MAGNESIUM DEFICIENCY AS THE CAUSE OF INJURY IN CEREALS

BY

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INTRODUCTION

The necessity of a suitable supply of magnesium to green plants is an acknowledged fact; the possibility, however, that a shortage of plant-available magnesium might exist in many fields has only recently occurred to agronomists. It was a disease of cereals, especially of rye and oats, which in 1918 for the first time drew the attention of practical growers in that direction, and since then the study of this disease has kept many minds and hands busy. Of late years many data have been gathered on the influence of magnesium content of the soil on various crops and on the amount of that element, required to obtain maximal yields, but these efforts have not, as yet, resulted in a clear understanding of the reason why different soils are so much at variety in this respect.

It soon appeared, that the magnesium content of soil samples gave insufficient information about the doses required and so the conceptions of "plant-available magnesium" and "magnesium requirement of soils" have been introduced and have led to many interesting investigations. The former property of the soil is approximated by extracting it with various solvents (mostly diluted hydrochloric acid or solutions of sodium acetate), the latter is usually estimated by means of biological methods: either by means of pot cultures or by using the mould *Aspergillus niger* and weighing the mycelium which develops under conditions where a known amount of soil is the only source of magnesium (NIKLAS c.s. (27, 28, 29)).

Both methods have their merits, but it is hard to make out to what extent the amounts estimated correlate with the varying requirements of different plants.

In our investigation we have focussed our attention to the range, where magnesium supply falls below the minimum required and injury in plants begins. As for cereals this injury is known in the Netherlands as "Hooghalensche ziekte" (Hooghalen disease) and we have tried to establish definitely the relation between this disturbance in cereals and a deficient supply of magnesium.

I. SUMMARY OF LITERATURE

By the name of "Hooghalen disease" HUDIG and MEYER in 1918 (10) designed a series of disturbances in the growth of cereals occurring on acid sandy and peaty soils. When the disease occurs in a merely slight degree and bears an often transient character, it is recognised in oats and rye by the occurrance of a dark green mottling on the lighter green leaves, designed as "tigering". In serious cases the leaves turn various shades of yellow, whereas the development is very much retarded and lastingly disturbed. As a remedy fertilizers with an alcaline reaction were mentioned, such as $CaCO_3$. On the actual cause of the disease the authors do not express their opinion, however.

Although the name "Hooghalen disease" is not to be found in German literature, it is learned from several papers, i.a. by SCHMITT (33), JESSEN (17) and others, that similar symptoms also occur in Germany in cereals on acid sandy soils. These German investigators could record that kaïniet in its former constitution, i.e. with a content of 30 per cent $MgSO_4$, acted very favourably (see also 31), unlike the purified K-salts, such as KCl and K_2SO_4, which had an unfavourable influence. Another investigation (GEHRING, 7, 8) showed that a marked increase in the crop could be obtained on acid soils by the use of Mg-salts such as $MgSO_4$. GEHRING and also SCHMITT ascribe the poor growth of plants on acid soils to an insufficient supply of magnesium. They are of opinion that the magnesium in these soils is fixed chiefly by adsorption; a neutralization of the soil is supposed to mobilize the magnesium. The actual cause of this fixation and mobilization is left open.

VAN ITALLIE (11, 12, 13) has made an extensive study of the Hooghalen disease and its cause. He could state, that $MgSO_4$ can make the symptoms of mottling and chlorosis disappear, but that in most cases an increase in pH is required to obtain an optimal growth. Next to Mg-salts, $CaCO_3$ or a gift of nitrogen as $NaNO_3$ had a favourable effect. K- and NH_4-salts on the contrary acted unfavourably. VAN ITALLIE ascribes the cause of the "Hooghalen disease" partly to an insufficient magnesium supply to the plants, partly to a damage actually due to acidity. According to this author the injurious influence of a low pH on the magnesium supply is to be found in the fact that a high concentration of H-ions interferes with the uptake of Mg-ions, whereas the plant, as this concentration increases, requires more magnesium for its normal development. The latter conclusion is drawn from the fact, that $CaCO_3$ increases the amount of dry material, whilst the magnesium content of the plant may remain on the same level. We do not consider this argument as convincing, since it is very well possible that the larger amount of magnesium, taken up under the influence of a lime gift, may induce a better development, in which case the magnesium content, calculated on dry material, remains the same or even decreases. Similar symptoms are also known in the case of other nutrional elements, as for instance Mn and P, sound plants sometimes being lower in content of these elements than deficient plants.

The injurious influence of K- and NH_4-salts on plants affected with Hooghalen disease is the result of a disturbance in the uptake of magnesium, as can easily be derived from VAN ITALLIE's data.

Magnesium deficiency has been recognised as a cause of plant diseases in the U.S.A. as well. In Florida the trouble is found on slightly acid sandy soils in the north, where the citrus cultures are often affected by a lack of magnesium (3). Characteristics are a serious chlorosis and an early shedding of the leaves. Subsequently the fruits are shed whilst the branches show a die back of the ends. On analysis the chlorotic leaves were much lower in magnesium content than the green ones, whereas the potassium content was higher. Of importance is the fact, that, in a general way, the seedless varieties are much less affected by a lack of magnesium and of other minerals than the seedbearing ones; this fact may be ascribed to the high mineral content of the seeds.

In North Carolina, Virginia and apparently in a few other states, magnesium deficiency is often met with in tobacco, corn, soy beans and cotton [1]). In tobacco this injury is called "sand drown"; this name covers the actual facts, the disturbance occurring mainly on

[1]) Personal communication of L. G. WILLIS, North Carolina Agricult. Exp. Station.

sandy soils and following a heavy rainfall. The disease is recognized by a turning pale yellow of the tips of the lower leaves. From the tips and margins this colour spreads all over the leaves, only the veins and the tissue along them remaining green for a much longer period. Like it is the case for the Hooghalen disease, K_2SO_4 and $(NH_4)_2SO_4$ have an injurious effect, whilst $CaCO_3$ has a favourable influence. $NaNO_3$ on the contrary is without any effect. The injurious action of much rain is supposed to be due to a washing out of the magnesium from the soil (4, 5, 6).

Although the symptoms of mottling also occur in corn affected by a lack of magnesium, the typical symptom, according to WILLIS, is to be found in yellow longitudinal bands in the leaves (see also GARNER and co-workers, (5)). The elder leaves are always the first to show these symptoms. Mg-deficiency in soy beans induces like symptoms as in tobacco; here again chlorosis occurs, whilst the tissue along the veins keeps its normal green colour. Initially cotton shows a light fading of the green margins of the leaves and in the fields between the veins. Soon, however, in these yellowish green regions anthocyan is formed, and such leaves, safe of the veins, turn red.

This short summary may suffice [1]). It appears, that favourable results following a gift of magnesium salts could be recorded in several cases, on soils with a relatively low pH value, where crops show the described symptoms. This has led to the view, that the poor growth of plants on acid soils might be due to an insufficient supply of magnesium. Some investigators, however, suppose that it originates from the damage that is caused by an excessive concentration of H-ions or a too high content of aluminium, zinc, iron or manganese (35).

As to the favourable effect of neutralizing materials such as $CaCO_3$ and $NaNO_3$, no consensus of opinion exists. Some investigators record a very good result following such a neutralization, without any gift of magnesium salts (7, 8, 12). Several others on the contrary could merely note a slight improvement and are able to induce a normal development solely by the supply of Mg-salts (23). As to the explanation of this effect of neutralizing opinions vary as well: some authors assume it as acting on the soil, others as on the plant.

Our experiments were put up with the aim of establishing unto what extend a shortage of magnesium in soils and plants influences the occurrence of the Hooghalen disease.

It seemed to us, that the availability of the magnesium present in healthy and diseased soil was worthy of a careful consideration and that the effect of neutralizing had to be studied from this same point of view.

[1]) For a summary of literature see JAVILLIER (15, 16).

In the course of the investigations practically the same working scheme was followed which had been adopted for the investigations on the copper content in soils on which the "reclamation disease" occurs (25). It is to be wondered at, that none of all investigators who have studied the Hooghalen disease, has compared its symptoms with those of magnesium deficiency, as induced in water or sand cultures. So primarily a series of water cultures, containing increasing amounts of $MgSO_4$, was prepared. As has been indicated above, the concentration of K-, NH_4-, Ca- and especially H-ions are of influence on the occurence of the disease. So sets containing magnesium were provided with various ionic ratios of the latter ions. Because of the difficulty, caused by the need of keeping the pH-value of the cultures on a constant level, a range of sand cultures were prepared moreover, in which, as a buffering material, 10 per cent peat ("mosveen") had been added to the sand.

For the sake of comparison a number of jars were filled with soil from fields on which the Hooghalen disease occurred. In some of them the soil did not undergo any treatment, to others magnesium-free $CaCO_3$ or $MgSO_4$ had been added. Another series was prepared with soil from a plot in the experimental garden of the Microbiological Laboratory at Wageningen, which no longer bore normally developed plants because some years earlier this plot had been dressed with sulfur, which had resulted in a high acidification of the soil. Subsequently we determined in a large number of soil samples, known to produce either diseased or sound crops, the amount of available magnesium, using the mould *Aspergillus niger*. Moreover we studied the influence which an increase or decrease of the pH may have on this amount. Then the magnesium content has been estimated in diseased and in normal plant material. Finally investigations were carried out as to the cause of the very low content of available magnesium in acid soils and of the favourable influence of neutralizing agents.

II. COMPARISON BETWEEN THE "HOOGHALEN DISEASE" SYMPTOMS AND THOSE OF MAGNESIUM DEFICIENCY IN WATER AND QUARTZSAND CULTURES

a. Water cultures.

The nutrient solution used for these cultures had the following constitution:

KNO_3	0,5	g
NH_4NO_3	0,2	,,
KCl	0,1	,,
K_2SO_4	0,25	,,

$Ca_3(PO_4)_2$	0,5	g
$Fe_2(NH_4)_2(SO_4)_4 . 24H_2O$ . . .	0,175	,,
$MnSO_4 . 4H_2O$	0,0015	,,
$ZnSO_4 . 7H_2O$	0,0005	,,
H_3BO_3	0,0005	,,
$CuSO_4 . 5H_2O$	0,0004	,,
H_2O, distilled in glass apparatus	1000	ml

The salts used were "pro analysi" brands from KAHLBAUM and MERCK, and practically magnesium-free, as had been proven by the blank experiments. The culture vessels were Weck jars of 1 liter, which had been cleaned by means of nitric acid. The four plants per vessel were suspended in cotton-wool in holes in the lid, which consisted of paraffined plaster. The nutrient solution was renewed every four weeks. This experiment was carried on with oats (Zege) and in some cases with springwheat (v. HOEK) and barley (MANSHOLT). Magnesium was supplied as $MgSO_4 . 7H_2O$ in total amounts of 0, 0,26, 1,35, and 105 mg per pot in 1938 and of 0, 4, 10 and 50 mg in 1939. These amounts have been given gradually, along with the renewal of the solutions.

Next to this series, containing solutions with an about neutral reaction, series with a same range of amounts of magnesium have been prepared either with a low pH value or with higher concentrations of K- and Ca-ions.

In order to obtain a pH value of 4, H_3PO_4 was used in 1938, but in 1939 $Ca(H_2PO_4)_4$ was substituted for $Ca_3(PO_4)_2$. An excess of potassium was given, viz. 1 g KCl per liter, to another series Ca was added as $CaCl_2 . 6H_2O$ in an aequivalent amount. After renewal of the nutrient solution, both salts were again added in the same amount. A series in which nitrogen was present as $(NH_4)_2SO_4$ had to be discarded, as most of its plants had succombed at an early date, due to an unexplained reason.

Observations on the development of oats.

