32.4
S2	850.9	13.5	108.0	27.6
S3	795.25	14.25	168.0	22.5
S4	743.95	14.25	225.0	16.8
S5	695.75	14.25	279.0	11.1
S6	642.05	14.25	339.0	5.7
R2S1	892.45	26.25	54.0	27.3
S2	840.55	26.25	111.0	22.2
S3	788.95	26.25	168.0	16.8
S4	729.1	28.5	231.0	11.4
S5	674.8	28.5	291.0	5.7
R3S1	878.5	42.0	57.0	22.5
S2	830.2	42.0	111.0	16.8
S3	778.6	42.0	168.0	11.4
S4	726.6	42.7	225.0	5.7
R4S1	868.9	57.0	57.0	17.1
S2	813.5	57.8	117.0	11.7
S3	758.8	58.5	177.0	5.7
R5S1	858.6	72.7	57.0	11.7
S2	803.8	73.5	117.0	5.7
R6S1	849.6	87.0	57.0	5.7
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

TABLE XI.

Supplementary for Solutions of Type V.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each nutrient solution of type V, with an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	KNO_3 (1.0m.)	$CaSO_4$ (0.013m.)	$Mg(H_2PO_4)_2$ (0.1m.)
VR1S1	736.9	1.9	146.2	115.0
S2	579.6	2.1	315.4	103.0
S3	447.7	2.1	469.2	81.0
S4	254.0	2.1	676.9	67.0
S5	156.7	2.3	800.0	41.0
[S6*	.....	2.4	1100.0	24.0]
R2S1	730.4	4.1	161.5	104.0
S2	582.8	4.2	330.0	83.0
S3	413.3	4.3	515.4	67.0
S4	298.8	4.4	653.8	43.0
S5	39.4	4.8	930.8	25.0
R3S1	730.6	6.5	176.9	86.0
S2	589.0	6.6	338.4	66.0
S3	391.0	7.2	553.8	48.0
S4	214.3	7.4	753.8	25.0
R4S1	757.2	8.6	169.2	65.0
S2	591.1	9.1	353.8	46.0
S3	420.5	9.4	546.1	24.0
R5S1	766.9	11.2	176.9	45.0
S2	619.6	11.3	346.1	23.0
R6S1	777.5	13.9	184.6	24.0
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

* Solution VR1S6 cannot be made with a total concentration value of 1.0 atm.

TABLE XII.

Supplementary for Solutions of Type VI.

Volumes (cubic centimeters) of distilled water and of the single-salt stock solutions required to make one liter of each nutrient solution of type VI, with an osmotic value of 1.00 atmosphere.

Solution number.	H_2O	KH_2PO_4 (1.0m.)	$CaSO_4$ (0.013m.)	$Mg(NO_3)_2$ (1.0m.)
VIR1S1	832.6	2.0	153.8	11.6
S2	672.3	2.1	315.4	10.2
S3	489.2	2.2	500.0	8.6
S4	290.8	2.3	700.0	6.9
S5	69.7	2.4	923.1	4.8
[S6*	.....	2.6	1192.3	2.6]
R2S1	824.4	4.0	161.5	10.1
S2	648.5	4.4	338.4	8.7
S3	450.0	4.6	538.4	7.0
S4	166.3	5.3	823.1	5.3
[S5*	.....	5.4	1046.1	2.8]
R3S1	824.1	6.2	161.5	8.2
S2	640.3	6.8	346.1	6.8
S3	426.5	7.3	561.4	4.8
S4	189.4	7.9	800.0	2.7
R4S1	807.5	8.9	176.9	6.7
S2	624.5	9.3	361.5	4.7
S3	387.1	10.3	600.0	2.6
R5S1	798.6	12.0	184.6	4.8
S2	584.3	13.0	400.0	2.7
R6S1	790.6	14.6	192.3	2.5
Control, R3.77S1.09 (1.75 atm.)	961.8	18.0	5.2	15.0

* Solutions VIR1S6 and VIR2S5 cannot be made with a total concentration value of 1.0 atm.

Iron in the Nutrient Solutions. The source of iron is to be added (as ferric phosphate) to each nutrient solution *after it has been placed in the culture jar,* in every case. The bottle containing this phosphate is first to be thoroughly shaken, so as to furnish a uniform suspension, and then a sufficient amount of the suspension is to be transferred to the culture jar (with a 1-cc. pipette), so as to add approximately three mg. of the precipitate to each quart jar. After this addition the jars are ready for the plants.

