

PLANT NUTRITION STUDIES IN RELATION TO THE  
TRIANGULAR SYSTEM OF WATER  
CULTURES\*

HARRY F. CLEMENTS

(WITH SIX FIGURES)

A review of the literature bearing on the subject of plant growth in culture solution reveals the fact that criteria used in determining the optimum combination of salts have consisted of measurements of the external portions of plants. Length of tops and roots, green and dry weights of tops and roots, amount of water of transpiration, etc., have served as the means of describing the plants grown in the cultures by the various investigators.

In the course of this article, the term "best" plant will be encountered repeatedly. In order to define the term, it is necessary to consider the purpose for which the plant is grown. Obviously, a plant which is considered best for forage purposes will not be the best so far as seed production is concerned, and *vice versa*, but the very fact that some plants are grown for forage and others for seed, implies that they were capable of being developed in the one direction or the other by man. Thus, when a phenomenon, such as the physiological balance of salts for any particular plant, is to be studied it seems only logical that the plant should be considered from the viewpoint of the plant itself and not from that of the animal. Thus, the general term "best" or "normal" plant refers to one which, more specifically, produces sufficient vegetative growth to insure reproduction of the highest order.

With such a plant in mind, it will be well to consider the indices of normal plants which have been mentioned previously. Measurements of roots must yield conflicting results since GARNER and ALLARD (3) have shown that roots often respond with increased vigor to stimuli which suppress top growth. Obviously, roots can not provide for optimum reproduction, and since the tops are concerned with the survival of the species, attempts must be made to obtain measurements from them. Likewise, if roots become a limiting factor, that condition will be manifested in the expression of the tops.

The amount of transpiration water has been used as an indicator. Here, too, it seems certain that many obstacles will be encountered which will tend to confuse rather than to explain results. At best, the amount of water

\* Published with the consent of the Experiment Station Director.

which a plant will lose will depend on a great multiplicity of factors, such as the concentration of the culture solution, succulence of the plants, etc. It seems improbable, therefore, that such a criterion could serve the office of determining what constitutes a good plant.

When such measurements as length and weight of tops are taken, again the question arises: "Is the tallest or the heaviest plant necessarily the best one?" Apparently it is not. Thus, any plant which shows a tendency toward succulence will in all probability be taller than one which shows a tendency to be higher in dry material. Green and dry weights would be affected in a similar manner.

It becomes apparent that any attempt to determine a well balanced plant—one that develops its usual functions normally—must be based not only upon quantitative data but must also take into consideration something other than the external expression of the plant.

The possibilities of using measurements of the external features of plants as standard criteria, therefore, seem to be quite thoroughly exhausted. It is probable that criteria based on the combined measurements of the internal and external features will yield more fruitful results. KRAUS and KRAYBILL (5) have pointed out that the external appearance of a plant is simply an expression of an internal condition. GARNER and ALLARD (3), on the other hand, have demonstrated that the behavior of plants depends to a large degree on the relative length of light exposure. NIGHTINGALE (8), however, emphasized the fact that although light exposures of various durations modify the behavior of the plant, they first cause a change in the internal composition of the plant which then is followed by a response in external appearance. In view of these tremendously important generalizations, it seems that by combining measurements of both the external and internal conditions, a criterion of what constitutes a normal plant may be established. Any attempt to demonstrate that one's best plant must be his tallest, or his heaviest, from either a green weight or dry weight basis would probably end in failure as the result of unreliable criteria. True, there are cases in which a plant may be an excellent one and be the tallest of a series, but when groups of workers differently located and with different points of view use the same measurements, only confusion from the reader's point of view can be the result.

No attempt will be made here to present an extended review of the literature on this subject. TOTTINGHAM (13) in 1914 gave a thorough review of the literature in his paper, and since that time several hundred papers have appeared dealing with water cultures. Many phases of plant development and behavior have been approached and many valuable and interesting results obtained. Such topics as the effect of the H-ion concentration on growth, the absorption and effect of certain ions or salt combinations,

rate of absorption, and the application of biometry seem to have attracted the greatest share of interest. Many times the data of one man have seemed to be diametrically opposite to those of another who used exactly the same plant and approximately the same conditions of temperature and humidity. Some have supposed that external conditions could not be duplicated and have gone into the work of perfecting control chambers. It seems improbable that any system having such a great range of salt proportions could be disrupted by a few degrees difference in temperature or a few per cent. difference in humidity. If this were the fact, the triangular system would be absolutely useless in our research problems and all that could be obtained with this technique would be to show the uniqueness of the method itself without any illuminating facts concerning the growth and behavior of plants. It does, however, seem that the relative length of day has not been given proper consideration in this type of experimentation.

Thus, an attempt will be made in this paper to throw light on the organic nutrition of the plants grown in the triangle and to study the internal conditions in relation not only to the various combinations of salts, but also to the various exposures of light.

## Methods

### SEED TREATMENT

The Scotch Beauty field pea was used in this experiment for no other reason than that legumes, with few exceptions, have not been used in this type of experimentation. In order to obtain uniform seeds, the peas were selected according to their weight. Several hundred seeds were weighed separately, the distribution of these weights was obtained and the two central classes were established from the data. Approximately five thousand seeds were then selected ranging in weight from 0.2193 to 0.2445 grams. Before planting, these seeds were treated in a formalin solution (1:250) for thirty minutes, washed thoroughly in distilled water, and allowed to soak in distilled water for ten hours. They were germinated between folds of moist blotting paper in pans which were kept at a temperature ranging from 29° to 31° C. When the roots were approximately 2.5 cm. long the seedlings were transplanted to the culture jars. These jars were of the glazed crock type and had a capacity of twelve liters. Thus it was possible to grow forty seedlings in a single culture. The importance of large numbers of plants in a culture is obvious, and, according to HIBBARD and GERSHBERG (4), necessary. Five seedlings were planted into each of eight corks which were fitted into the cover of the crock.