The initial symptoms of magnesium deficiency in oats occurred as early as the second week. The leaves took on a light green shade and the darker green mottling ("tigering") appeared. In course of time the shade of the leaves turned more yellow, whilst the green mottling disappeared. In some cases the shades varied from a yellowish red to a rusty brown. Always the lower, i.e. the elder, leaves showed these symptoms in their severest form. The highly chlorotic leaves did not shrivel up immediately, but kept their normal turgidity for quite a period. The younger leaves very often rolled along the margins, what made them appear slender and spindly. During the experiment it

Fig. 1. Oats in water culture at pH 6.5. One week after the supply of 3 mg Mg to a diseased plant

Fig. 2. Oats in water culture at pH 4.5. One week after the supply of 3 mg Mg to a diseased plant

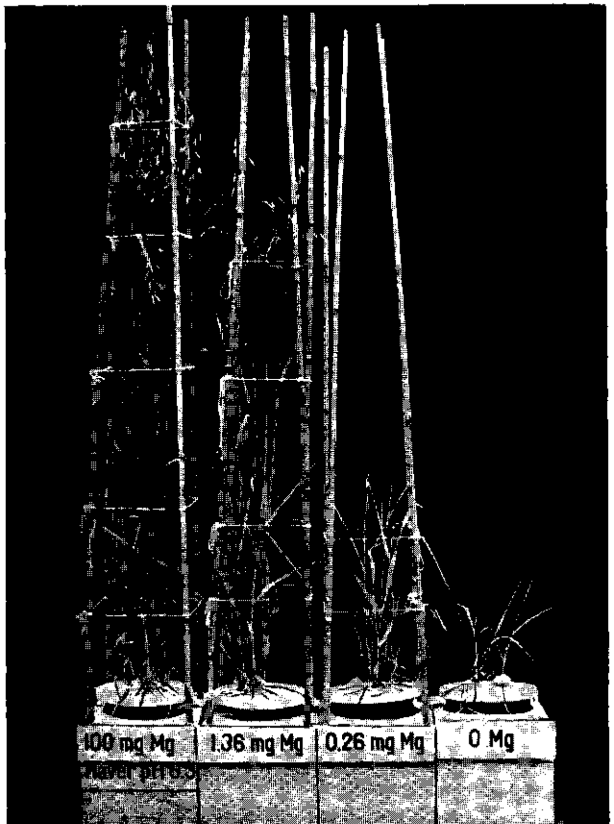

Fig. 3. Oats in water culture with increasing amounts of magnesium added. pH 6.5

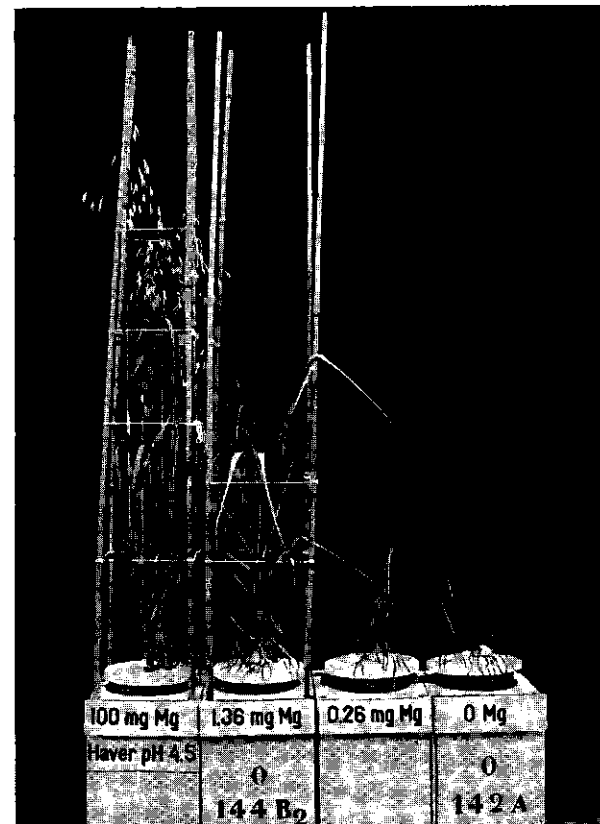

Fig. 4. Oats in water culture with increasing amounts of magnesium added. pH 4.5

could be noted, that plants which had been supplied only slightly with magnesium showed the yellowish green mottling more markedly than those without any supply of this element. So this mottling seems to be a mild symptom of magnesium deficiency. The latter fact became quite obvious, when plants affected with serious symptoms had been supplied with some milligrams of magnesium. After merely two days its effect, the green mottling, became apparent. It was particularly striking that leaves, which at the moment of magnesium supply were initiating their growth, showed the green mottling markedly (fig. 1 and 2); those which developed later were of the normal green colour, whereas the leaves already highly chlorotic, when magnesium had been added, showed practically no change. This gives the impression that the formation of chlorophyll, which requires magnesium, can only take place if the leaves are not yet fully developed.

As far as the amount of Mg, needed for a normal development, goes, it may be pointed out, that in the 1938 series only the largest gift sufficed to induce a normal plantgrowth (fig. 3). Even a supply of 3 mg Mg to cultures 2 months old, which had already received 1,35 mg, could not induce any grain formation (table 1).

Although the 1939 series, because of abnormal conditions, had to be brought to a close on July 1st, i.e. before the ripening, and so no production data could be recorded, several observations on the development could be made. Again in this series only those plants which had received the largest gift of magnesium were altogether free from deficiency symptoms. The plants which had been provided with 10 mg, although they had developed to nearly normal size, still showed the mottling. When 4 mg had been provided the green mottling was nearly gone and the leaves had taken on an even shade of light yellow.

Observations in the acid range.

In this range we have tried to arrive at and to maintain a pH value of 4–4,5. In the 1938 series this value was obtained by means of adding H_3PO_4 to the usual nutrient solution. It has to be emphasized, however, that it is not an easy task to keep the pH on a constant level, when no use has been made of dripping cultures, especially not if the growth is rapid. Although, as far as possible, a daily control was kept up, a few times the pH value happened to rise to a level relatively far above the one aimed at. In the series of 1939, where $Ca(H_2PO_4)_2$ had been substituted for $Ca_3(PO_4)_2$, a pH value was attained, lying just below 4. These cultures were to a much lesser extent subject to changes in the pH value than those of 1938.

In the acid nutrient solutions the root development was much impaired initially. The roots grew only for some mm down into the solution. Above the water surface they branched strongly, so that a very abnormal root system resulted. After some weeks roots were formed which seemed to be less sensitive to the low pH value and managed to grow into the nutrient solution. This was the case with plants, supplied with a sufficient amount of magnesium.

As to the development of the leaves in case of an insufficient magnesium supply, the growth was somewhat poorer when the acidity was high than when low, the magnesium concentration being the same (fig. 4). The small amount of magnesium, present in the nutrient solution, evidently could not be utilized sufficiently by the plants, be it because of the poor root system or of the high concentration of H-ions. In case of a sufficient magnesium supply the plants, raised in the acid solutions, did not show the usual green colour, but a particular chlorotic striation, i.e. light green along the veins (fig. 5). These symptoms, which were present not only in the 1938 water cultures, but also in the 1939 series and in the sand cultures with a high acidity, to be discussed later, are obviously not influenced by the magnesium supply, since they occurred in the cultures containing much magnesium as well as in those with a small dose; apparently they are due to a damage actually caused by acidity. What is the actual cause of this injury is not known yet. It has been assumed now and again that damage in acid soils may be due to intoxication by aluminium. In our case, however, this may be considered as out of the question, as the nutrient solutions did not contain any Al.

In order to ascertain unto what extent the assimilation of magnesium is influenced by the high concentration of H-ions, a number of the latter water cultures were supplied with small amounts of magnesium sulfate. This took place when the plants were almost 2 months old, and had developed a fairly good root system. It appeared that, notwithstanding the high concentration of H-ions, the plants answered just as quickly and in an equal measure to the magnesium added, as was the case in an about neutral solution. So apparently neither the taking up of Mg-ions by the roots, nor its assimilation in the plant is impaired by an excess of H-ions (cf. with the data for the production in table 1). So the unfavourable development of oats in acid solutions, low in magnesium, is likely to be due to an insufficient development of the root system.

Influence of an excess of K- and Ca-ions.

An excess of KCl somewhat stimulated the appearance of the symptoms of deficiency. The initial symptom in plants provided with an extra gift of K, however, is the occurrence of pale yellow green leaves, even in the plants with a maximum Mg supply. Sometimes the leaves took on a chlorotic striation, similar to the symptoms in cultures low in pH value. The extra K-gift had no influence on growth and dry matter production, nor did it inhibit the action of the slight amount of magnesium supplied to deficient plants, grown during two months. The supply of an aequivalent amount of Ca-ions, given as $CaCl_2 . 6H_2O$, especially enhanced the mottling symptoms. As to the general development, the dry matter production and the influence exercised by magnesium on deficiency plants, $CaCl_2$ did not show much effect.

Magnesium deficiency symptoms in wheat and barley.

In wheat and barley, too, the mottled leaves represent the mildest form of magnesium deficiency. In wheat the green spots are of more irregular shape than in oats. The light yellow regions may turn a whitish yellow, which gives the leaves a very marked mottled appearance. If the magnesium deficiency occurs in a more severe degree, no mottling whatever occurs. Wheat will be very spindly, the margins of the younger leaves being rolled up, whilst their colour is a pale yellowish green.

The characteristic symptom in barley, affected by magnesium deficiency, is the occurrence of large, longish, necrotic spots of a light grey shade, with dark edges on the lower leaves. The spots usually occur along the margins, but somtimes also in the central part of the leaves. They remind in a way of the necrotic spots, caused by manganese deficiency (grey speck disease) in oats.

Comparing the deficiency symptoms of the three cereals it is clear that the magnesium requirement of oats is smaller than that of wheat and barley. This is shown in table 1 (p.12), the crops of barley at the lower magnesium levels being relatively smaller than those of oats. Unlike oats, where the supply of small amounts of Mg sulfate to two months old deficiency plants had still caused a marked improvement in the growth, barley had not answered to so small amounts. The plants were already in such a poor condition, that no further intake of magnesium could occur.

b. Sand-peat mixtures

The quartzsand used for this experiment, held from the glass manufactory at Leerdam, was first heated in basins of cast iron, further treated with 10 per cent HNO_3 on a waterbath and finally washed with distilled water on a Buchner funnel until free from acids. The peat used was young "mosveen" (moss litter), treated with 1/10 N H_2SO_4 and afterwards washed with distilled water until the failing of any reaction with $BaCl_2$. Peat and sand were mixed in a ratio of 1 : 10. All jars were filled with 2 kg of the moist mixture. Glass cylinders were used with a capacity of 2,4 l, provided with glass tubes

TABLE I.

Yield in grams of dry matter of barley and oats, grown in nutrient solutions of various H-, K- and Ca-ion concentrations with increasing amounts of $MgSO_4 . 7H_2O$

Mg in mg	Oats		Oats		Oats		Oats		Barley	
	pH 6–6.5		pH 4–4.5		KCl		$CaCl_2$		pH 6–6.5	
	grain	straw	grain	straw	grain	straw	grain	straw	grain	straw
0	0	0,40	0	0,15	0	0,75	–	–	0	0,07
1 mg after 2 months . . .	0	1,90	0	0,29	0	0,80	0	1,48	–	–
0,26	0	2,58	0	0,47	0	2,16	–	–	0	0,43
0,26 + 2 mg after 2 months . .	0	3,14	0	3,24	0	4,11	0	4,26	–	–
1,35	0	5,40	0	3,50	0	5,13	–	–	0	2,50
1,35 + 3 mg after 2 months . .	0	8,24	1,22	5,73	0	10,87	0	9,73	–	–
105	8,17	12,07	6,66	9,80	8,0	11,60	9,82	11,94	8,69	9,56

reaching the bottom, for the sake of aeration. To each jar the following salts were supplied:

1	g K_2HPO_4	0,010	g $MnSO_4 . 4H_2O$
1	,, NH_4NO_3	0,005	,, $ZnSO_4 . 7H_2O$
0,5	,, Na_2SO_4	0,0025	,, H_3BO_3
1	,, $CaCl_2 . 6H_2O$	0,010	,, $CuSO_4 . 5H_2O$
0,025	,, $FeCl_3 . 6H_2O$		

This nutrient solution, compared with that of the water cultures, contains a considerably lower amount of potassium and an accordingly higher amount of other kations. This was done in view of the fact that, according to some investigators, and especially to VAN ITALLIE, a high concentration of K-ions in the soil may considerably lower the absorption of the magnesium by the plants.

This experiment was carried out in order to study the combined influence of pH value and magnesium supply on the development of oats. To that end vessels were brought up to various magnesium levels with increasing amounts of $CaCO_3$ [1]). Amounts were supplied of 0, 1, 2, 3 and 5 g to each vessel; magnesium was given as $MgSO_4 . 7H_2O$ in amounts of 0, 5, 10 and 50 mg respectively. Only the 0 and 10 mg series were supplied with the highest dose of $CaCO_3$.

The experiments have been started on March 3rd, 1939; the following pH values were found on May 5th, 1939 (in aequeous suspension with the aid of the glass electrode):

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[1]) Pure $CaCO_3$ was obtained by adding $CaCl_2 . 6H_2O$ to a Na_2CO_3 solution, both being p.a. brands. The $CaCO_3$ obtained in this way appeared to be free of magnesium, when examined by means of Aspergillus.