The preparation of the precipitated $FePO_4$ should proceed as follows: In a bottle with capacity of 2 liters or more place 500 cc. of a 0.04 vol.-mol. solution of KH_2PO_4 and add thereto, with agitation, a weak solution of ferric nitrate [$Fe(NO_3)_3$], continuing the addition until no more precipitate is produced. There should thus be formed about 0.02 gram-molecule of $FePO_4$, which should remain in suspension for considerable time, eventually settling to the bottom of the bottle.

Now fill the bottle nearly full of distilled water, shake thoroughly and stand aside till nearly all the liquid is clear. Carefully siphon off the clear liquid without loss of the precipitate, and repeat the filling, shaking, settling and decanting process several times, to wash the $FePO_4$ free from KNO_3 and any excess of $Fe(NO_3)_3$ that was originally added. The washing should be thorough, so that 1 cc. of the wash-water, evaporated at from 50° to 60° C., in a porcelain dish, shows no residue. Now transfer all the precipitate, with its water, to a graduated liter-flask and add distilled water to make a liter. Preserve in a tightly stoppered bottle. When shaken thoroughly the suspension thus formed should contain approximately 0.02 g.-mol. of $FePO_4$, about 3 g. per liter, or 3 mg. per cubic centimeter. It will be well to prepare enough $FePO_4$ to last for a year or two of this work.

Enough of the uniform suspension is to be added to each quart jar at each filling to give 3 mg. of $FePO_4$. Of course it will not dissolve appreciably, but it will furnish a constant and adequate supply of iron. This material is not to be added to the germination solution, however, as has been said.

Repetitions of the Experiments. It is planned that all of the sets of salt proportions (excepting the three that are impossible with that concentration) will be tested at least once with an osmostic value of 1.00 atm. The tests are to be carried out by solution types, so that different cooperators may be working with different types at the same time. The preliminary experimentation for any cooperator may thus consist in the testing of

one set of 19, 20 or 21 cultures (and the triplicate control). As soon as a type group has been tested once, the best seven solutions are to be selected, and all future work in this part of this project is to be confined to these best seven sets of salt proportions. This feature of the plan soon eliminates two-thirds of the solutions, and it is based on the assumption that the best seven solutions in any experiment will probably include the single *very best* solution of that type in any other experiment, no matter when or where such other experiment may be carried out. While climatic conditions may shift the position of the best physiological balance of the entire group in the series of the best seven sets of salt proportions, it is at least improbable that they will shift this position to one outside of this series of seven. If it should develop that none of the solutions of some type give even fair growth—as compared to the control—then the whole of that type may be eliminated. It is thus seen that the repetition will deal with at most no more than forty-two different sets of salt proportions. These repetitions are to be carried out repeatedly and under a great variety of climatic complexes, and it is really with these forty-two (or fewer) sets of salt proportions that the present phase of our campaign is to deal..

When the repetitions are taken up, other total concentrations are to be introduced, besides the one having an osmotic value of 1.00 atm. Detailed plans in this connection may be postponed till later, however. Plans for dealing with various logically possible complications in other aspects of the research may also be deferred till such complications actually arise from experimental results.

SAND-CULTURES OF WHEAT.

Introduction. The sand-culture method to be used is essentially that described by McCall (1916). The important feature is that the nutrient solution is renewed at frequent intervals, in much the same way as with water-cultures. As in all experimentation of this cooperation, it is necessary that essential details be alike in all experiments if the results are to be comparable. The nutrient solutions to be used are the same as those for water-cultures, so that there are one hundred and twenty-six different sets of salt proportions in sand-cultures to be compared, besides the general control, with Shive's solution IR5C2 (1/10-increments, 1.75 atm.). This last is our solution IR3.77S1.09 (see table I). The following plan is based on a memorandum furnished the committee by Dr. McCall and Dr. Shive.

Sand. The sand to be employed should be as nearly like that used by McCall as is possible. (A sample may be obtained from Dr. A. G. McCall, Maryland Agric. Exp. Sta., College Park, Md.) This sand shows about 98 per cent. of SiO_2. The grains are not much rounded (artificially crushed quartz should not be used in the present cooperation nor should thoroughly rounded sand be used). The mechanical analysis (by sieves) shows the following proportions as to diameters of particles: 1.0 to 0.5 mm., 1.4 per cent.; 0.5 to 0.25 mm., 86.4 per cent.; 0.25 to 0.10 mm., 11.5 per cent.; 0.10 to 0.05 mm., 0.7 per cent. It has a water-holding capacity of 31 per cent., on the dry-weight basis (Hilgard method, with a column one cm. high. (See Hilgard, Soils, page 209). It should be remembered that the washing process removes much of the most finely-divided material, so that the data just given are applicable to the sand *after* washing. A sand that does not satisfy these requirements may be corrected by addition or removal of proper amounts of certain groups of particles (using sieves). The water-holding capacity should agree with that stated above, within a range of *plus* or *minus* one per cent.; that is, it may have any value between thirty and thirty-two per cent. (dry-weight basis).

