

# WATER-CULTURES WITH CLAY SUSPENSIONS AND WITH NUTRIENT SOLUTIONS

by

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(With plates II—IV)

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## CHAPTER I.

### The Use of Water-Cultures in Plant Physiology in the Past.

Water-cultures as a research method for experiments concerning vegetation have been known already for a considerable time. Probably the first who did accurate work on this line was John Woodward, professor of „Physick” in Gresham College. In the years 1691 and 1692 he made his remarkable experiments, which were published — septimo anno imprimatur — in the Philosophical Transactions of the Royal Society of London for the year 1699.<sup>1)</sup>

In contrast with Van Helmont, Boyle and Bacon he was of opinion that „vegetables owe their growth and augment not to the water but to the earthy matter sustained in it.”

Van Helmont<sup>2)</sup>, who made the first quantitative

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<sup>1)</sup> John Woodward, Some Thoughts and Experiments Concerning Vegetation. Philosophical Transactions of The Royal Society of London. Vol. XXI, 1699. Number 253, p. 193—227.

<sup>2)</sup> Johan Baptista van Helmont, Opera omnia. Frankfurt 1682, p. 104.

experiment about this question, planting a shoot of a willow of five pounds in 200 pounds of dried soil and growing the willow for five years, found that after that time, the willow had augmented to 170 pounds, whilst the soil after drying showed only a diminution of weight of two ounces. He consequently concluded, that all the substance of the tree had arisen from the rainwater alone, with which the soil had been wetted.

Woodward however remarks, „that it is hard to bake „such a quantity of earth with such accuracy as to reduce „it twice to just the same dryness and that every water, „notwithstanding its clearness, contains terrestrial matter”. In order to get a better insight into this question he grew some plants, especially spearmint (*Mentha viridis* L.) in different kinds of water and suspensions. To give a clear impression of the way in which those first watercultures were arranged, of the care and exactness of the author and the results he obtained, his description of the experiments of 1691 and his results with spearmint in 1692 follow here.

TABLE 1.  
Experiments of Woodward with spearmint in 1692.

Source of water	mark	Number of days	weight in grains				grains of water transpired for one grain gain in weight	average	
			weight of plants		gain in weight	transpired water			
			when put in	when taken out					
Hyde park conduit . . . . .	H	56	127	255	128	14190	110,9	102,7	
	I	56	110	249	139	13140	94,5		
Hyde park conduit + 1 ½ ozs garden mould ..	K	56	76	244	168	10731	63,9	58,2	
	L	56	92	376	284	14950	52,6		
Hyde park conduit	distillate	M	56	114	155	41	8803	214,7	214,7
	residue	N	56	81	175	94	4344	46,2	46,2

„I chose several Glass Viols, that were all, as near as  
 „possible, of the same shape and bigness. After I had  
 „put what water I thought fit into every one of them,  
 „and taken an Account of the weight of it, I strain'd and  
 „ty'd over the Orifice of each Viol, a piece of Parchment,  
 „having an hole in the middle of it, large enough to  
 „admit the Stem of the Plant I design'd to set in the  
 „Viol, without confining or straightning it so as to impede  
 „its Growth. My intention in this, was to prevent the  
 „enclosed Water from Evaporating, or ascending any  
 „other way than only thorow the Plant to be set therein.  
 „Then I made choice of several Sprigs of Mint, and  
 „other Plants, that were, as near as I could possibly  
 „judge, alike fresh, sound and lively. Having taken the  
 „weight of each, I placed it in a Viol, ordered as above:  
 „and as the Plant imbibed and drew off the Water, I  
 „took care to add more of the same from time to time,  
 „keeping an Account of the weight of all I added. Each  
 „of the Glasses were, for better distinction, and the more  
 „easy keeping a Register of all Circumstances, noted with  
 „a different Mark or Letter, A, B, C & c and all set in  
 „a Row in the same Window, in such manner that all  
 „might partake alike of Air, Light, and Sun. Thus they  
 „continued from July the Twentieth, to October the  
 „Fifth, which was just Seventy Seven Days. Then I took  
 „them out, weigh'd the Water in each Viol, and the  
 „Plant likewise, adding to its weight that of all the Leaves  
 „that had fallen off during the time it stood thus. And  
 „Lastly, I computed how much each Plant had gain'd:  
 „and how much Water was spent upon it.”

The experiment of Table 1 took place from June 2 to  
 July 28, 1692. Controlglasses with a stick instead of a  
 living twig did not lose any water.

Woodward arrives in his experiments at the following  
 conclusions:

„In Plants of the same kind, the less they are in Bulk, „the smaller the Quantity of the Fluid Mass in which „they are set is drawn off.”

„The Plant is more or less nourish'd and augmented in „proportion as the Water in which it stands contains a „greater or smaller quantity of proper terrestrial Matter „in it”.

„Vegetables are not form'd of Water: but of a certain „peculiar terrestrial Matter”.

„Water serves only for a Vehicle to the terrestrial „Matter which forms Vegetables: and does not itself make „any addition unto them.”

From these experiments made in 1692 it is obvious that the more „terrestrial Matter” the water contains, whether this matter originates from garden mould or from the concentrated or natural spring water from Hydepark itself, the greater is the proportional increase of the plants and the smaller the quantity of water necessary to obtain the same gain in weight.

More than half a century afterwards Duhamel du Monceau<sup>1)</sup> showed in a convincing way the possibility of plants passing in a normal way through the whole cycle of their physiological processes without their roots coming in contact with any other matter than natural water. He made seeds of *Vicia Faba* L. germinate between humid sponges; when the roots had a length of little more than an inch the seedlings were placed in the mouth of carafes, only the roots coming in contact with the water. Duhamel used for his water-cultures water of the river Seine after filtering it through sand. In this way he got plants of *Vicia Faba* three feet high with beautiful leaves and flowers. Some of them even fructified

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<sup>1)</sup> M. Duhamel du Monceau, *La physique des arbres*. Paris, 1758, 2ième partie, p. 202.

and sunlight acted in the processes of assimilation and dissimilation. On the origin and composition of the ash in plants and its necessity for vegetable life he spent a and gave some small fruits. He repeated his experiments with *Aesculus Hippocastanum* L., the horse-chestnut, *Prunus Amygdalus* Stokes, the almond-tree and *Quercus Robur* L., the oak-tree. These experiments too succeeded exceedingly well. One of his oak-trees he even grew for eight years; the tree got 4 or 5 lateral branches and a height of 18 inches and only succumbed by lack of water during a long absence of Duhamel.

Concerning the growth of his trees on Seine-water only, the author remarks:

„Mais je dois avertir qu'il n'y a pas d'apparence que ces arbres eussent pu faire dans la suite de grands progrès: ils avoient poussé plus fortement les deux premières années que s'ils avoient été dans une bonne terre; les productions de la troisième & de la quatrième année étoient encore assez belles; mais depuis ce temps les pousses diminuoient tous les ans, & n'étoient presque plus sensibles, quoique les arbres continuassent à se garnir de belles feuilles”.

About the problem whether the dry matter of plants is formed out of water or terrigenous material he pronounces no decided opinion, but concludes:

„Je ne me suis proposé que de prouver, que l'eau la plus pure & la plus simple qui puisse se trouver, peut fournir aux plantes la nourriture qui leur est nécessaire, sans m'embarasser d'expliquer comment les parties de ce fluide deviennent solides”.

Not until chemistry had made important progress and had established quantitative methods it became possible to get a clearer insight by physiological experiments in the formation of dry matter by the plant.

In the footsteps of Ingenhousz and Senebier it

was Théodore de Saussure who by well-arranged physiological experiments combined with accurate chemical methods settled what part water, carbonic acid, oxygen great deal of work and his insight into this problem ranks high above that of his age. For some of his researches in this line he made use of water-cultures. About his work published in 1804 <sup>1)</sup> „Recherches chimiques sur la végétation” in which he laid down the results of his investigations, Julius Sachs <sup>2)</sup> remarks:

„Die Geradheit und kurz angebundene Art, mit durchschlagender Sicherheit quantitative Resultate zu Tage zu fördern, die Consequenz und durchsichtige Klarheit des Gedankenganges sind es vorwiegend, die uns bei der Lectüre dieses Werkes, sowie auch bei Saussure's spätern Schriften, ein Gefühl von Vertrauen und Sicherheit einflößen, wie kaum ein anderes Werk seit Hales bis auf die neueste Zeit”.

And the reader of today too, more than half a century after the pronouncement of Sachs, will hesitate what to admire more in the work of De Saussure: his keenness and clearness in putting the problems, the efficiency and accuracy of his experiments or the soundness of his conclusions.

That plants do not want only water and carbonic acid for their development and the production of seed, De Saussure proved by putting seeds of *Vicia Faba* L., *Pisum sativum* L., *Phaseolus vulgaris* L. and *Nasturtium officinale* R.Br. in funnels filled with pure sand or horse-hair and watering them with distilled water, the excess

<sup>1)</sup> Théodore de Saussure, *Recherches chimiques sur la végétation*. Paris, 1804 (Translated into German in the series of Ostwald's *Klassiker der exakten Wissenschaften*, 15 and 16 as „Chemische Untersuchungen über die Vegetation”).

<sup>2)</sup> Julius Sachs, *Geschichte der Botanik vom 16ten Jahrhundert bis 1860*. München 1875. Drittes Buch p. 538.

of the latter running down. The plants often flowered, but fruits never ripened, though he repeated this experiment for five consecutive years. (Recherches, p. 245).

With the same object he afterwards grew seedlings of *Vicia Faba* on medicine bottles, filled with distilled water. He did not use sand, because he did not feel sure, whether the root juice might not attack stones itself, „l'érosion que les lichens semblent faire quelquefois aux rochers, en est un indice". (Recherches, p. 305) The broad beans remained during 2½ months in the open air in the sunshine sheltered from rain. They made small flowers, then began to droop and were harvested. The air-dry material from these 41 plants was only 6,6 % more than the weight of the beans before the experiment. The quantity of ash was 424 mgs or only 16,3 % more than was found in the same number of seeds, analysed as such. This small increase of ash cannot be supposed to have been formed in the plant from air or water, as former investigators thought, for, as De Saussure says: „Si l'on „considère avec quelle promptitude un corps quelconque „exposé à l'air libre se recouvre de poussière, par l'immense „quantité de corpuscules qui flottent dans notre atmos- „phère: si l'on remarque que les quarante et une plantes „de fève, ont offert pendant près de deux mois à ces „corpuscules un arrêt de plus d'un pied carré de surface, „on doit être moins surpris que cette addition existe que „si elle n'existait pas." (Recherches, p. 307).

Whether roots of plants take from salt solutions salt and water in the same or in another proportion as these are given, was investigated too. He dissolved 803 mgs of different salts to the liter and found that when rooted plants had taken up half the volume of the solution, at the same time only 4 to 17 % of the salt had entered the roots, from one salt having been taken a larger part than from another. From a solution of coppersulphate however

water and salt were taken in the same proportion as they were offered, whilst the roots were very soon disorganised. When the roots of the plants were cut, the other salts too entered the plant in the same proportion to the water as they were given. (Recherches, p. 248).

After De Saussure progress in this part of science was only slow. The humus theory prevented, especially in Germany, the progress of ideas concerning the taking up of mineral components from the soil by plants. After the publication in 1840 of Liebig's „Report on Chemistry in its Application to Agriculture and Physiology” the humus theory was gradually abandoned and the mineral theory about the nourishing of vegetation by the soil got its chance.

Manuring with mineral fertilizers entered the focus of interest in agricultural circles. The composition of the ashes of plants was already wellknown and now the problem was put, which of these components were necessary for the development and which were superfluous.

In Germany after 1850 a number of agricultural experiment stations were established, the oldest in 1851 at Möckern in the kingdom of Saxony; many others followed in rapid succession, and this problem found naturally its way on the plan of work of these institutions. In the oldest volumes of the periodical „Die landwirtschaftlichen Versuchs-Stationen” initiated in 1859, numerous descriptions of investigations on this subject are found. As a method suitable for this researches the water-culture was chosen. J. Sachs<sup>1)</sup> accounts for this choice in this way:

„Die Wahl der Methode wird durch den Umstand „hinreichend gerechtfertigt, dasz es unmöglich ist, ein „festes Medium, z.B. Sand, Kohle u.s.w. so rein darzu-

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<sup>1)</sup> J. Sachs, Vegetations-Versuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernährungsbedingungen von Mais, Bohnen und anderen Pflanzen. Landw. Vers. Stat. Bd 2, 1860, p. 220.



„stellen, dasz diese Medien an die Wurzeln gar nichts  
 „mehr abgeben. Da Vegetationsversuche auch fast immer  
 „in groszer Anzahl angestellt werden müssen, so ist die  
 „Herstellung des nöthigen Quantums gereinigten Sandes  
 „und gereinigter Kohle mit sehr viel Zeitaufwand, Mühe  
 „und Kosten verbunden.“

„Durch die Gegenwart eines festen Mediums, mit  
 „welchem die Wurzeln in Berührung stehen, wird eine  
 „unmittelbare Behandlung der eigentlichen Ernährungs-  
 „fragen unthunlich, weil dabei die Porosität, Adhäsion,  
 „Absorption, die ungleiche Feuchtigkeit u.s.w. auf die  
 „zugesetzten Nährstoffe unbekannte Entschlüsse äuszern.  
 „Endlich ist eine genaue Beobachtung der Wurzeln  
 „während der Vegetation wünschenswerth, aber in einem  
 „festen Medium unthunlich“.

It took however several years and much experimenting before the scientists succeeded to grow in water-culture thriving plants which equalled and surpassed those from the fields. At the outset spring-water was given as nutrient medium, to which some salts were added. In the description of his first experiments with *Phaseolus nanus* L. Knop<sup>1)</sup> records that he added some potassium nitrate and a smaller quantity of magnesium sulphate to a great vessel with spring-water. With this solution a number of smaller vessels of one and a half liters were filled and to each was added about one gram of calcium ammonium phosphate.

Sachs<sup>2)</sup> tried a.o. the method of fractionised solutions: with this method the plants were put by turns in two nutrient liquids of different composition, one containing

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<sup>1)</sup> W. Knop, Ein Vegetationsversuch. Landw. Vers. Stat. Bd. 1, 1859, p. 196.

<sup>2)</sup> J. Sachs, Vegetations-Versuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernährungsbedingungen von Mais, Bohnen und anderen Pflanzen. Landw. Vers. Stat. Bd 2, 1860, p. 226.

all the bases and acids which were to be used, except phosphoric acid, the other the phosphoric acid and the other components, except those bases, which give a precipitation with phosphoric acid.

During the experiments to find a nutrient liquid of a good composition and suitable concentration, many particulars were noticed which were of interest for the prosperous growth of plants. Knop<sup>1)</sup> records e.g. that in proportion as the different plants grew in the nutrient medium the reaction of the solution became from neutral more and more alkaline; at the same time the plants became chlorotic.

Stohmann<sup>2)</sup> observed that solutions, which were at the outset acid, became alkaline after growing maize in it for some time. He advised in that case to give a fresh solution or to add some drops of diluted phosphoric acid as soon as the reaction became neutral. This proceeding, says he, facilitates the cultivating of plants in nutrient liquids considerably: he grew maizeplants, some of which became 7 feet high; one bore 370 ripe grains with full germinative faculty.

The gradual progress in the results obtained with water-cultures in strikingly illustrated by Friedrich Nobbe,<sup>3)</sup> who published a survey of the yields of buckwheat in water-culture at Chemnitz. From the most thriving buckwheatplant of his experiment he calculated each year the proportion of the air-dry weight of the whole plant to the weight of one fruit; he also counted from these plants the number of ripe fruit. The figures were as stated below.

<sup>1)</sup> W. Knop, Quantitativ-analytische Arbeiten über den Ernährungsprocesz der Pflanzen. Landw. Vers. Stat. Bd. 3, 1861, p. 300.

<sup>2)</sup> F. Stohmann, Über Vegetationsversuche in wässrigen Lösungen. Landw. Vers. Stat. Bd. 4, 1862, p. 66.

<sup>3)</sup> Friedrich Nobbe, Über die Entwicklungsfähigkeit und Tragweite der Wassercultur-Methode. Landw. Vers. Stat. Bd. 10, 1868, p. 3.

	multiplum in proportion to one fruit	ripe fruit
1862	215	20
1863	550	162
1864	1130	304
1867	4786	796

The buckwheatplant of 1867 had an air-dry weight, roots included, of 120 grams, of which 22,6 grs were ripe fruit. The plant had a height of 2,74 meters (the best buckwheatplant of the same variety grown in a field yielded only 7/9 times as much air-dry matter and considerable fewer ripe fruit). These plants were grown in glass cylinders of 3 liters each. As nutrient medium a solution in springwater of 4 aeq. KCl, 4 aeq.  $\text{Ca}(\text{NO}_3)_2$ , 1 aeq.  $\text{MgSO}_4$  was used, which was so far diluted, that the plant received at the start a solution of 1 : 4000 and later on of 1 : 1000, which latter solution was replaced five times by a fresh one of the same composition. To these solutions were added periodically small quantities of ferric phosphate and potassium phosphate.

