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INVESTIGATIONS ON THE NITROGEN NUTRITION OF PEA PLANTS

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Introduction. Pea plants, like other members of the *Leguminosae*, generally derive the nitrogen required for their proteins and other nitrogenous compounds from the gaseous nitrogen fixed by the nodules. Extensive investigations on the biochemistry of the nitrogen fixation have been carried out by Virtanen *et al.* ⁸⁾, ⁹⁾ and by Wilson *et al.* ¹⁰⁾.

The question whether the nodules of leguminous plants are fully capable of supplying their hosts with nitrogen fixed from the atmosphere, or that an additional absorption of combined nitrogen from the soil may still improve the growth of the plants, is important not only from a theoretical point of view but also in respect of the growing of these crops in practice.

The bad results sometimes obtained by Dutch farmers with the growing of peas, gave rise to an investigation of the effect of nitrogenous fertilizers on the growth of pea plants on certain soils. In addition to field experiments, investigations with pea and oat plants in nutrient solutions with increasing amounts of nitrogen were carried out. In other experiments the value of different nitrogen compounds as a nitrogen source for peas growing in nutrient solutions without *Rhizobium* was determined.

In experiments with nutrient solutions and with soil the influence of zinc and boron on the fixation of gaseous nitrogen was investigated.

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Experimental methods. In the experiments with culture solutions a medium of the following composition was used:

distilled water	1 l	MnSO ₄ .4H ₂ O	1 mg
KH ₂ PO ₄	325 mg	ZnSO ₄ .7H ₂ O	0.25 mg
Na ₂ HPO ₄	70 mg	CuSO ₄ .5H ₂ O	0.25 mg
MgSO ₄ .7H ₂ O	250 mg	H ₃ BO ₃	0.25 mg
CaSO ₄ .2H ₂ O	250 mg	Na ₂ MoO ₄ .2H ₂ O	0.05 mg
FeCl ₃ .6H ₂ O	25 mg		

In preparing these solutions water distilled in a glass apparatus was employed in most cases.

The pea seeds were germinated in quartz sand previously cleaned with nitric acid and washed free from acid with distilled water. The nutrient solutions were periodically aerated. They were changed every three or four weeks.

Effect of different amounts of anorganic nitrogen compounds on the growth of pea plants in nutrient solutions. In order to elucidate the behaviour of pea plants to anorganic nitrogen compounds, experiments with culture solutions supplied with different amounts of ammonium nitrate were carried out. „Mansholt” peas were transplanted into glass jars of 700 cc capacity on April 18, 1942, each jar holding three plants. On April 25 ammonium nitrate was added in amounts of 0, 20, 50, 100 and 200 mg N per jar respectively.

In order to prevent the development of nodules the jars and the nutrient solutions as well as the pea seeds were disinfected. Although no special precautions were taken in this experiment to keep the solutions free from spontaneous infection, no nodules developed.

On May 1 the first symptoms of nitrogen deficiency were observed: a light green shade of the leaves and a decrease in growth. On May 11 a good many of the lower leaves of the nitrogen-deficient plants had died, the dead tissue showing a gray white colour.

At the latter date one set of cultures was harvested. The fresh weights were determined and the plants were analysed for some nitrogenous compounds. Of the remaining cultures the nutrient solution was changed; the concentration of the nutrient salts was doubled, except that of ammonium nitrate which was given in the same amounts as before.

Some plants grown without nitrogen and some with 20 mg of

nitrogen were supplied with 23.3 mg of nitrogen in the form of ammonium sulphate and as calcium nitrate respectively, to find out to what extent nitrogen-deficient pea plants respond to anorganic nitrogen compounds. Precautions were taken to prevent an acidification and an alkalisation of the culture solution by these salts.

On May 21 a second set of pea plants was harvested and on May 28 a third. The fresh weights of the plants are recorded in table I. At both dates again some nitrogen-deficient plants were supplied with 23.3 mg nitrogen in the form of ammonium sulphate and calcium nitrate respectively.

TABLE I

Influence of nitrogen on the yield of pea and oat plants									
mg of nitrogen per jar *)	Time of harvest	Peas				Oats			
		Fresh weights in g		Dry weights in g		Fresh weights in g		Dry weights in g	
		Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots
0	May 21(20)	1.66	6.02	—	—	0.34†)	—	—	—
20	„	5.79	8.59	—	—	4.03	2.94	—	—
50	„	9.15	9.96	—	—	8.26	3.92	—	—
100	„	14.06	8.76	—	—	11.21	3.91	—	—
200	„	15.—	10.58	—	—	10.62	3.89	—	—
0	May 28	1.25	5.26	0.54	0.33	0.73	0.86	0.14	0.05
20	„	6.88	9.73	1.25	0.65	9.13	5.76	1.59	0.56
50	„	10.70	10.75	2.—	0.91	16.33	5.46	2.67	0.50
100	„	20.36	10.78	3.45	0.93	24.70	9.77	3.20	0.77
200	„	24.43	10.91	4.88	0.79	21.35	8.01	3.03	0.75

*) these amounts were added two times

†) weight of total plants

On June 10, after photographs of the plants had been taken, the experiment was brought to an end. The pea plants without nitrogen had completely died. The same was true of the greater part of the plants with the low rate of nitrogen (see fig. 1).

These results were in contrast with those of a similar experiment with oat plants which was carried out simultaneously. Although without the addition of nitrogen the development of the oat plants was much poorer than that of the peas, probably due to the much lower amount of nitrogen in the oat seeds, these plants were able

to complete their normal life cycle. Pea plants apparently require a considerably higher nitrogen level in their tissues than oats and other cereals do. When the nitrogen content comes below this level the pea tissue turns yellow and dies shortly afterwards. Nitrogen-deficient oat leaves, however, although showing a yellow colour and often dead tips, remain alive until the seed has ripened.

The different behaviour of pea and oat plants to nitrogen was not only apparent in the deficiency symptoms but also in the response of the deficient plants to newly introduced ammonium and nitrate nitrogen. Pea plants generally responded very badly to the



Fig. 1. Pea plants in nutrient solution without *Rhizobium* with different amounts of ammonium nitrate (1 : without nitrogen, 2 : 40, 3 : 100, 4 : 200 and 5 : 400 mg N per culture; added in two parts).

added nitrogen (see fig. 2 and 3). Leaves showing a light green shade were unable to retake a normal green colour following the addition of the nitrogen compounds. Only young emerging leaves responded to the added nitrogen. The light green leaves of the oat plants, on the contrary, readily turned normal green when the nutrient medium was supplied with nitrogen (see fig. 4).

Analysis of the nitrogen fractions of pea and oat plants grown with different amounts of ammonium nitrate. In order to obtain some information concerning the cause of the above-mentioned differences be-

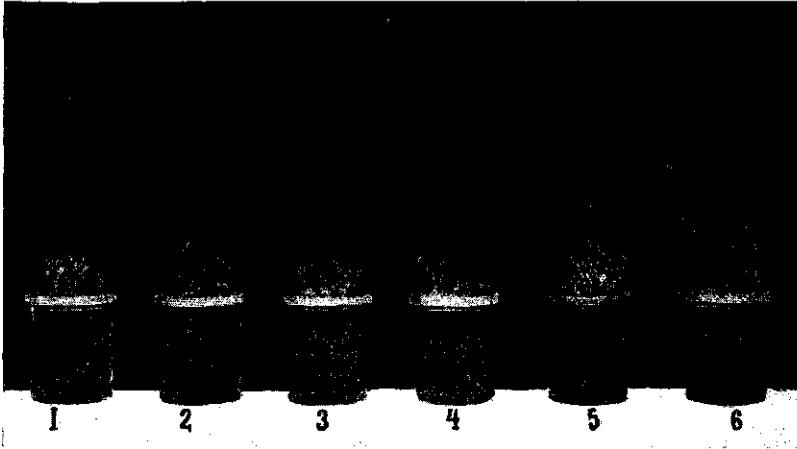


Fig. 2. Nitrogen-deficient pea plants without *Rhizobium*, supplied with 23.3 mg N on May 28 (1 and 2), on May 20 (3 and 4) and on May 13 (5 and 6) To the pots 1, 3 and 5 the nitrogen was added in the form of $(\text{NH}_4)_2\text{SO}_4$, to 2, 4 and 6 as $\text{Ca}(\text{NO}_3)_2$. Photograph taken on June 10.

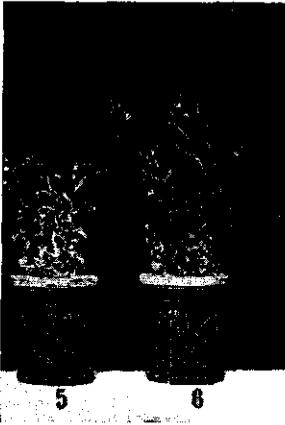


Fig. 3. Pea plants grown with 20 mg of N in the form of NH_4NO_3 without *Rhizobium*; supplied with 23.3 mg N as $(\text{NH}_4)_2\text{SO}_4$ (5) and $\text{Ca}(\text{NO}_3)_2$ (6) on May 13. Photograph taken on June 11.