The solutions used were those having an osmotic pressure of one atmosphere used by SHIVE (11). The combinations of salts used were as follows:

$\text{Ca}(\text{NO}_3)_2$ ,  $\text{MgSO}_4$ , and  $\text{KH}_2\text{PO}_4$ . Iron was added in the form of iron tartrate. It was found that when other iron salts were used they invariably caused a partial precipitation of the other culture salts. Twenty-five cc. of a 0.2 per cent. solution of the iron salt were added to each culture at the time that the solutions were changed. Likewise, an equal quantity of 0.2 per cent. solution of  $\text{MnCl}_2$  were added to the cultures to overcome a tendency on the part of the plants to be chlorotic.

The twenty-one culture jars were placed in a greenhouse on a rotating table which was kept in continuous operation during the entire experimental period. The solutions were changed once each week, and the experiment was continued for about seven weeks. At the end of each series the following data were obtained: green and dry weights of tops and roots, respectively, length of tops and roots, and various forms of carbohydrates of the tops, ash content of tops and roots, total nitrogen of the tops, and the nitrate content of both roots and tops. As the experiment progressed some of these indices were omitted after it was evident that they would aid little in the interpretation of results. The plants of all three series were free from disease.

#### CHEMICAL METHODS

A. DESICCATION.—Drying the plants was effected in an oven heated to  $90^\circ\text{C}$ . for the first half hour to insure killing all enzymes, after which the temperature was reduced to  $65^\circ\text{C}$ ., following the recommendations of TOTTINGHAM and LINK (6).

B. CARBOHYDRATES.—Three-gram samples of the dried material were used in the carbohydrate analyses. These samples were subjected to eighteen hours of continuous ether extraction. They were allowed to dry and were then subjected to a two-hour extraction in 90 per cent. ethyl alcohol.

Between the time that series 1 and 2 were run, collateral work with other plants showed that preserving the samples in alcohol instead of drying, considerably reduced the variation one found between two portions of the same sample. Hence, in series 2 and 3, approximately one half of the peas from a single culture was placed in alcohol immediately after length and weight measurements were taken. Such samples were later ground in a plate grinder, more fresh alcohol added, and the whole refluxed for two hours. The rest of the procedure was the same as in those cases where dried material was used.

a. *Simple sugars and sucrose*.—Following the alcohol extraction, the mixture was filtered. The filtrate was evaporated almost to dryness under reduced pressure which was so regulated that the alcohol boiled at never more than  $50^\circ\text{C}$ . The residue was taken up in water. Sometimes it was

difficult to get out all of the material and in such cases the water was either warmed or some ether was added. This watery extract was then clarified with Horne's dry lead. Disodium phosphate was used in deleading, following the recommendation of ENGLIS and TSANG (2). They find that carbonates, sulphates, and oxalates cause losses of reducing substances while disodium phosphate causes practically no loss. Their findings were also verified in this laboratory. After filtration, the filtrate was diluted to 500 cc.

For the sucrose determinations, aliquots of the above volume were treated according to the chemical method outlined in the Official Methods.

The reducing power of these solutions was obtained by the use of the SHAFFER-HARTMAN (10) modification of the MUNSON-WALKER method. Although there seems to be some question in the minds of the Standardization Committee (1) of the American Society of Plant Physiologists concerning the use of this method, there appears to be no definite criticism of the method. In this laboratory, the method was compared with the official MUNSON-WALKER method and showed every indication of being the more reliable of the two. Furthermore, in the process of clarification, even after the most severe filtration, there always remained some precipitated lead salt in a fine degree of suspension. After long standing this settles out. If the gravimetric method is used, the solutions must be allowed to stand until this has settled out; otherwise this, too, would be weighed as cuprous oxide. On the other hand, this suspension does not in any way affect the iodometric method. Although the permanganate method is accurate, it entails considerable routine and where many analyses are to be made, time becomes a limiting factor. It was possible in this laboratory for a single person to complete twenty analyses in an hour. It is felt that this method should be given a wider use. So, in spite of the recommendations of the Committee, the SHAFFER-HARTMAN method was used in this laboratory.

b. *Starch*.—The residue from the alcohol extraction was washed off the filter paper with water and the mixture was boiled for five minutes to thoroughly gelatinize the starch; it was then cooled, and 10 cc. of fresh saliva added. The mixture was allowed to digest over night in an incubator heated to 37° C. This digestion is usually complete after a much shorter digestion, but the routine was so arranged that this digestion began at the end of the day and was allowed to continue until the next morning. The mixture was filtered and H<sub>2</sub>SO<sub>4</sub> was added to the filtrate in such quantities that the latter represented a 2.5 per cent. solution of the acid. The solution was then heated for 1.5 hours on a boiling water bath. This solution was cooled, neutralized with NaOH, and again just turned to the acid side, after which the reducing power was determined. Although it seems to be the general practice to clarify these solutions, it is not being done in

this laboratory for the one reason that analyses made on aliquots, some of which were clarified and others not clarified, gave no difference in reducing power. It may be necessary to clarify when the gravimetric method is employed, but not when the volumetric method is used.

c. *Hemi-celluloses*.—The residue obtained from the starch filtration was washed off the filter paper with 2.5 per cent.  $H_2SO_4$ , so that approximately 400 cc. of the acid were used. The mixture was then placed on a boiling water bath and allowed to remain there for two and a half hours, filtered, neutralized, turned acid, made to volume and analyzed. Here, again, the solution was not clarified.