Already in 1865 Sachs <sup>1)</sup> describes in his „Handbuch der Experimental-Physiologie der Pflanzen“ rather circumstantially the technical details of the water-culture methods. He also relates the results obtained up to that time and in what way problems about the nutrition of the plants, the indispensability of the separate elements, the possibility to substitute one element by another etc. by means of water-cultures can be solved. Prescriptions for the composition of a nutrient medium however he does not give, but he writes that the experiments prove that a rather

<sup>1)</sup> Julius Sachs, Handbuch der Experimental-Physiologie der Pflanzen, 1865 (Band 4 des Handbuches der Physiologischen Botanik). Die Nährstoffe der Pflanzen, p. 113—125 und p. 141—155.

large latitude exists in the proportion and variation of the salts used, with which a vigorous growth can be obtained. When only the necessary bases and acids are present, he considers of secondary importance in what form the salts are given. He only considers desirable to give more potassium than sodium, more calcium than magnesium and always very little iron (and manganese as the case may be); further that the compounds of sulphuric, nitric and phosphoric acid predominate the chlorides. He also advises to renew the liquid repeatedly, especially from older plants in order to prevent the reduction of sulphate into sulphide and alteration of acid into alkaline.

The different prescriptions for nutrient liquids in modern physiological handbooks are all of much younger date. An extensive compilation with full particulars about their preparation, their pH, the salts which are to be used etc. is found with Hiltner<sup>1</sup>). Most of the prescriptions in his book originate from German investigators.

Besides the prescriptions of German origin there are many by Americans, e.g. those by Livingston, Livingston and Tottingham, Shive, etc. All these nutrient liquids contain as necessary elements K, Ca, Mg, Fe, P and S; further N, most as nitrate, but sometimes as ammonium, or as both. Some contain Cl too, when one of the metals is given as chloride, e.g. the older prescriptions according to Sachs and Knop and a few others. Other elements, though rather common in the plants in small, sometimes even in important quantities as Na, Cl, Si, Mn and Zn had been proved superfluous, except perhaps NaCl for the true halophytes. In present handbooks and manuals on physiology one always finds these

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<sup>1</sup>) E. Hiltner, *Wasserkultur und Vegetationsversuch*. (Inserted in Honcamp, *Handbuch der Pflanzenernährung und Düngerlehre*, Bd. 1, 1931, p. 642 seq.

seven elements stated above together with C, H and O enumerated as the necessary ones.

A constant point of discussion has been which solution was the most suitable for a given plant or in general. Many times a solution which an investigator had found the best for a special plant gave unsatisfactory results in other hands. Lundegårdh<sup>1)</sup> e.g. records that a solution which was the best for wheat according to Shive<sup>2)</sup> gave only half as much dryweight as two other nutrient liquids which he applied at the same time. Such experiences are numerous.

After 1900 it has been found by different investigators, that small dressings of salts of manganese, zinc, boron, iodine etc. often stimulated the growth of plants in the fields. Bertrand<sup>3)</sup>, who published in 1905 good results with a dressing of manganese on an oats-field proposed to call those manures, of which only very small quantities are beneficial for plant growth „engrais complémentaires” or „engrais catalytiques”.

Javillier<sup>4)</sup> repeated the experiments of Raulin with *Aspergillus niger* Cr.; he ascertained that a zinc-percentage of 1 : 10.000.000 till 1 : 100.000 gave an optimal growth. As he found that all green plants held zinc, he cultivated wheat in sterile water-cultures with and without zinc<sup>5)</sup> and found with a percentage in the solution of 1 : 5.000.000

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<sup>1)</sup> H. Lundegårdh, *Die Nährstoffaufnahme der Pflanze*, 1932, p. 96.

<sup>2)</sup> J. W. Shive, *Journ. Agric. Research*, 21, 1923, p. 701.

<sup>3)</sup> G. Bertrand, *Sur l'emploi favorable du manganèse comme engrais*. *Compt. rend. Acad. Sc. T.* 141, 1905, p. 1255.

<sup>4)</sup> M. Javillier, *Sur l'influence favorable de petites doses de zinc sur la végétation du Sterigmatocystis nigra* V. Tgh. (*Aspergillus niger* Cr.) *Compt. rend. Acad. Sc. T.* 145, 1907, p. 1212.

<sup>5)</sup> M. Javillier, *Le zinc chez les plantes*. *Ann. de l'Inst. Pasteur*, T. 22, 1908, p. 720.

till 1 : 250.000 an increase of dryweight, though the latter dose had already a detrimental effect on the roots.

After that Mazé experimented circumstantially with maize in sterile cultures, to which he added besides the usual elements manganese, zinc and silicon. He describes in details the symptoms of manganese and zinc deficiency. When the manganese was omitted <sup>1)</sup> each subsequent leaf had a lighter colour till the leaves were entirely chlorotic. The rim of these chlorotic leaves was corrugate and every subsequent leaf was smaller than its predecessor.

When the zinc was lacking entirely, the roots were no longer able to regulate the absorption of the elements; incrustations sprung up on the leaves, the plant wilted and died; the percentage of ash turned out to be abnormally high.

Next Mazé began a series of experiments with maize to investigate the indispensability of Al, B, F, I, and As <sup>2)</sup>. The salts for the nutrient liquid had been specially prepared for this purpose. Besides the customary elements the plants received also Si, Mn and Zn and next the five elements mentioned above. The first experiments failed, as he had added so much of these elements that they had a harmful effect; in later experiments these elements together without the As turned out to stimulate the formation of dry matter. When boron was absent, the plants did not develop; on the other hand As (2 mgs of sodium arsenate per liter) affected the growth very unfavourably.

How far each of the three elements Al, F and I separately furthered the growth could not be concluded with certainty, as the As present had a disturbing effect

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<sup>1)</sup> P. Mazé, *Recherches de physiologie végétale*, Ann. de l'Inst. Pasteur, T. 28, 1914, p. 36—44.

<sup>2)</sup> P. Mazé, *Recherches d'une solution purement minérale capable d'assurer l'évolution complète du maïs cultivé à l'abri des microbes*. Ann. de l'Inst. Pasteur, T. 33, 1919, p. 139.

on growth. Granting that the three acted favourably together, we are nevertheless of opinion, that the conclusion of Mazé, that each of the three was necessary for the development of the maize was premature.

The indispensability of the elements above stated has been investigated especially in England and America. Some of these experiments are mentioned in chapter III. They prove evidently that very small quantities of many of these elements are indispensable for a complete development and fructification of green plants, but the toxic dose is very soon reached. In future, especially in chapter III, in which these problems are more extensively discussed, those elements of which more considerable quantities are necessary for plant life are included under the indication „principal elements”; by „auxiliary elements” those elements are meant, of which only very small quantities have been proved indispensable.

These auxiliary elements may account for the discrepancies so often experienced between the results with the same nutrient medium by different investigators. Generally the quality of the chemical compounds used for the nutrient solutions is not stated. Normally we may take it for granted that the usual „pure” qualities were used, which contain traces of auxiliary elements. As these „pure” qualities are manufactured in different factories, the degree of purity of course varies. *So the nutrient liquids prepared by different investigators which were supposed to be alike, may have been to the plant in reality very dissimilar.* The less pure the nutrient salts were, the better will have been the results from the water-cultures. This in many cases may be the explanation why experiments in which nutrient media prepared on the same formula were used, had such a different outcome.

Some investigators got better results with a nutrient solution made with tapwater than when prepared with

distilled water according to the same formula. „So wurde <sup>1)</sup> „an der Pflanzenphysiologischen Versuchsstation Tharandt „unter F. Nobbe in Fällen, bei denen es die Versuchsfrage gestattete, das weiche Leitungswasser verwendet. „Es hatte sich herausgestellt, dass bei Verwendung von „solchen die Kulturen besser gedeihen als bei Gebrauch „von destilliertem Wasser“. Hiltner <sup>2)</sup> used at Munich hard tapwater, after neutralizing it with sulfuric acid to prevent chlorosis and writes about the results: „In solchem „mit Schwefelsäure neutralisiertem Wasser gedeihen die „Pflanzen meist wesentlich besser als bei Verwendung „von destilliertem Wasser“. These better results with natural water than with distilled water may be ascribed to the traces of auxiliary elements which are always present in natural water.

When the nutrient solution was more frequently changed, better results were often noticed. With a nutrient solution of principal elements + different quantities of boron Brenchley and Warington <sup>3)</sup> got far better results in all cases when first every fourth and afterwards every other day the nutrient solution was changed, than when this happened first every 16th and afterwards every 8th day. A frequent change of solution without addition of boron gave even better results than a normal change when the optimal quantity of boron was added. This outcome (and similar ones with frequent changing were noticed by other investigators) may be ascribed to the traces of auxiliary elements which came to the disposal of the plants in a four times larger quantity of solution.

<sup>1)</sup> E. Hiltner, Wasserkultur und Vegetationsversuch. In F. Honcamp, Handbuch der Pflanzenernährung und Düngerlehre. 1931, Bd. I, p. 644.

<sup>2)</sup> *ibid.* p. 651.

<sup>3)</sup> W. E. Brenchley and K. Warington, The Role of Boron in the Growth of Plants. *Annals of Botany*, 41, 1927, p. 171.



In consequence of the introduction of traces of auxiliary elements in nutrient solutions of principal elements only, the results about the comparative efficiency of these solutions will often have to be mistrusted, when these solutions were prepared from the usual „pure” salts, especially when the solutions were frequently changed. Unknown quantities of auxiliary elements may have been introduced by one or more of the salts. In considering experiments on the efficiency of combinations of the same salts in different quantities one should keep in mind that differences in efficiency may partly be due to the fluctuating quantities of auxiliary elements, entered as impurities. In order to be sure that the results of those former experiments about the efficiency of different nutrient solutions are correct, many of these experiments should be repeated using the purest possible salts of the principal elements, while at the same time to all nutrient solutions the same quantities of auxiliary elements should be added.

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## CHAPTER II.

### **The Growing of Plants on Clay Suspensions.**

The plant absorbs the mineral constituents necessary for its development and fructification from the soil solution, which derives these constituents directly or indirectly from the mineral soilparticles, especially from the smallest: the clay fraction of the soil. It was my intention to investigate the relation between the quantity of „available” mineral constituents found by analysis in the clay particles of a soil and the quantity of organic and mineral matter of plants grown on the same quantity of clay particles when circumstances had been favourable for the plants to absorb all the available constituents.

So the finest particles of a clay soil were separated from the coarser ones — the sand and the silt fraction — and the clay particles with the water necessary for the dispersion and separation were used for growing plants, in the same way as plants are grown in solutions of a number of mineral salts in water-culture. In this way the plants were able to absorb all the mineral constituents from the soil solution and the suspended clay would replace them as long as „available” constituents were present. In order to be sure that the clay could be completely exhausted by the plant grown in it, the quantity of clay had to be just sufficient for the development of the plant, not more, and other circumstances as temperature and light had to be favourable. The clay soil of which the suspension was prepared, did not contain much nitrogen, consequently the plant had to be supplied with some more in order to become able to exhaust the clay suspension as far as possible. As no mineral matter should be introduced to the suspension the nitrogen had to be given in the form of ammonium nitrate; for the regulation of the pH of the suspension nitric acid and ammonia were to be used.

Through the kind offices of dr. D. J. Hissink, Director of the Institute for Soil Research (Bodemkundig Instituut) at Groningen, I received a sample of a young heavy sea-clay from the Experimental Farm at Nieuw-Beerta. It contained no calcium carbonate. As dr. Hissink kindly informed me, the upper soil of plot No. 2, from which the sample had been taken, contains 4,3 % humus, 26,4 % particles with a diameter from 2 mm to 0,02 mm (coarse and fine sand <sup>1)</sup> ), 69,3 % particles with a diameter smaller than 0,02 mm (silt and clay) and 0,79 % interchangeable

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<sup>1)</sup> For method of analysis cf. Intern. Mitt. Bodenkunde 4, 1914, p. 30; also inserted in O. Lemmerman, Methoden für die Untersuchung des Bodens, p. 23 (1932).

CaO<sup>1</sup>). The soil is said to have little or no need of a dressing of lime, phosphoric acid or potash.

On receipt of the soil it was too dry for treating it immediately with water in order to get a suspension; so it was first dried at room-temperature, broken up and sifted with a 2 mm sieve; it contained practically no gravel. 20 kgs of the air-dry fine earth were used for the suspension. The fine earth was mixed in successive parts with distilled water to a rather humid paste which was kept one day in a closed vessel in order to prevent desiccation. Some drops of water were added to small parts of the paste in a large mortar and then the paste was kneaded with a pestle till the water became absorbed. Then little by little more water was added and kneaded into the paste till it became unctuous and next treacly. Afterwards it was diluted with more water and stirred on a strained piece of muslin with  $24 \times 32$  meshes to the  $\text{cm}^2$ . On the muslin remained coarse parts of humus and some coarse sand, which were removed.

The remaining treacly mass in the mortar blends much more easily with another part of the soil paste than distilled water does, which often spatters away during the first kneading with the soil paste.

The dense percolate of 10 kgs of fine earth was put in a large glass vessel, stirred with water to a homogeneous liquid of about 28 liters and left standing. In two days four strata had detached themselves. At the surface there was a transparent layer of 2 cms in consequence of the presence of electrolytes in a sufficient concentration to coagulate the soil colloids. Then came a layer of 8 cms of a light brown colour, containing only a small quantity of dry matter; next a layer of 4 cms with especially colloid

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<sup>1</sup>) For method of analysis cf D. J. Hissink, Intern. Mitt. Bodenkunde, 12, 1922, p. 81; also inserted in E. J. Russell, Soil Conditions and Plant Growth, 5th ed., 1927, p. 139 and 459.

matter, clay and silt and fourthly, at the bottom a layer of 11 cms, containing sand mixed with silt, clay and soil colloids <sup>1)</sup>).

In order to separate soil colloids and clay particles from the coarser material, the suspension was siphoned over in glass cylinders of about 25 cms high. The two upper layers were siphoned over together, stirred and left standing for 24 hours; the remainder was siphoned over in glass cylinders which were half filled and diluted with the same quantity of distilled water; after stirring the cylinders were left standing for 24 hours. In this way I got 4 cylinders from which each successive one contained more coarse particles than the previous one.

After 24 hours the uppermost 20 cms of the first cylinder were siphoned over and collected in a glass bottle of 25 liters; from the remainder the suspension was siphoned over in a clean cylinder, stirred with the upper 20 cms which were siphoned off from the second cylinder and left standing for 24 hours. The deposit from the first cylinder was stirred with some distilled water, the remaining suspension of the second cylinder was added with part of the upper layer of the third cylinder and some more distilled water and after stirring, the cylinder was left standing for 24 hours. In this way the colloid matter and the clay particles in the soil suspension smaller than 0,002 mms <sup>2)</sup> were separated from the deposits containing coarser particles. The upper layer of the first cylinder was collected each time when the deposit did not contain any more visible particles; in the other case the upper 20 cms were stirred again and siphoned over for collection of the suspension next day.

The percolate of the other 10 kgs of air-dry fine earth

<sup>1)</sup> cf. H. Gessner, Die Schlämmanalyse (Band 10 der Kolloidforschungen in Einzeldarstellungen) p. 39 and 157 seq.

<sup>2)</sup> cf. *ibid.* p. 186.

was treated in the same way. The total result was 167 liters of suspension. This was thoroughly mixed in a large metal vessel of 250 liters and kept in large glass bottles. The air-dry fine earth contained 4,92 % moisture, so 114 grams of dried soil gave one liter of the suspension. The pH of the air-dry soil, of the suspension etc. were determined colorimetrically with indicators according to Clark and Lubs in the „Hellige Comparator”, which enables us to determine the pH without any difficulty in coloured or opalescent liquids. In the soil the pH was determined after the method of Arrhenius<sup>1)</sup>; in the suspension this was done by filtering a little of the stirred suspension. In both determinations the first part of the filtrate was not used.

With bromothymol blue the pH was: in air-dry soil 7,3 and in the suspension 7,4.

The suspension contained 0,503 % of dry matter, dried at 105° C.

The experiments were made with wheat, buckwheat and rice. To dr. M. J. Sirks, botanist of the Institute for Plant Breeding (Instituut voor veredeling van landbouwgewassen) at Wageningen I am much obliged for providing me twice with grains of a pure strain of Japhet summer wheat (*Triticum vulgare* Vill.) In 1930 I received a quantity of grains of Japhet (6) first harvest got from one ear. These grains were used in that year for preliminary tests. In 1931 I received 25 ears of Japhet (8) which were used for the experiments in 1931 and 1932.

The fruit of buckwheat (*Fagopyrum esculentum* Moench) which I used in 1931 were the remainder of a lot used in other botanical investigations and came from Metz & Co, Berlin-Steglitz.

In the Botanical Garden at Amsterdam every year a plant of *Victoria regia* Lindl. is grown in a large basin,

<sup>1)</sup> O. Arrhenius, Kalkfrage, Bodenreaktion und Pflanzenwachstum, 1926, p. 92 seq.

together with a.o. some rice varieties, the grains of which are yearly harvested and laid out next year. The earliest ripening variety, *Oryza sativa* L. var. *mutica*, was chosen for the experiments.

Wheat and buckwheat were grown together in a spacious glass-house in the Botanical Garden; the site of the glass-house was east-west and it was exposed all day to the sun. Its back was composed of two pieces of gunny cloth, which could be opened for ventilation purposes. The back of the glass-house was screened by a row of shrubs, to shelter it from the wind, when part of the back of the house was opened. In windy weather only the upper part of the gunny cloth was opened in the middle by day and the lower parts were pinned together; in calm weather part of the backwall was removed. On sunny days the glasshouse was screened from about 10 a.m.—5 p.m. to prevent excessive heat. The air-temperature in the glass-house was controlled by a maximum-minimum thermometer among the plants, which was protected against the sunrays. Another maximum-minimum thermometer was suspended on a level with the roots in a cylinder filled with water only.

