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MANGANESE TOXICITY IN FIELD AND MARKET GARDEN GROPS*

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1. INTRODUCTION

The question of manganese toxicity of the soil as a practical problem in agriculture and horticulture was encountered quite accidentally.

In a field of sandy soil of light texture adjacent to the Laboratorium voor Microbiologie at Wageningen in 1922, a series of 40 permanent experimental plots of 42 m² were laid out. They are well separated by sunken cement plates. Since 1922 these plots have received various fertilizer treatments; each plot received a similar manurial treatment each year. Each plot has always been grown with the same

*) Since 1948 this investigation has been carried out under the National Council for Agricultural Research T.N.O. (Applied Scientific Research).

crops, which however, have been varied yearly. The plots which will be mentioned in the following account are a triplicate series of six, supplied with various amounts of limestone (0-5-10-15-30-60 kg) termed as I_0, I_5—I_{60}, II_0, II_5—II_{60}, III_0, III_5—III_{60} and two plots in duplicate which either have received chilean nitrate (Ch) or ammoniumsulphate (AS) as a nitrogen fertilizer. Under war conditions, however, when chilean nitrate was unavailable, ammoniumsulphate had to be substituted for it. These various treatments have of course greatly influenced the pH of the plots. In Table I the data are

TABLE I

pH of experimental plots												
Series of limed plots	I				II				III			
Date	1946 24/7	1947 20/5	1948 29/5	1949 6/5	1946 24/7	1947 20/5	1948 29/5	1949 6/5	1946 24/7	1947 20/5	1948 29/5	1949 6/5
60 kg l.	6.65	5.15	5.6	6.3	6.57	5.15	5.8	6.2	6.43	5.25	5.8	5.8
30 ,,	5.75	5.0	5.2	6.1	5.85	4.95	5.7	5.95	5.85	4.9	5.3	5.1
15 ,,	5.15	4.5	4.95	5.0	5.15	4.42	4.95	5.7	5.40	4.9	5.1	4.9
10 ,,	4.92	4.32	4.45	5	5.00	4.3	4.95	5.0	5.75	4.95	5.05	5.05
5 ,,	4.95	4.0	4.7	5.1	4.8	4.55	4.85	5.4	5.00	4.45	4.75	4.6
0 ,,	4.42	3.8	4.15	4.8	5.1	4.45	4.8	5.1	4.83	4.4	4.75	4.55
Ch. 1	5.97	4.5	5.55	5.8								
Ch. 2	6.65	4.9	5.15	5								
AS 1	4.57	4.05	3.6	5.15								
AS 2	4.55	4.1	4.85	4.3								

recorded. Since the differences between the limed plots tended to decrease in spring 1949 a fresh addition of limestone in appropriate proportion was made to each of them. In May 1940 war conditions prevailed in the Netherlands and the raising of nutritional crops was the immediate need. Half of each plot was sown with *Phaseolus vulgaris* (Var. b r u i n e b o o n), the seeds of which provide a food rich in protein, and the others halves of the plots were kept in potatoes. Up till 1945 both these crops were grown alternately on the half plots.

Early in the summer of 1940 in the more acid plots an injury to the foliage of the young bean plants was noted. In the leaves a marked marginal chlorosis extending into the intervenal areas occurred (Fig. 1). When older, the leaves became somewhat crinkled and were spotted with small yellowish and later whitish areas (Fig. 2 en 3).

Finally minute brown necrotic spots appeared (Fig. 4) and the petioles of the seed leaves and of the first trifoliate leaves were speckled with small superficial purple-brownish spots. Severely injured plants remained stunted and produced hardly any flower or seed. Less injured plants were often seen to recover later in the season, when only traces of the initial injury still occurred in the older leaves. Owing to the injury in the young stage, the final yield was reduced. In all successive years the same symptoms could be noted.

The literature then available did not furnish any description of this injury, so its cause was quite unknown.

2. THE CAUSE OF THE INJURY IN BEANS (*Phaseolus Vulgaris*)

a. Experimental induction.