Fig. 5. Oats at pH 4.5 (100 mg of Mg supplied). Symptoms of injury by acidity

Fig. 6. Oats in a sand-peat mixture, supplied with 3 g of $CaCO_3$ per vessel and increasing amounts of magnesium: from right to left: 0, 5, 10 and 50 mg

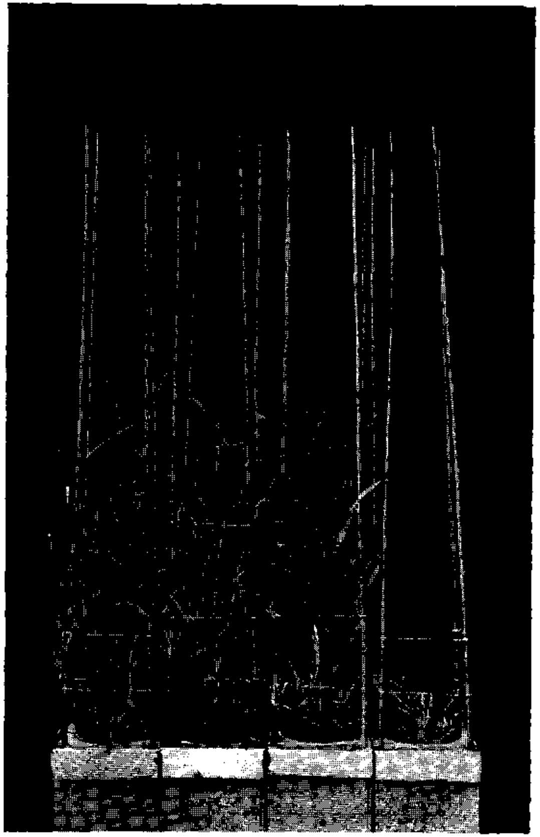

Fig. 7. Oats in sand-peat mixture without magnesium. From right to left: 1, 2, 3 and 5 g $CaCO_3$

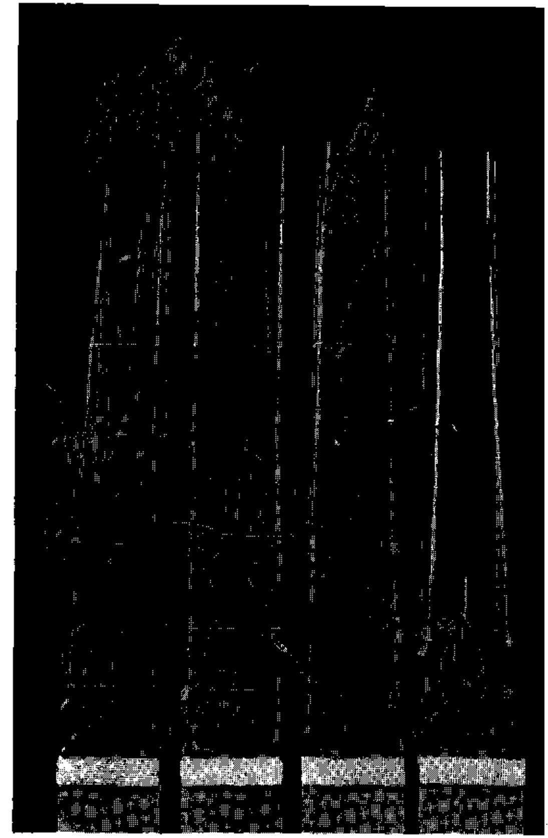

Fig. 8. Oats in a sand-peat mixture supplied with 10 mg magnesium. From right to left: 1, 2, 3 and 5 g $CaCO_3$

TABLE 2.

Influence of the addition of $CaCO_3$ on the pH-level in a sand-peat-mixture

$CaCO_3$ in g per vessel	pH (watery suspension)	
	0 mg of Mg	10 mg of Mg
0	3,50	3,46
1	4,12	4,22
2	4,55	4,70
3	5,45	5,66
5	6,64	6,92

Observations on the development (compare figs. 6, 7 and 8).

The cultures without $CaCO_3$ did not show any development; practically no roots were formed, whilst the colour of the leaves was of a pale greenish yellow. In some cases longitudinal yellow stripes occurred. As is described above this striation was also noted in water cultures having a low pH value. Hardly any effect of the Mg supply on the development of these cultures could be observed.

In the cultures supplied with 1 g of $CaCO_3$ the plants developed far better initially: the roots showed a normal length, but on the leaves a very marked yellow striation occurred. Subsequently the growth lagged behind and several of the lower leaves died off. In these cultures, too, the poor growth was due to the high acidity, for it was equally poor either with and without a supply of 50 mg of Mg to each jar. In the presence of 2 g of $CaCO_3$, however, hardly any damage had occurred, safe for a slight striation of the leaves, and the plants developed in a fairly normal way, as long as a sufficient amount of magnesium was present. In this series it was the magnesium content which acted as a regulating factor: without any magnesium added the same symptoms could be observed as had been recorded in the magnesium-free water cultures: a poor plant development, a pale yellowish green shade of the leaves, which were somewhat rolled up, whilst the well known mottling occurred. The cultures provided with 5 mg of Mg showed a much better growth, whilst the mottling was almost lacking. A higher magnesium dose, however, still increased the crop, as shows table 3. Although slightly, yellow stripes still occurred in this series. Even in the cultures supplied with 3 g of $CaCO_3$ some striation was still visible. Solely a gift of 5 g of $CaCO_3$ was sufficient to prevent these symptoms. The production data in table 3 have also been inserted in fig. 9.

This experiment shows that, even in the presence of a sufficient amount of magnesium, a fairly normal plantgrowth in the most acid ranges, the pH value amounting to 3,5 and 4,2, is impossible. This result does not agree with that of the water cultures, where a satis-

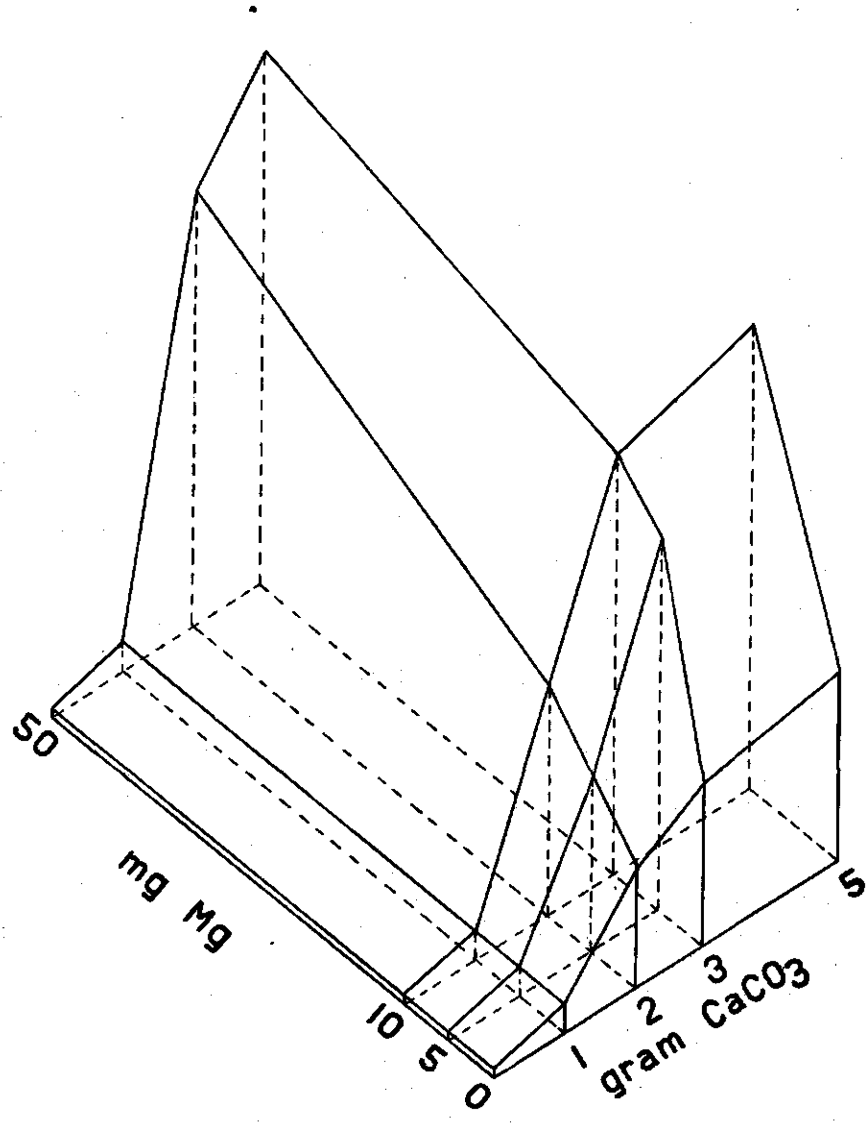

Fig. 9. Yield of crop (oats) in sand-peat mixtures, in connection with the addition of $CaCO_3$ and magnesium

TABLE 3.

Influence of the acidity and the supply of magnesium on the yield of dry material of oats (sand-peat mixtures)

$CaCO_3$ in g per vessel	no Mg		5 mg Mg		10 mg Mg		50 mg Mg	
	grain	straw	grain	straw	grain	straw	grain	straw
0	0	0,08	0	0,17	0	0,12	0	0,12
1	0	1,07	0	2,0	0	2,51	0	5,92[1]) 1,56[1])
2	0	8,51	6,07	12,42	8,13	12,26	12,87	14,12
3	0,70	10,06	8,58	15,83	11,57	16,06	17,73	15,46
5	0,75	11,59	–	–	13,14	17,10	–	–

[1]) The data of both vessels are given, because they do not coincide.

factory growth could be obtained even with a pH value of about 4. The cause of this discrepancy is still unknown.

In case of an initially moderate supply of $CaCO_3$ and $MgSO_4$ the production can as well be raised by means of a greater dose of $CaCO_3$, keeping magnesium on the same level, as by adding more Mg, whilst the pH value is left unchanged. Since in many natural soils, on which the Hooghalen disease occurs, corresponding values of about 4,5 are found, the favourable action induced by $MgSO_4$ and $CaCO_3$ in these soils may easily be accounted for. The highest production has been obtained with the largest supply of $CaCO_3$, matching a pH value of 6,5–7, and the highest Mg level. Such a pH value will be too high for many soils containing humus, because here the occurrence of manganese deficiency (grey speck disease) might be induced. Its symptoms, however, did not occur in this experimental set.

c. *Cultures in diseased soil*

In the course of 1938 we visited many fields on which the Hooghalen disease occurred, in order to get a clear picture of the symptoms and to collect soil samples for experimental cultures and for the estimation of their content of magnesium by means of *Aspergillus niger*, to be described in chapter III. These observations in the field made it clear, that the symptoms of the disease, occurring on many acid sandy and peaty soils, fully corresponded with the symptoms, induced in the water and quartzsand cultures by magnesium deficiency. In mild cases the mottling preponderates, in severe cases the leaves take on an even pale yellowish green colour, sometimes accompanied by a fading into brownish yellow of the leaf tips. The youngest leaves are narrow and spindly, and the plant development is much impaired.

The symptoms of injury due to acidity, such as occurred in water and sand cultures with a low pH value, could never be observed by us in cereals, affected with Hooghalen disease. According to VAN ITALLIE, however, they may incidentally occur. The symptoms in wheat and barley showed some difference, when compared with oats: on wheat the green mottling was usually courser and the spots were more irregular in shape, in barley the occurrence of brownish grey necrotic spots on the lower leaves was characteristic. The symptoms in both cereals agreed exactly with those, induced by magnesium deficiency in water cultures. To get better acquainted with the aetiology of the disease, we prepared in 1938 a provisional set of cylinder glasses according to NEUBAUER, filled with a number of "diseased" soils. To some of these cultures either $MgSO_4$ or CaO [1]) had been added. Again white oats was taken as a test plant. The same set of glasses was planted once again with oats in 1939, in order to study the after-effect of magnesium sulfate. Merely observations of the development were made; no production data were collected.

In the vessels without $MgSO_4$ or CaO nearly all of the oats were affected with the Hooghalen disease. The symptoms, however, disappeared after $MgSO_4$ had been added (see fig. 10) and in most cases a normal development resulted, notwithstanding the low pH value of the soil. In some cases however, although the symptoms had disappeared, the plants continued to grow poorly: they were of a blueish green. A supply of CaO effected a definite improvement in most cases. Further examination lacking it must be left open whether the poor plant growth in these single cases has to be ascribed to a damage by acidity or to an incidental poisoning by iron or aluminium. The fact that the aspect of the plants in the acid sand was entirely different from that in the water cultures does not suggest acidity as cause of the injury.

The soils on which in 1938, following the supply of magnesium, a normal development and grain formation had been recorded, produced a healthy crop in 1939 as well, without any further supply of magnesium. Moreover in 1939 a series of cultures of oats in cylinder glasses was put up, in order to study the influence not only of $MgSO_4$ but of magnesium-free $CaCO_3$ as well. In filling these vessels full attention has been paid to the need of a good structure of the soil. In order to uphold an even level of humidity water was supplied several times daily. The soil, whenever it had become silted, has been loosened over and again. In these experiments 4 sandy soils were used with a pH value of 4,1, 4,6, 3,9, and 5,4 respectively. Since the amount of soil was limited, glass cylinders were used which could contain merely $\frac{1}{2}$ and 1 kg of soil. As a basal manuring these vessels were provided with $\frac{1}{2}$ g NH_4NO_3 and 1 g K_2HPO_4 to each kg of soil. $CaCO_3$,

[1]) Later we observed, that this product contained 1% magnesium, for which reason these experiments are only mentioned incidentally.