It may be well here to make a few remarks regarding this almost entirely unknown group of carbohydrates, the hemi-celluloses. In a general way, it is known that pentosans (such as the xylans and arabans), hexosans (such as galactans, mannans, and glucosans), and such mixed polymers as the pectic bodies and mucilages, fall into this group. Beyond this practically nothing is known. In later papers, it will be shown rather definitely that this group is actively concerned with the normal nutrition of plants, and furthermore varies under some conditions of environment as much as starch. The water-holding capacity of all of the members of this group is of sufficient importance to warrant a detailed study of the group. The unfortunate thing about the reports regarding this group is that there are nearly as many methods of extraction as there are reporters. It is true that only relative results can be obtained, but until chemical methods are devised for the quantitative separation of this very important group of reserves, it will be best to adopt a uniform means of extraction, so that one worker's results may be compared with those of another.

C. NITROGEN.—a. *Nitrate nitrogen*.—Nitrate nitrogen determinations were made on aqueous extracts from three-gram samples of dried material. Devarda's method was employed.

b. *Total organic nitrogen*.—Total nitrogen contents were determined according to the Official Kjeldahl method, using three-gram samples.

D. ASH.—Ash determinations were made on two-gram samples. The dried material was placed in alundum crucibles, thoroughly mixed with 10 cc. of glycerol alcohol (1:2), and incinerated until subsequent weighings showed no further losses.

#### CONDITIONS OF THE EXPERIMENT

Three series of peas were run. Series 1 was started on the 29th of January, 1926, and discontinued March 20. Series 2 was used as a check on series 1, and was run from February 4, 1927, until March 24. Series 3 was begun immediately after the second series was completed. It ran from March 26, 1927, until May 24. Light from eleven 200-watt lamps was pro-

vided for series 1 and 2 from four o'clock in the afternoon until midnight of each day. Series 3 was grown under the normal conditions of light. In other words, series 1 and 2 were grown under long day conditions while series 3 was grown under a shorter light exposure; an exposure, however,

<u>3.085</u>	SERIES 1	<u>.280</u>
<u>3.625 2.956</u>		<u>.319 .260</u>
<u>3.428 3.030 3.236</u>		<u>.344 .273 .290</u>
<u>3.308 3.480 3.536 3.495</u>		<u>.327 .300 .339 .326</u>
<u>3.362 3.231 3.072 3.150 3.485</u>		<u>.327 .292 .299 .296 .342</u>
<u>3.070 3.442 3.350 3.225 2.877 2.589</u>		<u>.302 .336 .314 .315 .297 .271</u>
AVE. GREEN WT. PER PLANT-TOPS		AVE. DRY WT. PER PLANT-TOPS
<u>1.825</u>		<u>.087</u>
<u>1.848 1.733</u>		<u>.084 .088</u>
<u>1.888 1.814 1.776</u>		<u>.102 .091 .088</u>
<u>1.923 1.925 1.921 1.868</u>		<u>.097 .093 .094 .099</u>
<u>1.858 1.718 2.002 1.775 1.835</u>		<u>.082 .078 .107 .090 .096</u>
<u>1.575 1.639 1.626 1.698 1.578 1.430</u>		<u>.066 .076 .077 .082 .082 .069</u>
AVE. GREEN WT. PER PLANT-ROOTS		AVE. DRY WT. PER PLANT-ROOTS
<u>45.5</u>		<u>29.2</u>
<u>54.8 48.6</u>		<u>28.6 31.9</u>
<u>58.7 52.3 51.6</u>		<u>28.2 27.0 27.3</u>
<u>58.5 56.0 56.5 57.4</u>		<u>30.4 27.6 28.6 25.9</u>
<u>53.4 53.9 52.3 53.5 54.9</u>		<u>29.7 26.6 27.8 27.4 24.2</u>
<u>58.5 56.9 57.8 55.8 53.3 47.8</u>		<u>30.0 30.4 26.8 25.0 26.5 24.0</u>
AVE. LENGTH (CM) PER PLANT-TOPS		AVE. LENGTH (CM) PER PLANT-ROOTS
<u>1.29</u>		<u>1.11</u>
<u>1.38 1.45</u>		<u>1.20 1.28</u>
<u>1.35 1.31 1.57</u>		<u>1.28 1.26 1.44</u>
<u>1.47 1.37 1.41 1.31</u>		<u>1.23 1.59 1.62 1.48</u>
<u>1.35 1.27 1.25 1.48 1.51</u>		<u>1.41 1.42 1.41 1.72 1.43</u>
<u>1.25 1.33 1.27 1.26 1.20 1.08</u>		<u>1.08 1.29 1.45 1.42 1.63 1.42</u>
NITRATES-PERCENT DRY WT-TOPS		NITRATES-PERCENT DRY WT-ROOTS

Fig. 1. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

that approached the normal day for the growth of peas. The temperature of the greenhouse was kept around 20° C. and the humidity remained relatively low.

As the reader looks at the triangles shown in figs. 1-6, the left hand corner represents the high MgSO<sub>4</sub> corner; the right hand corner the high