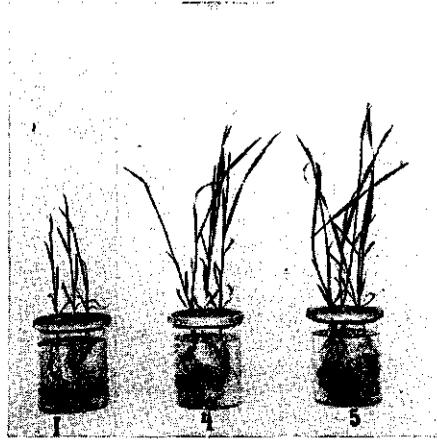


Fig. 4. Nitrogen-deficient oat plants supplied with 23.3 mg N as $(\text{NH}_4)_2\text{SO}_4$ (pot 4) and $\text{Ca}(\text{NO}_3)_2$ (pot 5) on May 20. To pot 1 no nitrogen was added. Photograph taken on June 11.

tween pea and oat plants, chemical analyses of the fresh tissues of these plants were carried out.

Proteins were precipitated by heating the ground plant tissue for 15 min. above 90°C and after cooling, adding trichloroacetic acid. Protein and soluble N were separated by filtration and both fractions were analysed for total N according to Kjeldahl-Lauro. Soluble N estimated by this method does not include nitrate-N.

Nitrate-N was determined according to the xylenol method (1).

Ammonia-N was distilled under reduced pressure at 40°C after adding a borate buffer of pH 9.

Amide-N was determined by hydrolysing an aliquot part of the filtrate in 5% H₂SO₄ and distilling the ammonia formed. NH₄-N originally present has to be subtracted.

The results of these analyses are reported in table II.

TABLE II

Nitrogen fractions in mg per 10 g of fresh plant tissue											
mg of nitrogen per jar	Time of harvest	Protein-N		Soluble-N†)		Nitrate-N		Ammonium-N		Amide-N	
		Leaves + stems	Roots								
0	Peas	21.1	12.1	10.2	5.9	0	0	1.0	0.4	2.1	0.6
20	May 11	25.5	17.6	6.3	9.2	0	0	0.5	0.3	1.0	1.5
50		32.8	20.5	3.-	12.3	0	0	0.3	0.9	1.5	1.0
100		47.1	18.3	14.8	15.7	0	0.2	0.2	0.9	2.5	1.8
200		48.6	20.9	23.7	16.8	0.3	0.7	0.4	0.9	5.-	2.7
0	Peas	36.3	9.8	19.8	5.-	0	0	0.3	0.1	2.-	0.5
20	May 21*)	43.7	14.2	7.8	6.2	0	0.6	0.6	0.4	2.2	0.3
50		52.5	20.9	12.4	11.1	0	0.3	0.3	0.6	—	0.7
100		37.5	24.5	18.1	17.-	0	0.6	0.2	0.6	2.-	0.6
200		58.4	24.7	26.7	21.4	0.1	0.9	0.3	1.1	3.9	1.7
0	Oats	—	—	—	—	—	—	—	—	—	—
20	May 20*)	28.3	11.1	8.1	4.3	0	0	0.7	0.7	0.6	1.0
50		35.7	12.2	11.9	5.8	0.3	0	0.3	0.3	0.7	1.9
100		45.3	15.5	17.9	14.8	2.8	0.8	0.3	0.8	3.6	3.7
200		49.3	17.-	24.3	20.1	5.1	1.8	0.6	1.-	5.6	7.3

*) two applications of nitrogen

†) except nitrate

It can be seen that pea plants showing heavy symptoms of nitrogen deficiency are very low in protein nitrogen. The content of soluble organic nitrogen of leaves plus stems, however, is much higher than in plants with a moderate nitrogen supply. Apparently a

considerable part of the proteins is converted into soluble organic nitrogen compounds. Although the breaking down of the proteins in the pea leaves undoubtedly is connected with the dying down of the leaves and stems, it may be a result and not the cause of this process.

Nitrate nitrogen was very low in the pea plants, even when these were growing in solutions with high applications of ammonium nitrate. Apparently after absorption it is readily converted into organic nitrogenous compounds. In oats, however, a considerable accumulation of nitrate in leaves and stems occurred under the same circumstances.

Ammonia-N was very low in all cases.

Amide nitrogen was low in plants supplied with a moderate amount of ammonium nitrate. It increased considerably with higher applications of nitrogen. In oats this rise was more evident than in peas. This was true for leaves and stems as well as for roots. As the latter three fractions were not or only very little higher in the nitrogen-deficient pea leaves and stems, the high concentration of soluble nitrogen must be due to the high content of some other fraction, presumably aminoacids.

Field experiments with dressings of nitrogenous compounds to pea plants. As the pea plants growing on certain soils showed an appearance of nitrogen deficiency, as observed in the above-described experiments with nutrient solutions, field experiments with application of different amounts of nitrogen were carried out. The experimental fields were laid out on clay soils on which the plants showed the above symptoms as well as on soils with a normal plant growth. „Unica” peas were used in most of these experiments. The seed was inoculated with an effective strain of *Rhizobium* before being sown.

In 1943 three experimental fields were laid out on soils on which in previous years the pea growth was inadequate, (field numbers 730, 757 and 770), and two on normal soils (field numbers 755 and 756). The soil of field 770 was a moderate clay that of the other fields was a sandy clay. The pH-value of these soils was about 7, the phosphate and potassium contents were moderate.

A basic dressing of 200 kg of P_2O_5 as superphosphate and 160 kg of K_2O as potassium chloride per ha was given to the fields 755, 756 and

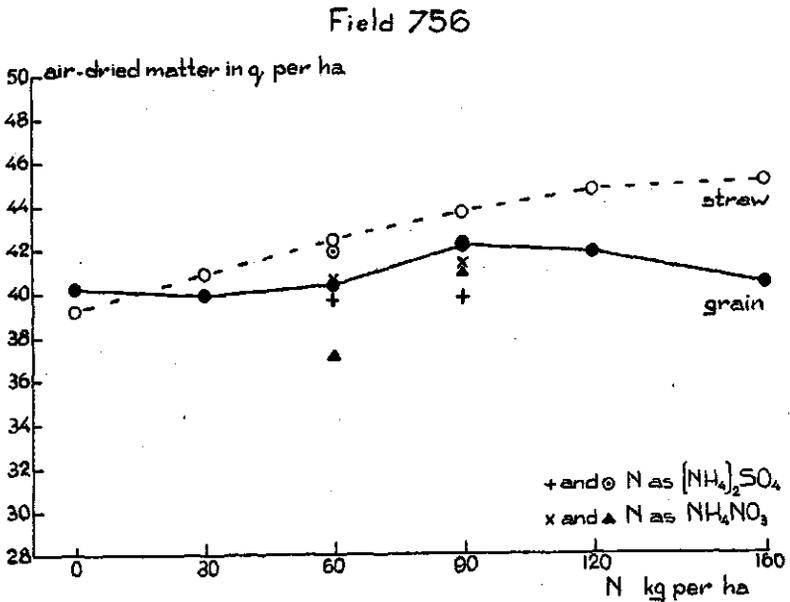
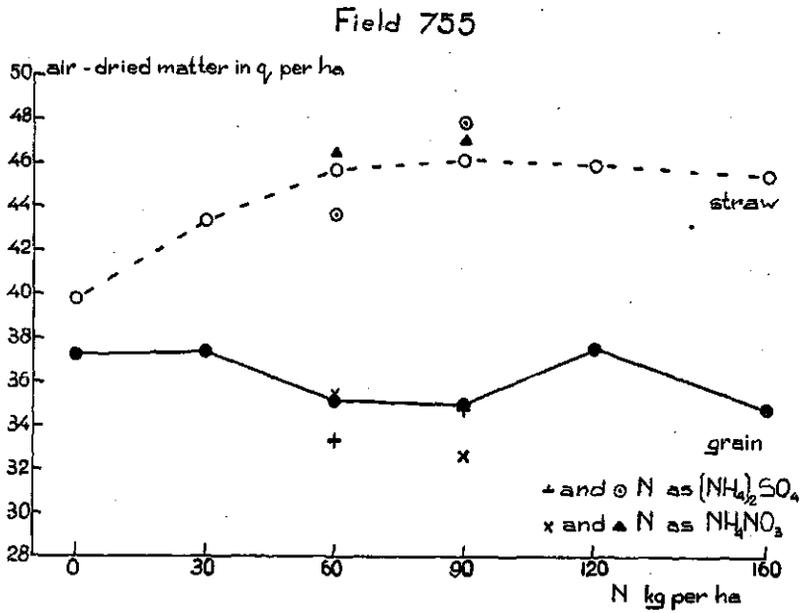


Fig. 5 and 6. Effect of different amounts of calcium nitrate on the yield of peas grown on a clay soil. Some plots were supplied with $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 respectively (1 q = 100 kg).

757. Field 730 was fertilized with 250 kg P₂O₅ and 125 kg K₂O per ha and field 770 with 100 kg P₂O₅ and 125 kg K₂O. Nitrogen was supplied as ammonium nitrate in applications of 0, 50, 100, 150, 200 and 250 kg of nitrogen per ha on field 730. On the fields 755, 756 and 757 nitrogen was added at a rate of 0, 30, 60, 90, 120 and 160 kg per ha as calcium nitrate. To some plots it was given as ammonium sulphate and ammonium nitrate respectively. Field 770 was dressed with ammonium nitrate at a rate of 50, 100, 150, 200, 250 and 500 kg of N per ha.