Before using, the sand is to be thoroughly washed, with tap or well water and then with distilled water. Washing is to be accomplished as follows: Fill a large glazed crock (about five-gallon capacity) about two thirds full of water and pour sand into this, stirring vigorously until crock is about two-thirds full of sand. Then direct a stream of water into the sand (as by inserting end of garden-hose nearly to bottom of crock), allowing water to overflow at top of crock; continue this washing (with violent agitation of the sand) until the overflow is free from sediment. Decant, refill crock with distilled water, stir sand vigorously, and decant again. Refill with distilled water, stir and decant once more. After second decanting of distilled water, spread sand on clean paper and allow it to become air-dry, avoiding dust or other foreign matter.

Preparation of Crocks. For culture pots, cylindrical half-gallon stoneware crocks (glazed within and without, but not dark-colored externally) about 14 cm. high and 14 cm. in diameter, are to be used. For definite reference, they should be serially numbered with paint, and solution designations may be marked on them with wax pencil.

Twenty-five hundred grams of air-dry washed sand is to be used for each crock, which should fill it to within about two centi-

meters from the top. A glass suction tube (with inside diameter about 4 mm., is to be placed vertically against the wall of the crock, its lower end resting on bottom of crock and its upper end extending about 1 cm. above the rim. This tube is to be placed before sand is poured into crock and will be held in position by the sand. The lower end of the tube is to be loosely plugged with glass-wool and tufts of the wool are to extend one or two centimeters beyond the tube, so that sand may rest on these and thus hold the plug in place.

A supply orifice is furnished by a paraffined paper cone (or a 100-cc. wide-mouth bottle with the bottom removed), standing at the center of the sand surface and embedded in it to a depth of about two centimeters. The paper cone should be about six centimeters high, four centimeters in diameter at its larger (lower) end and two centimeters in diameter at its smaller (upper) end. These cones are rolled from heavy paper, fastened by a pin or paper-clip, and are heavily paraffined.

Record the total weight of crock, tube, cone and sand. Cone is not permanently placed till after seedlings are in position.

The Seedlings. The seeds are to be soaked in germination solution and allowed to germinate on the germination net, just as for water-cultures, the shoots being four centimeters high when transplanting occurs. Selection is made just as for water-cultures. It is of course desirable that all seedlings be as nearly alike as possible.

Introduction of Seedlings. When seedlings are ready, some nutrient solution is poured on to the sand, to moisten it (a liter of solution should be ready, but only a little is used at the start), the surface is levelled and the selected seedlings are introduced, five in each crock. These are to be selected beforehand, as in the case of water-cultures. They are planted so that the grains will lie about one-half centimeter below the sand surface, equally spaced, in a circle half-way between the supply cone and the wall of the crock. A flat wooden dibble (like a spatula) about fifteen millimeters wide and having a sharp edge is convenient for setting the seedlings. (An ordinary pot label with broad end thinned serves will.) Care should be exercised not to injure the roots.

The supply cone is introduced in the center of the sand surface after the seedlings are in place, its larger end downward and embedded in the sand to a depth of about two centimeters. Then nutrient solution is poured in through the cone until the liquid surface is about one centimeter above the sand, and the

crock is slightly jarred to settle the sand about the roots of the plants and to give a flat surface for the wax seal.

An aspirator is now connected to the suction tube and the excess of solution is drawn off until the moisture content of the sand is reduced to about sixteen per cent. (dry-weight basis). The sand is then flooded a second time with nutrient solution, and the excess is again removed, as just described. The aspirator tube should be joined to the culture tube with a suitable bottle intervening as a trap to catch the solution that is removed. This bottle is to be marked so as to indicate when the desired volume of solution has been removed, a device that will save many weighings if the volume of solution added is known. The weight of the seedlings may be neglected and the required moisture content of the sand may have any value between fifteen and seventeen per cent. (dry-weight basis).

The Wax Seal. To hinder evaporation from the soil, the free sand surface is sealed with a mixture of eighty parts (by weight) of "Parawax" and twenty parts of "Vaseline, White" (Chesbrough brand). Have the mixture only warm enough to flow freely, otherwise the plants may be injured where their stems are in contact with the seal. Pour the wax over the sand surface, so as to form a layer from two to four millimeters thick, being sure that the wax forms a tight joint at its junction with the crock wall and with the supply cone, also that it makes good contact with the seedling shoots. (On wax seals, see: Briggs and Shantz, Bot. Gaz. **51**: 210-219. 1911.)

