THE VARIABILITY OF PLANTS GROWN IN WATER CULTURES¹

A. R. DAVIS

College of Agriculture, University of California

Received for publication November 9, 1920

During recent years, much emphasis has been placed upon the water-culture method as a means of determining the influence of salts upon plant growth. Because of its relative simplicity of composition and susceptibility to control, it has proved an especially valuable procedure in certain fields of agricultural research. Like other methods, however, it is not ideal. It possesses certain inherent errors which, if not recognized, may lead to a misinterpretation of data. In this study, it is desired especially to emphasize the extensive rôle plant variation may assume, even under the "controlled" conditions usually insisted upon in experiments involving culture solutions.

Most of the work of this sort involves the comparison of many diverse solutions, and where the relative salt requirements of plants are under consideration, such solutions may differ but little from one another. Where this is true, the results usually vary similarly, and the question arises as to whether fixed values can be assigned to differences of such degree when the material being compared shows extensive variation.

The general practice has been to base conclusions upon relatively few plants. Thus cultures, which as a rule contain 1 to 6 plants, are usually set up in duplicate, and the "growth efficiency" of solutions compared through the mean dry weights. Brechley (1) in a report on the effect of varying concentrations of copper sulfate and manganese sulfate upon the growth of barley, employed the mean of 20 replicate cultures of one plant each in her comparisons, while in later work (2) the mean of 10 plants was used. Stiles (11), studying the effect of different salt concentrations upon the growth of barley, rye, and wheat, likewise based conclusions upon the mean of 10 replicate cultures of one plant each. Tottingham (13), comparing the relative efficiency of many modifications of Knop's solution differing in total as well as in partial salt concentration, employed duplicates with 6 plants to a culture, Shive (7), following him in another phase of the same general investigation, set up single cultures of 6 plants each, and after several weeks repeated the experiment; corresponding cultures of the two series were then averaged, the mean dry weights being taken for comparison. Later Shive and Martin (10)

¹ This study was undertaken at the instance, and under the direction, of Dr. C. B. Lipman. The writer is indebted also to Dr. Geo. A. Linhart for kindly criticism, and to Dr. D. D. Waynick for suggestive preliminary work.

followed this same general procedure in working with young and mature buckwheat. For the mature phase, however, many more cultures than were required for the experiment were grown to the end of the "young" phase in a solution which had proved favorable, and from this excess the necessary number of cultures, uniform apparently insofar as the eye could judge, were selected for the second stage of the work. There are many other investigations which might be cited on this point, but these serve to illustrate the common practice.

The well established fact of biological variation under water-culture conditions has not been ignored, rather various expedients have been employed to minimize it. Thus the rotating table was employed by Shive (7) and others as a means of equalizing environmental influences. Seedlings have been very generally selected for uniform size and vigor and in certain cases [Brenchley (1)] seeds have been graded according to weight. Cultures have usually been made up of several plants, the idea being that less vigorous plants would offset more vigorous ones, and the total weight of any culture would not depart significantly from its replicates. In spite of such precautions, however, variability remains an important factor. Both Brenchley (1) and Stiles (11, 12) recognized that the number of plants which they employed was insufficient to give a mean of more than low accuracy, the former remarking that "owing to the individuality of plants, investigations dealing with small numbers cannot be depended upon," and the latter that, "plants growing in water cultures under exactly the same conditions are very variable." Both of these workers determined the probable error of the mean and interpreted results accordingly, but because of the limited number of determinations averaged and their rather high variability (40 per cent in Brenchley's work), the accuracy of this probable error is very questionable.

American workers have tacitly assumed that under the system of control adopted, variation is reduced to a negligible minimum; in other words, that the mean of few determinations will not depart sufficiently from that of many to vitiate conclusions. One need but examine the literature, however, to find many instances contrary to such an assumption. In many cases noted, where duplicate cultures have been employed, wide differences between them were apparent; in fact, such differences have been frequently observed to exceed that existing between the means under comparison. Where this is true, how much trust can we place in the mean? Certainly, under such conditions, means of duplicates cannot possess any high degree of accuracy, and differences attributed to efficiency of the solution may be due to variability of the plants themselves.

The first consideration, then, in comparing such variables as plants, is the determination of the trustworthiness of the mean; its limit of accuracy must be fixed. This will demand the consideration of a much larger number of replicates than workers have deemed necessary heretofore. Since the distribution of biological variables has been found to follow closely the law of prob-

ability, it is evident that the larger the number of variables averaged, the more accurate will be the arithmetic mean.² On the contrary, small numbers will give means which may depart more or less widely from the true mean, the extent of departure depending on the efficiency of the system.

In the statistical study herein reported, Shive's (7) extended work is taken as a basis. This was selected on account of the very careful attention the author gave to the many details, as well as because it considered the critical comparison of several solutions. Shive employed his well known three-salt solution in three different total concentrations, 0.1, 1.75 and 4.00 atmospheres; (the second of these, the 1.75-atmosphere concentration, is considered here). In these the partial molal concentration of salts was varied in increments of one-tenth, 36 different combinations resulting. A series of 36 cultures, each culture representing different partial concentrations of salts, was set up in 250-cc. bottles, the solutions changed every 3 days, and the experiment continued for 23 days on a revolving table. The greatest care seems to have been taken to have the culture solution the only condition of the environment differing in its influence upon the individual cultures. A careful perusal of the paper did not disclose whether duplicate cultures were set up or not. However, the experiment was repeated at a later date and the means of the dry weights of corresponding cultures in the two series taken for comparison of the efficiency of the solution. Results were plotted by the familiar triangle method as employed in physical chemistry, and on this triangle, areas of high and low yields were mapped out. Lying outside such areas were many results which might be considered medium in their value and these are especially important in a consideration of the data as a whole.

METHODS

In any study of frequency distribution, it is extremely desirable to have a sufficient number of variants to permit of an approximately normal distribution about the mean. In this study, we are dealing only with Shive's 1.75-atmosphere solution, and yet this alone contains 36 different combinations. It would manifestly be difficult to consider all of these statistically, nor is it necessary, since, in order to demonstrate the principle involved, one needs but to consider a few well chosen culture solutions of the 36 represented. For our work, modifications R_5C_2 , R_2C_6 , and R_1C_1 were chosen, these representing his so-called "best," his "poorest," and a "medium" solution,³ the last named lying between the highest and lowest-yield areas. These three had the following partial molal concentrations:

² This, however, as pointed out by Linhart (4), can be true only where the maximum deviation does not exceed the mean in magnitude.

³ The designations are those employed by Shive.