As was expected no response to the nitrogenous fertilizers was observed on the fields 755 and 756. The pea plants grew luxuriantly,

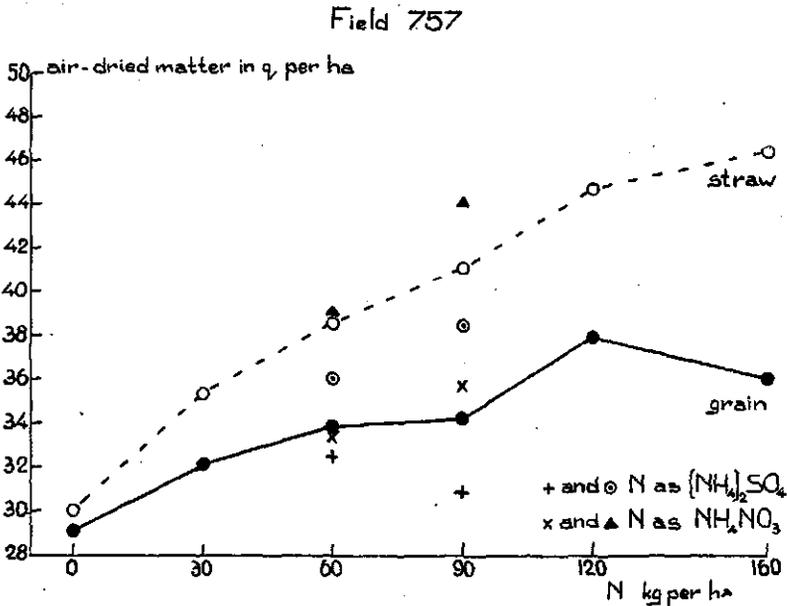


Fig. 7. Effect of different amounts of calcium nitrate on the yield of peas grown on a clay soil. Some plots were supplied with (NH₄)₂SO₄ and NH₄NO₃ respectively (1 q = 100 kg).

both those without the application of combined nitrogen as well as those supplied with nitrate, indicating that fixation of gaseous nitrogen by the nodules was fully capable of supplying the plants with nitrogen. As can be seen in fig. 5 and 6 only the yields of straw were somewhat increased by the nitrogen fertilizers*).

On field 757 the pea plants responded clearly to the added nitro-

*) The values plotted in Fig. 5—9 are average yields of two or three plots.

gen. On the nil plots a somewhat poor growth of the plants with a yellow green colour and a dying back of the lower leaves was observed. Supplied with combined nitrogen the peas grew more vigorously with a dark green shade of the leaves. The effect of ammonium sulphate was somewhat inferior to that of calcium nitrate and ammonium nitrate. Not only the yield of straw but that of seed as well was considerably increased by the nitrogen fertilizing (fig. 7). Obviously the fixation of N_2 by the nodules was inadequate to supply the plants with nitrogen.

The question arises to what extent the poor nitrogen fixation of the nodules in this experiment was due to a deficiency of some nutrient element which might have influenced the fixation process, without having affected the growth of the plants when supplied with combined nitrogen. Another possibility is that the formation of the nodules or the fixation of N_2 was suppressed by a parasite such as the larva of *Sitona lineatus* or by a bacteriophage.

Although this point will be dealt with in the following paragraphs, it may already be pointed out that no evidence was obtained that a mineral deficiency was the cause of the poor nitrogen fixation on this field. Applications of phosphate, potassium, boron and molybdenum had no effect on the growth and the appearance of the pea plants.

In a number of cases larvae of *Sitona lineatus* were found to have destroyed many nodules. It is unlikely, however, that these organisms alone were responsible for the poor nitrogen fixation. Although it was believed that bacteriophages played a part in the poor nitrogen fixation, no evidence was obtained that this was true.

Similar results as observed on field 757 were obtained by Miss J. C. Schreuder at this station on some other fields.

Highly significant responses to nitrogen dressings were also found on the fields 730 and 770 (fig. 8 and 9). On these fields, especially on the latter, the pea plants developed very poorly. Investigations carried out by Miss J. C. Schreuder revealed that the plants were infected by a *Fusarium* sp. Fertilizing with nitrogen compounds induced a much better growth of the peas, obviously due to a greater resistance against infection. When the soils were very heavily infected by *Fusarium*, however, only slight improvements in growth were obtained by nitrogen fertilizing (see fig. 9). Miss J. C. Schreuder in cooperation with Dr. F. C. Gerretsen

and the present writer who are studying *Fusarium* diseases of pea plants, will give a more detailed description of these latter experiments in a separate paper.

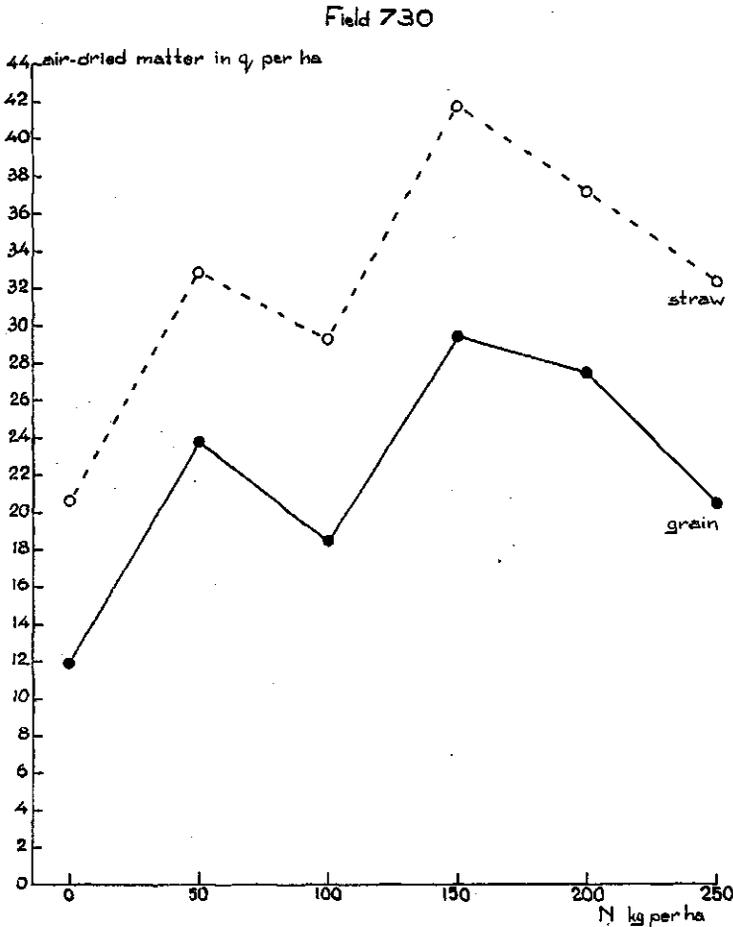


Fig. 8. Effect of different amounts of ammonium nitrate on the yield of peas grown on a clay soil infected by a *Fusarium* sp. (1 q = 100 kg).

The assimilation of different nitrogen compounds by pea plants.
 In the above-described experiments the nitrogen-deficient pea plants responded much better to nitrate than to ammonium nitrogen (see fig. 2 and 3).

To find out whether pea plants prefer nitrate to ammonium nitrogen an experiment with KNO_3 , NH_4NO_3 and $(NH_4)_2SO_4$ as the

sole source of nitrogen was started on May 28, 1942. The nutrient solution used in this experiment contained per litre 0.6 g of $\text{Ca}(\text{H}_2\text{PO}_4)_2$, 0.25 g of Na_2HPO_4 and 0.4 g of K_2SO_4 . Mg- and Ca-salts and minor elements were supplied in amounts twice as high as above-mentioned.

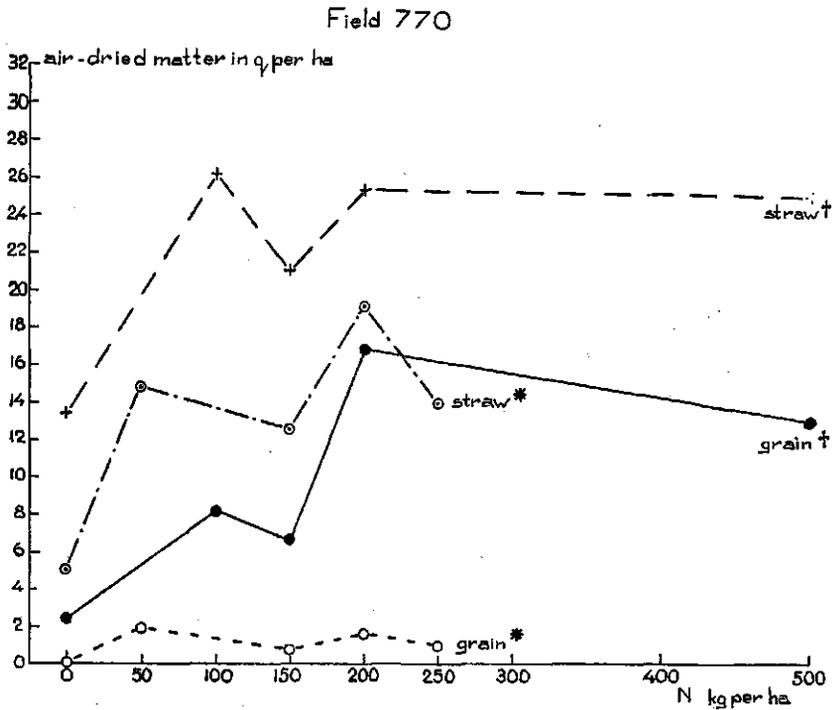


Fig. 9. Effect of different amounts of ammonium nitrate on the yield of peas grown on a clay soil infected by a *Fusarium* sp. *) is a heavily diseased part †), a moderately diseased part of the field. (1 q = 100 kg).

The pH of this solution was daily adjusted to a value of 6.0 with sulphuric acid and a dilute solution of sodium carbonate respectively.