	19.7		745
	<u>20.7</u> <u>20.6</u>	SERIES I	<u>.866</u> <u>1.14</u>
	<u>20.7</u> <u>22.1</u> <u>21.1</u>		<u>.783</u> <u>.919</u> <u>.632</u>
	<u>20.4</u> <u>21.8</u> <u>21.8</u> <u>25.6</u>		<u>1.24</u> <u>.386</u> <u>1.08</u> <u>.633</u>
	<u>18.7</u> <u>17.8</u> <u>20.6</u> <u>20.5</u> <u>22.4</u>		<u>.896</u> <u>1.33</u> <u>.379</u> <u>.590</u> <u>.183</u>
	<u>18.4</u> <u>19.1</u> <u>19.5</u> <u>19.6</u> <u>19.7</u> <u>21.0</u>		<u>.346</u> <u>1.54</u> <u>.785</u> <u>.782</u> <u>2.27</u> <u>1.03</u>
	ASH - PERCENT DRY WT.-ROOTS		SIMPLE SUGARS-PCT. DRY WT.-TOPS
	<u>1.30</u>		<u>.050</u>
	<u>1.03</u> <u>1.35</u>		<u>.096</u> <u>.357</u>
	<u>1.90</u> <u>2.88</u> <u>4.03</u>		<u>.236</u> <u>.336</u> <u>.096</u>
	<u>.566</u> <u>.703</u> <u>.969</u> <u>1.49</u>		<u>.133</u> <u>.086</u> <u>.123</u> <u>.086</u>
	<u>1.74</u> <u>1.66</u> <u>.790</u> <u>.766</u> <u>3.32</u>		<u>.213</u> <u>.219</u> <u>.393</u> <u>.583</u> <u>.073</u>
	<u>1.46</u> <u>1.47</u> <u>1.38</u> <u>1.87</u> <u>1.79</u> <u>.42</u>		<u>.186</u> <u>.186</u> <u>.480</u> <u>.186</u> <u>.439</u> <u>.558</u>
	SUCROSE - PCT. DRY WT.-TOPS		STARCH-PCT. DRY WT.-TOPS
	<u>9.11</u>		<u>.700</u>
	<u>8.79</u> <u>8.90</u>		<u>.700</u> <u>.700</u>
	<u>8.71</u> <u>9.00</u> <u>8.96</u>		<u>.929</u> <u>1.16</u> <u>.733</u>
	<u>8.97</u> <u>8.62</u> <u>9.32</u> <u>9.32</u>		<u>3.45</u> <u>4.44</u> <u>4.08</u> <u>2.08</u>
	<u>9.49</u> <u>9.65</u> <u>9.72</u> <u>9.89</u> <u>9.83</u>		<u>1.01</u> <u>2.21</u> <u>4.45</u> <u>4.33</u> <u>3.12</u>
	<u>9.87</u> <u>9.76</u> <u>9.39</u> <u>9.76</u> <u>10.34</u> <u>10.46</u>		<u>.986</u> <u>1.03</u> <u>1.30</u> <u>2.25</u> <u>3.12</u> <u>3.86</u>
	DRY MATTER-PCT. GREEN WT.-TOPS		HEMI-PCT. DRY WT.-TOPS
	<u>4.90</u>		<u>4.27</u>
	<u>2.98</u> <u>4.07</u>		<u>4.65</u> <u>4.72</u>
	<u>4.33</u> <u>4.62</u> <u>2.60</u>		<u>4.75</u> <u>4.64</u> <u>4.52</u>
	<u>3.85</u> <u>6.33</u> <u>6.82</u> <u>4.91</u>		<u>4.34</u> <u>4.64</u> <u>4.61</u> <u>4.47</u>
	<u>4.05</u> <u>5.91</u> <u>6.54</u> <u>6.80</u> <u>6.91</u>		<u>4.31</u> <u>4.35</u> <u>4.36</u> <u>4.32</u> <u>4.31</u>
	<u>3.76</u> <u>4.81</u> <u>4.78</u> <u>6.52</u> <u>8.40</u> <u>6.00</u>		<u>4.49</u> <u>4.39</u> <u>4.31</u> <u>4.26</u> <u>4.17</u> <u>4.06</u>
	TOTAL CARBOHYDRATES-PCT. DRY WT.-TOPS		NITROGEN - PCT. DRY WT.-TOPS

Fig. 2. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

Ca(NO<sub>3</sub>)<sub>2</sub> corner; and the top represents the high KH<sub>2</sub>PO<sub>4</sub> corner. The heavily underscored figures indicate the cultures which produced plants highest in that particular measurement.



Results and discussion

It will be interesting to compare the results of series 1 and 2. These were really checks on one another and should compare favorably. When

SERIES 1						SERIES 2					
<u>3.39</u>						<u>.352</u>					
<u>3.91</u> <u>3.99</u>						<u>.342</u> <u>.352</u>					
<u>4.41</u> <u>4.09</u> <u>3.62</u>						<u>.379</u> <u>.364</u> <u>.291</u>					
<u>4.14</u> <u>4.06</u> <u>3.54</u> <u>2.95</u>						<u>.354</u> <u>.342</u> <u>.317</u> <u>.297</u>					
<u>3.34</u> <u>4.12</u> <u>3.03</u> <u>3.84</u> <u>3.18</u>						<u>.321</u> <u>.402</u> <u>.285</u> <u>.363</u> <u>.302</u>					
<u>3.48</u> <u>4.06</u> <u>3.80</u> <u>4.11</u> <u>4.18</u> <u>2.24</u>						<u>.334</u> <u>.375</u> <u>.364</u> <u>.397</u> <u>.396</u> <u>.242</u>					
AVE. GREEN WT. PER PLANT-TOPS						AVE. DRY WT. PER PLANT-TOPS					
<u>1.92</u>						<u>.088</u>					
<u>1.84</u> <u>1.81</u>						<u>.079</u> <u>.076</u>					
<u>2.20</u> <u>2.05</u> <u>1.87</u>						<u>.076</u> <u>.099</u> <u>.075</u>					
<u>1.97</u> <u>1.78</u> <u>1.75</u> <u>1.60</u>						<u>.085</u> <u>.066</u> <u>.076</u> <u>.061</u>					
<u>2.00</u> <u>2.04</u> <u>1.73</u> <u>2.03</u> <u>1.56</u>						<u>.092</u> <u>.081</u> <u>.081</u> <u>.085</u> <u>.071</u>					
<u>1.58</u> <u>1.61</u> <u>1.93</u> <u>1.91</u> <u>2.05</u> <u>1.35</u>						<u>.069</u> <u>.070</u> <u>.083</u> <u>.079</u> <u>.080</u> <u>.128</u>					
AVE. GREEN WT. PER PLANT-ROOTS						AVE. DRY WT. PER PLANT-ROOTS					
<u>39.8</u>						<u>35.0</u>					
<u>63.3</u> <u>67.3</u>						<u>37.6</u> <u>32.0</u>					
<u>67.9</u> <u>66.3</u> <u>61.6</u>						<u>34.7</u> <u>27.9</u> <u>30.3</u>					
<u>62.8</u> <u>62.9</u> <u>61.5</u> <u>58.9</u>						<u>33.6</u> <u>32.7</u> <u>34.1</u> <u>27.3</u>					
<u>59.6</u> <u>65.5</u> <u>57.5</u> <u>65.4</u> <u>57.1</u>						<u>30.3</u> <u>29.2</u> <u>29.2</u> <u>27.1</u> <u>30.6</u>					
<u>61.4</u> <u>66.0</u> <u>65.1</u> <u>65.4</u> <u>65.4</u> <u>49.4</u>						<u>30.5</u> <u>37.7</u> <u>26.8</u> <u>30.2</u> <u>24.8</u> <u>19.7</u>					
AVE. LENGTH OF TOPS						AVE. LENGTH OF ROOTS					
<u>1.72</u>						<u>.295</u>					
<u>1.10</u> <u>1.30</u>						<u>.340</u> <u>.434</u>					
<u>1.01</u> <u>1.21</u> <u>1.29</u>						<u>.000</u> <u>.000</u> <u>.000</u>					
<u>.857</u> <u>.555</u> <u>.918</u> <u>.891</u>						<u>.479</u> <u>1.79</u> <u>.800</u> <u>.708</u>					
<u>1.11</u> <u>1.18</u> <u>1.18</u> <u>1.12</u> <u>1.37</u>						<u>.422</u> <u>.418</u> <u>.148</u> <u>1.21</u> <u>.311</u>					
<u>2.18</u> <u>1.26</u> <u>1.45</u> <u>1.18</u> <u>.696</u> <u>.715</u>						<u>1.78</u> <u>2.23</u> <u>1.57</u> <u>.670</u> <u>.471</u> <u>1.25</u>					
SIMPLE SUGARS-PCT. DRY WT. TOPS						SUCROSE-PCT. DRY WT. TOPS					