	KH ₂ PO ₄	Ca(NO ₃) ₂	MgSO ₄
R ₄ C ₄	0.0180	0.0052	0.0150
R ₂ C ₆	0.0072	0.0130	0.0150
R ₁ C ₁	0.0036	0.0026	0.0400

It is likewise true that the number of replicate cultures possible to consider in an experiment of this kind is limited by one's time and equipment, since the changing of solutions each three days for a large number of cultures is a considerable task. For our purposes, two series, "A" and "B", were set up, the first in quart Mason jars and the latter in 250-cc. bottles. Thirty-three cultures (165 plants) for each of the three culture solutions were arranged for Series "A" and 50 (300 plants) for Series "B." Such numbers, of course, were purely arbitrary, but it was felt they would be sufficient for the purpose at hand.

Culture solutions

Baker's analyzed chemicals were employed throughout. Stock solutions of single salts were made up and from these dilutions were made sufficient for a single change. Throughout this work, the importance of subjecting all cultures in any one series to the same culture-solution environment was emphasized and to this end the total amount of solution required for the 33 or 50 cultures, as the case might be, was made up in one lot and this thoroughly mixed several times before being placed in the final containers.

Seedlings

Instead of the Fulcaster wheat used by Shive, Tottingham, McCall, and others, Sonora wheat of selected strain and high germination was employed. This was obtained from the Division of Agronomy of the University of California. Shive's method of germination was used, many more seedlings than necessary being germinated, and only uniform, vigorous seedlings selected for cultures. This latter point was especially stressed, each seedling being carefully measured and all those not coming within the limits of 5 ± 1 cm. rejected.

Glassware

As stated above quart Mason jars were used for series A and 250-cc. wide-mouth bottles for series B. These were cleansed with chromic-sulfuric cleaning mixture, thoroughly rinsed and covered with opaque paper, white side out. Corks were given a very thin coating of "parowax."

Setting up experiments and changing solutions

The two series were set up in the greenhouse, series A on long benches, the position of the jars being changed several times a week in accordance with a

definite plan; series B was placed on revolving tables. Light conditions were excellent and the jars were arranged so as to prevent the shading of one culture by another. Likewise the jars were sufficiently removed from each other to obviate unequal inter-humidity effects. The unheated condition of the greenhouse gave rise to slightly higher variations in day and night temperatures than would have been true had heat been applied (table 1), but such differences were not significantly greater than those reported by Shive and since they affected all plants alike, they were not held to be important.

TABLE 1
Temperatures

	SERIES "A"	SERIES "B"
	°C.	°C.
Mean maxima	26.8	20.8
Maximum	32.5	29.0
Mean minima	9.4	5.2
Minimum	5.5	1.0

In changing solutions, the following procedure was adopted. The total amount of solution required for a single modification in series A, say for R_5C_2 , was 33 liters. This amount was made up from the strong single-salt stock solutions and thoroughly shaken. The cork and plants were placed in an extra bottle containing the solution, the old solution emptied out of the jar and the new lot measured in. The plants were then replaced. In series B the total amount for a change was about 12.5 liters. This was made up in a similar way and the change performed as above. The amount of transpiration was ascertained by measuring the old solution before discarding it.

Criterion of growth

Dry weight of the entire plant and of the top alone was taken as the criterion of growth. After harvesting, the plants were dried at 90°C. for 48 hours, and then to constant weight at 105°C. The weighings for series A were made to 0.01 and in series B to 0.001 gm.

Calculations

The statistical method presented by Davenport (3) and in general use in biometry, was employed except for the calculation of theoretical curves. The latter were calculated according to an equation recently obtained by Dr. G. A. Linhart. Since these methods are taken up *in extenso* by Davenport, by Dr. Linhart (4), and in various texts, it is not thought necessary to go into a mathematical treatment of them here.

TABLE 2
Total weights, series A*

NUMBER	R ₃ C ₃		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	gm.	gm.	gm.	gm.	gm.	gm.
1	2.64	0.49	2.36	0.22	2.00	0.44
2	2.50	0.35	2.33	0.19	1.57	0.01
3	1.92	0.23	1.96	0.18	1.64	0.08
4	2.06	0.09	2.05	0.09	1.57	0.01
5	2.00	0.05	1.66	0.48	1.76	0.20
6	2.13	0.02	2.47	0.33	1.61	0.05
7	2.09	0.06	1.99	0.15	1.38	0.17
8	2.34	0.19	1.94	0.20	1.53	0.03
9	2.24	0.09	2.33	0.19	1.34	0.22
10	2.06	0.09	2.02	0.12	1.34	0.22
11	2.18	0.03	2.13	0.01	1.57	0.01
12	2.18	0.03	2.13	0.01	1.40	0.16
13	2.27	0.12	2.11	0.03	1.57	0.01
14	2.33	0.18	2.29	0.15	1.80	0.24
15	2.11	0.04	2.13	0.01	1.56	0.00
16	1.83	0.32	1.87	0.27	1.51	0.05
17	2.25	0.10	2.17	0.03	1.75	0.19
18	2.39	0.24	2.04	0.10	1.33	0.23
19	2.12	0.03	2.01	0.13	1.29	0.27
20	1.95	0.20	2.00	0.14	1.49	0.07
21	2.18	0.03	2.19	0.05	1.58	0.02
22	1.71	0.44	2.28	0.14	1.47	0.09
23	1.89	0.26	2.04	0.10	1.45	0.11
24	1.93	0.22	2.15	0.01	1.53	0.03
25	2.27	0.12	2.28	0.14	1.69	0.14
26	2.08	0.07	2.22	0.08	1.57	0.01
27	2.07	0.08	2.23	0.09	1.75	0.19
28	2.23	0.08	2.39	0.25	1.41	0.15
29	1.92	0.23	2.19	0.05	1.62	0.06
30	2.23	0.08	2.46	0.32	1.49	0.07
31	2.46	0.31	1.81	0.35	1.76	0.20
32	2.17	0.02	1.93	0.21	1.75	0.19
33	2.12	0.03	2.46	0.32	1.51	0.05
Mean	2.15 ± 0.023 gm.		2.14 ± 0.023 gm.		1.56 ± 0.018 gm.	
σ	0.194 ± 0.017 gm.		0.195 ± 0.017 gm.		0.156 ± 0.013 gm.	
C.V.	9.02 ± 0.76 per cent		9.10 ± 0.091 per cent		10.0 ± 0.83 per cent	
P.E.	0.131 gm.		0.131 gm.		0.105 gm.	

* 33 cultures, 5 plants per culture; experiment continued 5 weeks; solution changed every 3½ days; results in dry weights per culture.