Manipulations During the Culture Period. At the end of each 3½-day period each culture is weighed, and sufficient distilled water is added (through the cone) to bring the sand moisture content back to the original sixteen per cent., and record is kept of the amount of water needed. Then the excess liquid is removed (aspirator) until the moisture content is about ten per cent. Then the sand is again flooded with fresh solution and the excess is once more removed, this time leaving the moisture content at sixteen per cent. The culture is then ready for the succeeding 3½-day period.

From the records showing the amounts of distilled water added to any culture are to be obtained data of transpirational water loss. It will be seen that the amount of salt absorbed by the plants is neglected (as though the plants absorbed only water from the solution), but the error thus introduced will be more truly negligible than the one that would be encountered if nutrient solution were used in place of distilled water.

Growth Phases. These are the same as for water-cultures with wheat. For the reproductive phase the plants will require mechanical support. (See McCall, Jour. Amer. Soc. Agron. **10:** 127-134. 1918.)

Location, Exposure, Climatic Records, etc. These are all the same as for water-cultures with wheat.

The Plant Records. These are the same as for water-cultures, excepting that the root weights require special manipulation. The wax seal is removed and the contents of the crock are placed on a wire sieve (about ten meshes to the inch), through which the free sand is washed with a stream of water. Then the roots are severed from the tops, as in the case of water-cultures, and the tops are treated as before described. The root systems of all plants from the same crock (including the adhering sand) are then brought together, dried at 102° C., and the dry weight for each culture is determined. Then each mass of roots is ignited in a weighed porcelain crucible till all organic matter has been removed, the weight of the remaining mineral matter is determined, and this value is subtracted from the original dry weight of roots and sand, to give approximately the dry weight of the roots alone. The errors introduced by this method are negligible in this sort of work. (See McCall, Soil Science **2:** 223. 1916.)

CULTURES WITH SOY BEAN.

Experimenters who wish to work with soy bean are requested to communicate immediately with the committee. While the general procedure for this plant will be similar to that for wheat, yet some details must necessarily be different, and many of these are not yet worked out by preliminary experimentation.

THE SUB-DIVISION OF THE PROJECT.

Introduction.—The following notes may serve as suggestions, to aid cooperators in choosing their problems in the project. It is hoped that cooperators will choose for their work those portions of the plan in which they feel most interested. At the same time, the committee will be glad to aid in this connection, and it may be necessary (in order that all parts of the plan may receive attention) for the committee to make special requests at a somewhat later time.

In the first place, it is strongly urged that *every experiment* include at least three cultures with Shive's best solution for the

early growth of wheat, the solution called *control* in the preceding pages. It is largely through these controls that climatic conditions and different groups of solutions tested at different times and places are to be compared. At a later time it may become expedient to adopt some other solution as general control, but the one mentioned is the logical one to use in the early stages of our studies.

The following paragraphs aim to present some of the most promising ways of grouping the one hundred and twenty-three solutions that are first to be tested (osmotic value, 1.00 atm.).

Subdivision by Types. Each set of 21 (or 19 or 20) solutions (tables I-VI) furnishes a logical group for experimentation. Such a group includes (with the triplicate control) 22, 23 or 24 cultures, which is a convenient number for an experimenter who does not devote nearly all his time to the work. It may be noted that types V and VI involve some physical-chemical difficulties not encountered with the others, and those who have had experience with this sort of difficulty are urged to undertake the study of these two exceptional types. Type I is the one on which most of the earlier work has been done.

Of course it will be desirable, where possible, to carry out each experiment with these type groups in duplicate, triplicate, etc., and an experimenter may thus give his entire time to the solutions of a single group.

It will also be desirable, especially where two or more experimenters can work together, that the full sets of several solution types (or even all six of them) be tested simultaneously, in which case a single triplicate control will serve for all sets.

Subdivision Into Groups Smaller Than Type Groups. For smaller groups than are represented by the six different types of solution, any solutions may be selected, either merely at random, or because of a personal interest. It will be especially desirable to study and compare in this way just those solutions that have already given good promise. For the seedling phase of growth Shive's (1915) results and those of Livingston and Tottingham (1918) are of special interest as indicating promising solutions. A study of Shive's graphic summary (page 390) for his optimal concentration will suggest small groups of solutions of type I that may be profitably compared. Table 2 of Livingston and Tottingham may furnish similar suggestions for type III. Many other sources of similar suggestions will doubtless become available as soon as a few experiments has been carried out, and such

suggestions may already exist in unpublished or published records.