The rice was cultivated in the *Victoria regia* hothouse after the middle of May. The hothouse is heated, except on warm summer days, and the water of the basin is kept constantly at about 29° C., so maximum- and minimum air-temperatures and water-temperatures in the hothouse were read only for about three weeks. The purpose of the control of the temperature was firstly to prevent too high temperatures in the glass-house in which the wheat and buckwheat were grown and secondly to establish the correlation between the daily range of the air-temperature and the temperature of the water in the cylinders. In table 2 are given the results of the temperature-readings. The maximum-temperature of the water in the cylinders with wheat and buckwheat always remained 4 till 5° C.

lower than the maximum air-temperature, while the minimum-temperature remained 2 till 3° C higher. With the rice these differences were slighter.

The mean temperature of the water in the cylinders in which wheat and buckwheat were grown, was during July, the hottest month of the growing-period, 5° C. lower than of the water in the cylinders with rice. It is probable that a higher temperature in the clay suspension makes a greater quantity of mineral compounds available. In that case the rice would be able to absorb more mineral compounds and form more organic matter than the wheat and buckwheat.

TABLE 2.

Air-temperature and water-temperature in the glass-houses in 1931.  
In Celsius degrees.

		Wheat and buckwheat			Rice
		Second half of May	June	July	22nd July — 10th Aug.
air	{ mean daily maximum.....	24,7	26,2	28,1	32,3
	{ " " minimum.....	11,5	11,8	16,8	21,8
	{ absolute maximum.....	30	31	36	36
	{ " minimum.....	8,5	10	11,5	18,5
water	{ mean daily maximum.....	20,6	21,5	23,7	28,6
	{ " " minimum.....	14,0	14,6	18,2	23,3
	{ absolute maximum.....	24	27	32	32
	{ " minimum.....	11	12,5	14,5	19,5
air	mean daily range.....	13,2	14,4	11,3	10,5
water	" " ".....	6,6	6,9	5,5	5,3

The plants were grown in glass-cylinders with feet of resistant glass, 30 cms high with a diameter of 10 cms and a capacity of 2,1 to 2,2 liters. The cylinders were closed with covers of American Redwood (*Sequoia semper-*

virens Endl.) because this kind of wood does not warp when moistened. In the covers grooves were cut fitting to the rim of the cylinder. In the centre of the cover of  $1\frac{1}{2}$  cms' thickness a borehole was made of 16 mms diameter for the buckwheat, 24 mms for the wheat and 26 mms for the rice; the preliminary tests had shown that rice wanted more room for its stool than wheat. Into the wooden cover a piece of wire of 25 cms long was screwed encased in a piece of tape, the selvages of which were joint and which was provided with bone rings at a height of 8, 16 and 24 cms. When necessary the plants were tied to the wire with raffia.

In a drying oven the covers were heated at  $70^{\circ}$  C. and then immersed in molten paraffine of about the same temperature (melting-point  $56^{\circ}$  C.) where they remained under the surface for half an hour.

Corrugated cardboard was wrapped twice round the cylinders, over which was put a layer of white kitchen-paper extending 1 cm above the wooden cover. On the wooden covers too white kitchen-paper of the same shape was fastened; corrugated cardboard and kitchen-paper together protected the cylinders against the light and against too great fluctuations of temperature. On the cylinder and on the kitchen-paper of cover and cylinder the number of each plant was noted.

For germination porcelain pots were used of one liter covered with a plate of glass. Inside was put a fitting square made of a glass bar, mounted on three glass feet of 5 cms. With molten paraffine a piece of aseptic gauze with 100 meshes to the  $\text{cm}^2$  was stretched across the glass square<sup>1)</sup>. The soaked seeds were put on the gauze at

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<sup>1)</sup> Method of K. Zylstra, De hoofdwortel van eenige graansoorten. Verslagen van landbouwkundige onderzoekingen der Rijkslandbouwproefstations. No. 26, 1922, p. 27.



regular distances of about 1 cm, and distilled water was added till nearly the whole piece of gauze was in contact with the water and the seeds were just touched by it. As soon as all the seedlings bore roots, the water was partly removed till the surface no longer reached the seeds.

When the seedlings had developed sufficiently, they were lifted out of the gauze. The tissue of the gauze is very loose and the roots can easily be loosened from the threads without injuring them. The seedlings were held in position in the bore-holes of the cylinder-covers by means of non-absorbent cotton-wool, which was wrapped round them, so that the grains of the wheat and the rice were placed at the bottom of the cotton-wool about 1 cm above the watersurface. The young seedlings got a loose tuft of cotton-wool; as the plants grew heavier, more cotton-wool was wrapped round, to prevent the plants from sinking

In preliminary tests the influence of components soluble in water from the glass of the cylinders on the gain in weight of the seedlings had been investigated. Grains of summer wheat Japhet (6) were laid out for germinating in the way mentioned above, pot and tripod were paraffined. Five seedlings were transplanted in paraffined and an equal number in unparaffined cylinders. To the distilled water of everyone of the ten cylinders 40 mgs of ammonium nitrate (14 mgs of N) were added so that if the plant would absorb mineral matter, dissolved by the distilled water from the glass, nitrogen could not become minimum-factor for the development of the plants. The first root appeared on April 9, the plants were transplanted after 8 days and harvested when 86 days old. During their growth they showed no symptoms of illness whatever. A clear, though not large difference was noticeable between the plants grown in paraffined and in unparaffined cylinders in favour of the last as is shown in table 3.

TABLE 3.

Averages for wheat plants 86 days <sup>1)</sup> old, grown in cylinders with 2,1 liters of distilled water and 40 mgs of ammonium nitrate.

	in unparaffined cylinders.	in paraffined cylinders.
Height to top of uppermost foliage leaf of the stalk .....	23,4 cms	18,4 cms
number of leaves.....	8,8	8,4
"  "  ears .....	1,0	0,4
"  "  spikelets .....	2,2	0,4
"  "  roots .....	13,0	11,3
weight of air-dry superterranean parts .....	100 mgs	79 mgs
weight of roots .....	21 "	19 "
"  "  total plant .....	121 "	98 "

The average air-dry weight of a seedling on the day of transplanting was 50 mgs, the average air-dry weight of a grain was 57 mgs.

The differences are small enough to be neglected. Moreover in this experiment the cylinders were new, while in the following experiments all the cylinders had been used for at least two months, consequently the quantity of matter, soluble in water, especially of potassium, had diminished again <sup>2)</sup>).

In the preliminary experiments wheat had been grown on solutions according to Knop and to Pryanishnikov, both prepared with tapwater, containing considerable

<sup>1)</sup> The age of the plant is calculated throughout from the day on which the first root appeared; the coleoptile will be counted as first leaf; the height of the superterranean parts of wheat and rice is measured from the grain; the roots are cut off at about 2 mms from their point of issue; the remainder of the grain is left with the stool.

<sup>2)</sup> E. Hiltner, *Wasserkultur und Vegetationsversuch*. In F. Honcamp, *Handbuch der Pflanzenernährung und Düngerlehre*, 1931, Bd. I, p. 629.

quantities of calcium bicarbonate. After some time the developing young leaves were chlorotic <sup>1)</sup>). When however a sufficient quantity of diluted acid was added, the young leaves, appearing about three days later, had a bright green colour again. A pH of about 5,5 prevented the appearance of chlorosis.

Consequently the pH of the clay suspension was lowered. By adding 2,5 cms<sup>3</sup> of normal nitric acid to one liter of suspension the original pH of 7,4 dropped to 5,2. All the plants grown on clay suspension in 1931 started with a pH of 5,2 in the root medium.

The necessity of lowering the pH of the suspension when ammonium nitrate is given as nitrogen-manure, became still more obvious the next year, when wheat was grown on suspension with the original and the lowered pH. Some days after the ammonium nitrate was added, the plants with the original pH got leaves of a very pale green sickly colour and the plants developed poorly <sup>2)</sup> <sup>3)</sup>). After some weeks the colour of the leaves gradually grew more normal, because the plants by the excretion of CO<sub>2</sub> and the absorption and exchange of ions had lowered the pH of the suspension themselves <sup>4)</sup>). The final result however was smaller plants with one ear only, while the same plants with lowered pH bore two ears.

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<sup>1)</sup> E. Hiltner, Wasserkultur und Vegetationsversuch. In F. Honcamp, Handbuch der Pflanzenernährung und Düngerlehre, 1931, Bd. I, p. 651.

<sup>2)</sup> W. Mevius, Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. *Planta* VI, 1928, p. 377.

<sup>3)</sup> W. Mevius und H. Engel, Die Wirkung, etc. II, *Planta* IX, 1930, p. 1.

<sup>4)</sup> D. N. Prjanischnikow, Ammoniak, Nitrate und Nitrite als Stickstoffquelle für höhere Pflanzen. *Ergebnisse der Biologie*, Bd. 1, 1926, p. 407.

In order to compare growth and yield of wheat, buck-wheat and rice on declining quantities of suspension four series of six plants were grown from each of these species. The plants of these series received quantities of suspension in the proportions 27 : 9 : 3 : 1. The suspension was supplemented with distilled water and ammonium nitrate. To simplify matters in this publication each series of plants of this experiment will be indicated with the proportional quantity of suspension received; so e.g. the 27 series means the series which received 27 times as much suspension as the 1 series.

In this way the quantities of suspension given per plant were:

the 27 series	4,—	kgs,	containing	20,12	grams	of	dry	matter.
„ 9	„	1,333	„	„	6,71	„	„	„
„ 3	„	0,444	„	„	2,23	„	„	„
„ 1	„	0,148	„	„	0,74	„	„	„

As the capacity of the cylinders was only 2,1 liters, the first series had to start with 2 kgs of suspension. After some time this suspension was changed for 2 kgs of fresh suspension. Through the employed suspension an air-current was blown and the pH was brought back to about 5,2. Whenever the transpired water had to be replaced, each plant should receive an equal quantity of the suspension used, till the whole quantity was finished.

All the cylinders received a quantity of nitrogen as nitric acid in proportion to the quantity of suspension; extra nitrogen was added as 0,1 normal ammonium nitrate. The quantity of soluble nitrogen present as nitric acid and ammonia in the suspension, may be neglected: per kg of suspension it was less than 2 mgs, for the greater part as nitric acid.

The quantities of nitrogen given to the different series in mgs per plant were

	as nitric acid	as ammonium nitrate	total nitrogen
to the 27 series . . . .	70	70	140
” ” 9 ” . . . .	23	70	93
” ” 3 ” . . . .	8	70	78
” ” 1 ” . . . .	3	70	73

Besides these 24 plants of each of the species of plants employed, some more plants of each species were grown for comparison. Of the wheat two were grown on the same liquid as the 27 series but without the clay particles. For that purpose 10 cms<sup>3</sup> of normal nitric acid were added to two bottles each containing 4 kgs of suspension. After some days the clay had settled and the liquid on the top was siphoned off. Per cylinder 2 kgs of this liquid, which was only slightly opalescent, was used; the remainder afterwards was used for changing. To these 2 kgs of liquid 70 mgs of nitrogen were added as ammonium nitrate, so that — except for the clay particles — these cylinders corresponded with those of the 27 series.

Four plants of each species were grown on a nutrient solution. As the concentration of dissolved mineral constituents in the clay suspension was low and ammonium nitrate was added to supply the want of nitrogen, a nutrient solution was chosen with a rather low salt concentration and ammonium nitrate as nitrogen-source, as described by Pryanishnikov. Instead of a solution of ferric chloride, cristallized ferric ammonium sulphate was used. This salt contains 11,6 % iron and is not hygroscopic as ferric chloride is, so it is well suited for dosing small quantities of iron. Moreover a quantity of nitric acid was added to lower the pH to about the same number as that of the clay suspension. 10 liters of nutrient solution were prepared as follows. In about 5 liters of distilled water were dissolved:

$\text{NH}_4\text{NO}_3$ .....	2,4 grs
KCl .....	1,6 „
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ .....	1,23 „

In a mortar were rubbed with distilled water and afterwards added to the solution

$\text{CaHPO}_4 \cdot 2 \text{H}_2\text{O}$ .....	1,72 grs
$\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$ .....	3,44 „

With 9 cms<sup>3</sup> of normal nitric acid the pH was lowered from 6,5 to 5,3, by which the solution became clear. Finally were added:

$\text{Fe}(\text{NH}_4) \text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$ .....	0,15 gr
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Then distilled water was added up to 10 liters.

The ammonium nitrate was „puriss. pro analysi Merck”; the other salts were of the customary „pure” qualities for pharmaceutical use.

In this solution without further addition two plants of each species were grown; two other ones started on the same solution, to which a liquid was added after some days, containing in solution boric acid and manganese chloride and in suspension calcium fluoride. To these cylinders were added:

$\text{H}_3\text{BO}_3$ .....	2 mgs (B	0,36 mg)
$\text{CaF}_2$ .....	2 „ (F	0,97 „ )
$\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ .....	4 „ (Mn	1,15 „ )

These compounds were added to the three species on May 9. The wheatplants were at that time 27 days old and had stood in the cylinders already for 17 days; the buckwheatplants were 14 days old with one day in the cylinders; the riceplants were 12 days old with 3 days in the cylinders. As I saw in the water-cultures of prof. Pryanishnikov at Moscow, the influence of this addition proved beneficial to the plants, especially in the long run.

The clay particles in the cylinders gradually settled, a small part adhering to the roots. Every two or three days the wooden covers with the attached plants were lifted, moved carefully up and down in order to loosen the adherent particles from the roots and put on an empty cylinder. The suspension was stirred energetically to bring all the clay particles in suspension again and to stimulate the absorption of oxygen. Then the transpired water was replaced and the plant was put back in its cylinder. The plants growing on nutrient solutions were treated in the same way in order that a fair comparison could be made.

Some days before harvesting the clay suspension of two of the riceplants turned bluish-grey by reduction. These afterwards proved to be the plants with the highest air-dry weight. On harvesting a thin layer on the water-surface, as well as the upper part of the glass wall and the roots were rust-coloured. The roots of these two plants were not used for analysis. With none of the other plants grown on clay-suspension reduction of the clay occurred; all the other suspensions kept their yellow-brown colour till the end.

#### *Experiments with wheat.*

There was a great difference in the weight of the well-developed grains of the summerwheat Japhet (8): from about 35 mgs to about 70 mgs. Upon weighing the grains those of 53 to 63 mgs were selected, the average weight being 57,53 mgs. They were washed and soaked in distilled water for one night and laid out at regular distances in the same position in such a way that the first root could grow without bending through the aseptic gauze. The first root appeared on April 13, the seedlings were transplanted in the cylinders after 8 days, all showing

one well-developed green leaf above the coleoptile and mostly 5 roots.

In the beginning no difference between the four series grown on suspension was visible. After 20 days all the plants had four leaves <sup>1)</sup>. On the 21st day the first lateral shoot sprouted in the plants of the 27 series. On the 36th day the 27 series got fresh suspension. After 46 days the differences were already very marked, as table 4 shows.

TABLE 4.

Wheat plants of 46 days old.

	number of leaves on the main stalk	mean distance from grain to top of 6th leaf	mean number of lateral shoots	mean number of assimilating leaves	mean of withered leaves
27 series ..	8	51 cms	3 5/6	16	2
9 " ..	7½	45 "	2 4/6	10	3
3 " ..	7	34 "	1 1/6	6	3
1 " ..	7	25 "	0	3	3

All the plants of every series had the same number of leaves on the main stalk. The differences in length of the sixth leaf between plants of the same series were small, e.g. with the 27 series the longest leaf was 52, the shortest 50 cms. The oldest leaves of the main stalk had withered; the laminae were cut off and gathered from each plant separately, to be weighed afterwards together with the harvested plant. After 56 days all the plants got another dressing of ammonium nitrate: respectively 70, 70, 28 and 28 mgs of nitrogen for the 27, 9, 3 and 1 series.

After 72 days in all the plants of the 9 and 3 series the ear appeared; in the two other series the place of the still

<sup>1)</sup> The coleoptile is always included.



enclosed ear could be seen and the ears appeared two days later in the plants of the 27 series; in the plants of the 1 series this did not occur until after some days more.

After 84 days one plant of each series was photographed (plate II, fig. 1); one was sufficient, as the six plants of each series resembled each other very closely. In the 27 series all the plants bore 3 ears; in the other series all the plants bore 1 ear, except one plant of the 1 series, which had none. That the plants belonging to each series were very much alike, appears from the following table 5.

TABLE 5.

Wheat grown on suspension harvested at the age of 93 days.

	number of roots	top of uppermost leaf of the stalk height in mms	average air-dry weight of superterranean parts in mgs		ratio the 1 series taken as a standard
			weighed	less 90 mgs	
1 series ...	14,0 ± 0,58 <sup>1)</sup>	388 ± 5,8	382 ± 18	292	1
3 " ...	23,2 ± 0,65	583 ± 8,4	900 ± 26	810	2,78
9 " ...	35,0 ± 0,77	815 ± 6,7	2210 ± 23	2120	7,26
27 " ...	51,2 ± 2,12	892 ± 12,5	5683 ± 27	5593	19,16
4 kgs of solution only .	36 ± 3	795 ± 3	2310 ± 10	2220	7,60

If the average air-dry weight of the superterranean parts of wheat-plants grown in unparaffined and in paraffined cylinders without any addition of mineral matter (90 mgs, cf. page 52) is subtracted from the average air-dry weight found here, the numbers in column 5 express the quantity in mgs of air-dry matter formed with the aid of elements from the suspension. Column 6 shows the ratio calculated between the quantities of air-dry matter, the amount of the 1 series taken as a standard. Among the series them-

<sup>1)</sup> Standard error calculated according to the formula  $m = \pm \sqrt{\frac{\sum v^2}{n(n-1)}}$

selves the ratio, calculated from the amounts in column 5 is:

$$5593 : 2120 = 2,64$$

$$2120 : 810 = 2,62$$

$$810 : 292 = 2,78$$

In each case the triple quantity of suspension has enabled the plant to form about 2,7 times the amount of air-dry matter. The two plants grown on 4 kgs of solution only without the clay bore one ear each and resembled closely the plants of the 9 series, as appears from table 5. The differences between these two plants and the 27 series are to be ascribed to the presence of the clay particles.