TABLE 3
Top weights, series A

NUMBER	R ₃ C ₃		R ₃ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	gm.	gm.	gm.	gm.	gm.	gm.
1	2.04	0.35	1.83	0.12	1.64	0.34
2	1.90	0.21	1.85	0.14	1.31	0.01
3	1.63	0.06	1.54	0.17	1.36	0.05
4	1.52	0.17	1.62	0.09	1.29	0.01
5	1.74	0.05	1.40	0.31	1.45	0.15
6	1.67	0.02	1.98	0.27	1.34	0.04
7	1.68	0.01	1.58	0.13	1.14	0.16
8	1.88	0.19	1.58	0.13	1.29	0.01
9	1.81	0.12	1.80	0.09	1.09	0.21
10	1.67	0.02	1.61	0.10	1.09	0.21
11	1.80	0.11	1.71	0.00	1.25	0.05
12	1.73	0.04	1.74	0.03	1.17	0.13
13	1.73	0.04	1.71	0.00	1.35	0.05
14	1.92	0.23	1.81	0.10	1.59	0.29
15	1.75	0.06	1.72	0.01	1.35	0.04
16	1.35	0.34	1.51	0.20	1.27	0.04
17	1.76	0.07	1.76	0.05	1.46	0.16
18	1.84	0.16	1.62	0.09	1.12	0.18
19	1.63	0.06	1.65	0.06	1.08	0.22
20	1.53	0.16	1.54	0.17	1.25	0.05
21	1.67	0.02	1.70	0.01	1.31	0.01
22	1.37	0.32	1.78	0.07	1.25	0.05
23	1.50	0.19	1.61	0.10	1.19	0.11
24	1.58	0.11	1.71	0.00	1.27	0.03
25	1.71	0.02	1.86	0.15	1.40	0.10
26	1.62	0.07	1.79	0.08	1.27	0.03
27	1.66	0.03	1.81	0.10	1.44	0.14
28	1.78	0.09	1.90	0.19	1.16	0.14
29	1.48	0.21	1.72	0.01	1.30	0.00
30	1.87	0.18	1.87	0.16	1.27	0.03
31	1.48	0.21	1.48	0.23	1.48	0.18
32	1.55	0.14	1.55	0.16	1.46	0.16
33	1.98	0.31	1.98	0.27	1.33	0.03
Mean	1.69 ± 0.018 gm.		1.71 ± 0.017 gm.		1.30 ± 0.016 gm.	
σ	0.153 ± 0.013 gm.		0.14 ± 0.012 gm.		0.135 ± 0.011 gm.	
C.V.	9.05 ± 0.76 per cent		8.24 ± 0.69 per cent		9.66 ± 0.80 per cent	
P.E.	0.10		0.094		0.090	

DISCUSSION OF DATA

It is stated in the law of errors that when a number of variants are considered, large deviations from the mean will occur relatively less frequently than small ones; moreover, when a sufficiently large number of such variants

are taken, deviations will fall equally on either side of the mean. This is true, of course, only under ideal conditions where chance is not interfered with in any way. One can plot such data, frequency against measurement, and obtain the so-called frequency curve, the apex of a normal curve representing the true mean and departures from that mean falling equally on either side. In the case of a small number of variants, it is not likely that perfect symmetry would be obtained, since the law of chance would not have fair play, and more experimental values might fall on one side of the true mean than on the other. This would give the so-called "skew," or asymmetric curve. Such unbalanced distribution is not peculiar to few variants. Indeed, it is a common arrangement of biological data even where many variants are considered. In the data given in this paper, the deviation of individual determinations from the mean is relatively small, i.e., it never exceeds the mean in magnitude, while the distribution of such determinations about the arithmetic mean is in all cases such as to permit the construction of a symmetrical curve (fig. 5 to 10).

In case the extent of one's experimental data falls short of the ideal for the symmetrical curve (the usual condition), and yet is sufficiently extensive to give an approximation of symmetry, the normal curve can be calculated, and thus the experimental can be related to theoretical values. It is obvious that in such a comparison, the arithmetic mean may not coincide with the theoretical as indicated by the apex of the calculated curve; if there is a divergence, it must be assumed that the theoretical mean is the true one, since the law of probability demands symmetry. It becomes entirely possible, then, that one may fall into error in comparing different series of determinations through their arithmetic means alone, even although a relatively large series may be considered, since these may neither indicate the true mean nor show the influence of variability. The chances for misleading conclusions become much greater, however, when few determinations are averaged, particularly when stress is laid upon slight differences, for here, as will be shown later, the arithmetic mean may vary greatly from the more nearly true mean of a large series. In the data herein presented, the variants considered, when grouped into classes give approximation of symmetry to the plotted frequency polygon, and from these, normal curves have been calculated which show graphically the extent of variability. Probable error,⁴ standard deviation, and the coefficient of variability, have been determined for plants grown in

⁴ The probable error of a single determination is given as that value lying on either side of the mean within which fifty per cent of the determinations of the series should lie. To find the probable error of a number of variants, use is made of the standard deviation, which is expressed as $\sigma = \frac{\sum d^2}{N}$, where $\sum d^2$ is the sum of the squared deviations, N the number of variants, and σ the standard deviation. The probable error, PE , is then found by the formula $P.E. = 0.6745 \frac{\sigma}{\sqrt{N-1}}$, where 0.6745 is a constant. [See Davenport (3) and Merriam (6).]

each of the three solutions, and comparisons made in the light of these limiting factors. Assuming that the mean of any one solution series is accurate within its probable error,⁵ we have a basis for judging the reliance which can be placed upon the means of duplicate cultures, or of any number which may be chosen at random from the series in question.

Series A

In this series, the culture (5 plants) rather than the individual plant is taken as a unit, thus following the common practice. Later, the variability shown by individual plants will be treated separately. The experimental data are given in tables 2 and 3, and are self-explanatory.

In Shive's original results, the values obtained for the means of two cultures are given as follows:

	R_2C_2	R_3C_3	R_1C_1
Dry weight, gm.....	0.5704	0.4842	0.4104
With R_1C_1 as unity.....	1.39	1.18	1.00

Referring to table 2, however, we find the means for 33 cultures of R_2C_2 and R_3C_3 practically coincide. This is graphically shown in figures 1 and 2 where the corresponding calculated curves are compared. The curves show the existence of considerable variability for the cultures in question, which has resulted in an overlapping of data; in the case of the first two solutions being practically complete, and with R_1C_1 relatively slight. Even under the accepted conditions of control, the range due to variation is seen to be considerable and offsets completely any apparent difference between arithmetic means. It is evident that were we comparing means of duplicates, our conclusions might have been otherwise, since they would be influenced by the position occupied by the cultures represented within the area of the curve in question. A glance at the figures will show at once that such chance distribution might give results comparable to those of Shive, to those herein presented, or different from either in that R_2C_2 might be concluded the better solution. This point will be considered more in detail later.