Nitrogen was added to an amount of 25 mg N per jar containing 600 cc of the basic nutrient solution. In these solutions the pea plants with KNO_3 and NH_4NO_3 showed a normal development. Supplied with ammonium sulphate, however, the growth of the plants was very poor with withered lower leaves (see fig. 10). These

withering symptoms commenced with a wilting at the margins of the leaves. As the pH of the nutrient solutions with the three nitrogen compounds was practically the same, i.e. 6.—, it was clear that the poor growth of the plants supplied with ammonium sulphate had something to do with the ammonium salt as such. The possibility exists that under these circumstances pea plants were unable to assimilate ammonium nitrogen. In the following experiment the assimilation of different nitrogen compounds was studied more in detail.

Sterile pea and oat plants were grown in 100 cc Erlenmeyer flasks containing a nitrogen-free nutrient solution.

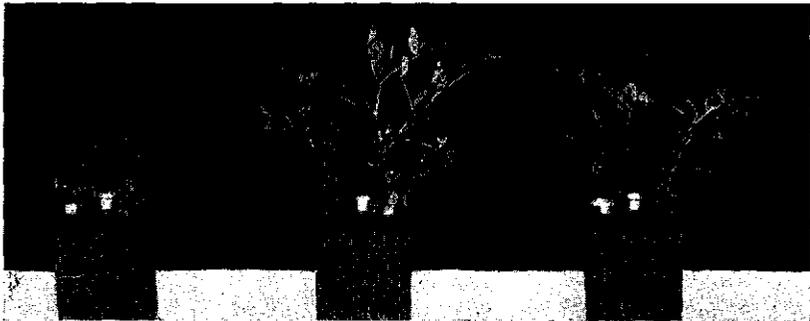


Fig. 10. Pea plants in nutrient solution without *Rhizobium*; 1 is supplied with $(\text{NH}_4)_2\text{SO}_4$, 2 with NH_4NO_3 and 3 with KNO_3 .

When symptoms of nitrogen deficiency became apparent, 11.6 mg of nitrogen per plant in the form of ammonium sulphate, calcium nitrate and asparagine respectively were added. In addition a phosphate buffer was added to keep the pH as constant as possible.

After some days the plants were harvested and analysed for protein, soluble organic nitrogen, nitrate and ammonia. Similarly to the results of the above-mentioned experiments, the pea plants supplied with ammonium sulphate showed a poor growth with many dead leaves; those with nitrate and asparagine had a much better appearance. During the last days of incubation the asparagine solution of the pea plants became infected by bacteria what resulted in the formation of ammonia (**).

** In a later experiment with sterile cultures a good response of pea plants to asparagine was found.

The oat plants responded similarly to the different nitrogen compounds; the solutions with asparagine remained sterile during the whole incubation period.

The results of this experiment are given in table III.

TABLE III

Assimilation of different nitrogen compounds by pea and oat plants							
Plants	Nitrogen compound added	Assimilation period in days	Fresh weight of 6 plants in g	Protein-N of 6 plants in mg	Soluble org. N+NH ₄ -N of 6 plants in mg	NH ₄ -N of 6 plants in mg	NO ₃ -N of 6 plants in mg
Oats	Control, no N	4	1.59	3.48	2.40	0.03	—
„	Ca(NO ₃) ₂	4	1.46	4.42	5.08	0.12	0.20
„	(NH ₄) ₂ SO ₄	4	1.36	4.26	4.01	0.09	0.00
„	(NH ₄) ₂ SO ₄ *	4	1.59	4.19	4.54	0.16	—
„	Asparagine	4	1.25	4.43	4.20	0.13	—
„	Control, no N	10	1.26 †)	2.46	2.17	0.11	—
„	Ca(NO ₃) ₂	10	2.13 †)	7.05	7.37	0.11	—
„	(NH ₄) ₂ SO ₄	10	1.82 †)	7.11	7.21	0.21	—
„	(NH ₄) ₂ SO ₄ *	10	1.81 †)	5.40	4.81	0.15	—
Peas	Control, no N	9	10.08	20.84	14.96	0.47	0
„	Ca(NO ₃) ₂	9	9.68	32.76	17.40	0.36	2.6
„	(NH ₄) ₂ SO ₄	9	7.62	27.51	23.50	2.40	0
„	(NH ₄) ₂ SO ₄ *	9	8.76	25.64	17.63	1.18	0
„	Asparagine	9	8.94	34.74	29.—	0.86	0

*) these plants were not exposed to direct sunshine.

†) 5 plants harvested and analysed.

These data show that in spite of the bad growth of the pea plants supplied with ammonium sulphate, the content of protein and of soluble organic nitrogen had considerably increased, thus indicating that under these circumstances pea plants were able to assimilate the added ammonium nitrogen.

A comparison of the plants supplied with nitrate and ammonium nitrogen shows that in the latter case the ratio protein-N to soluble-N was much lower than with nitrate, whereas a considerable amount of ammonium-N was present. As it is a well-known fact that plants with a non-acid or slightly acid cell sap are unable to accumulate ammonium nitrogen without damage, it is very probable that the high concentration of ammonium nitrogen was the cause of the wilting and necrosis of the pea leaves. The fact that the plants growing at reduced light intensity which, showed less necrotic

leaves, had a considerably lower content of soluble and ammonium nitrogen, is in accordance with the above hypothesis. The plants supplied with asparagine had a high content of soluble N and of protein. Ammonia was relatively low, however, and damage of the leaves was not observed.

The oat plants assimilated the different nitrogen compounds to the same extent; symptoms of damage were not observed.

The question now arises why in the pea plants of this experiment the absorbed ammonium nitrogen was partly accumulated as such and not quantitatively converted into organic nitrogen compounds. As it was expected that the pH of the nutrient medium was an important factor in the assimilation of ammonium nitrogen, an experiment at different pH-values was carried out in 1943.

Assimilation of ammonium nitrogen by pea plants at different pH-levels. In the nutrient solution used in this experiment the concentration of magnesium and calcium sulphate and of minor elements was twice as high as the concentration of the elements above-reported.

For the adjustment of different pH-values the following mixtures of KH_2PO_4 and K_2HPO_4 were used:

pH 5:	272 mg of KH_2PO_4	per jar,	containing	600 cc of nutrient solution
pH 6:	218	„ „ „	+ 71.3 mg of K_2HPO_4	
pH 7:	82	„ „ „	+ 249 „ „ „	
pH 8:			356 „ „ „	

The potassium content of every jar was brought up to the same level with potassium sulphate.

“Unica” peas were transplanted into the glass jars on July 20, every jar containing three plants. On July 31 5 mg of nitrogen as ammonium sulphate was added to four cultures of every pH range.

On August 3 again 10 mg of ammonium nitrogen was given and on August 7 again 25 mg. One set of cultures was supplied with potassium nitrate.

At the latter date clear differences were shown. At pH 5 the plants supplied with ammonium sulphate were poorly developed with many dead lower leaves. Of the higher leaves the margins were bent down. Supplied with nitrate these symptoms did not occur and the plants made a good growth.

At pH 6 an only slightly better appearance of the NH_4 -plants was observed than at pH 5. At pH 7 and 8, however, a normal development occurred without any damage of the leaves. This result clearly shows that the assimilation of ammonium nitrogen by pea plants depends on the pH of the nutrient solution. Only in media with a neutral or alkaline reaction these plants are able to use ammonium sulphate as a source of nitrogen. In media with a pH of 6 or lower, however, this compound is very toxic. It is very probable that the contradictory results of various authors in respect of the nitrogen nutrition of leguminous plants (P a r d o ?) are due to a different pH of the nutrient medium used.

After being supplied with another 50 mg of nitrogen per jar as ammonium sulphate and potassium nitrate respectively on August 21, the plants of this experiment were harvested on August 25. At this date a great part of the leaves of the NH_4 -plants growing at pH 5 was wilted. At pH 6 also a considerable damage of the leaves was observed. At pH 7 and 8 the plants grew quite normally without any damage of the leaves.

The yields of the plants (fresh weights) are recorded in table IV.

TABLE IV

Effect of ammonium sulphate and potassium nitrate on the yield of peas grown in nutrient solutions at different pH				
pH of nutrient solution	Nitrogen compound supplied	Leaves fresh weight in g	Stems fresh weight in g	Roots fresh weight in g
5	$(\text{NH}_4)_2\text{SO}_4$	0.91	1.55	4.—
5	KNO_3	3.19	3.56	4.97
6	$(\text{NH}_4)_2\text{SO}_4$	2.10	1.99	4.93
6	KNO_3	3.80	2.94	6.83
7	$(\text{NH}_4)_2\text{SO}_4$	5.93	4.35	7.16
7	KNO_3	6.—	4.77	8.56
8	$(\text{NH}_4)_2\text{SO}_4$	5.36	3.85	6.13
8	KNO_3	6.55	5.67	10.42

These results clearly show the essentiality of a non-acid reaction of the nutrient solution for the development of pea plants supplied with ammonium nitrogen. Although the NO_3 -plants gave higher yields at pH 7 and 8 than at lower values, they made a normal growth in the latter case, without necrotic leaves.

The fresh tissues of these plants were analysed for ammonium nitrogen by adding a borate buffer and distilling the ammonia

under reduced pressure at 40°C. The results of the analyses are recorded in table V.