It is not necessary that the plan of subdividing by solution types should necessarily involve the testing of all the solutions of a given type at once. The solutions may be graphed on the triangular diagram and then the diagram may be arbitrarily divided into two or more regions, each region being considered separately. Thus, the first ten or eleven solutions of any one of the tables as here given may be considered as a first group, the remaining solutions of that type constituting a second group, to be tested after the first.

An experimental comparison of the physiological properties of any single solution given in tables I to VI, with the control solution, will constitute a valuable step toward the working out of the plan. It is hoped that many workers who are not able to devote a great deal of time to this project will nevertheless join in the work, even by testing a single solution. Indeed, the study of the relation of climatic conditions to growth may be greatly advanced if a large number of experimenters will carry out cultures with the control solution alone. If one could do no more, it would be well worth while to get the plant measurements for a number of control cultures, at any time during the year, of course obtaining at the same time the climatic data mentioned in the plan. The growth of wheat in Shive's best solution for this plant might thus become a sort of common criterion by which different climatic complexes (for different seasons and for different stations) might be compared, in terms of their influence on the physiological processes of wheat.

As the work goes forward the committee will furnish further suggestions as to the subdivision of the plan, upon request.

REPORTS OF RESULTS.

As has been said, all cooperators are strongly urged to send to the committee all results obtained just as promptly as possible. Forms for such reports will be supplied to cooperators on request.

SUSCEPTIBILITY TO ATTACK BY BACTERIA OR FUNGI.

Emphasis has been recently placed on the observed fact that a poorly balanced fertilizer treatment may not only produce smaller or less well-developed plants than does a more nearly perfect treatment, but such poor treatment may frequently also

produce plants of apparently satisfactory appearance, which nevertheless are very unsatisfactory on account of a high degree of susceptibility to injurious attacks by parasitic organisms. Plant pathologists agree that the problem thus suggested is of very great fundamental importance and that definite information in this general connection should be made available as rapidly as possible. It is seen that this problem involves both pathology and physiology. Susceptibility to the attacks of a certain parasitic fungus or bacterial form may be considered as one of the physiological characters that need to be understood for agricultural plants, and it is especially important that the relations between such susceptibility and the salt treatment of the plants in question should receive attention.

It is consequently planned that cooperators in the present project who are familiar with the experimental methods of plant pathology may undertake experimentation in this field. Of course such experimentation must needs deal with the plants employed for the simply nutritional aspect of our problem; for the beginning, with "Marquis" wheat and soy bean.

It is planned that suitable groups of solutions may be employed in either water-culture or sand-culture and that when the plants have reached a proper stage of development they may be all tested for different degrees of susceptibility by being similarly inoculated with the fungus or bacterium. The final records in this sort of study will of course deal with the relative amounts of infection and with the relative degrees of injury produced by infection.

In order that the results may be standardized, it is necessary that the plants be grown according to the details of the foregoing plan for work on salt nutrition itself, and it is practically necessary that these inoculation experiments include uninoculated controls having the same solution treatments as are given to the inoculated cultures. The uninoculated cultures will then furnish the regular plant measurement (of the foregoing plan), with which similar data from the inoculated cultures are to be compared. For an illustration, a duplicate water-culture series with solutions of type I may be carried out until the end of the third week of the seedling phase of wheat. At that time the visual observations on the plants are to be made with special care, after which one culture with each solution is to be inoculated and the cultures are to be continued to the end of the five-week period of this phase. Final observations and measurements are to be made on all cultures. It is very essential that special attention be given

to the climatic conditions at the time of inoculation and later; it may be desirable to read the atmometers daily or even more frequently, and to obtain thermograph tracings of the temperature and special notes on the degree of cloudiness. The committee will be glad to discuss forms of experiment in this field with any cooperator who is interested. The details of such experiments need to be worked out for each special case.

CHEMICAL COMPOSITION OF THE PLANTS AS RELATED TO THE CHARACTERISTICS OF THE CULTURE MEDIUM.

Besides the plant records mentioned in the preceding pages, it is planned that the plants of selected cultures may be subjected to chemical analysis, to determine the proportions of some of their constituents, and that the results of these analyses may be correlated with the characteristics of the media in which the plants have been grown. Those who so desire may take part in this aspect of the project by communicating with the committee, which will attempt to make arrangements by which one cooperator may grow the plants while another performs the analyses. These analyses are planned to deal with both mineral and organic constituents of the plants.

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