Zylstra<sup>1)</sup> counted the number of roots of Japhet summerwheat grown as a water-culture. For two plants he found after 66 days 47 and 55 roots; for three plants after 100 days 102, 92 and 91 roots, but these plants were all more vigorous and had made 4 to 11 stalks.

Except the first two or three small leaves cut away after 46 days, no leaves were taken away before photographing, though many withered. On the figure the drooping leaves are all withered.

A short time before the stalk shot up, this withering process suddenly accelerated, proceeding from the older to the younger leaves. On the figure the earbearing stalks have only two or three assimilating leaves. Nine days later, after 93 days, when the plants were harvested, only the ears and the uppermost foliage leaves of the stalks were still green, all the other leaves having withered. The grains were not nearly ripe yet, but it was obvious that the accelerated withering of the leaves had been caused by the fact that the nutrient medium was exhausted and the

<sup>1)</sup> K. Zylstra, De hoofdwortel van eenige graansorten. Verslagen van landbouwkundige onderzoekingen der rijkslandbouwproefstations. No. 26, 1922, p. 44—47.

components for the grainformation could only be got by withdrawing them from the leaves.

At regular intervals the plants got measured quantities of distilled water, suspension etc., measured with an exactness of about 5 cms<sup>3</sup>. For the calculation of the quantity of water transpired in forming one gram of dry matter no correction has been applied for the evaporation from the water-surface. This must have been small however, since it took three weeks after the plants had been put in the cylinders before the water had to be replenished for the first time. To compensate this error, neither has a correction been applied for the quantity of dry matter present in the seedlings at the moment of transplanting. As the quantity of added water will have been a bit greater than the transpired water, and the quantity of dry matter in the harvested plant a bit more than that formed during the time the lost water was measured, these two errors tend to compensate each other.

The differences in transpiration between the six plants of the 27 series were not large; the smallest amount was 1200 cms<sup>3</sup>, while the air-dry weight of this same plant, including the roots, was also the smallest: 6,22 grams. The greatest quantity was 1320 cms<sup>3</sup>, the air-dry weight of this plant being also the largest: 6,58 grams. The average quantity of transpired water was  $1267 \pm 17$  cms<sup>3</sup>, of air-dry material  $6,355 \pm 0,053$  grams, of dry matter (dried at 105° C.) 5,672 grams. For each gram of dry matter about 223 cms<sup>3</sup> water were transpired.

As the transpiration was smaller than I had supposed, it was not possible after the suspension had been changed to restore the whole mass of the liquid used to the plants. So the suspension was separated into two parts by settling; the part which contained the clay was entirely restored to the plants; of the solution as much as possible. Totally all the clay particles and  $\frac{1}{3}$  of the solution were restored.

*Experiments with buckwheat.*

Of the buckwheat fruit were selected having a weight of 18 to 23 mgs and an average weight of 20,67 mgs. They were treated in the same way as the wheat. In contrast with the wheatgrains which germinated all at the same time, the buckwheatfruits germinated very irregularly; this gave some difficulty in transplanting, which took place on May 4, when the seedlings had an age of 9 days (calculated from the appearance of the root).

The 34th day the differences between the four series were still very small, smaller than the differences between plants of the same series. The main root of half of the plants reached down to the bottom of the cylinders (more than 30 cms.). These plants were equally divided over the four series, all the other plants having a main root of a length from 18 to 28 cms. At this age all the plants were budding, though no bud was open yet. The average height of the stem from the surface of the cover to the top of the inflorescence came to  $12\frac{1}{2}$ , 12, 11 and 9 cms for the 27 series, 9 series, 3 series and 1 series resp.

After 35 days one plant of the 1 series opened its first flower; after 37 days 4 plants flowered (1 of the 9 series, 2 of the 3 series, 1 of the 1 series); after 41 days 3 of the 27 series flowered, 5 of the 9 series, 4 of the 3 series and 2 of the 1 series. Of 3 plants of the 1 series the cotyledons were withering, though of none of the other series, apparently a first sign that in this series the supply of mineral components through the nutrient medium was insufficient.

After 45 days the plants of the 27 series received their fresh suspension; the clay of the liquid used was gradually put back into the cylinders, till after 68 days all the clay had been restored to the plants. The transpiration of the buckwheat was smaller than that of the wheat: of the solution used only  $\frac{1}{6}$  could be restored.

After 65 days already some plants bore black fruit; these were not the plants that had flowered first, but some of those whose first flowers had been noticed on the 41st day. These first ripe fruit belonged to plants of the 27 and the 9 series.

After 66 days a fair specimen of each series was photographed (plate II, fig. 2). Of course the plants of one series did not resemble each other so closely as the wheatplants, because they had been grown from outside seed and not from a pure strain; yet the differences were not striking.

Of the 1 series not a single plant had a lateral branch: of the 3 series 3 plants had none, 2 had 1 and 1 had 2 short lateral branches; of the 9 series 1 had 1, 3 had 2 and 2 had 3 lateral branches; of the 27 series five plants had 2 and 1 had 3 lateral branches. Totally the 27 series had the same number of lateral branches as the 9 series, but in the 27 series all the branches were longer as the figure shows. The differences became still more obvious afterwards, when all the plants were harvested, by the number of ripe fruit and the air-dry weights of the whole plants (Table 6).

TABLE 6.

Buckwheat grown on suspension, harvested at the age of 86 days.

	number of ripe fruit	height of the plants in mms	average air-dry weight of super- terranean parts in mgs		ratio the 1 series taken as a standard
			weighed	less 35 mgs <sup>1)</sup>	
1 series ...	3,5 ± 0,34	300 ± 30	338 ± 24	303	1
3 " ...	8,5 ± 0,85	460 ± 48	738 ± 41	703	2,32
9 " ...	24,7 ± 3,0	612 ± 29	1822 ± 96	1787	5,90
27 " ...	45,7 ± 2,4	830 ± 39	3405 ± 179	3370	11,12

<sup>1)</sup> 1,6 × weight of one buckwheat-fruit: proportionally the same correction as applied for wheat.

Among the series themselves the ratio, calculated from the amounts in column 5 is;

$$3370 : 1787 = 1,89$$

$$1787 : 703 = 2,54$$

$$703 : 303 = 2,32$$

After 86 days the plants of the 1 series had withered almost entirely, a sign that the root medium was exhausted; those of the 3 series had withered for the greater part, the 9 series less and the 27 series least. As I could not know before, whether the quantity of available mineral matter would be influenced almost exclusively by the amount of suspension given or whether the time during which the roots were in contact with the suspension would have a marked influence too, I decided to harvest all the series at the same time, also for this reason that all the wheatplants had been harvested together too. From the final results may be concluded however that it would have been better if the harvesting of each series of buckwheat had been postponed till the plants had all but withered. Withered leaves and ripe fruit were gathered from each plant separately during the growing period in order to be weighed together with the plants after harvesting.

The differences in transpiration and in air-dry matter of the six plants of the 27 series were rather great; the smallest quantity of transpired water was 670 cm<sup>3</sup>, the air-dry weight of this plant (the lightest) roots included, being 3,01 grs. The largest quantity of transpired water was 1035 cms<sup>3</sup>; this plant had also the largest quantity of air-dry matter: 4,42 grs. The average quantity of transpired water per plant was  $924 \pm 47$  cms<sup>3</sup>; of air-dry matter  $3,81 \pm 0,19$  grs; of dried matter 3,423 grs.

For each gram of dry matter 279 cms<sup>3</sup> of water had been transpired.

*Experiments with rice.*

For these experiments grains were selected weighing from 21 to 26 mgs with an average weight of 24,35 mgs. The two paleae were not removed; after the swelling of the grains an opening appears near the foot of the outer palea, through which root and shoot push.

The grains were washed, soaked and laid out in the hot-house; root and shoot appeared on April 27. In contrast with wheat and many other Gramineae where the coleoptile is followed by a leaf consisting of vagina and lamina, rice-seedlings have two primary leaves, the first about  $\frac{1}{2}$ , the second about 2 cms long.

After 8 days the seedlings were transplanted with two primary leaves and two leaves consisting of vagina and lamina. After 43 days the plants of the 27 series received their fresh suspension; the pH of the suspension used had fallen from 5,3 to 3,7 during the 35 days in which the riceplants had been growing in it. After aerating and reestablishing the pH to 5,3 the clay and part of the solution were restored to the plants. All the clay was

TABLE 7.  
Riceplants at the age of 54 days.

	number of leaves <sup>1)</sup> on main stalk	average distance from grain to top of longest leaf in mms	average number of lateral shoots	average number of leaves <sup>2)</sup>
1 series .....	$8\frac{5}{6} \pm \frac{1}{6}$	$483 \pm 11$	0	$8\frac{5}{6}$
3 " .....	$9\frac{1}{2} \pm 0$	$717 \pm 7$	0	$9\frac{1}{2}$
9 " .....	$10 \pm 0$	$867 \pm 12$	$\frac{4}{6}$	$13\frac{2}{6}$
27 " .....	$11 \pm 0$	$1098 \pm 12$	$1\frac{1}{6}$	17

<sup>1)</sup> Including the two primary leaves.

<sup>2)</sup> Including the two primary leaves of the main stalk and the primary leaf of each lateral shoot.

restored on the 63th day; of the solution used only a small part, about  $\frac{1}{8}$  could not be restored.

On the 54th day the appearance of all the plants was noted down; the plants of each series closely resembled each other, while the differences between the series were already very marked as table 7 shows.

From the 27 series 5 plants had 1 lateral shoot (each with 5 leaves) and 1 had 2 lateral shoots (with 6 and 5 leaves); from the 9 series 2 had no lateral shoots at all and 4 had 1 lateral shoot (each with 5 leaves).

After 64 days some withered leaves were removed and gathered, because the laminae hung very low and made the watering of the plants rather difficult. Young rice leaves have an erect position, the laminae being in one line with the vaginae. When the vagina has entirely developed, the lamina turns down round the hinge, till it is at about a rectangle with the vagina; cf plate III, fig. 3 and plate IV fig. 2. The force in the hinge is remarkable. When for some reason the whole bundle of stalks of the riceplant is bound together by a thread of raffia in such a way that a lamina is tied up among them, the lamina breaks on the raffia after some days. One has to be careful when a riceplant is tied up, that the binding material leaves the laminae out.

After 77 days the panicles began to issue from the vaginae of the uppermost foliage leaves of the stalks; first of the 27 and 9 series, afterwards of the 3 series; the 1 series did not flower at all.

After 105 days all the plants were harvested.

The plants harvested from the 9 and 27 series bore dark yellow grains; that these grains were ripe may be concluded from their high average weight. All the harvested plants still had at least  $2\frac{1}{2}$  assimilating leaves; in contrast with the wheat, and the buckwheat of the 1 and 3 series, the plants gave no impression of serious starvation



at the moment of harvesting. The rice transpired far more water than the wheat and buckwheat; the smallest quantity was used by the two lightest plants: 3060 cms<sup>3</sup> (each) though the air-dry weight of the one was 7,76 grs and of the other 8,72 grs. The largest quantity of water, 3340 cms<sup>3</sup> was transpired by the plant with the largest air-dry weight: 9,59 grs. The average quantity of transpired water was  $3174 \pm 43$  cms<sup>3</sup>, of air-dry matter  $8,89 \pm 0,25$  grs, of dried matter 7,912 grs; so for one gram of dry matter about 401 cms<sup>3</sup> water were transpired.

TABLE 8.

Rice grown on suspension harvested at the age of 105 days.

	Number of roots	Number of ripe grains	Air-dry weight			ratio the 1 series taken as a standard
			of superterranean parts in mgs	of roots in mgs	of superterranean parts less 40 mgs <sup>1)</sup>	
1 series ...	22,0 ± 0,95	0	368 ± 13	142 ± 7	328	1
3 „ ...	35,7 ± 0,67	2,7 ± 0,58	1027 ± 20	385 ± 12	987	3,01
9 „ ...	63,3 ± 3,2	10,0 ± 1,08	2590 ± 47	803 ± 11	2550	7,77
27 „ ...	97,3 ± 2,5	60,7 ± 2,65	7240 ± 196	1648 ± 60	7200	21,95

When of the average air-dry weight of the superterranean parts 40 mgs is subtracted, which may be due to the mineral matter present in the grain, the numbers of column 6 remain. In column 7 the ratio between the quantities of air-dry matter is given, the amount of the 1 series being taken as a standard. Among the series themselves the ratio, calculated from these figures, is:

$$7200 : 2550 = 2,82$$

$$2550 : 987 = 2,58$$

$$987 : 328 = 3,01$$

<sup>1)</sup> 1,6 × weight of one rice-grain: proportionally the same correction as applied for wheat.

In each case the triple quantity of suspension has enabled the plants to form from 2,6 to 3,0 times the amount of superterranean parts.

At the harvest of the wheat and the rice the roots were cut off separately, leaving two or three mms root to the stool; the remainder of the grain too was kept with the stool. Of the buckwheat the main root was cut at the cover. The stems were hung in a large drying-oven and dried at 70° C.; they were dry in a couple of hours and were afterwards exposed to the air to become air-dry. The number of roots of the wheat- and riceplants which sprouted from the stool, were counted; the roots of each plant were put in distilled water separately, which was refreshed till the water remained clear. The roots of the wheat and the rice were then white, as no more clay particles adhered to them. The roots of each plant were bound together and hung in the drying-oven, where they were soon dry; next they were exposed to the air to become air-dry. The roots of the buckwheatplants could not be washed white; they kept their clay-colour, even when the water itself remained clear.

After determining the air-dry weight of roots, fruit, etc. of each plant separately, the superterranean parts of a series were cut and milled together and so were the roots. As far as the quantities of air-dry matter permitted to do so, the shoots and the roots of the 27 series were analysed separately; when the quantity of the roots was insufficient, part of the determinations had to be omitted.

In the main the method of analysis described by Maschhaupt<sup>1)</sup> was followed.

<sup>1)</sup> J. G. Maschhaupt, De invloed van grondsoort en bemesting op het gehalte onzer cultuurgewassen aan stikstof en aschbestanddeelen. (The influence of soiltype and manuring on the percentage of nitrogen and ashconstituents of our cultivated plants). Verslagen van landbouwkundige onderzoekingen der Rijkslandbouwproefstations. No. 22, 1918, p. 107.

As the plants were grown in glass-houses and on nutrient solutions it was unnecessary to take into account the adhesion of sand and clay.

Moisture was determined by drying at  $105^{\circ}$  C., till the weight remained constant.

Phosphoric acid was determined by destruction with strong sulphuric acid and nitric acid according to Neumann-Fleischmann and precipitation of the phosphoric acid as ammonium phosphomolybdate according to von Lorenz.

Determination of total-ash. In combustion dishes of hard porcelain  $2\frac{1}{2}$  grams of air-dry powder, forming a layer of about 5 mms were put in a muffle-furnace on a plate 3 cms above the bottom and heated first with a small, afterwards with a larger flame of a Teclu burner. Only one burner was used during the whole time of combustion and the combustion dishes remained on the plate; consequently they did not come in contact with the heated parts of the furnace themselves. When some ash had been formed, the contents were carefully stirred with a thick platinum thread; about an hour afterwards no more coalparticles were visible; the whole process of combustion was carried out with a minimum of heating.

In order to determine the bases 20 grams of air-dry matter were burnt in the same way, dissolved with hydrochloric acid and the silicic acid separated and weighed. Then this impure silicic acid was treated twice or thrice with ammonium fluoride till the rest was constant; the rest was then treated with hydrochloric acid and this solution added to the solution of the bases.

For determining calcium and magnesium these two elements were separated according to Richards, the calcium precipitated as calcium oxalate and weighed as CaO, the magnesium precipitated according to B. Schmitz and weighed as  $Mg_2P_2O_7$ .

In order to determine potassium and sodium these were weighed together as chlorides, the potassium afterwards was precipitated and weighed as  $K_2Pt Cl_6$ .

Iron and manganese were determined by colorimetric methods in the solution of the bases: iron as sulphocyanate and manganese by oxidizing it with nitric acid and lead dioxide to permanganate.

Manganese which according to the brown colour of a couple of the CaO-determinations precipitated with the calcium and magnesium, was after glowing determined colorimetrically and the weight amounts of CaO and  $Mg_2P_2O_7$  accordingly corrected.

Sulphate and chlorine were not determined as the quantities of air-dry wheat and buckwheat were far too small for a separate determination; no more was sulphate determined in the solutions of the bases, as often a considerable part of the sulphur escapes during the combustion.