In 1943 some light was thrown on the cause of the damage. In a variety of bean (van Tol's bruine boon) on certain soils the occurrence of an internal necrosis in the seeds had been noted; a phenomenon very much like "marsh spots" in peas, caused by a deficiency of manganese. In order to establish, whether this injury could be ascribed to a similar cause, solution cultures deficient in manganese were set up. At the same time a few vessels received a large supply of $MnSO_4$ (100 and 50 mg/l) in order to demonstrate the symptoms of manganese excess in beans. Quite unexpectedly the symptoms induced in the latter cultures appeared to be strikingly similar to those already noted in the field.

In the meantime the same injury had been observed in agricultural fields on sandy soil in the neighbourhood of Wageningen. Under normal conditions such fairly acid fields would not have been grown with beans, but there also war conditions had favoured the raising of this crop. Frequently the injury could be recognized at a distance by the bright yellow colour of the leaves. It appeared then that the French bean (dubbele prinsesseboon), the pods of which are used as a vegetable, was remarkably susceptible and in later years "dubbele prinsesseboon" has been chiefly grown as the experimental plant.

b. The manganese content of the leaves.

The first means of attack on the problem was the estimation of the amount of manganese in the foliage of healthy and of affected plants. The youngest fullgrown leaves were collected and comparable samples were analysed according to the method of M a r s h a l l [14]) amended by S t e e n b j e r g [24]).

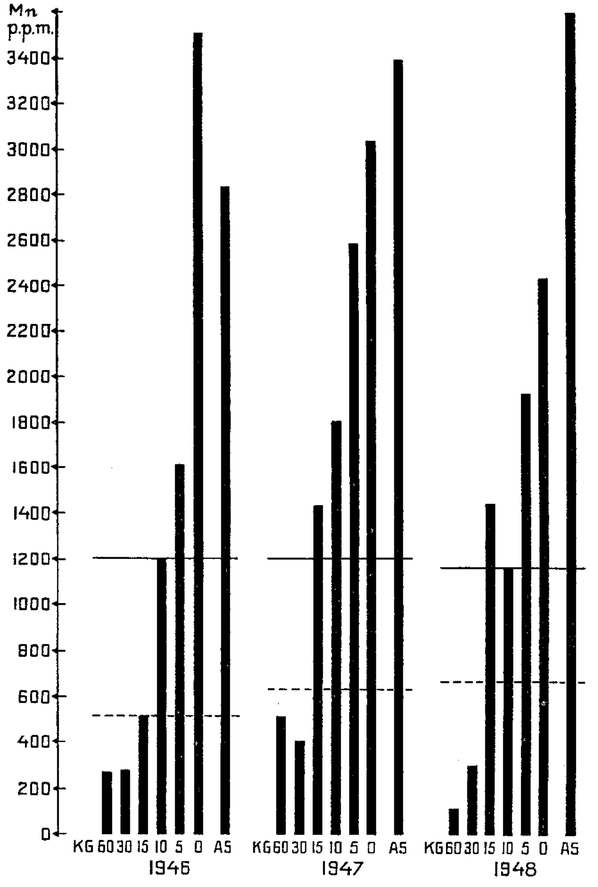
A sample of 0.5–0.05 g dry weight is transferred to a K j e l d a h l flask, a mixture of 5 ml of HNO_3 (5p) and 4 ml of H_2SO_4 (6p) are added and the flask is heated at first on a low flame, which is later increased, until the liquid is colourless. After the addition of 15 ml. dist. water the solution is rendered alkaline with 6 N NaOH. Conc. H_2SO_4 is then added until it is just acid and 1 ml is then added in excess. The solution is filtered into a 100 ml flask, 2 ml of 0.1 N $AgNO_3$ are added and the flask filled to the mark with distilled water. After filtering into a beaker, 1 ml of 6 N HNO_3, and a few mg of ammonium persulphate are added to the filtrate which is heated almost to the boil. After cooling the manganese as manganese permanganate is estimated in a P u l f r i c h photometer.

In the following account the content of manganese is always expressed as ppm of dry matter.

In the samples collected in 1943 a very marked difference in content appeared to exist between young healthy foliage (100—500 ppm) and injured foliage of the same age (1000—3000 ppm). (L ö h n i s [12]).