TABLE V

Ammonium nitrogen in pea tissues grown at different pH-values					
pH of nutrient solution	Nitrogen compound supplied	mg NH ₄ -N per 10 g of fresh tissue *)			
		Leaves		Stems	Roots
		Wilted but not necrotic tissue	Normal tissue		
5	(NH ₄) ₂ SO ₄	10.94	—	1.75	0.23
5	KNO ₃	0.40	0.05	0.10	0.04
6	(NH ₄) ₂ SO ₄	5.96	1.54	1.40	0.20
6	KNO ₃	—	0.00	0.02	0.07
7	(NH ₄) ₂ SO ₄	—	0.10	0.16	0.12
7	KNO ₃	—	0.04	0.22	0.22
8	(NH ₄) ₂ SO ₄	—	0.05	0.05	0.28
8	KNO ₃	—	0.00	0.01	0.18

*) average values of two determinations.

These data reveal a close connection between the occurrence of wilted leaves and the content of ammonium nitrogen. The higher the pH of the nutrient medium the lower this content. Thus it seems very probable that a ready assimilation of ammonium nitrogen by pea plants can take place only when the pH of the nutrient solution is 7 or higher. In the following paragraph the influence of the pH on the ammonia assimilation will be dealt with more in detail. As in the above-mentioned experiment the nutrient solutions were not kept completely sterile, the following one was carried out with sterile cultures.

Assimilation of ammonium and nitrate nitrogen by sterile pea plants. In this experiment 11 Erlenmeyer flasks were used as culture vessels. They were provided with rubber stoppers containing three tubes, one holding the plant, one for aerating the nutrient solution and a third for taking samples and adjusting the pH.

Germinated sterile peas were placed in the tubes which according to Gerretsen³⁾ were filled with paraffined cork filings containing a silver preparation (katadyne bolus, diatomaceous earth, covered with porous silver), which prevents the intrusion of moulds and bacteria into the nutrient medium, while the plants are not damaged.

The nutrient solution used in this experiment, had about the same composition as in the previous experiment, different mixtures of KH_2PO_4 and K_2HPO_4 being used to obtain pH-values of 5.2, 6.0, 7.0 and 7.6 respectively. 50 mg of nitrogen as KNO_3 , $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)_2\text{HPO}_4$ respectively were supplied on September 28. Some plants remained without nitrogen. As in the other experiments the pH-values were carefully maintained at the above values by adjusting with diluted sulphuric acid and a solution of sodium carbonate respectively.

The results of this experiment agreed well with those of the preceding one. At pH 5.2 the growth of the plants with $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)_2\text{HPO}_4$ was very poor with a wilting and afterwards a necrosis of the leaves. Supplied with nitrate normal leaves developed. At pH 6 the plants made a considerably better growth but many leaves still showed the symptoms of ammonium injury. At pH 7 and 7.6 normal plants were obtained. The fresh weights of the plants of this experiment are given in table VI. It should be stressed that these plants were harvested at different dates. The data of this table agree well with those of the previous experiment.

TABLE VI

Fresh weights of pea plants grown in sterile culture solutions at different pH-values									
Nitrogen compound supplied	Date of harvesting	pH = 5.2		pH = 6		pH = 7		pH = 7.6	
		Leaves + stems	Roots						
		in g	in g						
no nitrogen	Oct. 21	0.50	1.60	0.80	3.30	1.00	4.34	0.91	3.70
" "	" 25	0.52	2.30	0.78	4.55	0.47	2.06	0.49	2.81
KNO_3	" 20	1.78	3.48	1.45	3.39	2.53	4.60	1.14	2.31
	" 25	1.89	3.40	1.97	3.53	4.77	7.60	2.97	5.62
$(\text{NH}_4)_2\text{SO}_4$	" 15	0.83	2.30	1.38	4.28	1.86	3.53	1.56	3.55
	" 22	0.80	1.93	1.12	3.06	2.00	4.40	3.05	5.30
	" 23	0.53	1.30	1.40	3.77	2.94	7.50	3.80	6.70
$(\text{NH}_4)_2\text{HPO}_4$	" 18	0.95	3.08	1.32	2.83	2.16	5.47	2.28	4.53
	" 22	0.83	2.32	0.86	2.40	—	—	2.35	4.31
	" 22	1.14	2.84	0.96	2.25	1.85	3.05	3.55	7.62

In order to find out what might be the cause of the ammonia injury of the NH_4 -plants growing in acid media, starch tests were

carried out in leaves showing a different rate of damage. The leaves were boiled in alcohol to remove the chlorophyll and then treated with a solution of J₂ in KJ. The rate of blackening of the leaves being a measure of the starch content, estimates of this compound were thus made in a simple way. Table VII contains the results of these estimates.

TABLE VII

Influence of the pH of the nutrient medium and the nitrogen compound supplied on the starch content of peas growing in sterile solutions (Yields in table VI)			
pH of nutrient solution	Nitrogen compound supplied	Appearance of the leaves investigated *)	Starch test
5.2	no nitrogen	young leaves, pale green, margins somewhat necrotic	negative
5.2	KNO ₃	young leaves, no damage	highly positive
5.2	(NH ₄) ₂ SO ₄	young, light green leaves with necrotic margins	negative
5.2	(NH ₄) ₂ SO ₄	normal, without damage	moderately positive
5.2	(NH ₄) ₂ HPO ₄	heavy symptoms of ammonium injury	negative
5.2	(NH ₄) ₂ HPO ₄	normal, somewhat light green	slightly positive
6	no nitrogen	young, pale green leaves, no damage	moderately positive
6	KNO ₃	young, normal leaves	highly positive
6	(NH ₄) ₂ SO ₄	damaged	negative
6	(NH ₄) ₂ SO ₄	normal	moderately positive
6	(NH ₄) ₂ HPO ₄	damaged	negative
6	(NH ₄) ₂ HPO ₄	normal	moderately positive
7	no nitrogen	pale green, slightly damaged	positive
7	KNO ₃	normal	highly positive
7	(NH ₄) ₂ SO ₄	normal	„
7	(NH ₄) ₂ HPO ₄	normal	„
7.6	no nitrogen	pale green, slightly necrotic	positive
7.6	KNO ₃	normal green	highly positive
7.6	(NH ₄) ₂ SO ₄	normal green	„
7.6	(NH ₄) ₂ HPO ₄	normal green	„

*) 4-6 leaves of every sample were tested.

These data show that the poor growth of the ammonium-supplied plants and the rate of damage of the leaves are correlated with the starch content. Heavily damaged leaves are practically free from starch. The higher the pH of the nutrient medium the higher the starch content of the leaves.

The fact that the starch content of the plants growing in a ni-

trogen-free solution also depends on the pH of the nutrient solution, shows that it is the hydrogen ion concentration rather than the ammonium salt which influences the formation of starch. Since the assimilation of ammonium nitrogen requires the presence of carbohydrates to convert the absorbed ammonia into organic nitrogen compounds, it is probable that the poor assimilation of ammonium nitrogen by pea plants growing in acid nutrient media is due to the low carbohydrate level in the plant. As a result ammonia is accumulating to a concentration which is toxic to the cells.

To answer the question in what way the pH of the nutrient medium may influence the starch production in the leaves, pH determinations were carried out in leaf and root tissues of the above plants. The fresh plant tissues were ground in a mortar and in the pulp the pH was determined by a quinhydrone electrode. The results of these determinations, given in table VIII, show that the hydrogen

TABLE VIII

Influence of the pH of the nutrient solution on the pH of tissues of peas grown in nutrient solutions at different pH-values								
Nitrogen compound supplied	Leaves and stems				Roots			
	pH=5.2	pH=6	pH=7	pH=7.6	pH=5.2	pH=6	pH=7	pH=7.6
no nitrogen	5.89	5.93	6.07	6.17	5.56	5.61	5.73	5.83
" "	(5.65)*	(5.96)	(6.09)	(6.00)				
" "	5.40	5.80	5.92	5.94	5.48	5.55	5.70	5.90
" "	(5.40)		(5.96)	(6.02)				
KNO ₃	6.18	6.20	6.24	6.24	5.86	5.96	6.04	6.24
" "	6.10	6.13	6.40	6.32	5.69	5.80	5.91	6.12
(NH ₄) ₂ SO ₄	5.69	5.80	6.30	6.40	5.54	5.62	5.82	6.12
" "	(5.21)	(5.67)						
" "	5.90	5.82	6.20	6.36	5.58	5.53	5.68	5.93
" "	(5.43)	(5.50)						
(NH ₄) ₂ HPO ₄	5.86	6.04	6.33	6.48	5.80	5.88	6.00	6.16
" "	5.73	5.70	6.13	6.30	5.54	5.70	5.72	5.90
" "	(5.52)	(5.50)						

*) (): necrotic tissue.

ion concentration of the leaf plus stem tissue is influenced by the pH of the nutrient medium and by the nitrogen compound absorbed. In solutions containing ammonium sulphate or ammonium phosphate in which the hydrogen ion concentration varied from pH 5.2-7.6, the pH of leaf plus stem varied from 5.70 (5.20 in necrotic tissues) to 6.40. Plants supplied with KNO₃ although grown in a

nutrient solution of the same hydrogen ion concentration as those with ammonium sulphate had a considerably higher pH in the leaf plus stem tissues. The differences between ammonium and nitrate plants only existed in the acid media; in the neutral or alkaline solutions they were negligible.

These results are in contrast with those obtained in similar experiments with tomato and potato plants. The tomatoes were grown in nutrient solutions at the same range of pH-values as in the above experiment with peas. Nitrogen was supplied as ammonium sulphate and potassium nitrate respectively. No influence of the nutrient media on the hydrogen ion concentration of the leaf and stem tissues was exerted, however, a pH of 5.8 being found at all the pH levels and with both nitrogen compounds. In the ground root tissues the pH-values ranged from 5.6 in the acid media to 5.8 in the neutral and alkaline solutions.