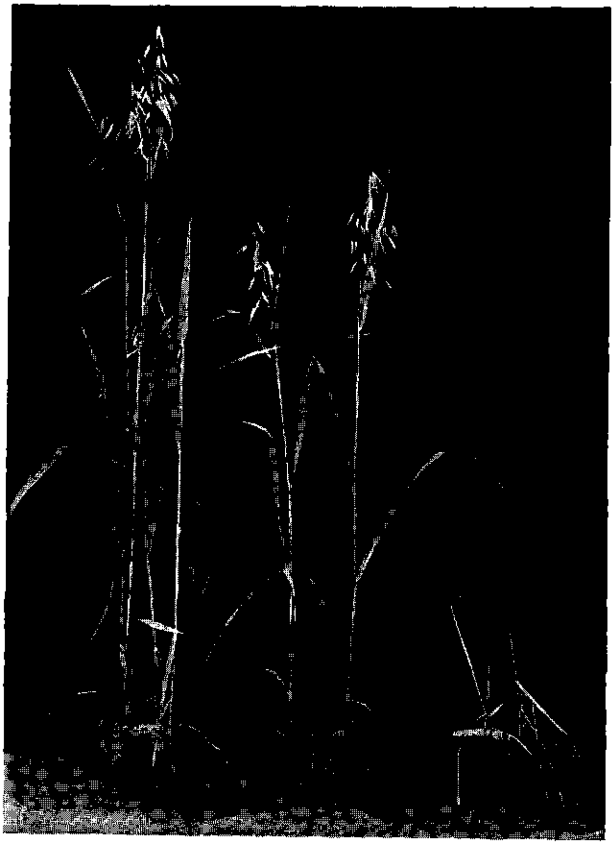

Fig. 10. Oats in acid soil. From right to left:
1. untreated
2. with CaO, containing Mg
3. with $MgSO_4$

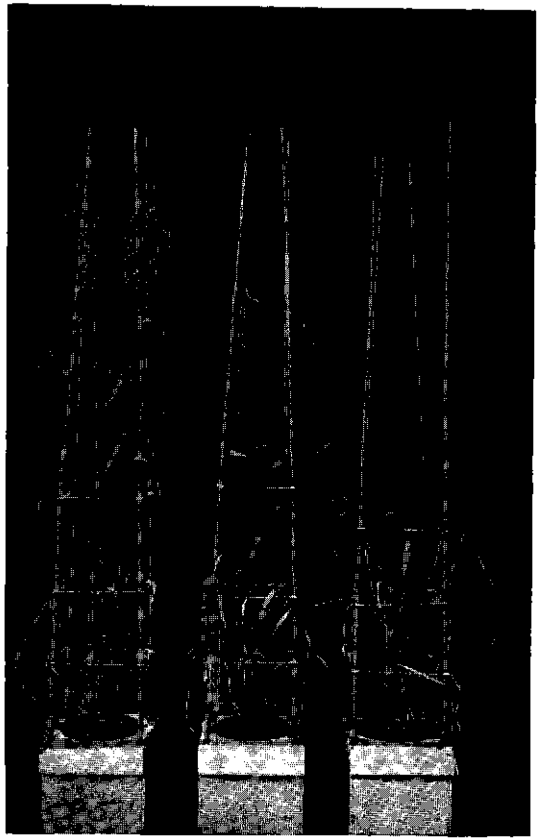

Fig. 11. Oats in acid soil. From right to left:
1. untreated
2. with pure $CaCO_3$
3. with $MgSO_4$

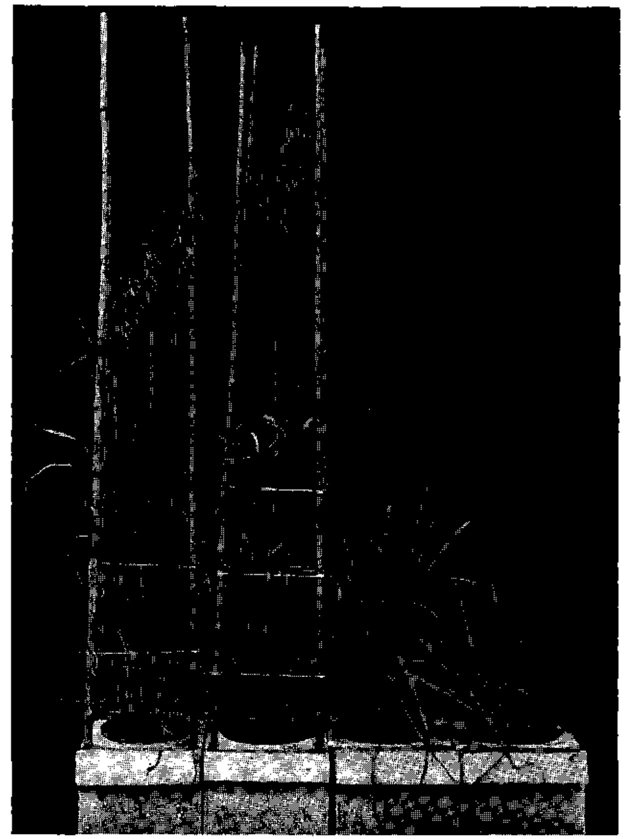

Fig. 12. Oats in acid soil. From right to left:
1. untreated
2. with $CaCO_3$
3. with $MgSO_4$
4. with $MgSO_4 + CaCO_3$

if supplied, was given in amounts of 2 g to each kg, $MgSO_4.7H_2O$ in amounts of 0,75 g to each kg of soil. White oats (Zege), planted 16-3-'39 and brought down to 3 plants per vessel, served as test plants.

Observations on the development.

It was obvious on May 15th that in soils with a low pH value the Hooghalen disease occurred very seriously. The initial mottling symptoms had vanished for the greater part and were substituted by an even greenish yellow shade. These leaves were rolled along their margins. Sometimes the colour of the lower leaves was fading into an orange yellow to red; this discoloration nearly always took its start from the tips of the lower leaves, as did the symptoms of approaching death, the youngest leaves always being least chlorotic. It was characteristic for the diseased plants that hardly any tillering occurred.

The plants provided with $CaCO_3$, however, although severely affected, developed better than the untreated ones. They tillered more abundantly, whilst the leaves, although still chlorotic, were more or less mottled. The plants supplied with $MgSO_4$ were excellent, without any chlorosis or mottling. Plants provided with $CaCO_3$ next to $MgSO_4$ were no better than those, supplied with none but $MgSO_4$.

In soil samples with a pH value of 5,4 oats showed only a slight mottling. A supply of $CaCO_3$ as well as one of $MgSO_4$ induced a normal green colour.

On June 6th the disease symptoms had in all of the plants grown less severe than on May 15th. As for those supplied with $CaCO_3$ the improvement was even more marked than in the untreated plants. It may often be noted in the field that the symptoms of disease grow less severe as the plant proceeds in development. Those supplied with $MgSO_4$ had shot their ears on May 15th, while in the other no ears had emerged yet. On June 21st in the plants supplied with $CaCO_3$ ears had shot. Merely the upper spikelets developed normally (fig. 11). Still later small ears emerged in the untreated plants, but hardly any grain developed, as is indicated in table 4.

These data indicate that on soils where the Hooghalen disease occurs a gift of magnesium may result in a very important increase of the crop. As a matter of fact a supply of $CaCO_3$ may lead to a marked improvement, but the result does not attain the level which $MgSO_4$ can induce. In the few cases where $CaCO_3$ had been supplied along with $MgSO_4$ the increase in production was somewhat higher than when solely $MgSO_4$ had been given. The fact, however, that in the latter case, where a salt is supplied which causes a decrease of the pH value of the soil, plant development and production are nearly normal, proves, that the poor growth is not caused by a low pH value,

TABLE 4.

Influence of $MgSO_4$ and $CaCO_3$ on the production of dry matter by oats, grown on diseased soil

Type of soil	Jars containing ½ kg of soil		Jars containing 1 kg of soil	
	Grain in g	Straw in g	Grain in g	Straw in g
Very diseased soil from Zelhem . . .	0	2,66	–	–
Same + $MgSO_4$ . . .	5,88	5,55	8,87	7,78
Same + $CaCO_3$ . . .	3,42	5,41	–	–
Same + $MgSO_4$ and $CaCO_3$	6,88	6,0	–	–
Very diseased soil from Ruurlo . . .	1,28	4,06	0,88	5,28
Same + $MgSO_4$ . . .	5,54	5,35	10,08	9,0
Same + $CaCO_3$ . . .	–	–	4,75	7,31
Very diseased soil from Gendringen .	2,27	3,85	3,32	6,62
Same + $MgSO_4$ . . .	6,53	6,22	9,72	9,35
Slightly diseased soil from Arnhem („Vrijland")	–	–	5,14	5,43
Same + $MgSO_4$ . . .	–	–	7,32	6,72
Same + $CaCO_3$ . . .	–	–	6,53	6,38
Same + $MgSO_4$ and $CaCO_3$	–	–	7,57	6,48
Sound spot in the diseased field	–	–	1,24	6,93

but by an insufficient supply of available magnesium. Among the Arnhem samples it was striking that a soil taken from a mildly diseased plot yielded a nearly optimal production after $MgSO_4$ had been added, whilst the crop on a sample originating from what was claimed a good plot came near to a failure, as a result of the occurrence of the "reclamation disease" (copper deficiency). Initially the latter plants developed well, with only slight Hooghalen disease symptoms, but when shooting began, the copper deficiency symptoms developed very markedly, being absent in cultures in soil which had been claimed "more diseased".

Apart from the latter samples an elaborate series of experimenting vessels was prepared with soil taken from an experimental plot of the Microbiological Laboratory at Wageningen, which some years before had been supplied with sulfur (cf. p. 7) and had become extremely acid. In this experiment glass jars have been used with a capacity of 1750 g of soil. As a basal manuring each vessel received: 0,5 g NH_4NO_3 and 1 g K_2HPO_4.

The following range has been prepared:

1. untreated.
2. with 5 g $CaCO_3$ per vessel.
3. with 2 g $CaCl_2 . 6H_2O$.
4. with 1 g $MgSO_4 . 7H_2O$.
5. with 5 g $CaCO_3$ + 1 g $MgSO_4 . 7H_2O$.
6. with 2 g $CaCl_2 . 6H_2O$ + 1 g $MgSO_4 . 7H_2O$.
7. with 20 ml of a saturated solution of $Na_2CO_3 . 2H_2O$.
8. with 1 g $MgSO_4 . 7H_2O$ and the ashes of 15 g of wheat and 15 g of oats, grown on fertile clay.
9. with 1 g $MgSO_4 . 7H_2O$ + 2 g $CaCl_2 . 6H_2O$ + 80 mg $FeCl_3 . 6H_2O$ + 20 mg H_3BO_3.
10. with 1 g $MgSO_4 . 7H_2O$ + 2 g $CaCl_2 . 6H_2O$ + 20 mg $MnSO_4 . 4H_2O$ + 20 mg $ZnSO_4 . 6H_2O$.

$CaCl_2$ was added because chemical investigation had proven the Ca-content of the soil to be very low. The ash of cereals, grown on a clay soil, which contained a large amount of the elements essential for plant growth, was added in order to establish whether acid soil, in addition to magnesium shortage, might show to be lacking in one or other important element. In order to neutralize their alcaline reaction, the ashes had been taken up in concentrated HCl, and then a Na_2CO_3 solution had been added in drops, up to the first hint of colour change in methyl red. Fe, Zn, Mn and B were added to investigate whether these elements had any influence, either favourable or injurious (SORAUER (35)).

Observations during development.

The mottling symptoms in the untreated plants occurred in a very early stage, i.e. when the second leave appeared. In the plants treated with $CaCO_3$ these symptoms initially occurred even more severely. Subsequently the latter plants developed somewhat better, but a normal production could not be attained at. $CaCl_2$ by itself was injurious; along with $MgSO_4$, however, it caused an increase in the grain production, whilst the straw production decreased. Na_2CO_3 was very injurious: no roots were formed, so the plants succombed at an early date. $MgSO_4$ induced altogether sound plants (fig. 12). This beneficial action without any doubt may be ascribed to the Mg-ions, since as a matter of fact the soil already contained an excess of SO_4-ions, thanks to the sulfur treatment.

The cultures provided with $MgSO_4$ + $CaCO_3$ initially lagged behind with those, which had been merely supplied with $MgSO_4$, but finally they arrived at a higher crop. The same holds true for the plants provided with the ash of cereals. Those supplied with Zn, Mn, Fe and B were slightly lower in development than those merely with Mg or Ca, especially as far as the straw production went.

In table 5 the air-dry matter produced has been inserted next to the pH-value, measured in watery suspension by means of the glass electrode, on June 1st, 1939.

TABLE 5.