In 1944—45 no further progress was possible, but since 1946 samples of bean foliage (P r i n s e s s e b o o n) have been collected from the 28 plots under investigation. The first samples were always taken as soon as symptoms of injury became conspicuous, generally during the development of the first trifoliate leaf and all plots were sampled simultaneously. In Gr. 1 the results obtained in 1946—48 in series I and AS_1 are presented. For the sake of conciseness the data for the plots, in series II and III and AS_2 which correspond well with those in Series I are not given. Although the amounts recorded in the same plot in various years may vary widely, the trend in the series of limed plots was always similar. The most striking result was, that the threshold values above which the foliage was always visibly affected (marked by a drawnout line in the graphs) was hardly seen to vary in the successive years. These values were in 1946 1210; in 1947 1211; in 1948 1167 and in 1949 1104. The values found in the healthy leaves ranged from 40—904, in injured leaves from 1104—4216. In the AS plots the injury was always the most severe and the corresponding content of manganese very high.

When the initial injury has not been very severe plants are apt to recover and in fully developed plants traces of injury may be noted only in the oldest leaves. Will the upper leaves of such plants contain



Gr. I. Content of manganese in the foliage of bean plants from the plots of Series I and AS_1.

——————— Lowest level of affected plants.

– – – – – – Highest level of healthy plants.

amounts of manganese below the level which causes injury in young plants? This frequently appeared not to be the case. In the tops of these healthy plants values might be found exceeding those recorded in young injured plants. These higher values ranged between 1296 and 1681. Hence more mature plants appear to tolerate higher amounts of manganese.

c. Solution cultures.

A close correlation between manganese content and the occurrence of injury does not, however, furnish strict proof for an actual causal relation. Shifts in the manganese content may easily be conceived as merely accessory phenomena, the actual cause, however, lying elsewhere. Further proof by means of solution culture was needed.

As the greenhouse of the Laboratorium voor Microbiologie had been destroyed during the war, experiments could only be run on a small scale and were dependent on weather conditions.

Glass vessels of 1 l capacity were used wrapped in corrugated cardboard. The lids were moulded from plaster of Paris and provided with holes.

4 plants were grown in each vessel. Tap water was used for the solutions and added daily. Solutions were renewed according to the rate of development of the plants. Where not otherwise indicated experiments were ru in duplicate.

The culture solutions used were von der Crone solution as modified by Bryan*) or a modified solution of Zinzadze (pH 5.5—6.5 †)) called solution II in the following account. Minor elements were always added. When 5 or 7.5 mg Mn/l was added symptoms similar to those occurring in the field were induced. (Fig. 5—7).