In the case of potatoes the pH of the soil in which the plants had grown varied from 4.2–7.0. The pH of the ground potato tubers only ranged from 5.80–5.95. The results of the experiments with tomato and potato plants are in good agreement with those reported by Hoagland and Davis⁴⁾ and by Mevius⁶⁾.

From the results of the experiments described in this paragraph it may be concluded that pea plants growing in acid nutrient media without combined nitrogen or supplied with ammonium phosphate or ammonium sulphate have a considerably higher hydrogen ion concentration in their leaf and stem tissues than those growing in neutral and alkaline solutions. Due to (or perhaps parallel with) this low pH the starch content of the leaves is low, and the assimilation of ammonia which requires a high carbohydrate level in the plant, is inadequate. Accumulation of ammonia takes place, followed by wilting and necrosis of the leaves and a poor growth of the whole plant.

The effect of zinc and boron on the fixation of nitrogen by the nodules of pea plants. The question whether or not a certain element is essential for the production of nodules or for the fixation of nitrogen, is not easy to solve. It can be done by growing the leguminous plants with increasing amounts of the element concerned, both without and with combined nitrogen. In the former case the nodules have to supply the plants with nitrogen. When the element has a specific

function in the fixation of nitrogen, differences between the two sets of plants may be expected due to a different nitrogen supply. However, when no differences can be observed, it is not sure that the element in question is not essential for the formation of nodules or the fixation of nitrogen. It only means that the requirements of the nodules are less than those of the other parts of the plant so that nitrogen fixation can take place. An exact observation of the habit and growth of the plants as well as of the nodules, in combination with nitrogen determinations carried out at the right moment may in this respect provide more information. This is particularly of importance in the case of elements in the absence of which deficiency symptoms occur, quite different from those of nitrogen deficiency.

In the following paragraphs some experiments will be described dealing with the influence of zinc and boron on the growth and nitrogen fixation of pea plants.

The importance of zinc in the nitrogen fixation by peas. To find out whether or not zinc is an important element in the fixation of nitrogen by the nodules of pea plants, culture solution experiments were carried out in 1943. In this experiment a nutrient solution of the following composition was used:

Water, distilled in a glass apparatus, 1 l

KH_2PO_4	0.279 g	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	15 mg
K_2HPO_4	0.193 g	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.6 mg
KNO_3	0.200 g	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.15 mg
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.108 g	H_2BO_3	0.15 mg
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.225 g	Na_2MoO_4	0.06 mg

“Weck” glass jars of 1.5 l capacity were used as containers. In every jar three germinated “Unica” peas were planted on March 27. The root system as well as the nutrient medium were inoculated with an effective strain of *Rhizobium*.

In the first days of May symptoms of zinc deficiency became apparent. The margins of the lowest leaves became necrotic, taking a gray white colour. Some days later the whole leaves had died. This process advanced upwards so that in the middle of May only the youngest leaves were still alive. The poorly growing plants were able,

however, to develop flowers and pods with some seeds. In contrast to other trace elements as for instance copper and boron, zinc apparently plays no part in the emerging of young leaves and in the seed formation.

The zinc-deficient plants developed many root nodules. Although these nodules were of a small size, probably due to the necrosis of a great deal of the plants, they fixed nitrogen from the atmosphere normally, as was deduced from the dark green colour of the living leaves, and from the fact that plants without combined nitrogen *) showed the same appearance as those supplied with 200 mg of nitrogen as KNO_3 per pot.

The yields of the plants of this experiment are given in table IX.

TABLE IX

Effect of zinc on the yield of pea plants				
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	N as KNO_3	Yields, dry weight in g		
		Seeds *)	Straw	Root
0	28	1.26	2.14	1.—
0	200	0.56	2.52	1.35
0.5 mg	28	— †)	7.44	2.18
0.5 mg	200	9.54	7.69	1.52

*) average yields of two and three pots respectively.

†) the plants of these pots were harvested before the seeds had developed.

The importance of boron in the formation of nodules and the fixation of nitrogen. In a preliminary experiment with pea plants in nutrient solutions, carried out in 1941, no development of nodules was observed in the absence of boron, notwithstanding that the culture solution as well as the root system were inoculated with an effective strain of *Rhizobium*. The fact that in this solution the secondary roots of the plants were very short, makes it highly probable that it is the plant rather than the bacteria which is affected by the lack of boron. This is in agreement with the fact that *Rhizobium*, similar to other bacteria, does not require boron, whereas pea plants need considerable amounts of it.

Due to the lack of nodules the nitrogen supply of the boron-deficient plants became inadequate; light green leaves appeared

*) the very small amount of nitrate nitrogen initially supplied, was used up in the first stage of growth.

and some weeks later the plants died from nitrogen deficiency. The typical symptoms of boron deficiency, which particularly can be seen in the young leaves and buds of the plant and which are quite different from the symptoms of nitrogen deficiency, were not observed.

Supplied with 0.5 mg of boric acid normal nodulation and nitrogen fixation occurred (table X).

TABLE X

Effect of boron on the nitrogen fixation of pea plants			
H ₂ BO ₃ mg per pot	Yields, dry weight in g		N in total plants in mg §)
	Tops	Roots	
0	1.10 *)	0.93	40.7
0.5	3.10 †)	1.04	110.8

*) average values of two cultures.

†) average values of four cultures.

§) the nutrient solution was initially supplied with a very small amount of combined nitrogen.

The results of this experiment are in full agreement with those of Branchley and Thornton²⁾ who in 1925 showed that boron is essential for the development of nodules on roots of *Vicia faba*.

In order to find out to what extent an application of combined nitrogen may decrease the boron requirement of pea plants, an experiment with increasing amounts of boric acid at two different nitrogen levels was carried out in 1942. "Unica" pea plants were transferred to glass jars containing 1.8 l of a nutrient solution on April 23.

This solution had the same composition as above-mentioned except that phosphate was supplied in a concentration of 180 mg of KH₂PO₄ and 60 mg of K₂HPO₄ per l.

Boric acid was added in amounts of 0, 10, 20, 150 and 1650 *) γ per culture. One set of cultures was supplied with 130 mg of nitrogen per culture in the form of KNO₃ and 30 mg N as (NH₄)₂SO₄, the other one received only 10 mg of nitrogen as KNO₃ and 5 mg as (NH₄)₂SO₄. As in the preceding experiments the roots of the plants and the nutrient solution of all the cultures were inoculated with an effective strain of *Rhizobium*.

After four weeks growth pronounced differences were observed.

*) the largest amounts were added in three or four parts.

In the absence of combined nitrogen *) the plants without boron and those supplied with 10 γ of boric acid developed only very small, light yellow, somewhat slimy nodules which apparently were unable to fix nitrogen. The leaves and stems of these plants showed pro-

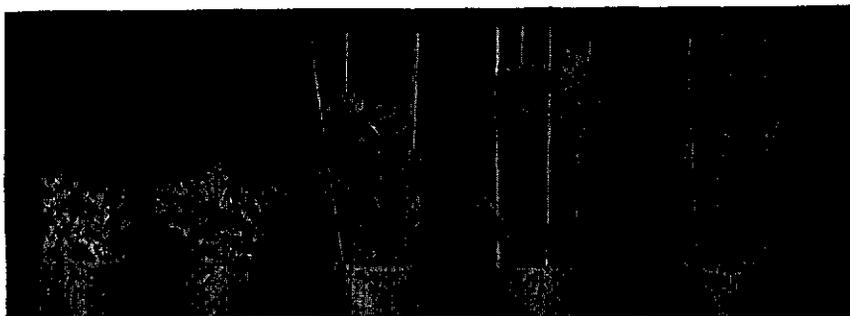


Fig. 11



Fig. 12

Fig. 11 and 12. Pea plants in nutrient solution with different amounts of boric acid (1: without B, 2 : 10, 3 : 20, 4 : 150 and 5 : 1650 γ of H_3BO_3 per pot).

Fig. 11: nitrogen supplied by the nodules. Fig. 12: plants supplied with combined nitrogen. The plants of the pots 1 and 2 in fig. 11 show pronounced symptoms of nitrogen deficiency, those of 3 and 4 in fig. 11 and of 1, 2, 3 and 4 of fig. 12 show symptoms of boron deficiency.

*) the very small amount of combined nitrogen initially-supplied to these cultures was used up in the first stage of growth.

nounced symptoms of nitrogen deficiency, but not the characteristic symptoms of boron deficiency. Some weeks later these plants died from nitrogen starvation.

The plants supplied with 20 γ of boric acid had many well-developed nodules on their roots and consequently they were well-supplied with nitrogen. The leaves were dark green and until June 1 the growth of these plants proceeded quite normally. At that date, however, symptoms of boron deficiency became apparent. The youngest leaves showed yellow margins while their tops often bent upwards and became necrotic. If this happened when the leaves were very young no further growth took place and usually the whole growing-point died off. Secondary buds developed and this resulted in a bushy character of the whole plant (see fig. 11 and 12).

Supplied with 150 γ of boric acid only light deficiency symptoms were seen in the youngest leaves. Some growing-points died, but in other cases normal blooming and fruit setting took place. With the highest amount of H_3BO_3 no deficiency symptoms were observed.

The plants supplied with combined nitrogen did not respond less to boron than those depending on the nitrogen fixation of the nodules. Only in the absence of boron and with the lowest amount of boric acid did these plants make initially a better growth, due to the fact that they did not suffer from nitrogen deficiency. Pronounced symptoms of boron deficiency became apparent, however, at an early date and as a result the plants developed very poorly. In contrast to the plants without combined nitrogen they remained alive for a considerable length of time.