Influence of the supply of various salts on the yield of dry matter of oats, growing in acid soil

Salts added to the soil	pH	Dry matter in g	
		Grain	Straw
Control	4,80	0	0,93
$CaCl_2$	3,97	0	0,95
$MgSO_4$	4,65	9,27	9,10
$MgSO_4 + CaCl_2$	4,46	11,90	7,29
$CaCO_3$	7,07	0,85	6,65
$MgSO_4 + CaCO_3$	6,85	11,24	11,40
$MgSO_4$ + ash of plants	4,75	13,91	9,83
$MgSO_4 + CaCl_2 + Mn + Zn$	4,46	9,80	5,67
$MgSO_4 + CaCl_2 + Fe + B$	4,40	9,17	6,12

These production data very clearly indicate the great discrepancy between the influence of $MgSO_4$ and of $CaCO_3$ in acid soil. The latter may induce a marked increase in growth; a normal development, however, is merely possible when magnesium has been added.

Discussion of the results gathered in II.

The results described above show that symptoms closely resembling those of the Hooghalen disease may be reproduced by magnesium deficiency. This not only applies to the rolling of the margins of the younger leaves and an early dying off of the elder ones, but also to the milder symptoms, e.g. the characteristic mottling. It is an important fact, that the symptoms in wheat and barley, as far as they deviate from those in oats, may be reproduced by magnesium deficiency as well. Although a treatment of the soil with $CaCO_3$ will usually result in a considerable improvement of the crop, as early as the first year, however, no normal production is to be expected. This may hardly be due to an insufficient neutralization of the soil by the $CaCO_3$, as a considerable amount of this finely dispersed material had been well mixed with the soil, some weeks before planting. Though an actual damage by acidity is very well possible in cereals, as could be recorded in the sand and peat cultures, we never observed its symptoms on the naturally acid soils, where we always meet with an insufficient magnesium supply of the plants. Accordingly the production may increase considerably by a supply of magnesium. An optimal crop, however, is not to be induced merely by this treatment in most cases;

to attain optimal development the pH value in the soil has to be increased.

We leave out of discussion here the question whether the picture of the injury to cereals, which agronomists call "Hooghalen disease", is completely covered by the symptoms of magnesium shortage observed in artificial cultures. Discussion of our results with some of our colleagues revealed their view that the symptoms, which could be cured merely by a magnesium gift, differed in some respects from those of the Hooghalen-sick crops on the field.

Nevertheless the fact remains, that the improvement in the development of the plants grown on diseased soils, could be brought about in most cases by magnesium alone. Only in a minor number of cases the addition of lime was necessary.

What, however, is the actual cause of the poor magnesium supply of plants on acid soils? First of all we tend to consider the possibility of a lack of available magnesium (GEHRING). On the other hand it can be conceived either that the plants may not be able to take up the magnesium present, or may need a greater amount in acid soils than is required in alcaline ones. It is van ITALLIE who emphasizes both latter possibilities.

Here the question arose for the first time: is there any occasion to suppose, that not all the magnesium present in the soil is available to plantgrowth, but that only part of it can be taken up by growing plants? And if this proves to be the case, which part of the total amount is available?

So the study of the availability of magnesium in these soils was needed. As method of investigation we made use of a biological one; the results obtained will be discussed in the next chapter.

III. MICROBIOLOGICAL ESTIMATION OF THE AVAILABLE MAGNESIUM IN HEALTHY AND IN DISEASE PRODUCING SOILS

Chemical methods to estimate the amount of available magnesium in soils are fairly numerous. They all are based on the exchange of the element to be estimated against other ions, added in abundance. Character and concentration of the exchange fluid are of primary importance in this method. In qualitative experiments it may in a sense be of no importance what is the solution used, so long as it is the same in each case, but in experiments concerned with the quantity of plant-available magnesium in various soils the choice of the solution is important.

In P. BRUIN's experiments (see O. DE VRIES (37)) 0,1 normal HCl was used. He estimated the "exchangeable magnesium" in 368 light, sandy soils in the Netherlands and found amounts varying between

4 and 60 mg of MgO in 100 g of dry soil. There was a sharp peak representing 8 mg, when the number of cases was plotted against the MgO content. So the fact was shown, that many sandy soils in the Netherlands are poor in available magnesium, when estimated by this method. It appeared that at least 6 mg were needed for a normal growth, but DE VRIES emphasizes the fact that in addition many factors have their influence upon the magnesium uptake by different plants, e.g. the amount of other cations present and the form of the nitrogen given.

LAVOLLAY also studied the exchange of magnesium ions (20, 21) and advocates the use of a solution of sodium acetate, because it is well buffered, so that its activity is not changed by H-ions of the soil, and a double exchange of ions is less to be expected than when ammonium acetate is used.

In choosing the concentration of the solution LAVOLLAY has considered the change of the ratio Ca/Mg which ensues, when this concentration is altered. It appears that 1/50 normal solution has certain advantages on stronger and weaker ones, consisting in a greater uniformity in the amount of magnesium solved from different soils as well as in the value of the ratio Ca/Mg. The method used by him is the following. 50 g of soil and 500 ml of 1/50 M. sodium acetate are subjected to a horizontal rotation during 3 hours, and after sedimentation the liquid is filtered off until clear. In this liquid Ca and Mg are estimated with the aid of 8-hydroxychinoline (20), after complete evaporation and destruction of organic material by heating with HNO_3. The excess of sodium ions does not interfere with the results.

LAVOLLAY's data show important differences depending on the character of the solute. So 1/20 normal HCl yields higher amounts than 1 M sodium acetate, and the latter solution extracts more than a 1/50 M. solution. LAVOLLAY indicates the amount extracted by a 1 M. solution as the "totalité du magnésium échangeable" and the amount exchanged by a 1/50 M. solution as the "cations fixés dans le complexe absorbant"; so he seems to express the view, that the former figure includes the amount dissolved from insoluble material by double decomposition with the acetate, whereas the latter is understood to contain merely the part which is present in the adsorption complex and is directly available. But it has not been established that merely the latter fraction is completely used, nor does it seem improbable that various plants can make use of part of the insoluble minerals. It therefore seems to us, that a reliable ratio between the chemically estimated amount of Ca and Mg and the plant-available quantity is lacking and that neither the methods used bij BRUIN and LAVOLLAY nor any other method using some different solution of chemicals is satisfactory in this respect.

As regards the figures obtained by LAVOLLAY, much importance is attached by him to the ratio Ca/Mg, which is obtained by using 1/50 M. sodium acetate. According to him the availability of the magnesium present would in a much higher degree depend on the value of this ratio than on the magnesium level itself. He even goes as far as to assert (p. 39) that a field, low in available magnesium and calcium needs no addition of the former, but that it is useful to a field of the same magnesium-level which contains much calcium.

Without objecting to the latter contention we are of opinion that the former can only be true to a very limited extent. Broadly speaking JAVILLIER and LAVOLLAY only consider as normal soils, where the ratio Ca/Mg, estimated by extraction with 1/50 M. sodium acetate is not considerably above 8 (expressed in milliaequivalents). Values below 8 would indicate fields with sufficient magnesium. Although we agree that a certain ratio of the said ions may be of value for the assimilation of the magnesium and that this may be interfered with when the ratio rises beyond a certain limit, our investigations have made it highly improbable, that the absolute amount of magnesium might not play its part.

Our first aim has been to find a method furnishing a better correlation between the amount of magnesium estimated in soil and that taken up by plants; to that end we have chosen a biological method, because we expected it to serve this end better than a chemical one.

We first of all tried the mould *Aspergillus niger*, which has proven of considerable value as a test organism in our earlier investigations on the relation of copper to the development of plants and microorganisms (25).

The need of magnesium for the growth and spore formation of this mould is known since 1894 (MOLISCH (24)); of later years (1913) JAVILLIER (14) has anew emphasized this fact. NIKLAS, POSCHENRIEDER and FREY (28) point out, that it was J. TRISCHLER who was the first to use *Aspergillus* in his investigations of the magnesium content of soils. In magnesium free solutions, to which a known amount of soil has been added, the growth of the mould is said to correlate well with the amount of available magnesium present in that soil; accordingly this amount is established by weighing the dry mycelium developed by the mould. E. SPINDLER (36) in his investigation of 22 different soils found a fair agreement between growth of the mould and the results of his field experiments.

In our experiments, carried out along somewhat different lines, a solution of the following composition was used.

H_2O, distilled in a glass apparatus	1000 cc
glucose	50 g
KNO_3	5 ,,

K_2HPO_4	2,5	g
$Na_2SO_4.10H_2O$	1,0	,,
$FeCl_3.6H_2O$	0,02	,,
$ZnSO_4.7H_2O$	0,01	,,
$MnSO_4.4H_2O$	0,003	,,
$Na_2MoO_4.2H_2O$	0,0015	,,
$CuSO_4.5H_2O$	0,001	,,

In later experiments 10 mg Ca (as $CaCl_2$ or $CaH_4(PO_4)_2$) has been added to all cultures. The calcium has no effect on the development in the lower and higher concentrations of Mg, but it induces a better development of spores in cultures with 75 and 100 mg Mg. In this range it renders the method more sensitive.

Before filling this solution into the steam sterilized 1 liter Erlenmeyer flasks, they were provided with 3 grams of dry soil, in order to moisten this dry material by the condensed steam, left by the sterilization. In this way the floating of dry soil particles on the 40 ml nutrient solution used for the experiment is prevented. Finally a suitable amount of mould spores is added, suspended in a few ml of sterilized water or nutrient solution, and the flasks are kept at 30 °C for 5 days. The development of the mould is then compared with a standard range of cultures in the same solution, containing 0, 25, 50, 75, 100, 150, 200, 300, 400 and 500 γ of Mg, given as $MgSO_4.7H_2O$. With no magnesium added, there is no development whatever [1]), with 25 γ a slight growth of sterile mycelium occurs and this is more abundant with increasing amounts up to 150 γ, where a development of black spores sets in. With 400 γ and above this amount formation of spores is complete, so that there is hardly any difference between 400 and 500 γ of magnesium (fig. 13).

It might be objected, that the difference between the so-called "chemical" methods, based upon the extraction of magnesium ions from the soil by a solution of an excess of other ions (H or acetate, in different concentrations) and this "biological" method, is only imaginary, because here, too, it is the amount of magnesium which the above solution of glucose and salts is able to extract from the soil added, on which the mould develops. Without denying the similarity it may be said, that it is difficult to see whether any correlation will exist between the amount of magnesium extracted from widely different soils by 0,1 normal HCl or 1/50 mol. acetate and the growth of, say, cereals. In particular it will be difficult to establish a borderline, separating rich from poor soils, in which the amount of other ions

---

[1]) NIKLAS and TOURSEL have found a fairly good development of Aspergillus niger in their blancs, doubtless so because the salts in the solution used were not free of magnesium (see the illustration on p. 95 of (29).

3

γ Mg

0

25

50

75

100

150

200

250

300

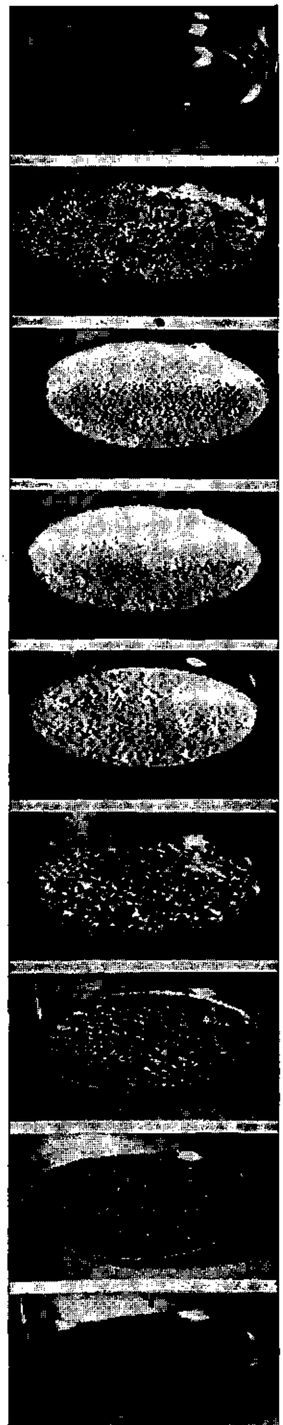

Fig. 13. Growth of *Aspergillus niger* in presence of increasing amounts of magnesium. Erlenmeyer flasks are used, containing 50 ml of the nutrient liquid

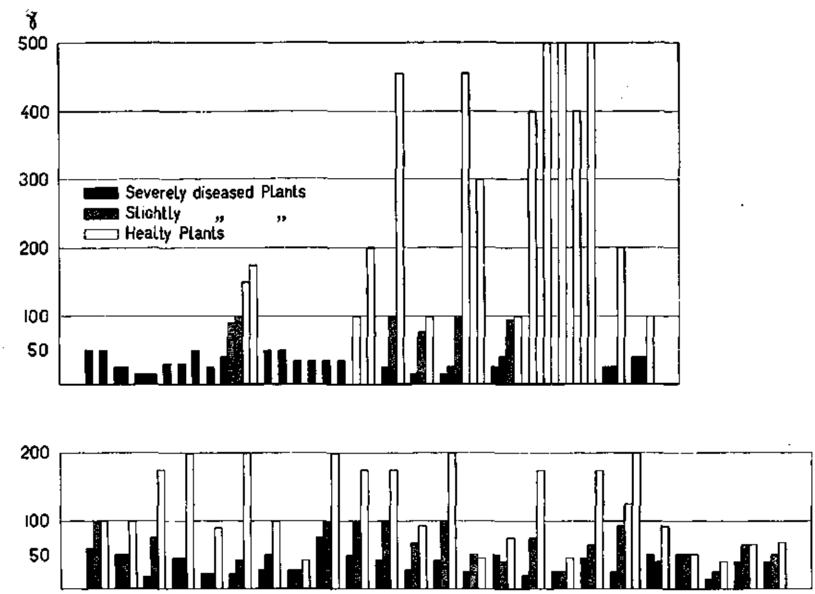

Fig. 14. Available magnesium in γ per 3 grams of soil

may vary so widely. As regards the growth of *Aspergillus* under the conditions described, wherein the contact with the soil lasts during the whole of the mould's development, the similarity with a plant growing in soil is doubtless more striking, and it is to be expected, and it will be shown lower down, that this development tells more about the ratio between available and unavailable magnesium in the soil than chemical methods are able to do. Moreover estimations and experiments to be described will show, that the relation between the amount of magnesium found and the occurrence of the Hooghalen disease is very close. Finally, large series of samples may be easily estimated at a time and the results are gathered after 4–5 days without any intermediate work.