In the case of R_1C_1 , we have a solution high in magnesium sulfate and relatively low in nitrate and phosphate salts. *A priori*, one would expect a yield lower than that given by the other two solutions, conditions which are realized in the analysis of the results. The mean of 33 cultures is here 1.56 gm. as

⁵ The mean of a number of variants cannot have a fixed value; the very fact of measurement introduces error. In biological data there is not only the error of measurement which may be relatively small, but errors as well due to an innate capacity to vary on the part of the organism. One must not only consider the mean in his calculations, but the probable error as well, since this, in the last analysis, fixes its accuracy. See Wood (14), Wood and Stratton (15), Merriam (6), Davenport (3) and texts on the law of probability, for discussion.

FIG. 1. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES A

FIG. 2. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES A

compared with 2.15 gm. for R_5C_2 , a difference of 0.59 gm., or about 27 per cent in terms of the latter solution. This difference is quite marked, and one might be justified in attaching significance to it. Referring to the curves in figures 1 and 2, however, it is seen that the overlapping, although slight, is such as to raise the question of its relative importance. How can one be certain that small numbers of cultures chosen at random from the series might not lie within this overlapping area? Since the ratio of this area to the area of the curves compared is small, it would be supposed that the chances for such an event occurring would be correspondingly slight. It is possible, however, to determine such odds when the probable error of difference between means is known.⁶ Thus, in this case, for example, we have the means of these two solutions as 2.15 ± 0.023 gm. and 1.56 ± 0.018 gm., respectively, and by the formula given, we find this difference of 0.59 gm. has a probable error of 0.029 gm.

TABLE 4
Table of odds—Differences in both directions

DIFFERENCE FROM THE MEAN IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERENCE OCCUR- RING UNDER UNIFORM CONDITIONS
1.00	1.41	1 to 1
1.25	1.76	3 to 2
1.44	2.03	2 to 1
1.71	2.41	3 to 1
1.90	2.68	4 to 1
2.00	2.83	9 to 2
2.05	2.87	5 to 1
2.50	3.53	10 to 1
2.93	4.13	20 to 1
3.00	4.24	22 to 1
3.20	4.51	30 to 1
4.00	5.66	140 to 1
4.90	6.93	1000 to 1
5.00	7.07	1350 to 1

Thus the actual difference between means is about 19 times its probable error. The significance of this value can be ascertained by reference to a table of odds, such as those included here under tables 4 and 5 which have been taken from Wood (14). In this case, the difference lies in one direction only, i.e., the effect of such a solution as R_1C_1 can be considered as varying in but one direction from R_5C_2 , and so table 5 must be utilized. Taking the value 19 to the second column of this table, the corresponding odds in the third column, in favor of this difference being due to something other than normal variation, would be seen to be enormous were they computed to so high a figure. Cer-

⁶ The probable error of difference is larger than that of either probable error alone, and is given by the formula: $A_1 - A_2 \pm \sqrt{E_1^2 + E_2^2}$, where A_1 and A_2 are the means, and E_1 and E_2 their respective probable errors.

tainly here this overlapping need not be considered and real inferiority may be assigned to the solution.

The above conclusions are made on the basis of 33 cultures where chance has had somewhat of an opportunity to locate a true mean. What would be the result were one to consider duplicates? In decreasing the number of cultures, the most apparent fact is the increase in the probable error, which

TABLE 5
Table of odds—Difference in one direction only

DIFFERENCE FROM THE MEAN IN ONE DIRECTION ONLY IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN ONE DIRECTION ONLY IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERENCES OCCURRING UNDER NORMAL CONDITIONS
1.00	1.41	3 to 1
1.25	1.76	4 to 1
1.44	2.03	5 to 1
1.58	2.23	6 to 1
1.71	2.41	7 to 1
1.81	2.55	8 to 1
1.90	2.68	9 to 1
2.00	2.83	10 to 1
2.48	3.50	20 to 1
2.70	3.81	30 to 1
2.89	4.07	40 to 1
3.00	4.24	44 to 1
3.03	4.28	50 to 1
3.44	4.85	100 to 1
4.00	5.66	290 to 1
5.00	7.07	2700 to 1

TABLE 6
Increase in probable error of the mean, with the decrease in the number of cultures—Series A

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R ₅ C ₂	R ₅ C ₅	R ₁ C ₁	R ₅ C ₂	R ₅ C ₅	R ₁ C ₁
	gm.	gm.	gm.	gm.	gm.	gm.
2	2.15-0.095	2.14-0.095	1.56-0.075	1.69-0.071	1.71-0.066	1.30-0.067
4	2.15-0.066	2.14-0.065	1.56-0.052	1.69-0.050	1.71-0.047	1.30-0.043
8	2.15-0.047	2.14-0.047	1.56-0.038	1.69-0.036	1.71-0.033	1.30-0.033
16	2.15-0.034	2.14-0.034	1.56-0.026	1.69-0.025	1.71-0.023	1.30-0.023
33	2.15-0.025	2.14-0.023	1.56-0.018	1.69-0.018	1.71-0.016	1.30-0.016

varies as the square root of the number of cultures averaged. This is shown in table 6, where probable errors have been calculated for various numbers of cultures up to the 33 making up the series. Here it is seen that where the probable error of the mean total weights of both R₅C₂ and R₅C₅ is 0.023 gm., it increases to 0.095 gm. for duplicate cultures within these two series. Should we select any two cultures at random from either R₅C₂ or R₅C₅, their average by our definition of probable error would have but an even chance of coming

within the limits 2.05–2.25 gm. When we undertake to compare means of such low degree of accuracy as is exhibited here, it is perfectly evident that the difference between them must be considerable before we would be justified in assigning even relative efficiency values to these solutions. In the case at hand, it is possible to determine just how great this difference must be. We have found that the probable error of difference between R_5C_1 and R_5C_2 becomes 0.134 gm. when the comparison is made through the means of duplicate cultures. To obtain an even chance that a difference is real, it must equal the probable error of difference in magnitude, or in this case 0.134 gm. For the security of a 10 to 1 chance (table 4), a difference between solutions must exist equal to 2.83 times the probable error of difference (column 2), or in this case, $2.83 \times 0.134 = 0.379$ gm. However, a 10 to 1 chance is not considered sufficient to give security of the degree desired in this sort of work. It is more customary to demand the reliance of 30 to 1 odds, and if this were desired, one would not be justified here in attaching significance to a difference less than 0.53 gm., or 24 per cent of the mean.

It was noted above that the inferiority of R_1C_1 , as shown by the mean of the series, was most evident. Is the larger probable error when duplicates are compared, sufficient to offset this difference in arithmetic means? The probable error increases to 0.078 gm. for duplicates and when this solution is compared with duplicates of R_5C_2 , we find a difference of 0.59 ± 0.13 gm. The ratio of difference to probable error becomes 4.54, which, when taken into the second column (table 5) gives chances approximately 30 to 1 that the difference is due to something other than normal variation.