Fig. 3. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

one considers the length of the tops and roots of the two series (figs. 1 and 3), it is doubtful whether or not he would be favorably impressed

since there is a lack of similarity and yet there seems to be a tendency for the highest plants to fall in the same side of the triangle, that is, on the left hand side. When the green weights are examined (figs. 1 and 3), there is a much closer resemblance between the two series, but even they do not check in the way one would like to have them. Dry weights, likewise, tend to be the highest in certain regions and yet they are quite different. This difference may be due to the fact that there were more cloudy days in the period in which series 1 was growing than in that in which the second series was run. It thus becomes obvious that a different means of measuring the plants must be employed. Nitrate determinations (figs. 1 and 3) reveal little in the way of tangible results. In the tops, the highest nitrate contents were found more or less scattered over the triangle. This is to be expected since there are several things which would account for the variability, such as rate of absorption, rate of assimilation, etc. The nitrates of the roots seem to be most abundant in the high  $\text{Ca}(\text{NO}_3)_2$  corner, and this might be expected, but surely this cannot be a measuring stick.

As the next attempt, the ash content of the roots was determined, and although the highest ash content centered on six cultures, it is still a question whether this would indicate good or inferior plants (fig. 2).

Sugar determinations seem to be more promising (figs. 2 and 3). Simple sugars do not yield any definite information except that little can be determined from them. This will be emphasized even more in a report to follow later. Sucrose likewise is erratic, although it shows a strong tendency to be highest in the lower part of the triangle. Starch (figs. 2 and 4) behaves in a similar manner in both series. Hemi-celluloses, too, seem to be most abundant in the lower part of the triangle. Total carbohydrates, that is, the sum of all the carbohydrates reported plus dextrins, are decidedly localized in the lower part of the triangle, and what is more, *they are the most abundant in plants grown in solutions which have the highest proportion of nitrates.* This, indeed, is a startling finding.

When total nitrogen determinations of the tops are examined (figs. 2 and 4), an extremely interesting thing is found. The highest amounts of organic nitrogen, on a per cent. basis, show an extremely well localized area, not where there is the highest nitrate content in the solution, but where there is the highest content of the potassium salt. This finding bears out the study of STOKLASA (12). A study of the relation of potassium to nitrogen assimilation under various exposures of light would, indeed, be fascinating. This is especially promising in view of the so-called radioactivity of potassium.

To show further that this question hinges around the nitrogen reserve, it will be well to call attention to another fact. With the gradual variation in the salt combinations used in any one row in the triangle, there must be

one culture which shows better salt absorption than any other culture in the same row. Thus, no matter how good or how poor a series of plants may be there must necessarily be one plant in this series which is either the best or the poorest. So in any row of the triangle one culture must either be the lowest in total nitrogen or the highest. With the gradual variation in the combination of nutrient salts there ought to be a gradual variation