In the clay suspension the quantities of available phosphoric acid and bases had to be determined. The usual method with soils is shaking it with the tenfold quantity of a 1 % or 2 % solution of citric acid. This seemed however not very suitable here. The clay suspension contained about five grams of dry matter per liter, so the quantities of available phosphoric acid and bases would be low, and so far bigger quantities of extract than used in a normal soil analysis would be necessary here. This big quantity of extract contains such a large amount of citric acid, that its destruction would cause much trouble. With a view to this difficulty the nitric acid method according to von Sigmond<sup>1)</sup> was chosen. To 25 grams of

<sup>1)</sup> A. A. J. von Sigmond, Zur Frage der Laboratoriumsmethoden zur Bestimmung des Düngerbedürfnisses des Bodens. Transactions of the second Commission of the International Society of Soil Science. Budapest 1929, Vol. A, p. 147, Inserted in: O. Lemmermann, Methoden für die Untersuchung des Bodens, 1932, p. 74.

soil is added so much normal nitric acid and distilled water to one liter, that after shaking it for half an hour on two successive days a solution results with an acidity of 0,01 normal. The determination of the necessary quantity of nitric acid was done with the slight alteration given by Jessen and Lesch.<sup>1)</sup> In this extract the same determinations can be made as in the ash of plants, using the same methods; phosphoric acid can be determined directly in the extract or in the extract free from silicic acid.

According to Von Sigmond's method were examined:

1. The *fine earth* which was used for making the suspension. The extract was made of 25 grams fine earth per liter. The results are expressed in mgs per 100 grs of the dried fine earth (the air-dry fine earth contained 4,92 % water).

2. The *suspension* as it was used for growing the plants. It contained 0,503 % of dry matter, about 5 grs per liter. The fine earth, from which the suspension was made, contained (see p. 44) 26,4 % of sand and 69,4 % of silt and clay. On making the suspension it was obvious that from this 69,4 % by far the greater part was silt and not more than the fifth part was clay. As the nitric acid from the coarser particles of the fine earth could affect the surface only, 5 grs of dry matter in the suspension would probably give a quantity of soluble matter of the same kind as 25 grs of fine earth. So the suspension was extracted as it was, and the quantities of soluble components per liter proved to be of the same kind as in the extract of the fine earth.

3. After growing the plants in the suspension sub 2 the *remaining clay* was gathered by settling. This thick suspension was analysed too. As it was rather exhausted,

<sup>1)</sup> W. Jessen und W. Lesch, Zs. für Pflanzenernährung, Düngung und Bodenkunde, A. Bd. 18, 1930, p. 225.

a large quantity of dry matter per liter was used; 23,16 grs, about the quantity in the extract of fine earth.

The results are all expressed in mgs per 100 grs of dry matter (table 9)

TABLE 9.

Components found by extraction according to Von Sigmond in mgs per 100 grams of dry matter.

	Si O <sub>2</sub>	CaO <sup>1)</sup>	Mg O	K <sub>2</sub> O	Na <sub>2</sub> O	Fe <sub>2</sub> O <sub>3</sub>	MnO	P <sub>2</sub> O <sub>5</sub>	bas. <sup>2)</sup>	end-acidity <sup>3)</sup>
1. fine earth ...	200	1108	180	48	26	14	42	8	52,5	10,8
2. suspension ..	747	1780	331	185	178	41	20	25	66,2	9,6
3. remaining clay	180	681	138	20	21	16	9	3	30,7	10,6

100 grs of dry matter of the suspension yielded more to the extract of all the components than 100 grs of fine earth, except manganese, of which only half the quantity was dissolved. The only possible reason for this can be, that in the fine earth the manganese content of the coarser particles is far larger than it is in the clay. Especially of K<sub>2</sub>O and Na<sub>2</sub>O, Fe<sub>2</sub>O<sub>3</sub>, P<sub>2</sub>O<sub>5</sub> and SiO<sub>2</sub> a far larger amount per 100 grams of dry matter was got from the suspension. 100 grs of dry matter of the remaining clay yielded far less of all the components than the suspension did. This must be ascribed in the first place to the exhaustion by the plants which grew in it, but also partly to the fact that by the gathering of the clay by far the greater part of the solution on the top of the clay had been thrown away.

In table 10 are put together the quantities of mineral

<sup>1)</sup> Hissink found in 100 grams of fine earth of the same plot 790 mgs of interchangeable CaO (by another method of analysis, cf p. 44).

<sup>2)</sup> bas.: number of cms<sup>3</sup> of normal nitric acid neutralised by 100 grs of dry matter.

<sup>3)</sup> end-acidity: number of cms<sup>3</sup> of normal acid present in one liter of the filtrated extract.

components found per plant of the 27 series. The roots have also been analysed, the amount of their weight however did not admit of the determination of all the elements, so CaO and MgO and  $P_2O_5$  of the buckwheat-plants were omitted. In comparison are given the quantities of the same components extracted according to Von Sigmond's method from the quantity of suspension used for one plant. The quantities of the various elements extracted by the three kinds of plants vary widely. These differences must be ascribed in the first place to the properties of the species, e.g. the large quantity of silicic acid in wheat and rice and the small quantity in buckwheat, which on the other hand has the greatest quantity of CaO.

External causes can however exist too. The buckwheat was harvested at a time when the 9 and 27 series did not yet show any sign of starvation. The supposition may be made, that these plants would have acquired a considerably larger quantity of mineral and of dry matter, if they had been allowed a longer growing-period.

The rice has absorbed far more mineral matter and formed more organic matter than the wheat. Though the greater part of the rice plants were withered at the moment of harvesting, they did not make such an impression of starvation as the wheat had done. As an external cause of the larger quantity of mineral compounds available for the rice, must be thought of the higher temperature of the nutrient medium in the hot-house (cf. table 2, p. 49) which must have enlarged the solubility of the various compounds of the clay.

We may take for granted that the wheatplants in my investigation (cf. table 10) exhausted the suspension with regard to those elements only which according to the analyses were present in abnormally small quantities. By a fortunate circumstance it was possible to compare the

TABLE 10.

Milligrams of the undermentioned components present in one plant and in the quantity of suspension given to one plant extracted by Von Sigmund's method.

	Dry matter 105° C.	Rest by combustion (ash)	Si O <sub>2</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	Fe <sub>2</sub> O <sub>3</sub>	MnO	P <sub>2</sub> O <sub>5</sub>
Suspension .....	20.120	18.080	150,2	358,5	66,5	37,2	35,9	8,2	4,1	5,1
Wheat .....	5.084	219,0	82,5	30,2	11,1	42,3	3,8	4,1	0,6	7,3
Buckwheat .....	3.064	171,4	11,4	59,0	15,2	20,7	2,0	1,4	1,8	6,0
Rice .....	6.498	498,4	226,1	55,6	46,8	52,8	14,4	10,6	9,5	10,6
Wheat .....	588	24,1	8,5	—	—	3,3	2,0	1,9	0,15	1,2
Buckwheat <sup>1)</sup> .....	359	27,1	9,8	—	—	2,2	0,7	2,8	0,07	—
Rice .....	1.414	70,2	34,5	—	—	4,1	3,2	8,1	0,10	1,2
Wheat .....	5.672	243,1	91,0	—	—	45,6	5,8	6,0	0,8	8,5
Buckwheat .....	3.423	198,5	21,2	—	—	22,9	2,7	4,2	1,9	—
Rice .....	7.912	568,6	260,6	—	—	56,9	17,6	18,7	9,6	11,8
Shoots of wheat (Maschhaupt) <sup>2)</sup>	5.084	371,3	120,8	16,0	6,8	91,7	5,9	3,1	—	26,6

<sup>1)</sup> The roots of the buckwheat could not be washed white, as the roots of the wheat and the rice, so that especially the quantities of Si O<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> will probably be too high.

<sup>2)</sup> Cf. p. 75.



analysis of the superterranean parts of the wheatplants grown in the suspension with an analysis, made in the same way of the same wheat-variety (Japhet summer-wheat) grown on a soil similar to and situated in the neighbourhood of the soil, of which the suspension was prepared.<sup>1)</sup> Maschhaupt harvested every fortnight a part of his experiment-plants in order to study the changes in the composition of the mineral components and of the nitrogen percentage. I took for the comparison the analysis of those plants which were harvested a fortnight after the appearance of the ears. This analysis seemed suitable, because the plants of the 27 series were harvested 18 days after the appearance of the ears. In the experiments of Maschhaupt the average dry-weight per plant was 5,575 grs; so the quantities of components found by Maschhaupt in this analysis were multiplied with 0,912 and in this way reduced to 5,084 grs, which represents the average dry-weight of the wheat-plants of the 27 series.

In table 10 at the bottom the quantities thus obtained are stated in mgs. When both wheat-analysis are compared, the plants cultivated in suspension appear to have only 27 % of the  $P_2O_5$ -percentage and 46 % of the  $K_2O$ -percentage of the plants grown in the soil: these components were evidently only present in anomalous small quantities, as may also be seen from table 11. On the other hand the plants grown in suspension had a considerable higher quantity of  $CaO$  and  $MgO$ ; they had a little more of  $Fe_2O_3$ , but less of  $SiO_2$  and  $Na_2O$ : more or less of the latter components between reasonable limits does not

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<sup>1)</sup> J. G. Maschhaupt, De samenstelling onzer landbouwgewassen in opeenvolgende groeiperioden (= the composition of our cultivated plants in succeeding growing-periods). Verslagen van landbouwkundige onderzoekingen der Rijkslandbouwproefstations, No. 27. 1922, p. 129.

influence the development of the plant. We may assume only for the quantity of  $P_2O_5$  and  $K_2O$  that the whole quantity available in the suspension is absorbed.

When the quantities absorbed by the whole plant are compared with those obtained by extraction according to Von Sigmond's method, the latter appear to be the lowest; these quantities are about 80 % of the  $K_2O$  absorbed and about 60 % of the  $P_2O_5$  absorbed; the

TABLE 11.  
Percentages in dry matter of plants grown in clay suspension.

		Ash	SiO <sub>2</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	Fe <sub>2</sub> O <sub>3</sub>	MnO	P <sub>2</sub> O <sub>5</sub>
wheat.....	{ stalks	4,31	1,62	0,59	0,22	0,83	0,08	0,08	0,012	0,14
	{ roots	4,11	1,45	—	—	0,57	0,35	0,32	0,026	0,20
buckwheat	{ stems	5,60	0,37	1,93	0,50	0,68	0,07	0,05	0,051	0,20
	{ roots <sup>1)</sup>	7,60	2,72	—	—	0,62	0,18	0,78	0,020	—
rice .....	{ stalks	7,67	3,48	0,86	0,72	0,81	0,22	0,16	0,146	0,16
	{ roots	4,97	2,44	—	—	0,29	0,23	0,58	0,007	0,08

TABLE 12.  
Percentages in ash of plants grown in clay suspension.

		SiO <sub>2</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	Fe <sub>2</sub> O <sub>3</sub>	MnO	P <sub>2</sub> O <sub>5</sub>
wheat.....	{ superterranean parts ..	37,7	13,8	5,1	19,3	1,7	1,9	0,28	3,3
	{ roots .....	35,2	—	—	13,8	8,5	7,9	0,63	5,0
buckwheat	{ superterranean parts ..	6,7	34,4	8,9	12,1	1,2	0,8	1,04	3,5
	{ roots <sup>1)</sup> .....	36,1	—	—	8,2	2,4	10,3	0,26	—
rice .....	{ superterranean parts ..	45,4	11,2	9,5	10,6	2,9	2,1	1,91	2,1
	{ roots .....	49,2	—	—	5,9	4,5	11,6	0,14	1,7

<sup>1)</sup> The roots of the buckwheat could not be washed white, as the roots of the wheat and the rice, so that especially the percentages of silicic acid and ferric oxide probably are too high.

extraction according to Von Sigmond's method does evidently not give results which are too high for available potassium and phosphoric acid. If however we take into account that a plant will never be able to deplete a soil of its available components so thoroughly as this is possible with the clay particles suspended in water, we may conclude that the quantities of  $K_2O$  and  $P_2O_5$  found according to Von Sigmond's method, give a fair expression of the quantities available in a soil.

The clay which remained after growing the above-mentioned plants in suspension was gathered by settling. In this clay wheat was grown once more. Two plants received per cylinder a quantity corresponding with 100 grams of dry matter and two got each a quantity of 33 grams. The plants developed normally and fructified, but they were slender and made no lateral shoots. The only visible difference between both sets was that the plants on 100 grams of clay produced 9 leaves, and those on 33 grams 8 leaves (coleoptile included). The quantity of dry matter produced (roots included) differed only slightly, of ash far more.

Clay	100 grs	33 grs
Air-dry grains per plant ..	115 mgs	130 mgs
Total dry matter " " ..	798 "	743 "
Ash " " ..	79 "	44 "
Organic matter " " ..	719 "	699 "
% ash in dry matter .....	9,92 %	5,87 %

From the fact that both sets of plants produced about the same quantity of grains and of organic matter must be concluded that in the suspension some noxious factor had originated, tending to check the assimilation as much as the greater quantity of mineral matter tended to stimulate it; perhaps we may think here of organic com-

pounds, originating from the roots of the plants which had grown in the suspension before.<sup>1)</sup>

In table 11 and 12 are given the percentages of the various elements in dry matter and in ash. From these tables it is also obvious that the percentages of potash and especially of phosphoric acid are exceedingly low. For comparison the percentages of phosphoric acid were also determined in the superterranean parts of the plants grown on the nutrient solution according to Pryanishnikov with the addition of boron, manganese and fluorine.

The percentages of  $P_2O_5$  found in the air-dry matter were: a larger percentage in the roots than in the stalks. For buckwheat and rice the reverse is true: the percentages in the shoots are higher; for the rice the percentage in the

wheat .....	0,73 %
buckwheat .....	1,81 %
rice .....	2,62 %

The percentages in buckwheat and rice are exceedingly high, especially in the latter.

When the percentages in dry matter of stalks and roots are compared, we come to the conclusion that the iron passes only very slowly from the roots to the stalks of wheat and rice, a much higher percentage remaining in the roots. It may be asked, whether this iron is localised especially at the outer side of endodermis and Casparian strip, by which, according to De Ruzf de Lavison<sup>2)</sup>

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<sup>1)</sup> See a.o. J. F. Breazeale, Effect of Certain Solids upon the Growth of Seedlings in Water-cultures. *Botanical Gazette*, 41, 1906, p. 54. Spencer Pickering, The Effect of one Plant on Another. *Annals of Botany*, 31, 1917, p. 181.

Bull. of the U.S. Dep. of Agriculture, Nos 28, 40, 74, 88, 108 and 164.

<sup>2)</sup> M. Jean de Ruzf de Lavison, Du mode de pénétration de quelques sels dans la plante vivante. Role de l'endoderme. *Revue générale de botanique*, 22, 1910, p. 225.

iron is arrested and through which it probably cannot pass in noticeable quantities.

With manganese wheat shows the same phenomenon: stalks is even twenty times as high as in the roots. The difference with wheat on this point is remarkable as is also the difference with the other mineral components in rice, of which none is contained in more than the threefold percentage in the stalks than in the roots ( $K_2O$ ). From these differences one may conclude that manganese is not arrested by the endodermis and the Casparian strip of rice, as has been found for iron and other heavy metals with many plants, nor by the pericycle, as is the case with baryum according to Colin et De Rufz de Lavison.<sup>1)</sup> That the percentage in the stalks is twenty times as high as in the roots would be an indication that manganese is rapidly fixed in the stalks, in this way causing a lasting ascent of all soluble manganese from the roots.

#### *Summary.*

Wheat, buckwheat and rice were grown in cylinders on a clay suspension as the only source of mineral constituents. As nitrogen sources some ammonium nitrate and some nitric acid were added (the latter in order to lower the pH of the suspension from 7,4 to 5,3). When sufficient clay was present plants could be grown from seed to seed in this nutrient medium without renewing it.

Wheat grown on the clay suspension with ammonium nitrate without nitric acid got yellow-striped leaves and a very sickly appearance. After some weeks the plants recovered by and by owing to the fact that during the growth the pH was lowered by the plants themselves.

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<sup>1)</sup> H. Colin et J. de Rufz de Lavison, Absorption comparée des sels de baryum, de strontium et de calcium. *Revue générale de botanique*, 22, 1910, p. 337.

By wrapping the glass-cylinders in corrugated cardboard and kitchenpaper the mean maximum-temperature of the nutrient medium in the unheated glass-house was 4 to 5° C. lower than that of the air; the mean minimum-temperature about 2° C. higher. (The mean daily range of the water-temperature was 6°,6 C. less than that of the air-temperature).

In paraffined and unparaffined new cylinders of resistant glass, each containing 2,1 liters of distilled water and 40 mgs of ammonium nitrate, wheatplants were grown. The air-dry grains weighed 57 mgs, the total air-dry weight of the plants, harvested at the age of 86 days was 98 mgs per plant from the paraffined and 121 mgs from the unparaffined cylinders.

Wheat, buckwheat and rice were grown on clay suspension in which the quantities of clay were dosed in the proportion 1 : 3 : 9 : 27; for wheat the yield of dry matter of each series was about 2,7 times as much as of each preceding series.

The proportional weights of the air-dry matter of the superterranean parts were:

for wheat: 1 : 2,78 : 7,26 : 19,16

for rice : 1 : 3,01 : 7,77 : 21,95

The air-dry weight of the superterranean parts of the 9 and 27 series showed for the wheatplants, which were grown from a pure strain, a far smaller standard error than for the buckwheat and the rice, where this was not the case.

When growing plants on clay suspension, the clay particles did not adhere to the roots of wheat and rice, as they did to the roots of buckwheat.

In the clay suspension and the fine earth the available components were determined according to Von Sigmund's method. Especially  $K_2O$ ,  $Na_2O$ ,  $Fe_2O_3$ ,  $P_2O_5$

and  $\text{SiO}_2$  were present in the clay particles in a far higher rate than in the fine earth.

The same components were determined in the harvested plants for the roots and shoots separately, and the quantities of mineral components per plant were compared with the quantities of available components present in the given quantity of clay suspension.