COMPARISON OF TOP WEIGHTS

There is considerable divergence of opinion among investigators regarding the relative value of total dry, top dry, or green weight, as a criterion of solution efficiency. The two former are employed as criteria here and when the means of 33 cultures are compared, no real difference in relative results is apparent (tables 2 and 3). In the comparison of top weights for duplicate cultures, however, the difference between R_1C_1 and R_5C_2 becomes less pronounced than where total weights were compared, thus:

$$1.69 - 1.30 \pm \sqrt{80^2 \text{ mgm.} + 67^2 \text{ mgm.}} = 0.39 \pm 0.105 \text{ gm.,}$$

$$\text{and } \frac{0.39}{0.105} = 3.71$$

This value gives less than a 20 to 1 chance that the difference is significant. This, again, illustrates the error of considering too few cultures. The striking disparity between the means of these two solutions indicates a real difference, but because of overlapping due to variation, and the low accuracy of means of duplicates, one cannot have a chance better than 20 to 1 that it is real, unless the probable error is decreased by averaging a greater number of cultures. Thus, for 33 cultures, the probable error of difference decreases to 0.026 gm., and shows without question the real inferiority of the solution.

TABLE 7
Total weight, series B*

NUMBER	R ₄ C ₃		R ₃ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	534	59	440	20	422	2
2	484	9	487	27	432	12
3	582	107	525	65	430	10
4	468	7	471	11	428	8
5	489	14	481	21	470	50
6	507	32	482	22	452	32
7	483	8	479	19	454	34
8	424	48	408	52	458	38
9	525	47	417	43	380	40
10	545	65	464	4	375	45
11	547	18	461	1	425	5
12	428	47	449	11	428	8
13	465	10	472	12	390	30
14	504	29	469	9	408	12
15	482	7	544	84	461	41
16	430	45	460	0	436	16
17	476	1	482	22	398	12
18	418	59	394	66	413	7
19	445	35	383	77	452	32
20	385	90	443	17	426	6
21	455	20	452	78	412	8
22	492	17	457	3	430	10
23	494	19	451	9	361	59
24	513	38	517	57	421	1
25	412	63	522	62	365	55
26	424	49	411	49	434	14
27	431	44	459	1	428	8
28	504	30	455	5	408	12
29	404	68	479	19	340	80
30	453	22	426	34	459	39
31	427	48	538	78	376	44
32	536	61	451	9	400	20
33	484	11	424	36	440	70
34	524	45	454	6	438	18
35	509	34	438	96	458	38
36	404	67	479	19	362	58
37	434	41	389	71	422	2
38	527	52	390	70	424	4
39	452	23	448	12	440	20
40	520	45	458	2	425	5
41	561	86	494	34	383	37
42	465	16	411	49	436	16
43	468	7	509	49	461	41

* 50 cultures, 6 plants per culture; experiment continued 23 days; solution changed every 3 days; results in dry weights per culture.

TABLE 7—Continued

NUMBER	R ₅ C ₁		R ₂ C ₂		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
44	510	51	504	44	313	107
45	505	29	477	17	483	63
46	445	38	460	0	500	80
47	425	51	433	27	416	4
48	445	37	455	5	369	51
49	489	17	498	38	423	3
50	510	35	440	20	445	25
Mean	475 ± 4.3 mgm.		460 ± 3.8 mgm.		420 ± 3.5 mgm.	
σ	44 ± 3.0 mgm.		40 ± 2.7 mgm.		37 ± 2.5 mgm.	
C.V.	9.30 ± 0.62 per cent		8.70 ± 0.58 per cent		8.81 ± 0.59 per cent	
P.E.	30 mgm.		27 mgm.		25 mgm.	

SERIES B

Total weight

In this series, attempt was made to duplicate as nearly as possible the methods and technique employed by Shive. The quart jars used in the preceding series were replaced by 250-cc. wide-mouth bottles; solutions were changed every 3, instead of every 3½ days, and the experiment was carried on for 23 days, instead of for 5 weeks. Six plants made up a culture and 50 cultures were set up rather than 33. The average temperature was lower than for series A (table 1), and this, together with the shortened growing period, resulted in lower yields. Other possible temperature influences will be discussed later.

Reference to figures 3 and 4 clearly shows the variation existing within each of the three solutions and the overlapping due to this variation. While the means for R₅C₂ and R₂C₂ do not approach each other so closely as is true in series A, the three curves considered together are more closely associated than in that series. The value of such curves is here demonstrated. Were one to consider separate means without taking variation into account, the conclusion might be reached that the three solutions showed real differences. However, where the data are plotted in the form of a curve and the variation shown graphically, one can see at a glance that the question of overlapping, as caused by variation, must be seriously considered.

Table 7 shows the means of the three solutions in this series to be 475 ± 4.3 mgm., 460 ± 3.8 mgm., and 420 ± 3.5 mgm., for R₅C₂, R₂C₂, and R₁C₁, respectively. In this case, an appreciable difference seems to exist between the means of R₅C₂ and R₂C₂, the relative difference, in fact, being approximately that noted by Shive. Clearly, if arithmetic means alone are compared, the latter solution must be assigned an efficiency value less than that of R₅C₂.

TABLE 8
*Top weights, series B**

NUMBER	R ₂ C ₂		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	359	36	286	14	291	1
2	343	20	309	9	309	19
3	409	86	357	43	297	7
4	328	5	320	20	299	10
5	380	57	333	33	320	29
6	344	21	323	23	311	21
7	332	10	322	22	313	23
8	283	60	281	19	307	17
9	352	29	286	14	251	39
10	350	27	330	30	256	34
11	313	10	307	7	288	2
12	290	33	294	6	282	8
13	316	7	311	11	262	28
14	332	11	305	5	282	8
15	319	4	349	49	311	21
16	300	23	313	13	305	15
17	332	11	315	15	277	13
18	272	51	265	35	283	7
19	300	23	257	43	321	31
20	272	51	294	6	292	2
21	317	6	306	48	277	13
22	349	26	321	21	305	15
23	347	24	300	0	253	37
24	343	20	338	38	295	5
25	286	37	316	16	263	27
26	293	32	282	18	304	14
27	305	18	298	2	302	12
28	332	11	300	100	279	11
29	263	60	324	18	235	55
30	282	41	289	17	320	29
31	306	17	359	53	269	21
32	350	27	309	3	284	6
33	330	7	282	24	218	28
34	355	32	310	4	257	33
35	342	19	294	12	318	28
36	278	45	308	2	247	43
37	302	21	255	51	316	26
38	354	31	269	37	297	7
39	312	11	305	1	309	19
40	345	22	318	12	299	9
41	384	61	329	23	247	43
42	314	9	263	53	297	7
43	322	1	339	33	321	31

* 50 cultures, 6 plants per culture; experiment continued 23 days; solutions changed every 3 days; results in dry weights per culture.