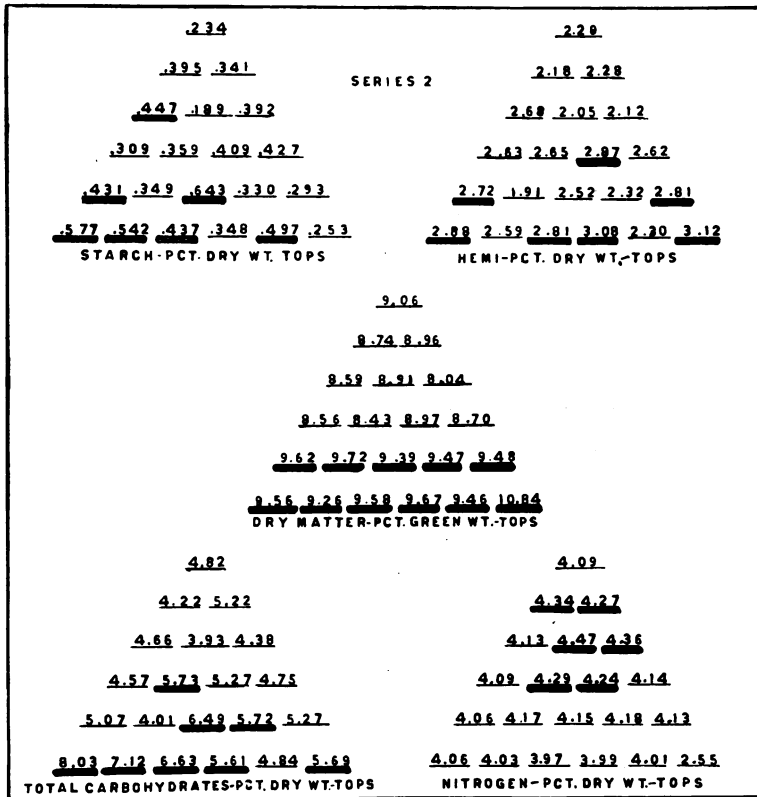


FIG. 4. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

in the total nitrogen contents in either one or two directions. This is especially outstanding in the total nitrogen results of all three series. Thus, the total nitrogen per cent. of series 1, the lowest line, reads 4.49, 4.39, 4.31, 4.26, 4.17, and 4.06. The row leading from the lower left hand corner to the upper corner reads 4.49, 4.31, 4.34, 4.75, 4.45, and 4.27. In the first row mentioned, the highest nitrogen assimilation occurred in the first culture and a gradual but definite decrease is found throughout the row until the last culture. In the second row given, the cultures with one exception in-

creased up to 4.75, and then decreased. It is extremely interesting to compare these trends in series 1 with those in series 2. Although all of the cultures ran lower in total nitrogen in series 2 than in series 1, these trends within the series show marked similarities. Thus in series 1, the high culture in the row on the left side of the triangle was the fourth culture up while in series 2 it was the fifth. In the row on the right, the fifth culture up was the highest while in series 2, the fourth culture was the highest. In the bottom row, the first culture on the left was highest, the one on the right was lowest, exactly as in series 2. It seems that these data show very definitely that the absorption and assimilation of nitrogen are markedly affected by the physiological balance of the culture solution. The trends in series 3 reveal the fact that a shorter light exposure very markedly affects the position of the highest culture in any one row. This seems to yield results favorable to the question asked by NIGHTINGALE and KRAUS (9): "Does light affect the fertilizer requirements of plants?" It seems very apparent that it does.

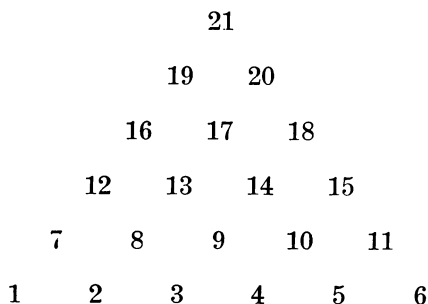
In a measure, now, we are able to generalize regarding the best culture solution, and regarding what we might term as the best plant or series of plants. KRAUS and KRAYBILL working with the tomato found a very definite relation existing between abundant organic nitrogen and succulence of plants, and between a high carbohydrate content and high dry matter. As either of these two groups of reserves became excessive, the plants no longer were regarded as normal because their reproductive process was hindered. When the plants were extremely high in nitrogen, many of their flowers failed to develop, while the general stature of the plant continued to enlarge. On the other hand, when the plants were extremely high in carbohydrates, some flowers developed and set fruit, but the vegetative growth of the plants was not sufficient to make efficient reproduction possible. Reproduction of the highest order was found only in those plants in which there was neither an excess of nitrogen nor an excess of carbohydrates. In other words, if these two reserves are moderately abundant in plants, the plant will express that internal condition by having vigorous vegetative development accompanied by fruitfulness. This, obviously, is what will be a normal plant. Although the tomato is a highly selected plant which must depend on artificial care for its proper development, it afforded a picture which has been verified time and again with other plants. The pomologist finds the same principles applicable to his fruit trees and regulates the reserve balance by proper pruning, fertilizing, etc. The florist practices it when he continually re-pots his plants until a short time before he wants them to flower. Then, he allows the plants to become "pot bound." This is nothing more than limiting root development and nitrate absorption and hence, correcting the highly vegetative state of his plants. The agronomist

realizes the application of the nitrogen-carbohydrate principle when he criticizes a field of grain as being too heavy in straw (too vegetative) or being too short (non-vegetative). The production of grain obviously is optimum when the straw is neither short nor long but intermediate. Thus, it seems only fair that this same criterion should be used in experimental plots and culture solutions.

In the triangles of series 1 and 2, the high nitrogen area is found in the top of the triangle, while the high carbohydrate area is found at the bottom. In view of the relation described above, it becomes obvious that the best plant of the series will not be found in the high nitrogen area, nor in the high carbohydrate area, but must be in a region which is intermediate with respect to these two groups of reserves. This region in the triangle, of course, will be between the high nitrogen and high carbohydrate areas. It was indicated above that plants high in nitrogen are high in moisture and those low in nitrogen and high in carbohydrates will be high in dry matter. A glance at the triangles representing dry matter in series 1 and 2 will not only verify this generalization but will show how closely the two series resemble one another.

The fact that the areas in which we find these reserves in greatest abundance are practically the same, for the first two series show rather decisively that here is a measuring stick which can be used to determine the region of the triangle in which best growing conditions prevail.

Thus, it is readily seen that this new criterion is far more satisfactory in determining the best cultures. Neither the total nitrogen nor the total carbohydrates can serve as indicators when taken alone, but when they are used in relation to one another, very definite information is obtained. Each maintains a definite area in series 1 and 2, and in view of the discussion here presented the best balanced plant cannot be found in either groups, but in the region between these areas. After this region is located, then such indices as length of the plants, their weights, etc., can select the best culture. Following is a diagram showing the numbering system:



In series 1, cultures no. 13, 14, 16, 17, 18, 19, 20 are eliminated from further consideration because of their high nitrogen content. Cultures no. 4, 5, 6, 8, 9, 10, 11, 13, 14 are eliminated because of their high carbohydrate content. Thus, cultures no. 1, 2, 3, 7, 12, 15, and 21 remain. Culture no. 21 is decidedly mediocre in its external appearance, being the shortest plant of the entire series. Of the remaining cultures no. 12 seems to be superior to the others in that it is high in green weight and dry weight of both tops and roots. Its tops are taller than those of any of the remaining cultures.