Since it is known, especially after the investigations of VAN ITALLIE (12), that addition of potassium to soils increases the need of magnesium and aggravates the disease, which is caused by a lack of this element and as it seemed possible, that with the mould also the requirement of magnesium might vary with a changing ratio of the ions in the solution, experiments were carried out in order to study the influence of the amount of potassium ions on the growth of mould.

We used a nutrient solution, in which $NaNO_3$ had been substituted for 3/5 of the KNO_3 and $Ca(H_2PO_4)_2$ for K_2HPO_4, and another solution to which an extra $\frac{1}{2}$ g of KCl had been given per 40 ml, and this was compared with a fourth one, where an aequivalent amount of $CaCl_2$ (723,8 mg) took the place of KCl.

It was proved that the magnesium requirement of the mould remained unchanged. Only the colour of the spores was slightly altered and the sterigmata were shorter. Decrease of the concentration of all inorganic salts, keeping the sugar concentration constant, likewise left the standard unchanged.

The influence of a change in pH was also estimated and it was found that a value as low as 2 increased the magnesium requirement a little: 200 γ of magnesium in this case resulted in a sterile mycelium, but the addition of an acid soil (pH 4,1) or its watery extract had no injurious influence. It was found on the contrary that the presence of the soil somewhat enhanced the development of the mould, especially with the higher dose of Mg.

Besides this mould, also *Azotobacter chroöcoccum* may be used for the estimation of the available magnesium in soils. To that end agar plates were used, which were freed of this element by a special method, described in a recent paper by one of us in the journal ANTONIE VAN LEEUWENHOEK (26). We refrain from going into details and results here.

Results of the experiments with Aspergillus niger.

The figures obtained with a great many different sandy and peaty soils, healthy and diseased, are collected in Table 6 and fig. 14. pH was measured in watery suspension (1 part on 2,5 parts of water) with the glass electrode.

TABLE 6. *Available magnesium in „diseased" and „healthy" soils*

No.	Origin of soil sample	Kind of crop in field	State of crop in field	State of Neubauer cultures (oats)	pH	Available Mg in 3 g of airdried soil (γ)
1	*Ede*	rye	much injured	much injured	4,3	< 50
	Ede + $MgSO_4$	–	–	sound	–	> 500
2	*Heuven a* [1])	oats	much injured	much injured	4,2	< 50
	Do.+$MgSO_4$ [2])	–	–	sound	–	> 500
	Heuven b	oats	slightly injured	injured	4,4	50
	Heuven c	oats	sound	sound	4,8	100
3	*Lunteren a*	oats	slightly injured	much injured	4,1	< 50
	Do. + $MgSO_4$	–	–	sound	–	> 500
	Lunteren b	oats	sound	slightly inj.	4,4	50
4	*Rheden a*	oats	much injured	much injured	4,0	< 50
	Do. + $MgSO_4$	–	–	symptoms gone, poor developm.	–	> 500
	Rheden b	oats	slightly injured	much injured	4,7	–
5	*Wolfheze a*	rye	much injured	much injured	4,2	50
	Do. + $MgSO_4$	–	–	sound	–	> 500
	Wolfheze b	rye	slightly injured	injured	4,5	–
6	*Ede*	oats	much injured	much injured	4,1	50
	Do. + $MgSO_4$	–	–	no sympt., poor dev.	–	> 500
7	*Rheden a*	oats	much injured	much injured	4,4	50
	Do. + $MgSO_4$	–	–	sound	–	> 500
	Rheden b	oats	slightly injured	much injured	4,5	50
8	*Gasselte*	wheat	sound	sound	5,5	> 500
9	*Marum*	oats	sound	sound	5,5	> 500
10	*Sappemeer*	oats	sound	sound	5,0	4–500
11	*Wageningen*	oats	sound	sound	5,0	100
12	*Wageningen*	oats	sound	sound	5,0	300
13	*Wageningen*	wheat	sound	sound	–	3–400
14	*Zuidlaren*	oats	sound	sound	–	200
15	*Wolfheze*	oats	much injured	much injured	4,6	< 50
	Do. + $MgSO_4$	–	–	sound	–	> 500
16	*Ede*	oats	much injured	much injured	4,6	< 50

[1]) a, b, c indicate different areas in a same field.

[2]) Added to the collected soil samples before putting them to the Neubauer test.

It may be concluded from these figures, that fields, showing the Hooghalen disease in their crops, contain a very low amount of available magnesium. pH of these soils is mostly low: 5 or even 4,5 and less. Soils of healthy fields, or healthy areas in diseased fields, show a considerably higher content of magnesium. The low pH is not the cause of the poor development of the mould; this is proven by the fact, that addition of $MgSO_4$ materially improves this growth, whereas magnesium-free $CaCO_3$ has no effect.

A second series of soils was collected from experimental plots which had been provided with increasing amounts of lime on the fields of Prof. HUDIG in Wageningen. The plots without any lime showed the disease badly. Moreover 6 acid soils, labelled 2, 22, 31, 51, WESTLAND and WILLEMSE, all equally diseased, were studied. The results are recorded in Table 7.

TABLE 7. *Available magnesium in soils of various H-ions concentration*

Soil sample	State of crop in field	Available Mg in 3 g of air-dried soil (γ)
L 1	much injured	<25
L 2	do.	<25
L 3	improvement as figures increase	<25
L 4		100
L 5		4–500
L 6		4–500
G 1	much injured	<25
G 2	do.	25
G 3	improvement as figures increase	50–100
G 4		100
G 5		100
G 6		100
H 1	much injured	<25
H 2	do.	<25
H 3	badly injured	<25
H 4	improvement as figures increase	25
H 5		100
H 6		4–500
H 7		4–500
H 8		4–500
Westland	badly injured	50
Willemse	,, ,,	50
pp 2	,, ,,	<25
pp 22	,, ,,	<25
pp 31	,, ,,	<25
pp 51	,, ,,	<25

The correlation between the occurrence of the Hooghalen disease and a low amount of available magnesium (50 γ and less) is again quite striking, and once more the addition of magnesium results in a better growth of Aspergillus.

The results of a third set of soil samples from all parts of the Netherlands are shown in table 8.

TABLE 8.

Available magnesium in samples taken from different areas in plots of oats with „Hooghalen disease"

No.	Origin of soil sample	State of crop in field	pH	Available Mg in 3 g of air-dried soil (γ)
1	*Eibergen* (A. Schepers) . . .	Badly injured	–	<25
2	Do.	Slightly injured	–	75–100
3	Do.	Sound	–	100–150
4	Do.	Sound (turnips ploughed under)	–	200
5	*Zelhem* (Wassinkbrink) . . .	Injured	4,10	<25
6	Do.	Slightly injured	4,63	<25
7	Do.	Sound	5,45	200
8	*Eerbeek* (H. J. Harmsen) . .	Badly injured	4,22	25
9	Do.	Slightly injured	4,51	25
10	Do.	Sound	5,81	75–100
11	*Klarenbeek* (H. Mulder)	Badly injured	4,51	25
12	Do.	Slightly injured	4,80	25–50
13	Do.	Sound	5,17	150
14	*Achterhoek, Wilp* (E. J. Hemeltjen).	Badly injured	5,20	50
15	Do.	Slightly injured	5,64	100
16	Do.	Sound	5,78	150–200
17	*Ruurloo* (A. Klein)	Badly injured	4,62	25–50
18	Do.	Slightly injured	4,83	25–50
19	Do.	Sound	4,87	100
20	*Lonneker* (J. Peuk)	Injured	4,40	50–100
21	Do.	Slightly injured	4,80	100
22	Do.	Sound	4,76	200
23	*Hellendoorn* (M. H. Vasseveld)	Badly injured	5,02	25
24	Do.	Slightly injured	4,47	25
25	Do.	Sound (?)	4,90	25–50
26	*Oud Ootmarsum* (Oude Vrielink)	Injured	4,43	25
27	Do.	Slightly injured	4,79	50
28	Do.	Sound	4,93	100
29	*Beuningen* (H. J. Groener) manured with 1500 kg dolomite marl	Injured	4,16	25
30	Do.	Slightly injured	4,29	75
31	Do.	Sound	4,68	150–200
32	*Voorst* (E. G. W. Dieken)	Badly injured	–	<25
33	Do.	Injured	–	<25
34	Do.	Sound	–	25–50
35	*Voorst* (J. F. Schepers) . .	Injured	4,38	25–50
36	Do.	Slightly injured	4,43	100
37	Do.	Sound	5,00	150–200

No.	Origin of soil sample	State of crop in field	pH	Available Mg in 3 g of air-dried soil (γ)
38	*Smilde* („dalgrond", peat soil)	Badly injured (?)	4,49	200
39	Do.	Slightly inj. (?)	4,88	200
40	Do.	Sound	4,52	150–200
41	„*Vrijland*", *Arnhem*	Badly injured	4,94	a trifle <50
42	Do.	Slightly injured	5,70	a trifle <50
43	Do.	Sound	5,15	a trifle <50
44	*Langendijk* (W. Leupen) Reclamation of moorland, very poor in humus, manured with 700 kg powdered burned lime.	Badly injured	–	0–25
45	Do.	Slightly injured	–	a trifle <25
46	Do.	Nearly sound	–	25–50
47	*Steggerden* (A. Spel) 2 days before sampling manured with „patentkali", [1]	Injured	–	100
48	Do.	Sound	–	200
49	*Vriezenveen* (J. Kakes)	Injured	5,04	<25
50	Do.	Slightly injured	5,73	50–100
51	Do.	Sound	6,08	150
52	*Twekkelo* (W. Spieler)	Injured	5,16	50
53	Do.	Slightly injured	5,27	100
54	Do.	Sound	5,12	100
55	*Heurne* (Jansen)	Injured	5,16	25
56	Do.	Slightly injured	5,32	50–75
57	Do.	Sound	4,72	75–100
58	*Te Kempel*	Injured	–	25–50
59	Do.	Slightly injured	–	a trifle <50
60	Do.	Sound	–	200
61	*A III*	Badly injured	–	25–50
62	*C III*	Slightly Injured	–	50–75
63	*B III*	Sound	–	150–200
64	*Hagedoorn, Epe*	Badly injured	4,16	<50
65	Do.	Slightly injured	4,29	<50
66	Do.	Sound	4,68	75–100
67	*Experimental farm, Haarloo* .	Badly injured	–	25
68	Do.	Nearly sound	–	75–100
69	Do.	Sound	–	100
70	Do.	Hooghalen disease caused by an excess of K	–	25–50
71	Ir. Cleveringa's experim. field on lime status, *Groot Graffel* II *Warnsveld* (Samples from $NaNO_3$ field, pH = 4,05) 1	Badly injured	–	25–50
72	2	Fairly normal development	–	50–75

[1]) A mixture of sulfate of potassium and sulfate of magnesium.