TABLE 8—Continued

NUMBER	R ₂ C ₂		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
44	297	26	328	22	206	84
45	339	16	307	1	329	39
46	310	13	303	3	345	55
47	288	35	284	22	282	8
48	298	25	296	10	266	24
49	340	17	339	33	286	4
50	341	18	284	22	299	10
Mean	323 ± 3.0 mgm.		306 ± 2.3 mgm.		290 ± 2.9 mgm.	
σ	31 ± 2.1 mgm.		24 ± 1.6 mgm.		30 ± 2.01 mgm.	
C.V.	9.60 ± 0.64 per cent		7.84 ± 0.52 per cent		10.35 ± 0.69 per cent	
P.E.	20.8 mgm.		16.1 mgm.		20.1 mgm.

FIG. 3. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES B

In comparing R_3C_2 and R_2C_5 , we find a difference of

$$475 - 460 \pm \sqrt{4.3^2 + 3.8^2},$$

or, 15 ± 5.8 mgm.

We do not know whether such a difference will always occur in the same direction, and hence, in the computing of odds, recourse must be had to table 4. The difference obtained is 2.58 times the probable error, a value which represents but a 4 to 1 chance that the discrepancy in means is due to a fundamental difference in solution and not to variability. To insure odds of

FIG. 4. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES B

30 to 1, the probable error of difference must be reduced to 3.3 mgm., or with the probable error as found a difference of at least 26 mgm. between the two means must exist. The disparity in means of R_3C_2 and R_1C_1 , is again sufficiently great to warrant the previous conclusions regarding its inferiority, its value being 55 ± 5.7 mgm.

From table 9, we find the probable errors of duplicate cultures become 21 mgm., 19 mgm., and 18 mgm., for the three solutions in order. Once more, there is but a 1 to 1 chance that the mean of any two cultures will fall within the probable error of the mean of the series. In the case of R_3C_2 and R_2C_5 , the difference in means is 15 ± 28 mgm. It is difficult indeed, to say that

this value means anything definite, for the probable error is of such magnitude as to annul completely the significance of difference. The question might arise that since there is an apparent difference between the solutions, and the probable error decreases with the number of cultures employed, all that is necessary is to decrease the probable error until such a point is reached as will give the security of chance desired. This might be done if one wishes to lay emphasis upon such small differences. It must be remembered, however, that the probable error decreases rapidly at first, then more and more slowly as the number of cultures is increased, and, to attain the accuracy desired, the number of cultures required might become prohibitive.

TABLE 9

Increase in the probable error of the mean, with the decrease in the number of cultures—Series B

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R_2C_2	R_2C_3	R_1C_1	R_2C_2	R_2C_3	R_1C_1
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
2	475±21.0	460±19.0	420±18.0	323±15.0	306±11.4	290±14.2
4	475±15.0	460±14.0	420±13.0	323±11.0	306±8.0	290±10.0
8	475±11.0	460± 9.8	420± 9.0	323± 7.4	306±5.8	290± 7.2
16	475± 7.3	460± 7.0	420± 6.2	323± 5.2	306±4.0	290± 5.0
32	475± 5.3	460± 4.8	420± 4.4	323± 3.6	306±2.8	290± 3.5
50	475± 4.3	460± 3.9	420± 3.6	323± 3.0	306±2.2	290± 2.8

THE RELATION OF THE MEAN OF DUPLICATE CULTURES CHOSEN INDISCRIMINATELY, TO THE MEAN OF THE SERIES IN WHICH THEY LIE

It has been shown above that odds in favor of the difference between two means being significant, decrease with a decrease in the number of samples averaged. Thus, for duplicate cultures, greater differences between the means of two pairs must be apparent than would be true for larger numbers. As stated in the introduction, the worker who employs duplicate cultures for comparative purposes assumes that no matter how many sets of duplicates he might use, the means would not vary enough from each other to vitiate his conclusions. The error of this assumption is most evident from the data in table 10. Here we have the mean of 33 cultures from series A as a check, and can select any number of pairs desired to see whether they approach this more accurate mean within reasonable limits. Sixteen pairs of cultures were selected by chance from the data for R_2C_2 of this series. Here, in a single solution and under a uniform set of conditions for all cultures, the means of these pairs so chosen range from 2.55 gm. to 1.82 gm., or 34 per cent of the mean of the series, 2.15 gm. Had we based our comparisons upon one of these random means and upon another chosen similarly from solution R_2C_3 , it is seen that the conclusions reached would have depended entirely upon the mean which chance happened to throw in our way. It might be stated

however, that, when sampling at "random," the chances of getting a pair of values representing a large deviation from the true mean are not so great as they would be for a small deviation. In this case, we may take the probable error of two cultures, which has already been calculated for solution R_5C_5 , i.e., 0.095 gm. Any haphazard selection, then, of the means of a pair of weights, such as given in the table, would have but a 1 to 1 chance of coming within the limits of 2.15 ± 0.095 gm. When we group these selected means in table 10 according to the relative size of their errors, we actually find that seven are found within such limits and nine outside, which is a rough approximation of the theoretical odds. The fact is here apparent in this experiment that one time out of two, we shall fall short of the true mean by at least one-tenth of a gram. Thus one would hardly be justified in attaching significance to a difference in the second, third, or fourth decimal place.

TABLE 10
Variations shown in the means of duplicate cultures chosen at random from series A

SELECTION NUMBER	VARIATION	SELECTION NUMBER	VARIATION
	gm.		gm.
1	2.22	9	1.97
2	2.18	10	2.05
3	1.97	11	2.37
4	2.15	12	2.25
5	2.34	13	2.09
6	2.00	14	2.55
7	2.16	15	1.99
8	2.28	16	1.82
Mean.....			2.15

VARIABILITY AS SHOWN BY INDIVIDUAL PLANTS

Although, as previously noted, certain workers have employed cultures of one plant each as the experimental unit, the common practice has been to use several plants to the culture. Conforming to this custom, we have in the foregoing discussion considered the culture as the basis for statistical treatment. It is perfectly obvious, however, that such collective units do not express the true variability of the individual plants, since the very act of grouping tends to offset any extreme errors which might be exhibited by the individuals alone. The literature has very little to say on this point as applied to water cultures. Brenchley (2) and Stiles (11), determined the probable error of the mean of 10 one-plant cultures. So few plants, however, cannot disclose the full extent of individual variation.

A difference of opinion exists regarding the relative advantages of cultures made up of one or several plants. The first condition gives optimum conditions for growth as far as interference by other plants is concerned, but the advantages of an average of several plants is lost.