With application of 20 γ and particularly with 150 γ of H_3BO_3 the symptoms of boron deficiency became apparent at an earlier date and more seriously than was the case with plants depending on the nitrogen fixation of the nodules. Obviously this was due to a better supply of nitrogen, which enabled the plants to make a somewhat quicker growth with a higher production of dry matter, and as a result a higher consumption of boron.

Although the plants supplied with combined nitrogen gave higher yields of dry matter than those deriving their nitrogen from the nodules the general trend of both yield curves is about the same (fig. 13).

The results of this experiment have shown that the boron requirement of the growing leaves and stems in general is considerably

higher than that of the nodules. Apparently a small amount of boron initially available in the culture solution is high enough to develop the nodules and once being formed nitrogen fixation proceeds normally, independent of the occurrence of pronounced symptoms of boron deficiency in the tops. Only when boron is totally absent in the nutrient solution when the nodules are developing, is the nodule-forming tissue more affected by the lack of this element than the tops. As a result normal nodules are not developed and the plants die from nitrogen deficiency.

Experiment with boron-deficient sand-peat

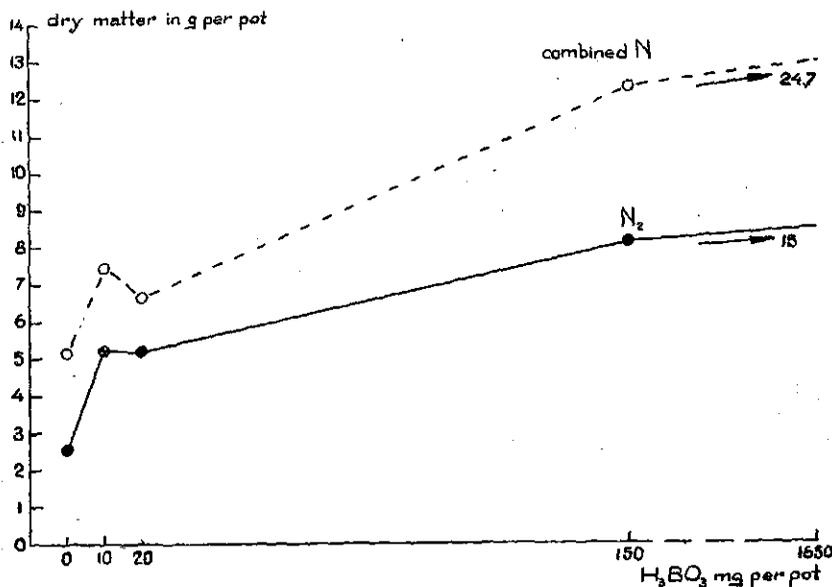


Fig. 13. Effect of different amounts of boron on the yield of pea plants grown in nutrient solution, inoculated with *Rhizobium*, with a very small and a normal supply of combined nitrogen respectively.

mixtures. In order to elucidate the behaviour of pea plants towards boron when growing in soils poor in this element, experiments with a sand-peat mixture were carried out.

The quartz sand used in this experiment was treated with 20% nitric acid and then washed free from acid with distilled water. The peat was in the same way treated with 5% sulphuric acid.

Six parts of sand were mixed with one part of peat. To this mix-

ture calcium carbonate was added to attain a pH-value of 6.2. Glass jars containing 2 kg of the mixture were used as culture vessels. Every pot was dressed with the following salts:

Ca(H ₂ PO ₄) ₂	1.— g	FeCl ₃ .6H ₂ O	25.— mg
K ₂ SO ₄	0.5 g	CuSO ₄ .5H ₂ O	10.— mg
(1.5 g when the small amount of nitrogen was added)		MnSO ₄ .4H ₂ O	10.— mg
MgSO ₄ .7H ₂ O	0.5 g	ZnSO ₄ .7H ₂ O	5.— mg
KNO ₃	0.2 g	Na ₂ MoO ₄ .2H ₂ O	2.— mg

As usual the experiment consisted of two sets of pots, one with only a small amount of nitrogen (0.2 g KNO₃ per pot) and one with 2.— g of KNO₃. Boron was supplied in amounts of: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.2, 3, 4, 5 and 10 mg of boric acid per pot. Germinated "Unica" peas inoculated with a suspension of *Rhizobium* were planted on March 23, 1943.

Very pronounced symptoms of boron deficiency were seen at an early date in the pea plants, not only in those where boron was absent but also in the plants supplied with 50, 100, 200 and 300 γ of H₃BO₃. An application of 0.5 mg of boric acid gave a considerably better development but with higher amounts of H₃BO₃ much higher yields were obtained (fig. 14). These results suggest that considerable amounts of boron were fixed by the peat.

In contrast to the above experiment with nutrient solutions, symptoms of nitrogen deficiency were not observed in the boron-deficient plants, notwithstanding the fact that the nodulation of these plants was very poor. Obviously the small amount of potassium nitrate gave an adequate supply of nitrogen to the poorly growing plants.

A remarkable difference was found between the two sets of plants. Those supplied with 2.0 g of potassium nitrate suffered much more from B-deficiency than the corresponding ones with 0.2 g (fig. 14). This different behaviour was still more clearly shown when pea plants were grown for the second time in the above pots in which, in the first experiment, plant growth totally failed. After the poorly developed plants of these pots had been removed, additional amounts of H₃BO₃ were given and on May 14 peas were planted for the second time. The yields of this second experiment are given in table XI. In the plants without nitrate, boron deficiency was only observed at a rate of 300 and 400 γ of H₃BO₃ per pot, while

supplied with nitrate even the plants with the highest amounts of boric acid were still slightly deficient in boron.

TABLE XI

Effect of boron on the yields of pea plants grown in sand-peat mixtures						
H ₃ BO ₃ γ per pot	Without combined nitrogen			Supplied with KNO ₃		
	Grain in g	Straw in g	pH of soil	Grain in g	Straw in g	pH of soil
300	3.02	11.12	6.75	—	6.30	6.90
400	2.—	8.—	6.65	0.38	7.68	6.95
500	7.25	8.85	6.70	2.35	8.02	6.95
600	7.22	8.65	6.90	1.68	8.85	6.85
650	11.47	12.32	6.65	0.13	6.45	6.95
700	10.95	12.10	6.65	1.82	11.—	7.—
750	9.25	8.98	6.95	1.82	9.83	7.—
900	8.10	10.37	6.70	3.—	8.33	7.10
1000	9.20	11.05	6.70	5.77	9.75	6.95

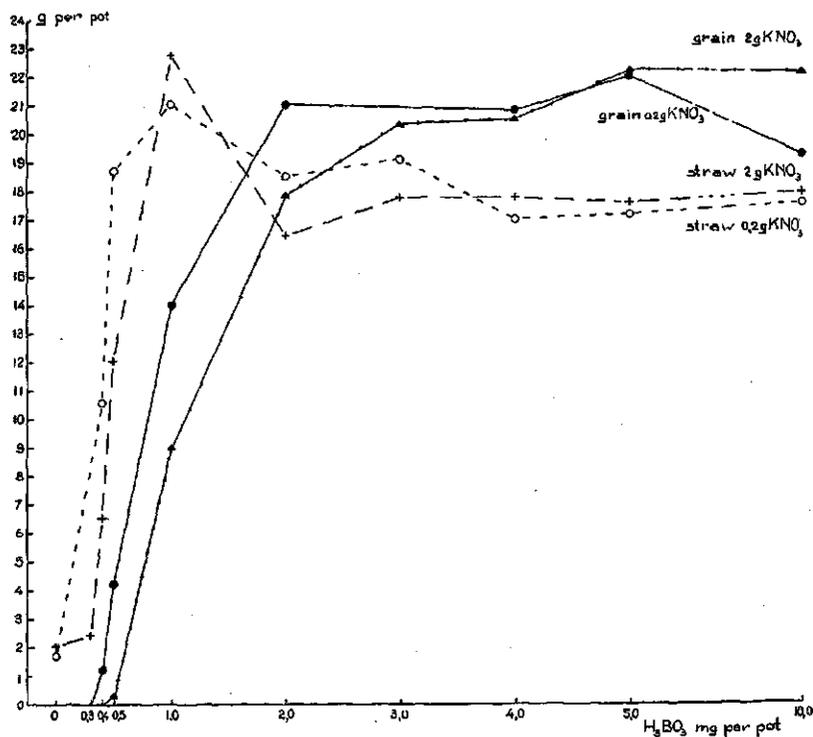


Fig. 14. Effect of different amounts of boron on the yield of pea plants grown in a sand-peat mixture, inoculated with *Rhizobium*, with a very small and a normal supply of nitrate respectively.

As it was suggested *) that the higher requirement of boron of the nitrate plants was due to a more alkaline reaction of the nutrient medium following the absorption of the nitrate, pH-values of the soil were measured after harvesting the plants.

As is seen from table XI the sand-peat mixtures to which nitrate was added had consistently higher pH-values. It may be expected that in the rhizosphere, from which the roots absorb the nutrients, the differences would have been more evident.

Experiments with soil. In the autumn of 1941 a pot experiment was carried out with 24 different soils from fields in the southern part of the Netherlands on which boron deficiency in beets had been observed, and from fields in the province of Groningen on which the growth of peas was inadequate.

Eight glass jars containing about 2.5 kg of soil per jar were filled with each soil. Per pot the following basic dressing was added: 0.5 g of K_2SO_4 , 0.5 g of $Ca(H_2PO_4)_2$ and 50 mg of $MnSO_4$. 2 pots remained without further dressing, 2 were supplied with 1.25 g of NH_4NO_3 per pot, 2 with 20 mg of boric acid and 2 with 20 mg of H_3BO_3 and in addition 1.25 g of NH_4NO_3 . Each soil was inoculated with 5 cc of a suspension of *Rhizobium*. "Mansholt" peas were planted on August 27, 1941.