In series two, which should approximate series one since light conditions were practically the same, the carbohydrates are most abundant in the lower part of the triangle while the high nitrogen plants are found near the top. Thus, cultures no. (fig. 4) 13, 14, 16, 17, 18, 19 and 20 are eliminated because of their high nitrogen content. Cultures no. 1, 2, 3, 4, 6, 9, 10 and 13 are eliminated because of their high carbohydrate content. Cultures no. 5, 7, 8, 11, 12, 15, 21 remain for further consideration. It is to be noted that of these, 7, 12, 15 and 21 were also found in the remaining group in series 1 after the poorest cultures were removed. Thus, it is possible to select a culture which showed good growth in both series. That there is some difference in the growth of the plants can well be explained by temperature and humidity conditions. Culture no. 21 shows strong tendencies to favor high carbohydrate formation in both series. Culture no. 15 is decidedly inferior in its external features in series 2. Culture no. 12 is superior to no. 7, in green and dry weight of tops and in length of tops and roots, and since no. 12 showed a decided advantage over the other cultures in series 1, it seems only just to select it as the best culture in which to grow peas under a long day exposure. This balance is made up of three parts of  $\text{KH}_2\text{PO}_4$ , one part of  $\text{Ca}(\text{NO}_3)_2$  and four parts of  $\text{MgSO}_4$ .

Now it will be of interest to see what effect a shorter day has on the relative positions taken by the highest carbohydrate and highest nitrogen cultures. Series 3 was run for that purpose. Beginning with the superficial measurements such as length of tops, roots, their green and dry weights (fig. 5), it is seen at once that our biggest plants are in an entirely different part of the triangle than they were in the previous series. Furthermore, when the region in which nitrogen is the highest is examined (fig. 6), it is seen at once that it has in a sense migrated to a lower position on the triangle nearer to the high nitrate corner. How has the high carbohydrate area responded? That has seemingly been switched around so that now instead of being in the lower right hand corner as in previous series, it is found in the left hand side of the triangle. Under these conditions, we must look for the best plant in a place different from that of the other series.

In applying the elimination process to this series (fig. 6), cultures no. 4, 9, 10, 13, 14 and 16 represent high nitrogen cultures. Cultures no. 1, 2, 3, 7, 12, 16, 17, 18, 19, 20 and 21 represent high carbohydrate cultures. Thus, the best plants must be found among cultures no. 5, 6, 8, 11 or 15. Culture no. 6 does not compare favorably with the other cultures so far as the external measurements are concerned (fig. 5). Culture no. 8 is rather

SERIES 3				
<u>4.49</u>				
<u>3.73 4.91</u>				
<u>4.01 4.39 4.25</u>				
<u>5.95 5.82 6.88 5.85</u>				
<u>6.44 6.52 6.49 7.12 6.66</u>				
<u>4.16 4.73 5.00 5.00 7.18 5.51</u>				
AVE. GREEN WT. PER PLANT—TOPS				
<u>2.65</u>				
<u>2.32 2.61</u>				
<u>2.29 2.19 2.23</u>				
<u>2.62 2.67 2.71 2.39</u>				
<u>2.90 2.93 2.88 3.01 2.83</u>				
<u>2.22 2.55 2.68 2.53 3.06 2.53</u>				
AVE. GREEN WT. PLANT—ROOTS				
<u>4.59</u>				
<u>3.90 5.17</u>				
<u>4.13 4.63 5.16</u>				
<u>4.59 4.76 5.32 5.59</u>				
<u>5.83 4.99 5.65 6.13 6.15</u>				
<u>3.95 3.91 3.95 4.03 6.14 7.13</u>				
AVE. LENGTH OF TOPS				
<u>.39</u>				
<u>.34 .59</u>				
<u>.40 .39 .41</u>				
<u>.79 .82 .98 .75</u>				
<u>.97 .73 .76 1.02 1.03</u>				
<u>.50 .53 .63 .70 .97 .62</u>				
AVE. DRY WT. PLANT—TOPS				
<u>.15</u>				
<u>.14 .17</u>				
<u>.14 .13 .14</u>				
<u>.16 .13 .14 .16</u>				
<u>.14 .16 .19 .19 .16</u>				
<u>.14 .13 .10 .15 .15 .14</u>				
AVE. DRY WT. PER PLANT—ROOTS				
<u>4.41</u>				
<u>2.47 2.92</u>				
<u>2.56 3.22 3.02</u>				
<u>2.37 2.89 3.06 2.91</u>				
<u>2.46 2.86 2.36 2.96 2.75</u>				
<u>2.45 2.48 2.63 2.79 3.08 2.25</u>				
AVE. LENGTH OF ROOTS				

FIG. 5. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

high in moisture while culture no. 11 is higher in dry matter than any other culture. Culture no. 15 shows a tendency for greater root development in proportion to tops and thus culture no. 5 is left. It is moderately high in dry matter and nitrogen. Its low content of carbohydrates is well explained by the fact that its top and root development is excellent. Thus, culture no. 5 apparently contains the optimum balance of salts in which to grow peas under an intermediate light condition. This balance is represented by one part of  $\text{KH}_2\text{PO}_4$ , five parts of  $\text{Ca}(\text{NO}_3)_2$ , and two parts of  $\text{MgSO}_4$ .