No.	Origin of soil sample	State of crop in field	pH	Available Mg in 3 g of air-dried soil (γ)
73	Plot with pH = 4,4	Fairly normal development	–	100
74	Plot with pH = 5,5	Normal development		150
75	Plot with pH = 6,95	Normal development	–	150–200
76	Ir. Cleveringa's experim. field in *Vorden* (Beunk) 1. no lime or Mg or potassium	Badly injured	4,98	0–25
77	2. CaO	Fairly normal development	6,69	nearly 50
78	3. CaO + $MgSO_4$	Normal development	–	100–150
79	4. $MgSO_4$	Symptoms gone, but poor development	4,52	100–150
80	5. Kencika	Injured	4,49	25
81	6. Kencika	Sound	6,52	100–150

These figures again demonstrate the close relation which exists between the occurrence of the disease and a low content of available magnesium. There are, however, a few exceptions, where soil from an apparently sound spot shows too low a figure and, conversely, where a field bearing a very diseased crop shows a relatively high one. In the former case it was found, that pH was fairly high, viz. above 5, and it is inferred, that this circumstance might have improved plant-growth, whereas the development of the mould has not increased in a same measure. In chapter II, table 4 and 5 more examples have been given, where increase of pH in pot experiments results in a better growth, whilst the experiments with Aspergillus niger have not shown the same trend. VAN ITALLIE concludes from his experiments that in such a case absorption of magnesium by root cells is facilitated. We, however, are of opinion, founded on the result of the experiments described in chapter II and in the data furnished by analysis of plant material, which will be presented in the next chapter, that a better development of the plant roots in less acid soils may mainly explain the improved absorption of magnesium.[1])

It may be added, that such a case appears to be very rare, and that the disease in the Netherlands is practically limited to acid fields.

[1]) That, however, the influence of pH on the Mg-uptake by the roots of corn and rye in a nutrient solution is not negligeable, is shown in a recent study by G. MICHAEL (Bodenk. u. Pflanzenern. *25*, 65, 1941).

TABLE 9.

Origin of soil sample	pH	Exchangeable by Na-acetate, 1/50 M. Ca mg p. 100 g.	Ca m. eq. p. 100 g.	Mg mg p. 100 g.	Mg m. eq. p. 100 g.	Ca/Mg mg	Ca/Mg m. eq.	Available Mg for Aspergillus niger in 3 g of air-dried soil (γ)	State of crop in the field
No. 32	6,3	46,2	2,310	4,630	0,386	10	6	250	normal
K 1	4,8	15,2	0,760	0,300	0,025	50	30	<25	injured
K 2	5,1	16,5	0,825	1,980	0,165	8	5	100	nearly normal
K 3	5,15	29,1	1,460	2,184	0,182	13	8	>200	normal
H 1	5,2	15,5	0,776	0,512	0,043	30	18	50	injured
H 2	5,3	18,2	0,910	0,693	0,058	26	16	75	slightly injured
H 3	5,0	16,5	0,825	0,905	0,076	18	11	150	normal
Sl 1	4,85	15,2	0,759	0,905	0,076	17	10	<25	badly injured
Sl 2	4,7	12,9	0,645	1,030	0,086	13	7,3	25	slightly injured
S 1	4,0	5,3	0,264	0,023	0,0019	230	139	0	sterile
S 2	4,3	7,26	0,363	0,021	0,0017	346	213,5	<25	badly injured
S 4	5,4	10,6	0,530	0,400	0,033	26	16	150–200	normal
B 5	4,4	13,0	0,650	0,198	0,016	66	41	<25	badly injured

To ensure a better comparison between the results of the chemical investigations of our samples and the data established along biological lines, we present a table containing the amounts of Ca and Mg, determined with LAVOLLAY's method, the results of the estimation with *Aspergillus* and the state of the crops (table 9).

It will be seen, that in most cases diseased soils show a higher ratio Ca/Mg than healthy ones; the figure 8, however, regarded by LAVOLLAY as lying on the border line between those two groups, can not be held to be a reliable limit. The data for the available magnesium correlate, however, in a same measure with the occurrence of the disease, as has already been indicated. A content of 50 γ or less in 3 g of soil in all probability indicates a soil which will induce disease.

We conclude that the insufficient magnesium supply to plants on acid soils is primarily caused by a shortage of available magnesium and may only secondarily be attributed to difficulties in the uptake of Mg caused by too high a level of H- or K-ions.

It appears that this uptake of magnesium is influenced also by the nitrogen fertilisation. Investigations now being carried out by one of us (E.G.M.) at the Agricultural Experiment Station at Groningen show the influence of nitrogen fertilization, qualitatively (NH_4^+ or NO_3^-) as well als quantitatively, on the magnesium uptake by cereals. Whether these phenomena are to be attributed to an "antagonism" between these ions or to an acidification due to a preferential absorption of NH_4-ions, has still to be investigated.

IV. INVESTIGATIONS ON THE CONTENTS OF CALCIUM AND MAGNESIUM IN HEALTHY AND DISEASED PLANT MATERIALS

The magnesium content of plant material was estimated with 8-oxychinoline as well as biochemically with *Aspergillus*. In using the chemical method LAVOLLAY's description (20) was followed with merely slight modifications. The plant material is incinerated in small porcelain troughs at 500–600 °C. The ashes are taken up in hydrochloric acid. After the addition of a few drops of methylred solution, a few drops of acetic acid and an excess of ammoniumoxalate, the solution is neutralized with carbonate of soda, until the red colour of the methylred begins to fade. After heating on a waterbath during 10 minutes the calciumoxalate can be filtered off and Ca is estimated in the usual way with $KMnO_4$. The filtrate is evaporated until 10–20 ml are left; these are transferred to a centrifugal tube of 100 ml which is provided with a 2,5 per cent solution of 8-oxychinoline in 4 per cent acetic acid. After adding a few drops of thymolblue solution the liquid is neutralized by means of the gradual addition of a saturated solution of sodium carbonate. Most of the metals, Mg included, are precipitated (the voluminous flocks often make it difficult to observe exactly the moment when the indicator changes its colour). The mixture is heated during 10 minutes on a waterbath, then cooled down and centrifugated. The clear fluid is drawn off by means of a thin glass tube.

Now the Mg-chinolate in the precipitate must be separated from the other chinolates present. To that end a mixture of 1 vol. of chloroform and 4 vol. of alcohol, alcalized with a 0,4 per cent ammonia solution, is added and the mixture is heated during 1 minute on a waterbath. The Mg-salt is insoluble, the other chinolates are solved. After cooling and centrifuging the clear liquid is again drawn off. This procedure is repeated twice and the precipitate is brought on a SCHOTT's glass filter, covered with a thin layer of pure cellulose. After the liquid has passed through, the filter with the precipitate is dried till the solvents have evaporated; then the Mg-chinolate is solved in 10 per cent hydrochloric acid. After this the chinoline in this solution is estimated by the bromine method. Stoppered bottles are used of ca. 300 ml capacity, containing glass tubes of ca. 50 ml. The hydrochloric solution is transferred to the bottle and an excess of KBr-solution is added. The tube is provided with a solution of KJ and an exactly measured amount of a known $KBrO_3$-solution is added to the liquid in the bottle. Now the bottle is stoppered and its contents are mixed during 3 minutes without contamination with the KJ solution in the tube. By that time the bromation is complete and the excess of bromine is estimated by transferring the contents of the tube into the main solution; the iodine formed is titrated with 0,1 norm. thiosulfate and starch solution.

For the biological estimation of Mg in the ashes, the solution neu-

TABLE 10.

Magnesium and calcium in plant matter from crops raised in experimental vessels

Origin of soil	State of plants in experim. vessel	Plants matter	Yield p. pot (g)	Ca (mg)	Mg in mg		Mg per kg dry matter (g)
					Aspergillus method	Oxychinoline method	
Zelhem	badly injured	grain	0	–	–	–	–
		straw	2,19	2,75	0,4	0,30	0,137
Zelhem	badly injured	grain	0	–	–	–	–
		straw	3,14	2,70	0,4	0,39	0,124
Do. + $CaCO_3$	injured	grain	3,19	–	1,25	1,62	0,508
		straw	5,10	11,5	0,3	0,22	0,043
Do. + $CaCO_3$	injured	grain	3,64	–	1,25	1,68	0,462
		straw	5,72	13,5	0,45	0,80	0,140
Zelhem + $MgSO_4$	sound	grain	6,01	–	6,9	7,35	1,223
		straw	5,60	2,8	5,5	6,97	1,245
Do. + $MgSO_4$	sound	grain	9,08	–	12,5	12,85	1,415
		straw	7,67	3,2	8,0	8,80	1,147
Do. + $MgSO_4$	sound	grain	8,66	–	10,0	12,80	1,478
		straw	7,90	–	9,0	9,21	1,166
Do. + $CaCO_3$ and $MgSO_4$	sound	grain	7,02	–	8,8	9,60	1,367
		straw	6,14	10,0	5,5	6,89	1,122
Do. + $CaCO_3$ and $MgSO_4$	sound	grain	6,77	–	7,5	7,42	1,096
		straw	5,85	8,5	4,0	4,86	0,831
Do. sound spot in diseased field	sound	grain	3,69	–	3,3	3,74	1,014
		straw	3,74	4,8	1,0	0,66	0,177
Do. sound spot in diseased field	sound	grain	3,91	–	5,0	4,47	1,143
		straw	3,73	5,0	1,6	1,59	0,426
Klein, Ruurlo	badly injured	grain	1,28	–	1,0	0,68	0,531
		straw	4,06	2,4	0,4	0,38	0,097
Do.	badly injured	grain	0,88	–	0,8	0,57	0,648
		straw	5,28	–	0,7	0,51	0,097
Do. + $CaCO_3$	injured	grain	4,34	–	4,1	3,67	0,846
		straw	7,36	–	0,5	0,58	0,079
Do. + $CaCO_3$	injured	grain	5,17	–	4,1	3,87	0,749
		straw	7,27	–	0,5	0,46	0,063
Do. + $MgSO_4$	sound	grain	5,54	–	6,7	7,0	1,264
		straw	5,34	3,0	4,5	6,73	1,260
Do. + $MgSO_4$	sound	grain	10,08	–	10,0	12,63	1,253
		straw	9,00	5,6	5,5	8,17	0,901
OP, „Vrijland" Arnhem	slightly injured	grain	5,18	–	5,0	5,48	1,058
		straw	5,41	6,0	0,4	0,35	0,065
Do.	slightly injured	grain	5,10	–	4,1	4,54	0,890
		straw	5,45	6,2	0,4	0,30	0,055
Do. + $CaCO_3$	sound	grain	6,63	–	5,8	6,0	0,905
		straw	6,47	11,0	0,4	0,31	0,048
Do. + $CaCO_3$	sound	grain	6,43	–	5,8	4,85	0,754
		straw	6,3	9,1	0,6	0,34	0,054

tralized with carbonate of soda is transferred to the Erlenmeyer flask containing 40 ml of the usual nutrient solution for *Aspergillus*. By comparing the growth of this mould with a standard series the amount of magnesium is found. The results of this method are less accurate than those of the chemical method, but the former one has the advantage that it allows of the examination of a large number of samples in a short time.

The material used for the estimation of magnesium was taken from pot experiments in 1939. The results are tabulated in table 10 (see p. 32).

It may be concluded from these data, that diseased plants contain very little magnesium. The plants from the healthy spots and those from the slightly diseased OP-soil contained considerably more of this element. It is noted that nearly all of the magnesium is contained in the grain: the magnesium level of the straw is 10–15 times lower. In the plants from soils treated with $MgSO_4$, however, the contents of straw nearly equals that of the grain.

Although the magnesium level in the plants from soils treated with $CaCO_3$ is still fairly low, the total quantity of magnesium taken up by these plants is considerably higher than the amount in the plants from the untreated soils.

V. CAUSES OF THE SMALL AMOUNT OF AVAILABLE MAGNESIUM IN ACID SOILS, AND OF ITS INCREASE FOLLOWING NEUTRALIZATION

In order to decide whether the low content of available magnesium in acid soils results from a shortage of the element or from the non-availability of the magnesium present, five soil samples were heated for two hours at 500–600°, and the ashes were investigated by means of *Aspergillus*. Notwithstanding the fact that the available amount in the original soil was low, 25 γ per 3 g at most, the content of the ash of 3 g of the various samples was considerably higher, viz. 150, 600, 450, 900 and 150 γ. Thus it is clear that calcination sets free a large amount of magnesium which presumably had been bound in organic (humic?) material. Another possibility viz. the splitting of the soil particles so as to form new cleavage surfaces of magnesium bearing minerals, seems hardly probable in these sandy soils.

So the question arose, whether the low amount available has to be attributed to a fixation under the influence of an excess of H-ions (as Gehring (8) contends) or that the initial amount has disappeared with increasing acidity.

In order to answer this question increasing quantities of normal H_2SO_4 were applied to a sound soil ("dalgrond"), so that the following acidities were reached: 4,1, 3,9, 3,6, 2,8, 2,2, The mixtures were left during two days in this moist condition, then were dried and investigated by means of the *Aspergillus* method. The results proved that the amount of available magnesium was left unchanged, hence no fixation of the element had taken place. In the sample with the lowest pH the mould had even slightly better developed than in the others. In a second series the mixtures were left moist during 2½ months before drying, but neither in this case anything like a fixation could be recorded. When, however, these acid samples were leached with distilled water, until no further SO_4-ions occurred in the filtrate, nearly all of the magnesium appeared to be lost. This fact indicates that the injurious action of a low pH does consist in a leaching-out of the available magnesium.