Due to the warm weather in September and October 1941 a fairly good growth of the pea plants took place. Although in many cases a beneficial effect of the combined nitrogen was observed, a response to boron was only in a few cases perceptible. On one sandy soil this was particularly true (see fig. 15). Without application of boron the nodules were small with an abnormal pale white yellow and sometimes black brown colour. Supplied with boric acid normal nodules with a reddish brown colour developed.

The experiment was repeated in the spring of 1942 with five soils, on which the plants more or less had responded to boron. As in the preceding year glass jars were used containing about 2.5 kg of soil. As a basic dressing the following salts were added per pot: 0.5 g K_2SO_4 , 0.5 g $Ca(H_2PO_4)_2$, 0.2 g $MgSO_4 \cdot 7H_2O$. The plan of this experiment was as follows:

- a) no boron, 62.5 mg of ammonium nitrate.
- b) 20 mg of H_3BO_3 , 62.5 mg of ammonium nitrate.

*) see Lehr (5).

- c) no boron, 1250 mg of ammonium nitrate.
- d) 20 mg of H_3BO_3 , 1250 mg of ammonium nitrate.

On two soils, a sandy and a clay soil, the plants responded to boron. Deficiency symptoms became only apparent, however, after the plants had already grown for nearly two months. The growth of the plants slackened and the leaves showed a light green and later a yellow colour. They ripened at an earlier stage than those supplied with either boron or nitrogen and gave lower yields (see table XII). These symptoms resembled those of nitrogen deficiency.

TABLE XII

Effect of boron on the yields *) of pea plants grown in soil								
Soil	62.5 mg of NH_4NO_3 per pot				1250 mg of NH_4NO_3 per pot			
	No boron		20 mg of H_3BO_3 per pot		No boron		20 mg of H_3BO_3 per pot	
	Grain in g	Straw in g	Grain in g	Straw in g	Grain in g	Straw in g	Grain in g	Straw in g
Clay soil	5.25	4.60	7.45	6.92	4.20†)	6.17	10.05	8.82
„ „	5.60	4.60	8.05	6.40	7.13	7.94	8.62	8.45
Sandy soil	4.97	4.45	9.22	9.17	5.89†)	6.12	9.19	7.46
„ „	4.71	4.94	10.30	7.10	13.—	11.12	2.55†)	5.16

*) dry weight.

†) the low yield of these pots was due to an irregular germination of the seeds.

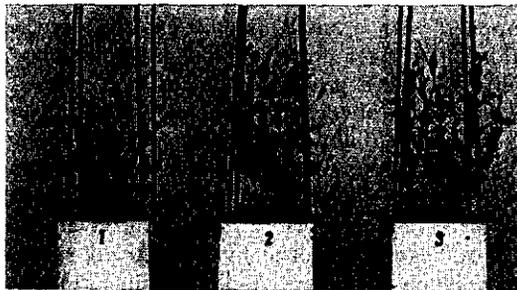


Fig. 15. Effect of boron and combined nitrogen on the development of pea plants growing in a sandy soil. 1: without B and combined N, 2: 20 mg of H_3BO_3 per pot (no N), 3: 1.25 g of NH_4NO_3 per pot (no B).

Although many nodules were present, their colour was black and they were shriveled at the time of harvesting. Supplied with 20 mg of H_3BO_3 normal nodules occurred. When NH_4NO_3 was applied, practically no nodules had been developed.

The most important result of this experiment was, however, that no typical symptoms of boron deficiency were observed in the tops of the plants growing without application of boric acid. The blooming and fruit setting were quite normal and the lower yields of seeds and straw were only due to the early ripening of the plants owing to an inadequate fixation of nitrogen by the nodules. It is not clear why in these soils the boron requirement of the nodules seems higher than that of the tops, while in the experiment with nutrient solutions in general an opposite result was found. It may be that the development of nodules in the soil started relatively late when most of the available boron already had been absorbed by the plants.

Summary

The effect of different amounts of ammonium nitrate on the development of pea plants growing in nutrient solutions in the absence of *Rhizobium* was investigated. It was found that these plants behaved to nitrogen quite differently from oat plants which were grown in a similar set of culture solutions. Nitrogen-deficient pea plants became yellow and died some weeks later. This was in contrast to oat plants which were able to complete their life cycle in a solution free from nitrogen.

Nitrogen-deficient oat plants responded much more readily to newly introduced nitrogen than pea plants.

Tops of pea plants with pronounced symptoms of nitrogen deficiency had a considerably higher content of soluble nitrogen and less protein-N than plants supplied with a small amount of combined nitrogen. With higher applications of nitrogen protein as well as soluble nitrogen increased.

In field experiments with peas no response to combined nitrogen was observed on soils on which always a good growth of these plants took place. On soils with a poor pea growth in previous years a clear response to the supplied nitrogen was observed. In one case the inadequate fixation of nitrogen was partly due to the larvae of *Sitona lineatus* which destroyed the nodules. On two other fields the plants growing in the absence of combined nitrogen were more heavily attacked by a *Fusarium* sp. than those well-supplied with nitrogenous fertilizers. As a result the latter plants gave much higher yields.

In nutrient solutions of pH 6 the effect of some nitrogenous compounds on the growth of pea plants was investigated. Ammonium nitrate and potassium nitrate provided a much superior source of nitrogen than ammonium sulphate. Plants supplied with the latter compound developed poorly and had many wilted and necrotic leaves. It was shown that the poor growth of these plants was not due to their inability to assimilate ammonium nitrogen. The ratio soluble N to protein N was much higher in the plants supplied with $(\text{NH}_4)_2\text{SO}_4$ than in the nitrate plants. In addition the concentration of ammonium nitrogen in the former plants was high.

In culture solutions of different hydrogen ion concentrations a ready assimilation of ammonium sulphate by pea plants without necrosis of the leaves was found at pH-values of 7 and higher. The ammonia content was very low in these plants, while at pH 6 and 5 many necrotic leaves were observed and the NH_4 -content of the leaves and stems was considerably higher.

In a similar experiment with sterile plants it was found that the leaves of pea plants growing at pH 5.2 and 6, and supplied with ammonium sulphate or ammonium phosphate had a very low content of starch. In the wilted leaves practically no starch was found. The pH-values of the ground leaves and stems of these plants were considerably lower than those of plants supplied with KNO_3 or with $(\text{NH}_4)_2\text{SO}_4$ in neutral or weakly alkaline solutions, which had a much higher starch content in their leaves. It was concluded that the necrosis of the leaves and the poor growth of the pea plants supplied with $(\text{NH}_4)_2\text{SO}_4$ at pH-values of 6 and lower were due to an injury by ammonia, which accumulated owing to an inadequate assimilation of this compound in leaf tissues without starch and with a relatively high hydrogen ion concentration.

Pea plants growing in a zinc-deficient culture solution inoculated with *Rhizobium* developed very poorly with many necrotic lower leaves. Blooming and formation of seeds took place relatively normally. Although the nodules were small they were able to supply their hosts with nitrogen.

The effect of boron on the growth and nitrogen fixation of pea plants was investigated. In agreement with the results of Brenchley and Thornton with broad beans it was found that no nodules developed on the roots of peas growing in nutrient solutions in the absence of boron. Due to this the plants died from nitrogen deficiency. Supplied with small amounts of boric acid normal nodules developed, which fixed nitrogen independently of the fact that pronounced symptoms of boron deficiency became apparent at an early date in the tops of these plants. To obtain normal plants considerably higher amounts of boric acid had to be applied.

Supplied with combined nitrogen the pea plants required somewhat higher amounts of boron than in the absence of this compound, probably due to a higher production of dry matter.

In an experiment with sand-peat mixtures pea plants supplied with potassium nitrate required considerably higher amounts of boron than plants supplied by the nodules. Presumably the more alkaline reaction of the peat in the former case had decreased the availability of the boron.

In experiments with natural soils a response of the pea plants to boron was found in some cases. In contrast to the experiments with culture solutions no symptoms of B-deficiency were seen in the tops of these plants. The fixation of nitrogen was decreased, however, and due to this the plants ripened at an earlier date and gave lower yields.

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INVESTIGATIONS ON THE NITROGEN NUTRITION OF PEA PLANTS

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Introduction. Pea plants, like other members of the *Leguminosae*, generally derive the nitrogen required for their proteins and other nitrogenous compounds from the gaseous nitrogen fixed by the nodules. Extensive investigations on the biochemistry of the nitrogen fixation have been carried out by Virtanen *et al.* ⁸⁾, ⁹⁾ and by Wilson *et al.* ¹⁰⁾.

The question whether the nodules of leguminous plants are fully capable of supplying their hosts with nitrogen fixed from the atmosphere, or that an additional absorption of combined nitrogen from the soil may still improve the growth of the plants, is important not only from a theoretical point of view but also in respect of the growing of these crops in practice.

The bad results sometimes obtained by Dutch farmers with the growing of peas, gave rise to an investigation of the effect of nitrogenous fertilizers on the growth of pea plants on certain soils. In addition to field experiments, investigations with pea and oat plants in nutrient solutions with increasing amounts of nitrogen were carried out. In other experiments the value of different nitrogen compounds as a nitrogen source for peas growing in nutrient solutions without *Rhizobium* was determined.

In experiments with nutrient solutions and with soil the influence of zinc and boron on the fixation of gaseous nitrogen was investigated.

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