This, perhaps, helps in a measure to explain some of the inconsistencies which are encountered in the literature regarding the plants grown in the triangular system. We are contending not only with a variety of salt combinations but also with the way plants respond to these combinations in building their reserves. These reserves then seemingly dictate the external expression of the plant. Further than that, we are contending with the

SERIES 3.									
<u>5.34</u>					<u>.93</u>				
<u>4.81 4.95</u>					<u>.44 .00</u>				
<u>4.62 4.72 4.84</u>					<u>.33 .16 .00</u>				
<u>4.86 4.69 3.66 4.08</u>					<u>.48 .13 .00 .00</u>				
<u>4.66 5.69 5.11 4.11 2.95</u>					<u>.68 .13 .58 .27 .52</u>				
<u>6.07 6.25 5.07 4.23 3.46 5.29</u>					<u>.96 .71 .35 .42 .17 .00</u>				
SIMPLE SUGARS					SUCROSE				
<u>8.6</u>					<u>5.34</u>				
<u>9.2 11.8</u>					<u>4.02 2.25</u>				
<u>9.9 8.9 9.6</u>					<u>4.02 6.14 3.98</u>				
<u>13.2 14.1 14.3 13.1</u>					<u>4.41 2.96 2.96 1.83</u>				
<u>15.0 11.2 11.7 14.3 15.6</u>					<u>4.63 .70 .85 .77 .88</u>				
<u>12.1 11.2 12.6 14.3 13.5 11.6</u>					<u>2.66 5.63 3.91 1.70 1.38 2.18</u>				
DRY MATTER					STARCH AND HEMI				
<u>11.61</u>					<u>4.06</u>				
<u>9.27 8.20</u>					<u>4.63 4.13</u>				
<u>8.97 11.05 8.82</u>					<u>5.08 4.35 4.29</u>				
<u>9.75 7.77 6.62 5.51</u>					<u>4.89 5.45 5.56 4.24</u>				
<u>9.97 6.52 6.54 5.15 4.35</u>					<u>4.44 4.83 5.93 5.57 4.40</u>				
<u>9.69 12.59 9.23 6.35 5.01 7.43</u>					<u>4.17 4.45 4.83 5.10 4.20 4.17</u>				
TOTAL CARBOHYDRATES					NITROGEN				

Fig. 6. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

effect that light has on modifying the response of plants to the combination of salts.

Following such studies it becomes apparent that the triangular system has the extremely important rôle in plant physiology of showing the effect that light has on modifying the fertility requirements of plants in the various sections of the country and at various seasons of the year. Furthermore, the aid of the triangle will be of inestimable value in studying the effect of the various elements on such functions as nitrogen assimilation and carbohydrate accumulation and utilization.



The writer wishes to express his gratitude to Dr. E. A. BESSEY for his criticisms of the manuscript, and to Dr. R. P. HIBBARD for the use of his laboratory and equipment.

### Summary

1. Three series of peas were grown in the triangular system of water cultures. Series 1 and 2 were grown at the same time of the year, the former in 1926, the latter in 1927. Series 3 was run in 1927 but at a date later than series 2.
2. From data presented, it is obvious that such criteria as length of plants, green and dry weights, etc., cannot give a correct idea of the best balance of salts for the growth of plants.
3. Indices such as highest ash content and highest nitrate content are presented, but are shown to be erratic in their positions and hence unreliable.
4. This index unreliability is likewise true of the soluble forms of carbohydrates.
5. Data are presented which show that  $\text{KH}_2\text{PO}_4$  has a very marked influence on nitrogen assimilation.
6. The areas in which nitrogen and carbohydrates are highest, respectively, assume very definite positions in the triangle.
7. The positions of high nitrogen and high carbohydrate areas are taken at opposite sides of the triangle and leave an area in which the best plants of the triangle may be expected to be found.
8. It is shown that a shorter exposure to light results in different positions being occupied by the best cultures. This must be expected in view of the behavior of the two large groups of reserves found in the plants which were grown under shorter light exposures.
9. A possible rôle of the triangular system of water cultures in future research regarding fertilizer requirements is mentioned.

MICHIGAN STATE COLLEGE,  
EAST LANSING, MICH.

### LITERATURE CITED

1. COMMITTEE for the Standardization of Chemical Methods, Report of Plant Physiol. **2**: 195–204. 1927.
2. ENGLIS, D. T. and TSANG, C. Y. The clarification of solutions containing reducing sugars by basic lead acetate. The effect of different deleading agents. Jour. Amer. Chem. Soc. **44**: 865–867. 1922.
3. GARNER, W. W. and ALLARD, H. A. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871–920. 1923.

4. HIBBARD, R. P. and GERSHBERG, S. The salt requirements of Marquis wheat in water culture for the vegetative phase of development. Michigan Exp. Sta. Tech. Bull. 64. 1-28. 1924.
5. KRAUS, E. J. and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Sta. Bull. 149. 1-90. 1918.
6. LINK, K. and TOTTINGHAM, W. E. Effect of the method of desiccation on the carbohydrates of plant tissues. Jour. Amer. Chem. Soc. 45: 439-447. 1923.
7. DOOLITTLE *et al.* (Editing Committee). Official and tentative methods of analysis of the association of official agricultural Chemists. 2d ed. Washington, D. C., 1928.
8. NIGHTINGALE, G. T. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Exp. Sta. Res. Bull. 74. 1-68. 1927.
9. ————— and KRAUS, E. J. Does light affect the fertilizer requirements of plants? Wisconsin Exp. Sta. Bull. 362. 52-54. 1924.
10. SHAFFER, D. A. and HARTMAN, A. F. The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. 45: 365-390. 1920.
11. SHIVE, J. W. A three salt nutrient solution for plants. Amer. Jour. Bot. 2: 157-161. 1915.
12. STOKLASA, J. Ist das Kaliumion an der Eiweissynthese in der Pflanzenzelle beteiligt? Biochem. Zeitschr. 73: 107-60. 1916.
13. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. 1: 133-245. 1914.