The method of Von Sigmond for determining the available quantities of phosphoric acid and bases proved in the clay suspension to give results rather too low than too high for phosphoric acid and potash. This results from the fact that wheat which had withered entirely some time before the grains were ripe, on account of deficiency of these two constituents, was found to contain more phosphoric acid and potash than resulted from Von Sigmond's method in the same quantity of suspension.

From the same quantity of suspension rice absorbed far more mineral components than wheat. For lime the quantity was not yet twice, for silicic acid, sodium and iron it was three times as much; for magnesium four times and for manganese even twelve times the quantity absorbed by a wheat plant. Though the wheat exhausted the suspension, as far as potash and phosphoric acid are concerned, yet the rice was able to absorb about 25 % more potash and about 40 % more phosphoric acid than the wheat had done. This greater absorption may be ascribed to the fact that the temperature of the nutrient medium for the rice was always at least  $5^\circ \text{C}$  higher than for the wheat, which probably has resulted in a greater solubility and as a consequence a faster and larger absorption of the mineral matter. It would be worth while to investigate whether extraction according to Von Sigmond's method would yield larger quantities of available components at a higher temperature than at room temperature.

For trials concerning the influence of various salts or other components on plants, a clay suspension in some cases may be preferred to the usual nutrient solutions, as the nutrient components are not present in higher but in lower concentration than in the soil solution, whilst in the nutrient solutions used in most experiments described in literature this concentration is far much higher than in most soils. Besides the presence of the clay makes the circumstances far more comparable with those in the soil, while these circumstances can be constantly controlled much more effectively than with experiments in soil.

So may for instance the effect of brackish water on plants probably be studied better in a clay suspension than in a nutrient solution of salts. In the first place, while no soluble salts besides those of the brackish water are added and in the second place while in the suspension part of the Na from the seawater will be exchanged for Ca, just as is the case with soil, while all the Cl of the sea-water remains in solution. The composition of a solution of nutrient salts, to which seawater is added, will show much difference with a clay suspension to which the same quantity of sea-water has been added, while the latter may be sooner compared with a brackish soil as regards the prevailing conditions.

### CHAPTER III.

#### **The influence of Auxiliary Elements in Nutrient Solutions on the Development of Plants.**

During the preliminary tests in 1930 a couple of wheat plants were grown on the solution after Knop and on that after Pryanishnikov. The solutions were made of tapwater and salts of the pure quality for pharmaceutical use.



Difficulties caused by chlorosis which after some time appeared, especially in consequence of the use of tapwater, were set right by reducing the pH under 5,5 with some 0,1 n. acid. The wheat developed reasonably well, but the vaginae, laminae and later the stalks lacked the firmness which marks the wheat grown on soil. The ears were impotent to undo themselves from the vaginae of the uppermost foliage leaves of the stalks: their tops stuck, which than were bent while the stalks grew on; consequently they had to be loosened by hand (cf. plate III, fig. 1).

The plants grown on these two different solutions diverged little in size and number of ears and were alike in the limpness of their bodies.

In the experiments with wheat, buckwheat and rice of 1931 recorded in chapter II plants were grown on nutrient solution by the side of those grown on suspension. The solution after Pryanishnikov was used with and without addition of compounds of boron, manganese and fluorine.

To each cylinder of 2 liters were added:

$H_3BO_3$ .....	2 mgs	(= 0,36 mg B)
$CaF_2$ .....	2 „	(= 0,97 „ F)
$MnCl_2$ 4 $H_2O$ ....	4 „	(= 1,15 „ Mn)

The buckwheatplants to which the auxiliary elements were added when they were put in the cylinders, were the first to show a marked difference. Before long the new internodes of the plants without the auxiliary elements became very short, leaves and stems grew red and fleshy; the plants did not flower and made very short lateral branches. On the other hand those plants with auxiliary elements developed normally and produced ripe fruit.

At the time of the harvest the buckwheatplants without auxiliary elements reached a height of about 10 cms, with

a few lateral branches of at most 4 cms long; the lateral branches broke off easily at the stem. The air-dry superterranean parts weighed 1,12 grs per plant, the roots 0,07 gr; the quantity of water transpired was 410 cms<sup>3</sup> per plant or calculated on a gram of air-dry matter 344 cms<sup>3</sup>.

The buckwheat grown with auxiliary elements was at the time of the harvesting about a meter high with three lateral branches; about one hundred of ripe fruit were produced per plant with a fair average weight; the air-dry superterranean parts weighed 5,63 grs per plant, the roots 0,48 gr; the quantity of water transpired was 1560 cms<sup>3</sup> or calculated per gram of air-dry material 255 cms<sup>3</sup>.

One half of the wheatplants on the nutrient solution after Pryanishnikov got the addition of the auxiliary elements after 27 days, when they had been in the solution for 17 days. During the first weeks after this addition no marked difference in growth between the plants with and without auxiliary elements was visible, both series developing normally and producing a strong number of shoots, but the former plants were firmer than the latter at the time the ears had to be formed. Of the plants without auxiliary elements the oldest shoots did not develop, the stalks remained short and limp and no ear appeared (cf. plate III, fig. 2, no. 28).

The plants with auxiliary elements had long and solid stalks; after 77 days the ears appeared; the plants looked very sound (cf. plate III, fig. 2, no. 29). The oldest shoots of the plants without auxiliary elements became yellow, wilted and withered; new shoots developed in their place, which neither produced an ear.

The plants were harvested after 114 days; those without auxiliary elements did not bear a single well-developed ear; the number of shoots per plant was on an average 30; when the shoots were pulled to pieces a few contained a

half-developed, empty ear. The plants with auxiliary elements each bore five ears with average weight of 5,5 grs per plant (the grains were not yet fully ripe) and six shoots which had not formed ears; the number of grains per plant was on an average 158. The dried plants without auxiliary elements could easily be crushed, because they were practically void of any solid matter.

The quantity of water transpired for the formation of 1 gram of air-dry matter was abnormally high with the plants grown without auxiliary elements, as may be seen from table 13. The quantity of water transpired for the formation of a gram of air-dry matter with the plants

TABLE 13.

Water transpired per gram of air-dry matter by wheat grown on different nutrient media.

Nutrient medium	cm <sub>3</sub> water transpired per plant	air-dry matter (stalks and roots) in grs	water transpired per gr. of air-dry matter in cms <sup>3</sup>
solution after Pryanishnikov without auxiliary elements...	4418 ± 18	6,37 ± 0,18	694 ± 17
same with B + Mn + F .....	4572 ± 67	16,83 ± 0,14	272 ± 6
4 kgs of suspension .....	1267 ± 17	6,355 ± 0,055	199,3 ± 1,4
1 <sup>1</sup> / <sub>3</sub> " " " .....	618	2,433 ± 0,026	254
4 " " suspensionsolution only .....	686 ± 15	2,60 ± 0,04	264 ± 2

grown in a nutrient solution with auxiliary elements is about the same as with those grown in the clay-solution; this figure on the contrary is with the plants grown without auxiliary elements about 2<sup>1</sup>/<sub>2</sub> times as high.

Mazé, who grew maize on nutrient solutions with a great number of auxiliary elements among which zinc and manganese were always present, whereas one of the others was in turn left out, found a quantity of water transpired

varying from 161 to 195 cms<sup>3</sup> per gram of dry matter. <sup>1)</sup>

An explanation of the exceedingly high quantity of water transpired per gram of air-dry matter by the plants grown without auxiliary elements may be found in table 14. It is evident, that the higher weight of the plants grown with auxiliary elements is especially due to the ears and the stems of the fertile stalks. On the contrary the weight of the laminae, to which the transpiration has chiefly to be ascribed, is higher with the plants grown without auxiliary elements, so that we may take for granted that their surface too was larger. <sup>2)</sup> The laminae of the plants grown without auxiliary elements however withered sooner than the other ones; so the transpiring surface of both groups at a given time may have been equal. Though transpiration and leaf-surface were about equal, the formation of organic

TABLE 14.

Air-dry weight of parts of wheatplants grown with and without auxiliary elements.

	air-dry weight in grs	
	without	with
	auxiliary elements	
Laminae .....	3,61 ± 0,31	2,75 ± 0,01
vaginae of fertile stalks .....	— —	2,35 ± 0,08
„ „ infertile „ .....	1,53 ± 0,06	0,39 ± 0,02
stems of fertile stalks .....	— —	4,62 ± 0,24
„ „ infertile „ .....	0,86 ± 0,04	0,26 ± 0,04
ears .....	— —	5,50 ± 0,76
roots.....	0,37 ± 0,05	0,96 ± 0,22
Total weight ....	6,37	16,83

<sup>1)</sup> P. Mazé, Recherche d'une solution purement minérale capable d'assurer l'évolution complète du maïs cultivé à l'abri des microbes. Ann. de l'Inst. Pasteur, T. 33, 1919, p. 154 seq.

<sup>2)</sup> B. E. Livingston, Relation of transpiration to growth of wheat. Botanical Gazette, Vol. 40, 1905, p. 178.

matter was evidently depressed in a high degree by the deficiency of auxiliary elements.

When the riceplants had been for three days in the nutrient solution they received at the age of 12 days the same quantities of auxiliary elements as the wheat and the buckwheat. This amount of auxiliary elements however turned out to be harmful; the plants with auxiliary elements developed fewer lateral shoots and consequently fewer leaves; the roots got a slightly yellowish brown colour and the oldest leaves withered. This stagnancy in growth is demonstrated most clearly in the quantity of water transpired: till the age of 61 days the transpiration per plant was 540 cms<sup>3</sup>, without auxiliary elements 710 cms<sup>3</sup>. In the long run however the auxiliary elements worked more favourably: the plants with auxiliary elements overtook the others and left them behind. This is also demonstrated by the quantities of water transpired: from the 61st day till the 124th day the transpiration per plant with auxiliary elements was 2700, without 1700 cms<sup>3</sup>.

As a consequence of the effect of the auxiliary elements, at first harmful, later beneficent, the differences between both series of riceplants were less marked than with the wheat and the buckwheat, and the differences among the plants of the same series were greater. The favourable effect of the auxiliary elements was demonstrated best in the fructification.

The riceplants without auxiliary elements were not limp as was the case with the wheatplants; the stalks were reasonably well developed and solid and so were the vaginae; both had a normal weight.

From this description it follows that the rice needed less auxiliary elements than buckwheat and wheat, and that the quantity added was already slightly poisonous for these 12-days-old riceplants. A similar effect did not show itself with the buckwheatplants which also received the auxiliary

elements immediately after being put in the cylinders, nor with the wheatplants, which however were then already 27 days old and so had considerably more roots than at the moment of transplantation and could probably endure more.

TABLE 15.

Rice grown on nutrient solution after Pryanishnikov with and without auxiliary elements.

Per riceplant	without	with
	auxiliary elements	
number of grains .....	9	92
weight " " .....	0,15 grs	1,76 grs
total air-dry weight .....	4,34 "	7,30 "
transpired water .....	2418 cms <sup>3</sup>	3240 cms <sup>3</sup>
" per gram of air-dry matter.....	557 "	444 "

In the literature on the investigations of the beneficent or harmful effect of auxiliary elements we find that special attention has been paid to the concentration of the auxiliary elements in the nutrient solution and the good or bad effect of this concentration always has been mentioned. Whether a certain solution is harmful or beneficent probably does not exclusively depend, however, on the concentration. The absorption of the auxiliary elements which are present in very low concentrations also will depend on the quantity of nutrient solution: the more solution, the more absorption. We may take for granted that the whole quantity of nutrient solution comes in contact with the roots because through the difference in day- and night-temperatures currents are always caused in the solution. In the experiments mentioned in this paper the solutions were moreover stirred every two or three days in order to give them the opportunity to absorb oxygen; by so doing the whole solution may in any case be con-

sidered to have come in contact with the roots. The effect, be it harmful or not and the dose desirable for the plant will moreover depend on the development of the plant. Just as the dose of strongly active chemical substances used for the medical treatment of animals or men is given more or less proportional to the weight of the body, so with the plants a similar proportion will probably exist between the desirable dose and the quantity of dry matter of the plant or perhaps the total active surface of the roots. Moreover a smaller quantity of certain elements will apparently suffice during the vegetative period than in the period of the development of the organs of propagation. Did not the wheatplants, which developed a plentiful number of shoots and leaves with the slight quantities of auxiliary elements found in the usual „pure” salts, fail to produce any flowers? With the small quantities of the auxiliary elements present in the salts used, the riceplants could however not only make their leaves, but also produce a small number of grains; the effect of a bigger quantity of the auxiliary elements resulted from the much greater number of grains produced by the plants, which were grown with the addition of auxiliary elements.

As of the three testplants grown, rice showed the least need of auxiliary elements, and as in the literature, as far as could be ascertained, rice did not occur among the plants for which the necessity of auxiliary elements had been established, the authoress resolved to try to grow rice entirely without auxiliary elements. At the same time could be examined, whether the presence of a smaller or greater quantity of auxiliary elements in the grains had a marked influence on the development of the plants. The grains harvested from the riceplants cultivated on the nutrient solutions with and without auxiliary elements afforded a good opportunity for this purpose. As it was only her intention to investigate how far rice would grow

without any auxiliary elements, and the measure to which auxiliary elements generally were useful, those elements had to be excluded as far as possible from some plants. To other plants those elements which had proved beneficial should be added, but in such quantities that a harmful effect was not to be feared.

In chapter I is mentioned how Mazé proved in 1914 the indispensability of manganese and zinc for maize and his description in detail of the symptoms of their deficiency. In 1919 he proved the indispensability of boron for maize too. From Mazé's paper the most favourable dose of manganese and zinc cannot be concluded, as he added 20 mgs of  $MnCl_2$  and  $ZnCl_2$  per liter; through the presence however of 2 grs of calcium carbonate per liter the manganese and the zinc both were nearly entirely precipitated. The boron was given as 4 mgs of borax per liter.

Warington<sup>1)</sup> proved the indispensability of boron for a great number of plant species; in her experiments a concentration of 1 : 12.500.000 gave the best results with barley grown 92 days; with barley grown 71 days a concentration of 1 : 100.000.000 proved still more favourable. The symptoms of boron-deficiency with *Vicia Faba* are fully described by her in another publication.<sup>2)</sup> s'Jacob described the same symptoms as Warington when growing *Vicia Faba* without boron.<sup>3)</sup>

Brenchley<sup>4)</sup> and Warington experimented on a large

1) K. Warington, The effect of boric acid and borax on the broad bean (*Vicia Faba* L.) and certain other plants. *Annals of Botany*, 37, 1923, p. 629.

2) K. Warington, The changes induced in the anatomical structure of *Vicia Faba* by the absence of boron from the nutrient solution. *Annals of Botany*, 40, 1926, p. 27.

3) J. C. s'Jacob, Anorganische beschadigingen bij *Pisum sativum* L. en *Phaseolus vulgaris* L. 1927, p. 104.

4) W. E. Brenchley and K. Warington, The rôle of boron in the growth of plants. *Annals of Botany*, 41, 1927, p. 167.



scale on the symptoms of boron-deficiency and the degree of indispensability of boron for different plant-species. With *Vicia Faba* the apices continually died in default of boron and numeral lateral shoots were formed, which for the greater part died too. Many other plantspecies began to die at the apex of the shoot and failed to flower; the roots were short and stunted. The necessity of boron always became more manifest at the approach of the flowering period. For some plant-species boron appeared to have a favourable effect, but not to be indispensable; for some no effect could be established.

Swanback<sup>1)</sup> proved the indispensability of boron for tobaccoplants; two parts per million of boric acid in the nutrient solution gave an optimum growth.

Mes<sup>2)</sup> describes the symptoms of boron-deficiency with tobacco.

Johnston and Dore<sup>3)</sup> proved the indispensability of boron for tomato-plants. In default of boron the conducting tissues in the stem broke down, and they were able to prove that in consequence the total of sugars and starch was larger in the leaves and stems of the plants deficient in boron than in those of normal plants.

Johnston and Fisher<sup>4)</sup> continued the investigations of the necessity of boron for tomatoplants and found that the plants had to be supplied with boron throughout the

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<sup>1)</sup> F. R. Swanback, The effect of boric acid on the growth of tobacco plants in nutrient solutions. *Plant Physiology*, Vol. 2, 1927, p. 475.

<sup>2)</sup> M. G. Mes, *Fysiologiese siektesimptome van tabak*, 1930, p. 35.

<sup>3)</sup> E. S. Johnston and W. H. Dore, The influence of boron on the chemical composition and growth of the tomato plant. *Plant Physiology*, Vol. 4, 1929, p. 31.

<sup>4)</sup> E. S. Johnston and Paul L. Fisher, The essential nature of boron to the growth and fruiting of the tomato. *Plant Physiology*, Vol. 5, 1930, p. 387.

growing and fruiting-period; two weeks after the boron-supply was stopped the plants ceased growing and the fruit were covered with black spots; 0,5 mg. of boron per liter of the nutrient medium kept the tomatoes healthy.

Schmucker<sup>1)</sup> proved the indispensability of boron for the germination of the pollen-grains of *Nymphaea zanzibarensis* Casp.

The indispensability of manganese for garden peas was proved by Mac Hargue.<sup>2)</sup> In accordance with Mazé's description of his maize experiments he notes as a first effect of manganese deficiency that the young leaves get a yellowish instead of a normal green colour; soy beans and cow peas showed the same symptoms as garden peas.

Samuel and Piper<sup>3)</sup> found that the grey speck disease of oats was caused by manganese deficiency. In nutrient solutions without manganese oats showed symptoms which were similar to those of the grey speck disease in the field; the plants died in the seedling stage unless manganese was supplied. 0,25 mg of manganese per liter sufficed to prevent the disease.