The individual plants of series A were weighed separately and will here be treated as statistical units. Such data have not been placed in tabular form; it has been considered advantageous to treat them directly in connection with

FIG. 5. SOLUTION R_1C_2 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

the constructed curves. Thus figures 5 to 10 show not only the polygons for experimental data (broken lines), but the calculated frequency curves and the original determinations from which the curves were constructed. These latter are shown as small circles at the base of the polygon making it possible to read

off directly the value of every determination of the 165 considered. In figure 5, for example, the separate determinations for R_5C_2 are so indicated. In plotting the experimental polygon, the weight values have been grouped

FIG. 6. SOLUTION R_2C_1 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

into classes, the distribution being such as to give an approximation of a normal curve. These classes are indicated by the heavy separating lines at the base of the figure, and the frequencies of determinations included in them are shown by the ordinate values. The various calculated values for the six

curves are given in table 11 where they may be compared. It is seen that here, as in the original series A the mean weights for solutions R_5C_2 and R_2C_5 are the same. In this case, the curve for R_2C_5 is the flatter of the two, show-

FIG. 7. SOLUTION R_1C_1 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

ing a wider range, i.e., a larger coefficient of variability. In fact, these coefficients of variability for both total weights and tops are shown to increase inversely as the values attributed to the solutions by Shive (7). Whether this fact is significant or not remains to be shown. It might indicate that

the real difference between these two solutions lies in the extent of variability which they exhibit rather than in their means. If this be true, R_3C_3 might be called less efficient than R_3C_2 . However, we know practically nothing regard-

FIG. 8. SOLUTION R_3C_2 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

ing the effect of different culture solutions upon the variability of plants grown in them and certainly at the present time are not warranted in drawing distinctions on such a basis. Further study is required on this point.

In figures 11 and 12, this more extensive variation shows itself in an increased overlapping of the three curves as compared to that evident in figures 1 and 2. The difference between R_5C_2 and R_1C_1 remains significant, however, even

FIG. 9. SOLUTION R_2C_5 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

when compared through the probable error of 10 plants. This probable error becomes 11 mgm. for R_5C_2 , and 12 mgm. for R_1C_1 and from these the probable error of difference on the basis of 10 plants is ± 16 mgm.

FIG. 10. SOLUTION R_1C_1 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

TABLE 11

Condensed table of values, individual plants—Series A

	WEIGHT OF COMPLETE PLANT			WEIGHT OF TOPS		
	R_4C_2	R_2C_3	R_1C_1	R_4C_2	R_2C_3	R_1C_1
Mean (mgm.).....	438 ± 3.0	438 ± 4.0	310 ± 3.9	350 ± 4.0	350 ± 4.8	260 ± 4.0
P.E. (mgm.).....	37	49	39	49	61	50
σ (mgm.).....	55 ± 2.0	73 ± 2.7	58 ± 2.2	73 ± 2.7	91 ± 2.4	75 ± 2.7
C.V. (per cent).....	12.8 ± 0.48	17.0 ± 0.65	18.7 ± 0.72	20.8 ± 0.80	26.0 ± 1.08	28.8 ± 1.15

GENERAL DISCUSSION

It is seen from the results reported herein that plants grown in culture solutions exhibit considerable variability. Indeed, this is of such magnitude as to cast serious doubt upon the practice of drawing conclusions from the means of relatively few cultures. Whenever a worker concerns himself with material of so varying a nature as living organisms and seeks to compare the effect of differing environments, he must first of all ascertain the variation expressed by that material under the condition of the experiment. That such variations cannot be determined with any reasonable degree of accuracy with few variants is patent, and, because of this fact, the average of duplicates can have little meaning unless relatively large differences are being considered. Moreover, arithmetic means of even large numbers of determina-

FIG. 11. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

tions of this sort cannot be considered as having fixed value; they are from the very nature of the case only accurate within certain limits, i.e., within their probable error. Until this error of probability is known, it is impossible to place absolute reliance upon the mean itself, no matter how many determinations are averaged. False values may be assigned them, and the conclusions formed be misleading.

In the culture solutions under discussion here, it is apparent that in neither series A, nor B, can a real difference between R_3C_2 and R_2C_3 be demonstrated, whereas had duplicate cultures been employed, the influence of variability upon their means could not have been determined, and solutions might have been given credit for differences due to variability. Thus in series B where

appreciable differences exist between the means of these two solutions, it would have been evidently misleading to have assigned definite values to the two arithmetic means. In Shive's (7) study, there are a number of solutions, such as R_2C_6 , which lie outside the high or low-yield areas as mapped out on his triangles; in fact approximately half of the total number of solutions considered are found within such limits. It is to be wished that our limitations of time and facilities were not such as to make impossible the consideration of all such "medium" solutions, since it is felt that many of them, when interpreted statistically, would show no difference from those making up the high-yield area. Certainly the data reported here would indicate that the designation of a "best" solution, even for a given set of conditions is

FIG. 12. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

open to doubt. In fact it would seem that, as far as dry yield is concerned, the plant may respond equally well to a number of solutions having relatively wide limits in both total and partial concentrations of salts. In this connection, Stiles (11) noted that where total salt concentrations of 1, $1/5$, $1/10$, and $1/20$ normal were compared, the probable error of the mean dry yield of 10 plants was of such magnitude as to nullify any differences shown by the means alone of the first three solutions.

In mapping out "high" and "low" yield areas, Tottingham (13), Shive (7, 8), and McCall (5) have set rather arbitrary limits. Thus in Shive's first paper (7), as well as in a later (8), the range between solutions giving highest and lowest values is divided into four parts, the upper one-fourth constituting

the high-yield area. In the first investigation, where yields of tops are compared, this high-yield area included but two solutions. From the data given herein, it is evident that, because of variability, such an arbitrary limit fails to give the true boundaries of the area in question. "High-yield areas" are really those made up of solutions giving yields which are not significantly different from those of the most efficient solutions. Thus, if we take R_5C_2 as such a standard, then those solutions would be excluded from the high-yield area whose mean yields give a difference from this standard large enough to be legitimately attributed to something other than normal variation. This would probably mean an enlargement of such areas, since undoubtedly certain "medium" solutions such as R_2C_5 would now have to be included.

McCall (5) in a study similar to Shive's (7) but in which sand was employed instead of water cultures, placed R_5C_2 in the low-yield area, while R_2C_7 , a solution quite markedly different in salt proportions, gave the highest yield. Shive (8), on the other hand, in testing out the effect of the relation of moisture in sand cultures to salt balance, again found R_5C_2 the "best" solution. Wherein rests the explanation for these two widely divergent results? The first thought is of the conditions under which the two experiments were conducted. Shive (9), investigating the influence of sand on a nutrient solution, concluded that if the sand is carefully washed, no material alterations in the physiological properties of the nutrient solutions is to be expected, especially if they are frequently renewed. Both Shive and McCall employed washed sand. It is assumed that such conditions for these studies were not exactly the same, and yet they could hardly have varied sufficiently to be held responsible for the large difference noted. Does it not indicate rather that variability is the causal factor, and that in making comparisons on the basis of duplicate cultures, as has been done here, one is actually comparing fluctuating values? How is one to know whether the relative value assigned to R_5C_2 in Shive's work or in McCall's investigations, reveals the true efficiency of this solution? Obviously the only way we can be certain of comparative values in a study of this sort is to treat the solutions statistically and this can be done only by considering sufficient cultures to give a mean of fair degree of accuracy. This would, of course, preclude the consideration of large numbers of solutions differing slightly in salt proportions, but since it is apparent that small differences in partial salt concentration have little physiological significance, it would seem better to consider few such modifications, using relatively large numbers of replicates for each rather than many modifications employing duplicates only. Conclusions based on the former would have the value of being fairly reliable, while those based upon the latter might be entirely misleading.