The same conclusion was reached by experiments with soil samples taken from the experimental plot in the garden of the Microbiological Laboratory in Wageningen, where sulfur had been given in increasing amounts, with the intention to induce the Hooghalen disease. This had been successful, as may be taken from the experiment cited above (see table 5), and the investigation with *Aspergillus* established the fact, that the increase in acidity caused by the addition of sulfur had reduced the amount of available magnesium to practically nothing (table 10*a*). A similar result could be attained when the non-treated soil (with ca. 200 γ of available magnesium per 5 g) had been treated during 18 hours with 1/20 normal hydrochloric acid and washed until free from acid. Growth of mould was then inhibited but it was restored when 500 γ of magnesium were given. The addition of magnesium-free $CaCO_3$, however, caused no improvement whatever. Incineration of the soil resulted in an increase of the available magnesium from less than 25 γ to 600 γ per 3 g.

These acidulated soil samples were also studied chemically and it could be proved, that the acidification, with sulfur as well as with mineral acids, had resulted in a nearly complete disappearance of the total calcium content. The total magnesium, on the contrary, had only slightly decreased and it proved to be only the plant- and mould-available part which had disappeared, whereas the non-available magnesium had remained. The figures taken up in table 10a substantiate these facts.

TABLE 10A. *Mg and Ca contents of soils provided with sulphur*

	pH	Ca in 100 g (mg)	Mg in 100 g (mg)	Available Mg in 5 g (γ)
s_1	4,0	5,2	20,4	0
s_2	4,3			25
s_3	5,1			100
s_4 (untreated)	5,4	86,3	21,9	200

This difference in the behaviour of calcium and magnesium is a fact of general occurrence, as is proven by the analyses of HASEBÄUMER, BALKS and BACH (9), abstracted in tables 11 and 12. These authors emphasize, that between pH and CaO-content of various soils (estimated with fuming HCl) there is a definite correlation, not, however, between pH and other elements, determined by the same means.

TABLE 11.

pH, CaO and MgO in various areas in a same field (Hasebäumer and co-workers)

pH in soil	CaO in % (conc. HCl)	MgO in % (conc. HCl)
5,9	0,460	0,291
5,5	0,305	0,398
5,2	0,240	0,341
5,0	0,230	0,387
4,6	0,123	0,350
4,5	0,115	0,350

TABLE 12.

pH, MgO and CaO (soluble in conc. HCl) in a number of soils from different fields (Hasebäumer and co-workers)

pH	CaO in %	MgO in %	Organic matter
6,6	0,36	0,33	3,31
6,5	0,33	0,32	2,42
6,2	0,23	0,31	2,61
5,7	0,28	0,33	3,32
5,6	0,31	0,38	4,14
5,4	0,18	0,32	3,50
5,4	0,22	0,48	2,83
4,7	0,15	0,35	3,32
4,7	0,10	0,16	6,50
4,6	0,14	0,39	4,42
4,6	0,07	0,85	1,87
4,2	0,09	0,10	19,02
4,2	0,08	0,15	27,50

If HASEBÄUMER and co-workers had determined the mould-available magnesium in stead of the HCl-soluble amount, they would doubtlessly have found, that there is also a correlation between magnesium and pH.

In order to see whether any correlation exists between the amount of available potassium and pH, the potassium which can be assimilated by *Aspergillus* was determined in a number of soils. To this end a nutrient solution of the following composition was used:

distilled H_2O	1000	cc
glucose	50	g
$NaNO_3$	5	,,
Na_2HPO_4	2,5	,,
$MgSO_4 . 7H_2O$	1,0	,,
$FeCl_3 . 6H_2O$	20,0	mg
$ZnSO_4 . 7H_2O$	10,0	,,
$MnSO_4 . 4H_2O$	3,0	,,
$Na_2MoO_4 . 2H_2O$	1,5	,,
$CuSO_4 . 5H_2O$	1,0	,,

This investigation went along the lines followed in the magnesium research, except that 5 g of soil were used in stead of 3 g. For the determination a standard series with known amounts of potassium was used. The results are taken up in table 13.

TABLE 13.

Occurrence of the Hooghalen disease and content of available Mg and K

Origin of the soil	State of plants	Available Mg in 3 g of air-dried soil (γ)	Available K in 5 g of air-dried soil (γ)
Vriezenveen (J. Kakes)	badly injured	<25	200–300
Vriezenveen (J. Kakes)	sound	150	a trifle <300
Ede D (pH = 4,6)	badly injured	<50	300–400
Rheden 3	badly injured	<25	500–1000
Wolfheze 2	badly injured	25	500–600
Rheden	badly injured	<25	500–1000
Zelhem (pH = 4,1)	badly injured	<25	a trifle <500
Zelhem (pH = 5,45)	sound	200	a trifle <500
Marum	sound	> 500	> 2000
Wolfheze P (pH = 4,2)	badly injured	50	200–300
Wageningen, H-series			
Prof. Hudig, H 1	badly injured	<25	600–800
Do. H 8	sound	400–500	800–1000
Do. L-series L 1	badly injured	<25	400–500
Do. L-series L 6	sound	400–500	500–600
Do. G-series	badly injured	<25	300
Do. G-series	sound	100	300–400
Do. Willemse	badly injured	50	500–1000
Do. Westland	badly injured	50	300

Although apparently a low pH may often cause a decrease in the amount of available potassium, the correlation is much less evident than in the case of magnesium. Whether the relatively high potassium content of acid soils is the result of a gift of potassium fertilizer or of the less extensive leaching out of the potassium, is left to future research to decide.

Although the results of the experiments described in this chapter do not indicate any fixation of magnesium in acid soils, it remains an open question whether the influence of a high concentration of H-ions during a long period and an alternating desiccation and moistening might not bring about such a fixation. In answering this question the work of KARDOS and JOFFE (18) will have to be considered, who could state that silica and magnesium have a much stronger affinity than silica and calcium, so Mg-silicates are more easily formed than Ca-silicates.

Now what is the reason that alcaline substances like $CaCO_3$ and $Ca(OH)_2$, or nitrogen compounds like nitrates which change the reaction of the soil in the alcaline direction, show a tendency to prevent the Hooghalen disease?

This favourable influence must be ascribed, for a part, to the fact that most of the substances cited contain magnesium as an impurity. In fact two of the brands of $CaCO_3$ appeared on analysis to contain 0,5 and 0,2 per cent of available magnesium. By fertilizing a field with 10.000 kg of these carbonates per ha 50 or 20 kg of magnesium would be added to the soil, a quantity sufficient for the magnesium requirement of the crop. Samples of CaO of various origin contained 1 and 0,05 per cent respectively, and in a same way we found 0,75 and 2 per cent for Kencika and silicalime. These differences in content of Mg may explain the fact, that the effect of these lime dressings is not always the same.

The effect of alcaline fertilizers is not restricted, however, to this magnesium content. This fact appeared from pot experiments with magnesium-free $CaCO_3$; although the pots with none but $CaCO_3$ gave a much lower yield than those with $MgSO_4$, when compared with the untreated pots they had markedly improved in growth.

The total magnesium content of these "$CaCO_3$-plants" proved to be materially higher than that of the diseased plants on untreated soils, as is shown in table 10. As has been pointed out in chapter III, the addition of $CaCO_3$ never increased the amount of magnesium, available for the mould. Even when the treatment with $CaCO_3$ was prolonged or intensified by shaking, this holds true, although plant development was better.

These facts prove that the improvement following the liming of a field cannot be due to an increase in available magnesium. So the reason is not to be looked for in the soil but in the response of the plant to neutralization. VAN ITALLIE in studying the same question was led to the same conclusion, already cited in par. 1, that the elimination of H-ions helps to promote the magnesium uptake by the roots. Our own experiments with water cultures, described in chapter II, tend in a same direction; we could notice a somewhat more severe magnesium shortage in solutions with pH 4 than with pH 6. It is our opinion, however, that it is the disturbance in the root development in acid solutions which has the stronger effect, because the poorly developed roots will be slow in assimilating the low amount of magnesium.

Whether the better growth of the "$CaCO_3$-plants", cited above, may be due to a better developed root system or to an easier absorption of the element by the root hairs, remains an open question, as well as the question, whether a prolonged contact with neutralizing substances might not in the end result in the transition of part of the magnesium into a better available form. The above considerations on the effect of $CaCO_3$ on acid soils, producing diseased crops, only hold when the disease consists in a lack of magnesium and not in the damage actually caused by acid. As a matter of fact in the latter case no symptoms of disease will appear as soon as the excess of H-ions will be eliminated.

SUMMARY AND CONCLUSIONS

The disease of oats and other cereals, called "Hooghalen disease", initially recognized in 1918, is still frequent in the Netherlands and other European countries as well as in several parts of the United States. Because of its frequency on acid fields it has often been ascribed to the acidity as such, but several authors have emphasized the possibility, that a shortage of magnesium might be among its causes. Nevertheless the relation between such a shortage and the occurrence of the disease remained obscure; so we took up the task to study the effect of a lack of magnesium on the growth of cereals with various concentrations of H-ions.

It could be proved, that the symptoms occurring in the field could be exactly reproduced by means either of watercultures or of sand- and peat cultures, where Mg concentration was low; they vanished as soon as a suitable dose of magnesium was given. When the acidity was too high (pH below 4), however, magnesium by itself did not suffice for the production of sound plants and the symptoms of damage caused by acidity could be observed. If neutralizing substances like $CaCO_3$ or $Ca(OH)_2$ had been supplied, these symptoms gradually disappeared and those of a magnesium shortage remained.

In natural soils the acidity symptoms were never met with, but quite a number of soil samples were gathered from fields, where the characteristic symptoms of Hooghalen disease occurred markedly. Addition of small amounts of $MgSO_4$ in nearly all of the cases sufficed to cure or to prevent the disease. A few cases were left, where addition of $CaCO_3$ was needed to complete the effect of magnesium. The supply of $CaCO_3$ by itself resulted in a better development of the plants, but not in an abundant grain production. Therefore magnesium could not be dispensed with. As it could be presumed that only part of the magnesium present in soils (as determined by chemical methods) might be available to plants, we were led to the use of a microbiological method in determining this available portion. Therefore the development of *Aspergillus niger* in a magnesium-free solution was used, where the soil to be investigated was the only source of magnesium. A series of flasks with known amounts of this element served as a standard of comparison. It proved a satisfactory method to estimate the part of the total magnesium, which may be assimilated by *Aspergillus niger*. Healthy soils appeared to contain 100 γ or more per 3 g of soil. Now a striking parallelism was found to exist between the occurrence of the disease and the amount of the magnesium as available to the mould. For it was found, in an investigation of a great number of soils, producing either diseased or sound crops, that with hardly any exception the former contained less than 50 γ magnesium per 3 g of soil. So it may be assumed, that the availability for *Aspergillus niger* and for the cereals is nearly the same. The total amount in the soil was found when the soil had been calcinated initially at 500–600 °C. In this case all the magnesium present in the ashes can be assimilated by the mould. It was found that this amount is many times greater than the amount available in the natural soils: in healthy as well as in diseased soils the available part is only a fraction of the total. So it was found that in highly acidulated soil (pH 4, 0), where no trace of available magnesium could be detected, the total amount was 20,4 mg in 100 g, whereas the same soil, untreated (pH 5,4), and healthy, and containing 200 γ of available magnesium in 5 g, showed a total content of 21,9 mg in 100 g. Thus it is understood, why chemical determinations of magnesium, even in such extreme cases as this one, will fail to show the marked difference, which agrees with the condition of the crop.

The magnesium content of healthy and diseased plants and grain was in agreement with the above data. In a healthy crop it markedly surpassed the content of a diseased crop. This amount increased when magnesium had been given to the soil. When the soil had been neutralized with $CaCO_3$, the magnesium content of the vegetative parts and of the grain had increased as well. The mould-available amount of magnesium, however, was not materially increased by neutralization, but it could be stated, that development of the root system was improved by the decrease of H-ions. It is supposed that it is due to this improvement of the root system rather than to an increase in availability of the magnesium, that the plant can absorb more magnesium then when the soil is left acid. Nevertheless the yield of grain remains materially below a normal one, which is only reached as the result of an additional gift of magnesium.

From all data collected the following picture may be drawn of the happening in the fields which are deprived of their lime content by natural washing-out agencies and an insufficient supply of neutralizing fertilizers.

The dropping of the pH involves a loss of nearly all of the calcium and a fraction of the magnesium present. But this latter amount is the fraction which had occurred in an exchangeable form and thus had been the fraction available to plants. Most of the magnesium, however, seems to be bound up in organic materials, and thus will be freed by calcination, but cannot be assimilated directly. If the drop in pH continues, symptoms of a damage due to acidity, which can not be cured by a magnesium gift, may be expected. If the soil has been neutralized, plants may improve in development, without, however, a proportional increase of the crop, provided the fertilizer is free of magnesium; this is often not the case, however. Only the gift of a suitable amount of this element may restore the insufficient stock and induce the normal fertility. In the soil mere neutralization does not suffice to enhance the availability of the magnesium when in insoluble form, at least not within a short period. It remains to be investigated whether in the long run it may do so. It seems sound policy, however, not to wait for the outcome of this improvement but to bridge this period over by providing magnesium as a fertilizer.

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