Miller<sup>4)</sup> proved the necessity of manganese for tomatoes, tobacco, cabbage, wheat, oats and maize. For these plants too the most striking symptom of the manganese deficiency was the chlorosis.

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<sup>1)</sup> Th. Schmucker, Zur Blütenbiologie tropischer *Nymphaea*-Arten. II. Bor als entscheidender Faktor. *Planta*, 18, 1932, p. 641.

<sup>2)</sup> J. S. Mac Hargue, Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. *Journ. of Agric. Res.* Vol. 24, 1923, p. 781.

<sup>3)</sup> G. Samuel and C. S. Piper, Grey speck (manganese deficiency) disease of oats. *Journ. Agric. So. Australia*, 31, 1928, p. 696 and 789.

<sup>4)</sup> L. P. Miller, Manganese deficiency in sand cultures. *Amer. Fertilizer*, 68, 1928, p. 21.

The indispensability of zinc which was proved by Mazé for maize, was investigated by Sommer and Lipman<sup>1)</sup> and by Sommer<sup>2)</sup><sup>3)</sup> for a number of plants. These investigations were carried out with special precautions. In order to prevent dust falling on the plants they were cultivated in a small glass-house built within a larger one; the ventilators were provided with an apparatus to prevent the dust from entering; the cemented floor was kept dustfree. The utmost care was taken to use only the purest salts available and vessels which did not yield any trace of soluble components that might interfere in the experiments. In <sup>2)</sup> was proved the necessity of zinc for sunflowers and barley; in <sup>3)</sup> for wheat, buckwheat, broad beans and red kidney beans. Especially flowering and fructification failed in default of zinc; wheat and barley died already in the first growingperiod when zinc was lacking. The symptoms mentioned by Mazé when zinc was lacking (cf. p. 40) were not described by Sommer and Lipman.

Sommer proved that copper too is an essential for plant growth.<sup>4)</sup> Just as the investigation described above with zinc, this too was made with the utmost care. All the plants received besides the principal elements traces of Mn, Zn, Al, I, F, Na, Cl and B and partly Cu, partly not. The plants without copper showed less growth after two weeks then those with copper, those without copper

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<sup>1)</sup> A. L. Sommer and C. B. Lipman, Evidence of the indispensable nature of zinc and boron for higher plants. *Plant Physiology*, Vol. 1, 1926, p. 231.

<sup>2)</sup> A. L. Sommer, Further evidence of the essential nature of zinc for the growth of higher green plants. *Plant Physiology*, Vol. 3, 1928, p. 217.

<sup>3)</sup> A. L. Sommer, The search for elements essential in only small amounts for plant growth. *Science*, 66, 1926, p. 482.

<sup>4)</sup> A. L. Sommer, Copper as an essential for plant growth. *Plant Physiology*, Vol. 6, 1931, p. 339.

did not yield any fruit and bore hardly any flowers. For sunflowers, tomatoes and flax the necessity of copper was evident; 0,06 mg of copper per liter was sufficient. The quantity of copper is to be dosed carefully as Sommer found that 0,25 mg of Cu per liter already stopped the growth of the roots.

From the literature cited it appears that a considerable number of plants have been proved not to be able to fructify, in many cases even not to develop their vegetative parts in case of a total absence of boron, manganese, zinc and copper and that the presence of aluminium, fluorine and iodine is beneficial, if perhaps not necessary to plant development. Consequently these elements as well as sodium and chlorine (because they are always present in irrigation water and in every soil solution in comparatively considerable quantities) were added as auxiliary elements. The potassium phosphate used for the nutrient solution was „according to Sörensen” and the other salts were „pro analysi” in original packing of Schering-Kahlbaum. The salts used for preparing the solution with auxiliary elements were of the usual pure quality, as for my purpose there could be no objection to traces of other elements than those mentioned in the solution with auxiliary elements. The water was distilled from a tinned copper still with tinned tube and gathered in demijohns.

The formula of the nutrient solution was similar to that used by Sommer,<sup>1)</sup> the quantity of phosphor taken was a little higher in relation to the other elements and the solution was almost twice as weak in order to come up to natural circumstances. For the solution with auxiliary elements the formula given by Sommer in the same publication was used as a guide. A solution containing the auxiliary elements was made, from which, if necessary,

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<sup>1)</sup> Plant Physiology, 3, 1928, p. 217.

the desired quantity could be added to the cylinders with nutrient solution. Both formulae are given in table 16.

From the riceplants grown the year before on a nutrient solution without addition of auxiliary elements 13 grains, each weighing 15 till 20 mgs and together weighing 232 mgs were selected; the remaining grains, which weighed less, were rejected. Of the grains harvested from the plants grown with auxiliary elements also 13 grains were selected, each weighing 15 till 20 mgs and together weighing 234 mgs. These grains were washed, soaked and laid down to germinate as has been described. All grains germinated. After three days one sixth of the distilled water was exchanged for the nutrient solution to give the seedlings an opportunity to absorb the principal elements as soon as possible. After 7 days the seedlings which had all one leaf consisting of vagina and lamina and 4 to 5 roots, were put in the cylinders. Eight cylinders contained the nutrient solution of table 16 only; in four of them the seedlings of the poor grains were put, and in four those of the normal grains. To four other cylinders was added 1 cm<sup>3</sup> of the solution with auxiliary elements, consequently the addition of each element came to as many gammae as the solution contained mgs per liter. After 22 days another cm<sup>3</sup> was added, after 28 days three cm<sup>3</sup> and after

TABLE 16.

Nutrient solution and solution with auxiliary elements used for the growing of rice.

Salts with principal elements	mgs per vessel of 2,13 liter	mgs per liter
KNO <sub>3</sub> .....	960	450
KH <sub>2</sub> PO <sub>4</sub> .....	240	113
MgSO <sub>4</sub> . 7 H <sub>2</sub> O .....	600	282
CaSO <sub>4</sub> . 2 H <sub>2</sub> O .....	960	450
Fe(NH <sub>4</sub> ) (SO <sub>4</sub> ) <sub>2</sub> . 12 H <sub>2</sub> O .....	24	11

37 days 5 cm<sup>3</sup>. The traces of auxiliary elements which the young seedlings received at the start can in no way have

Solution with auxiliary elements.

Salts with auxiliary elements	mgs per liter	elements	mgs per liter
NaCl .....	2000	Cl	1214
H <sub>3</sub> BO <sub>3</sub> .....	100	Na	786
CaF <sub>2</sub> .....	100	F	49
MnCl <sub>2</sub> . ca 2 H <sub>2</sub> O <sup>1)</sup> .....	50	I	38
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> . 18 H <sub>2</sub> O .....	50	B	18
KI .....	50	Mn	17
CuSO <sub>4</sub> . 5 H <sub>2</sub> O .....	20	Cu	5,1
ZnSO <sub>4</sub> . 7 H <sub>2</sub> O .....	20	Zn	4,5
		Al	4,1

been harmful. After 17 days the influence of the additional auxiliary elements was already visible. After 20 days the eight plants without auxiliary elements had three laminae, the four plants with auxiliary elements had already four, the third lamina being much longer than with the plants without auxiliary elements. The plants were altogether more vigorous and the colour of the leaves was of a bright green, whilst the plants without auxiliary elements still retained the more yellowish green colour of very young rice seedlings; the roots of the plants with auxiliary elements were much longer than those of the others.

After 26 days the three series were well distinguishable. The plants from the poor grains had three laminae each of the fourth lamina 3 till 6 cms were visible; only the first lamina had a light but still normally green colour, the next two were yellowish; they had only four white roots longer than 3 cms (the roots which developed first)

<sup>1)</sup> The manganese salt was exsiccated over quick lime, the analysis gave 34,5 % of manganese (Mn).

and about seven brownish roots, shorter than 3 cms, which had not yet reached the nutrient solution.

The plants from the normal grains without auxiliary elements had three full-grown laminae; the fourth showed a length of from 8 till 18 cms; the first and second had a light but still normal colour; the third and fourth were yellowish; they had 5 white roots, longer than 3 cms and about six shorter ones, mostly of a brownish colour.

The plants grown from the normal grains with auxiliary elements had five laminae, all of a bright green colour, about 10 roots longer than 3 cms and about 5 short ones; all the roots were white. The longest roots of these plants were about 15 cms long, while those from all the plants without auxiliary elements only 7 cms.

After 31 days the first lateral shoot became visible in the axilla of the fifth leaf (with the third lamina) of the plants with auxiliary elements; four days later the second lateral shoot appeared in the axilla of the next leaf. The plants without auxiliary elements made no lateral shoot, but showed many symptoms of deterioration; the fourth and the fifth lamina were yellow with light yellow-green vertical stripes; the plants from the poor grains had still less green in their leaves than those of the normal grains. Some of the leaves had got brown spots and were withering. The length of the roots was about the same as after 26 days; those shorter than 3 cms had slightly increased in number; many of them had grown dark brown and were drying up or getting mouldy.

After 45 days the plants from the poor grains were on the point of dying; those from the normal grains without auxiliary elements were not looking so bad as those from the poor grains; the difference was however small; those grown with auxiliary elements were healthy and vigorous.

Three of each series of plants without auxiliary elements were photographed, described, dried and weighed. The

differences between the plants of the same series were rather considerable. Probably the quantity of auxiliary elements which the different grains of one series contained in themselves had not been exactly the same and these differences between the grains were all the more obvious now that the auxiliary elements were entirely lacking in the nutrient medium. The plants of the series grown with auxiliary elements closely resembled each other; they had the same number of leaves at the main shoot; all four had two lateral shoots, with the same number of leaves for each shoot; both length and vigour of shoots and roots were for all four plants the same.

From left to right in fig. 3 of plate III nos 1 till 3 had

TABLE 17.

Riceplants aged 45 days, grown without auxiliary elements (plants 1—3 from poor grains, plants 5—7 from normal grains) and with auxiliary elements (plant 4).

plant-number	length in cms		air-dry weight in mgs of			
	longest vagina	longest lamina	shoot and root	roots average	shoots average	
1	7	16	69	} average 63 ± 11	5	58
2	6	16	77			
3	5	11	42			
5	6	17	100	} average 139 ± 26	9	130
6	13	25	189			
7	8	19	128			
4	27	56	1600		265	1335

been grown from the poor grains and nos 5 till 7 from the normal grains, both without auxiliary elements; the middle plant had been grown with them. The differences between the three series are obvious and are stated clearly by the data in table 17.



Details of the appearance of the three series are shown in plate IV fig. 1, where the second, the seventh and the fourth plant of the foregoing photograph have been taken together. The laminae of the largest plant have been partly cut off as they covered the smaller plants. The difference in root-development should be especially noticed: the largest plant counted 63 healthy white roots; of the other plants no roots had reached the nutrient solution except the four of five oldest. It should be noted how the length of the subsequent vaginae and laminae continues to grow in the plants with auxiliary elements and remains the same in the other plants.

Length of vaginae:

plant 2: 3 — 4 —  $5\frac{1}{2}$  —  $4\frac{1}{2}$  —  $5\frac{1}{2}$  — 4 cms  
 „ 7: 4 —  $5\frac{1}{2}$  —  $5\frac{1}{2}$  — 8 „  
 „ 4:  $3\frac{1}{2}$  —  $7\frac{1}{2}$  — 11 — 14 — 21 — 17 — 27 cms

(The probable cause of the smaller length of the sixth vagina of plant 4 will be discussed later).

Length of laminae:

plant 2: 3 — 13 — 16 — ? — ? — ? cms  
 „ 7: 3 — 14 —  $16\frac{1}{2}$  — 16 — 19 — 18 „  
 „ 4: 3 — 14 — 23 — 29 — 38 — 45 — 48 — 56 cms

(The lengths of the three youngest laminae of plant 2 could not be measured in consequence of their crinkling and shrivelling).

As the plants grown without auxiliary elements developed, their chlorosis became more obvious, especially with those grown from the poor grains; moreover these chlorotic leaves were very thin and so were the nerves, which was the cause of their crinkling and shrivelling. The chlorosis of these plants was caused only by a deficiency of manganese: the pH of the nutrient solution after harvesting ranged between 4,6 (plant 3) and 4,9 (plant 6), so lack of iron

caused by a too high pH could not be thought of. Besides the fourth plant of both series had got a trace of manganese after 28 days; after 37 days the new leaf of the plant from the normal grain had a green colour and two young white and longer roots had been formed (the plant from the poor grain reacted in the same way but only many days afterwards, because its condition was so much worse). The plant recovered by and by, but never got a quite healthy appearance.

Of the plants described above it is clear that riceplants — just as other plants, for which this was investigated — cannot grow without auxiliary elements in their nutrient medium. The less the quantity of auxiliary elements in the grain, the earlier and the more striking are the symptoms of deficiency.

Besides the fact that without auxiliary elements riceplants cannot develop, it had to be proved that with these elements a prosperous growth and fructification can be obtained. Consequently the cultivation of the three remaining plants grown with auxiliary elements was continued.

In order to keep the plants in good health the pH had to be controlled. When nitrogen is given as nitrate only, the pH rises as soon as the growth becomes vigorous. As was observed above, the pH of the nutrient solution which at the start was 4,4 had risen in the cylinder with the most vigorous plant without auxiliary elements in 38 days only to 4,9. The plants grown with auxiliary elements suddenly showed chlorosis after 33 days (when they had stood 26 days in their nutrient solution); the sixth lamina of the main shoot and the first lamina of the first lateral shoot of all four plants which had just appeared at this moment, were yellow; with bromocresol purple the pH with three of the plants was determined at 5,9, with one plant it was still higher. With 5 cms<sup>3</sup> of 0,1 normal nitric acid the pH was brought down from 5,9 to 4,4.

Three days later the influence of the changed pH was already perceptible as the parts of the laminae which became visible were green again. The short time of the chlorosis left its mark on the plants: every following vagina was some cms longer than its predecessor, but the sixth vagina only of all four plants remained some cms shorter than the fifth. (cf. p. 99).

Every time when the transpired water had to be replaced the pH was tested and when it was too high, it was brought down again. When the solution gave a yellow colour with methyl red, so many cms<sup>3</sup> of 0,1 normal nitric acid were added, till the liquid became bright red. As the pH rose so rapidly it was brought down a number of times as low as 3,7; at the time of most vigorous growth it rose in three days to 6,0 and more; the highest pH noticed was 6,7. No harmful effect whatever was visible with the cultivated riceplants from these constant changes of the pH; the whole plants, roots included, looked extremely healthy and vigorous. The quantities of 0,1 normal nitric acid which had to be added were in close proportion to the quantities of transpired water. When for the first time 5 cms<sup>3</sup> of nitric acid were added, the quantity of transpired water was about 150 cms<sup>3</sup>; at the time of highest transpiration against 600 cms<sup>3</sup> of distilled water about 20 or 25 cms<sup>3</sup> of nitric acid had to be added every two or three days to keep the pH on the required level; the total quantity of transpired water when the plants were harvested was about 28 times the quantity of 0,1 normal nitric acid that had been added. Only once this proportion was obviously disturbed: when the first panicles were peeping out of the uppermost foliage leaves of the stalks the quantity of acid that had to be added diminished by half and more. This difference was evidently caused by the exhaustion of the nutrient solution; when this solution was renewed, the quantity of acid which the last time before the renewal

had been 5 cms<sup>3</sup> on 500 cms<sup>3</sup> of water now became 25 cms<sup>3</sup> on the same quantity of water.

An often repeated renewal of the nutrient solution seems superfluous, if sufficient auxiliary elements are present, the pH is kept under control and the solutions are stirred up every time before replacing the transpired water. But of course it is necessary to give a fresh solution when the liquid gets exhausted. During the growth of the riceplants the nutrient solution was five times renewed. These

TABLE 18.

Supply of the riceplants with principal and auxiliary elements.

age of plants in days	2,13 liters of solution of principal elements	cms <sup>3</sup> solution of auxiliary elements	extra mgs of Fe(NH <sub>4</sub> )(SO <sub>4</sub> ) <sub>2</sub> . 12 H <sub>2</sub> O
7	first	1	10
22		1	
28		3	
37		5	
49	second	10	20
56		10	
63	third	10	20
69		10	
79	fourth	10	20
86		20	
93	fifth	20	20
99		20	
105	sixth	20	
Total		140	100

The development of the riceplants has been chronologically put down in table 19.

TABLE 19.

Development of the riceplants; the age is calculated from the appearance of shoot and root.

Age of plants in days	
7	one lamina, 5 roots.
26	five laminae, 10 long roots, the longest about 15 cms.
31	first lateral shoot visible.
33	chlorosis visible, caused by pH of 5,9; nitric acid added for the first time.
35	second lateral shoot visible.
36	plants cured from chlorosis.
45	main shoot: 8 laminae, first lateral shoot 3 laminae; second lateral shoot 2 laminae; one of the plants had 63 roots (cf. plate III fig. 3).
47	two more lateral shoots appear.
51	another lateral shoot appears; the plants have 6 shoots (main shoot included) on an average.
61	average of 7 shoots
68	" " 10 "
74	first panicle of each plant detaches itself.
79	each plant has 6 or 7 panicles, all flowering.
84	1, resp. 2 young shoots, 2 cms long appear at the foot of the stool of two plants.
86	each plant has 9 panicles.
89	the oldest panicles begin to bend, the first grains become yellowish.
96	two of the plants have produced resp. one and three smaller panicles; the young shoots at the foot of the stool have still the same length as 12 days before.
99	the upper half of the six oldest panicles have become golden.
103	at the foot of all three stools two more lateral shoots have appeared of about 6 cms length; those which appeared on the 84th day have now grown to that length also.
105	the plants are photographed; of each plant the oldest laminae which have withered during the growing-period have been removed before; each plant has produced another couple of young shoots; most of the panicles are now golden all over.
113	the plants are harvested.

renewals, the supply of auxiliary elements, the addition of extra iron may be read from table 18. The first solution was used for six weeks, the following ones were each used for two weeks; when the fresh solution had been used for a week, auxiliary elements and iron were added once more.