In the comparison of culture solutions the importance of using seed of known parentage can hardly be overemphasized. Some varieties of wheat, for example, intercross readily under suitable conditions and the effect of this upon the variability of resulting plants may be marked. In this connection

it would be well worth while knowing whether variation is influenced significantly by different atmospheric environments, since it is usually only with difficulty that such conditions can be exactly duplicated. Again, we know nothing regarding the influence of different culture solutions upon variability. In such a series as Shive's, for example, where we find 36 modifications in partial molal concentration of the constituent salts, it would be of value to know whether any of these solutions increase or decrease the tendency of the plants growing in them to vary. It is expected that this point will be brought out in experiments now under way.

Comparison of many of the well known plant-culture solutions have been made from time to time, but it would seem that here a statistical comparison would be necessary in order to determine definitely their relative efficiency. Shive (7) for example, fixed such relative values for the conditions under which he conducted his experiment. It is doubtful, however, whether much reliance can be placed upon these assigned values, even under the limits which the author imposed, since an insufficient number of plants were employed to give a reliable mean. In fact, many of the studies heretofore made on culture solutions, while exceedingly important in their contribution to our knowledge of salt absorption, do not possess the convincing quality they would have if sufficient plants were employed to give a mean of known accuracy. Since plants do vary we must recognize the fact and allow for it in interpretations, otherwise conclusions may be faulty and future work built upon them prove to be wasted effort.

SUMMARY

The study herein reported concerns the variability shown by plants grown in water cultures and under the commonly accepted methods of control.

Three culture solutions were employed, selected from Shive's (7) 1.75-atmosphere series, and characterized by him as "best," "medium," and "poor" as regards growth efficiency. (The designations used by him were R_6C_2 , R_2C_6 , and R_1C_1 , respectively.) They differed only in the partial molal concentration of constituent salts.

Two series were set up, series A in quart "Mason" jars, consisting of 33 replicate cultures of 5 plants each, and series B in 250-cc. bottles, made up of 50 replicate cultures of 6 plants each. Dry weights of complete plants and of tops alone were employed as criteria of variability.

The following general results were obtained:

1. The variation exhibited by plants of both series was of considerable magnitude, the range being approximately 20 per cent on either side of the mean for culture weights, and about 50 per cent when individual plants were compared. When results were plotted in the form of frequency curves and the curves compared, this variation was of such extent as to show considerable overlapping of data for the three solutions in question.

2. The arithmetic means of the "best" and "medium" solutions, series A, were practically the same, and the calculated frequency curves completely overlapped, thus showing no significant difference in the growth efficiency of these two solutions. In series B, an apparent difference was shown between the means of these two solutions, but the probable error was of such magnitude as to prevent assurance that this was due to anything other than normal variation. In both series the "poor" solution gave a mean significantly different from the other two solutions, thus attesting its real inferiority.

3. No difference was apparent between dry weights of the complete plant and tops alone as criteria of comparison.

4. A chance selection of duplicate cultures from series A gave means varying widely in value. Thus where the mean of the series was 2.15 gm., means of 16 pairs selected at random ranged from 1.82 to 2.55 gm.

It may be concluded that:

1. In comparing the relative growth efficiency of various culture solutions, the limits of accuracy of the means compared must be fixed, i.e., their probable errors must be ascertained, otherwise differences due to variation may be attributed to differences in the efficiency of the solution.

2. The mean of few cultures can have at best but low accuracy; it may vary greatly from the more nearly true mean of a larger series. Such a mean is valueless unless large differences are being compared.

3. There is no one "best" solution, at least under the conditions of control possible to maintain at present; undoubtedly the range of solutions favorable to plant growth is relatively extensive.

REFERENCES

- (1) BRENCHLEY, WINIFRED E. 1910 The influence of copper sulphate and manganese sulphate upon the growth of barley. *In Ann. Bot.*, v. 24, p. 571-584.
- (2) BRENCHLEY, WINIFRED E. 1916 The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures. *In Ann. Bot.*, v. 30, p. 77-90.
- (3) DAVENPORT, C. B. 1914 Statistical Methods. John Wiley & Sons, New York.
- (4) LINHART, G. A. 1920 A new and simplified method for the statistical interpretation of biometrical data. *In Univ. Cal. Pub. Agr. Sci.*, v. 4, p. 159-181.
- (5) MCCALL, A. G. 1916 Physiological balance of nutrient solutions for plants in sand cultures. *In Soil Sci.*, v. 2, 207-253.
- (6) MERRIMAN, MANSFIELD. 1913 A Text Book on the Method of Least Squares. John Wiley & Sons, New York.
- (7) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *In Physiol. Res.*, v. 1, p. 327-397.
- (8) SHIVE, J. W. 1920 Relation of moisture in solid substrata to physiological salt balance for plants and to the relative plant-producing value of various salt proportions. *In Jour. Agr. Res.*, v. 18, p. 357-378.
- (9) SHIVE, J. W. 1920 The influence of sand upon the concentration and reaction of a nutrient solution for plants. *In Soil Sci.*, v. 9, p. 169-179.
- (10) SHIVE, J. W., AND MARTIN, WM. H. 1918 A comparison of salt requirements for young and mature buckwheat plants in water cultures and sand cultures. *In Amer. Jour. Bot.*, v. 5, p. 186-191.

- (11) STILES, WALTER 1915 On the relation between the concentration of the nutrient solution and the rate of growth of plants in water culture. *In* Ann. Bot., v. 29, p. 89-96.
- (12) STILES, WALTER 1916 On the interpretation of the results of water culture experiments. *In* Ann. Bot., v. 30, p. 427-436.
- (13) TOTTINGHAM, WILLIAM 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Res., v. 1, p. 133-245.
- (14) WOOD, T. B. 1911 The interpretation of experimental results. *In* Jour. Bd. Agr. (London), v. 18 (sup. no. 7), p. 15-17.
- (15) WOOD, T. B., AND STRATTON, F. J. M. 1910 The interpretation of experimental results. *In* Jour. Agr. Sci., v. 3, p. 417-440.