As may be seen in plate IV fig. 2 (taken 8 days before the harvest) the vegetative parts looked still vigorous, though most of the grains were ripe. At the time of the harvest only 9 or 10 laminae had been taken away from each plant because they had withered; these were the six or seven oldest from the main shoot and the one or two oldest from the two lateral shoots that had appeared first: for the greater part small laminae. The remaining laminae from the stalks with panicles, numbered 45 per plant on an average, were green and showed no signs of withering. As has been noted in the chronological survey of the development, young shoots of 2 cms length appeared at the foot of the stool after the 84 th day. They remained stationary till about the 100th day when half of the six oldest panicles were golden. At this moment they continued growing while at the same time some more shoots appeared and developed at the foot of the stool.

Soon afterwards some young shoots also appeared above the vaginae of higher leaves. When the harvested plants were dissected, in the vaginae of nearly all the leaves in which this was possible, a young shoot was found, often just peeping out of the vagina or a little too short to be visible without dissection. On an average each plant had 30 healthy vigorous young shoots. As table 20 with the weekly transpiration shows, the transpiration rose regularly every week till the panicles appeared. Then for three weeks the amount of water transpired sank to a lower level, but it rose once more when the panicles began to turn golden and young shoots developed. This sudden rise of

transpiration was not caused by outward conditions: during the week from day 90—96 were registered 41 hours of sunshine by the sunshinerecorder standing in the Botanical Garden; during the week from day 97—103 nearly the same number of hours: 39.

The roots were in healthy condition at the harvest.

TABLE 20.

Transpiration of three riceplants in succeeding weeks in cms<sup>3</sup>.

till	33	days	.....	450	68—	75	days	...	5760
	33—40	"	.....	630	75—	82	"	...	4770
	40—47	"	.....	1160	82—	89	"	...	4465
	47—54	"	.....	2290	89—	96	"	...	4650
	54—61	"	.....	2900	96—	103	"	...	6940
	61—68	"	.....	4575	103—	110	"	...	6060

The harvested plants were measured, described and dissected; the roots were counted and repeatedly washed with distilled water. All the vegetative parts were dried at about 100° C., spread on a table for some days to become air-dry and were afterwards weighed. The panicles were already nearly air-dry; they were spread on a table for a week and also weighed. The results are given in table 21.

From their vigorous appearance, the fact that after the ripening of the grains young shoots developed in almost all the axillae where this was possible, the transpiration which rose again after the grains were ripe, it may be concluded that the plants were still in the best possible condition, one might be tempted to say in abnormally good condition for plants with ripe seeds. The plants had produced a high weight of air-dry matter and a high number of hard grains. If we consider the ratio between the weight of paddy (panicles with hard and deaf grains) and the total weight of superterranean parts without the

TABLE 21.

Riceplants grown with auxiliary elements, harvested at the age of 113 days.

Serial number of the plants (cf. plate IV fig. 2) .....	94	95	96	mean	
length of longest roots in cms ....	22	22	23		
largest distance top of uppermost foliage leaf of the stalk to grain in cms .....	112	128	122		
largest distance top panicle to grain in cms .....	107	103	105		
number of panicles .....	9	10	12		
"  "  young shoots .....	31	27	31		
"  "  roots .....	269	266	238	257	± 10
"  "  hard grains .....	825	974	1026	942	± 60
"  "  deaf grains .....	108	111	91	103	± 6
weight of hard grains in grs .....	19,42	22,40	24,07	21,96	± 1,36
"  "  deaf " " " .....	0,30	0,32	0,27		
"  "  panicles without grains ..	0,99	1,09	1,17	1,083	± 0,052
"  "  laminae in grs .....	7,59	7,97	7,33	7,630	± 0,186
"  "  vaginae " " .....	6,84	7,64	6,96	7,147	± 0,249
"  "  stems " " .....	9,96	10,72	10,66	10,447	± 0,244
"  "  roots " " .....	2,58	2,62	2,43	2,543	± 0,058
"  "  young shoots in grs ....	4,72	5,16	3,81	4,563	± 0,397
total air-dry weight .....	52,40	57,92	56,70	55,67	± 1,67
total cms <sup>3</sup> of transpired water .....	15120	15940	15320	15460	± 246
per gram of air-dry matter transpired cms <sup>3</sup> .....	288	276	270	278	± 5,3
total cms <sup>3</sup> added 0,1 n. HNO <sub>3</sub> ...	527	649	487	554	± 48,7
per cm <sup>3</sup> 0,1 n. acid transpired cms <sup>3</sup>	28,7	24,5	31,5	28,23	± 2,03
average weight of hard grains in mgs	23,5	23,0	23,5		
weight % of paddy <sup>1)</sup> on total air-dry (except roots and young shoots) .....	45,9	47,5	50,6	48,0	± 1,38

<sup>1)</sup> paddy: the whole panicles with hard and deaf grains.



young shoots (because these did not belong to the growing-period of the grains), it is obvious that the percentage of paddy is very high. These results and especially the high percentage of paddy are due to the well-chosen dosage of auxiliary elements. As regards this dosage it may be observed that in the experiments the first very small dose (very small in order to be sure that it would do no harm) might have been followed after a few days already by a second and a third, as soon as it was perceptible that

TABLE 22.

Quantities of auxiliary elements given per plant and per liter solution.

elements	mgs which may have been maximally absorbed by a riceplant of ca 55 grs	mgs per liter maximally present in the nutrient solution	mgs per liter present from the 7th to 22nd day
Cl	170	22,8	0,57
Na	110	14,8	0,37
F	6,8	0,92	0,023
I	5,4	0,72	0,018
B	2,48	0,33	0,008
Mn	2,42	0,32	0,008
Cu	0,71	0,095	0,0024
Zn	0,63	0,085	0,0021
Al	0,57	0,076	0,0019

the roots were growing well. From the analysis of the plants grown on soil suspension it is evident that rice absorbs comparatively much manganese (cf. table 10 and 11) which is transported rapidly from the roots to the shoots. The quantity of manganese in the solution of auxiliary elements might have been three-times as high as it was now without any fear of doing harm. From table 16 and 18 may be calculated what were the maximal

quantities of each auxiliary element that might have been absorbed by one of the riceplants of 55 grs during the whole growing-period.

These quantities have been put down in the second column of table 22. In the next column have been given the highest possible concentrations of the auxiliary elements during some of the last weeks, when a week after the renewal of the nutrient solution a second portion of auxiliary elements had been added. For most of the elements the concentration must have been lower, as part of the quantities given the week before will have been already absorbed. Of none of the auxiliary elements mentioned (except Na and Cl) more than 1 mg per liter was present at any time. The quantities of Na and Cl agree with those found in soil-solutions of cultivated fields. Russell e.g.<sup>1)</sup> gives some typical analyses of drainage water. One of the parcels of the Broadbalk Field at Rothamsted manured with dung contained in the drainage-water 21 mgs of Cl and 10 mgs of Na; parcels without manure contained about half of these two elements.

In the last column is given the concentration in mgs per liter of the auxiliary elements during the first two weeks in which the riceplants stood in the nutrient solution. This very weak concentration proved already sufficient to give marked differences between the plants with and without this addition. This becomes quite intelligible, when we compare these figures with those of the second column. The quantities maximally absorbed by one plant had been quite sufficient to provide for 1000 grains. Consequently one grain contained less than one thousandth part of the amounts noted in column two. In one liter of solution was three times as much at the start, in the whole

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<sup>1)</sup> E. J. Russell, *Soil Conditions and Plant Growth*, 5th Ed., p. 196.

cylinder of 2,13 liters at least 6 times as much as could possibly be contained in one of the harvested grains. If the roots were able to absorb for instance one sixth of the auxiliary elements present in the solution, the plants would contain already about twice as much as the seedlings grown without auxiliary elements and the difference in appearance caused by such small quantities is sufficiently explained.

*Summary.*

*(For the summary of the first part see p. 79).*

Besides the elements K, Ca, Mg, Fe, P, S and N of which the plant absorbs larger quantities from the soil, traces from some other elements, B, Mn, Zn and Cu and probably also Al, F and J are indispensable for the development and fructification. In this publication the elements of which the plant absorbs larger quantities are called „principal elements” and those of which traces suffice already to give prosperous growth and fructification are called „auxiliary elements”. As also iron is necessary in traces, it might be regarded as an auxiliary element too, the more so, as it becomes already harmful when present in small quantities. To simplify matters however, the above-mentioned grouping is used.

In a nutrient solution after Pryanishnikov, prepared from the usual „pure” salts and distilled water, the development of wheat, buckwheat and rice was much less than with the addition of boron, manganese and fluorine. This difference was largest with the buckwheat; neither buckwheat nor wheat flowered; with rice the difference was smallest; there the plants without auxiliary elements yet produced some germinative grains; the riceplants with auxiliary elements showed slight symptoms of poisoning at the start as a consequence of this addition.

The influence of the addition of B, Mn and F is clearly

shown on plate III, figs. 1 and 2. All three wheatplants were grown on a nutrient solution according to Pryanishnikov, made from „pure” salts for pharmaceutical use. For wheatplant 28 in fig. 2 distilled water was used for the solution: neither a solid stalk nor an ear appeared. For the wheatplant in fig. 1 tapwater was used and so traces of auxiliary elements were added: stalks and ears were formed, but were so limp, that the ears could not undo themselves from the vagina of the uppermost foliage leaf of the stalk; for wheatplant 29 distilled water was used as for 28, but boron, manganese and fluorine were added; the plant made hard, solid stalks and five well-developed ears: so, the larger the quantities of auxiliary elements present, the better the development of stalks, ears and grains.

Though plants 28 and 29 transpired the same quantity of water, the quantity of air-dry matter of 29 was  $2\frac{1}{2}$  times larger than of 28 on harvesting; this difference was especially due to the ears and solid stems of 29; the weight of the laminae of 28 being even larger than of 29.

After these experiments the grains of the riceplants produced with and without auxiliary elements were used in a new experiment; they were grown on a solution of the purest salts of principal elements only. Both series were soon chlorotic in default of manganese and began to die after 45 days. The difference however was important; the plants cultivated from the richer grains (richer in the meaning of containing more auxiliary elements) were more robust and had twice the dry weight of those from the poorer grains (139 and 63 mgs per plant).

Along with these experiments rice was also cultivated in the same solution, but with the addition of the auxiliary elements B, F, Cl, I, Na, Al, Mn, Cu and Zn; this solution gave a most favourable growth and produced flourishing plants, which weighed at the harvest 55 grams airdry

each and had 22 grams of ripe grains (nearly 1000 grains per plant), the percentage of paddy was 48 %.

A very small dose of the auxiliary elements was given at the start in order to be quite sure to prevent any injurious effect; this dose was raised as the plants grew, till it reached an amount 20 to 40 times as much as at the start, which is an analogon of the fact that poisonous medicaments are given to man and animal also in proportion to the weight of the body. This way of dosing is probably preferable to the other, which provides for a constant quantity of auxiliary elements in the nutrient solution; the plant is able to use and probably to stand more auxiliary elements in proportion as its weight and the active surface of its roots increase. After the grains were ripe the rice made new shoots in all axillae where this was possible, without the old leaves withering; this vigour can only be ascribed to the constant addition of auxiliary elements.

### *Conclusions.*

Plants can be grown from seed to seed on a clay suspension without any other addition than some ammonium nitrate.

Wheat exhausted the used clay suspension only in respect of potash and phosphoric acid. The quantities of these two components absorbed by one wheatplant were a bit higher than the quantities of „available“ potash and phosphoric acid found by Von Sigmond's method in the quantity of clay suspension used per plant.

For trials concerning the influence on plant growth of brackish water, salts of alkalies or alkaline earths etc. a clay suspension may in some cases be preferred to the usual nutrient solution. In a clay suspension the concentration of the nutrient components is very low, so that it may be neglected, which is not the case in the usual

nutrient solution. Moreover in a clay suspension an exchange of cations will take place, just as is the case in a soil. Part of added Na, K and Mg ions will be exchanged for cations from the clay particles, in particular for Ca. When much sulphate is added, part of the calcium sulphate will precipitate after the exchange of cations, while Cl and NO<sub>3</sub> ions will remain in the same concentration in which they were added. For investigations about the harmfulness of brackish water and of the Cl ion the use of a clay suspension may offer particular advantages.

Auxiliary elements are indispensable for the development and fructification of plants. This fact can especially be shown with seeds from plants grown themselves with insufficient quantities of these elements.

When making investigations with water-cultures or manuring trials with sand-cultures one should keep in mind that optimal growth is only possible when well-dosed quantities of auxiliary elements are added. When this is neglected the higher yields in particular will be depressed.

The results obtained and the conclusions drawn from investigations with water- and sand-cultures might at times have been far different, if next to the principal elements these auxiliary elements had been added.

The foregoing investigations were carried out in the Laboratory for Plant Physiology of the University of Amsterdam. To its Director, prof. dr. Th. Weevers, the authoress wishes to express her hearty thanks for the kindly interest and some good hints she got in the course of the investigation.

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PLATE II.

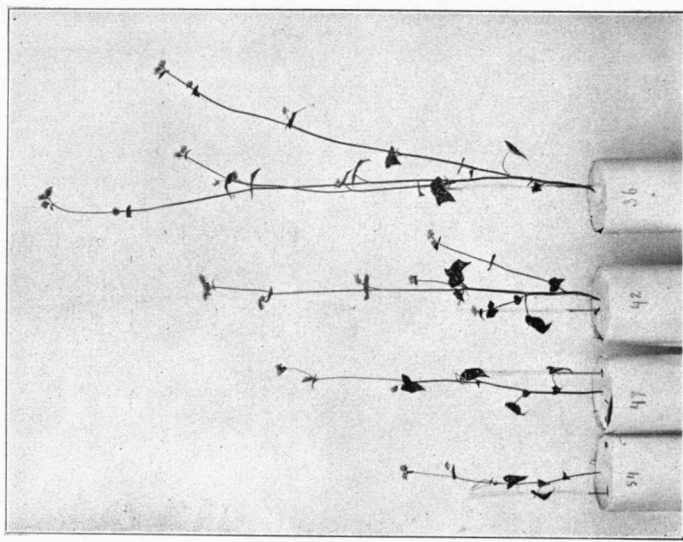


Fig. 2. Buckwheatplants, age 66 days  
grown on quantities of clay suspension in the proportions 1 : 3 : 9 : 27.

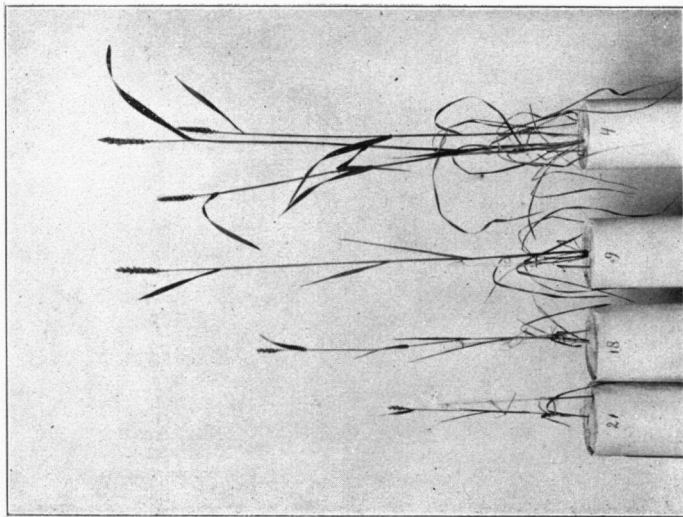


Fig. 1. Wheatplants, age 84 days  
grown on quantities of clay suspension in the proportions 1 : 3 : 9 : 27.



Fig. 1. Wheatplant grown in tapwater with "pure" salts, age 91 days.

Fig. 2. Wheatplants grown in distilled water with "pure" salts, 28 without, 29 with Mn, B and F, age 84 days.

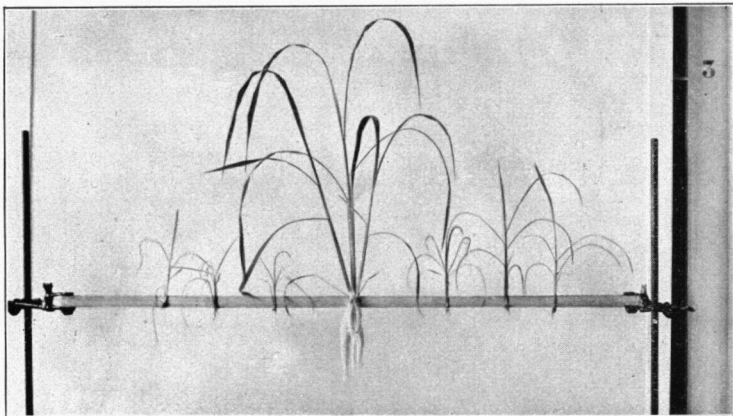


Fig. 3. Riceplants age 45 days; 1-3 at the left grown from poor grains, 4-7 grown from normal grains without, the middle plant with auxiliary elements.



PLATE IV.



Fig. 1. Riceplants 2, 7 and 4 from Plate III, fig. 3.



Fig. 2. Riceplants grown with auxiliary elements, age 105 days.