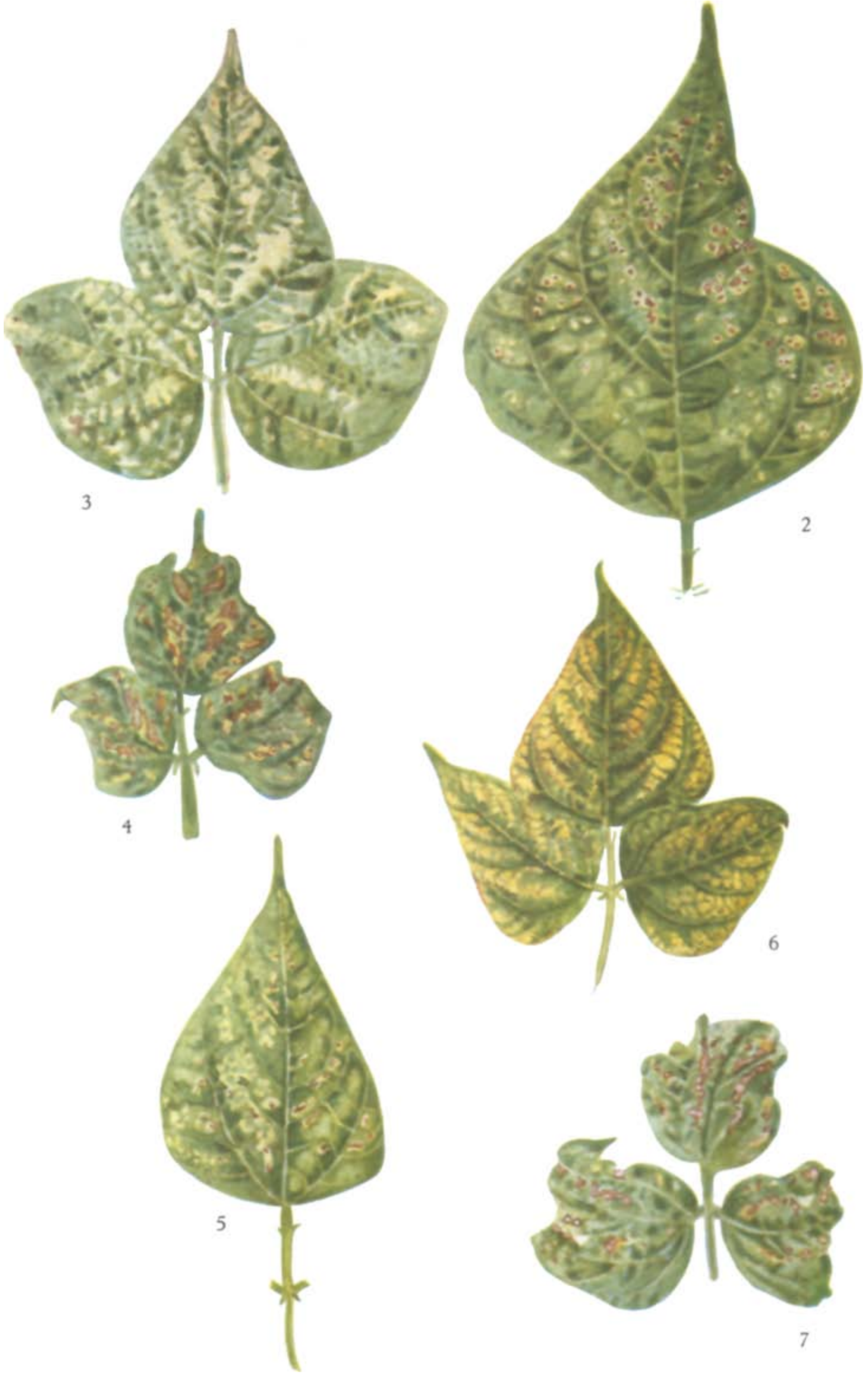
When these experiments were run in late summer however, results were sometimes erratic and the susceptibility of the plants appeared to be less than in early summer.

1949 provided better conditions for such experiments. A small warehouse had been built which was put into use at the end of May and early in the spring also cultures were grown in the properly heated and lighted culture compartments of the Laboratorium voor Plantenphysiologisch Onderzoek. Culturing under these conditions offered an explanation of difficulties met with in former years.

On Febr. 27th cultures in von der Crone solution provided with 0.5, 5 and 10 mg Mn/l had been set up. By March 8th none of these plants had shown any signs of injury, although the doses of 5 and 10 mg were high enough to warrant the expectation of the occurrence of signs of damage. On March 9th the temperature in the afternoon rose so high that it was feared that the plants might wilt so they were moved to a more moderately heated compartment of

*) Von der Crone solution: KNO_3 1 g; $CaSO_4$ 0.2 g; $MgSO_4.7H_2O$ 0.2 g; $Ca_3(PO_4)_2$ 0. 2 g; $FePO_4$ 0.2 g; per l. tapwater. Minor elements added to all solution cultures: H_3BO_3 0.5 mg; $CuSO_4$ 0.125 mg; $ZnSO_4.7H_2O$ 0.5 mg.

†) Solution II: KNO_3 0.5 g; NH_4NO_3 0.2 g; KCl 0.36 g; $Ca_3(PO_4)_2$ 0.5 g; $MgSO_4.7H_2O$ 0.25 g; $Fe(NH_4)_2(SO_4)_4.24H_2O$ 0.175 g.



2. Bean (Prinsesseboon), Unlimed plot. — 3. Bean (Prinsesseboon), Unlimed plot.
4. Bean (Prinsesseboon), Unlimed plot. — 5. Bean (Prinsesseboon), 10 mg Mn/l.
6. Bean (Prinsesseboon), 7.5 mg Mn/l. — 7. Bean (Prinsesseboon), 7.5 mg Mn/l.

the greenhouse. On March 10th all those plants which had been supplied with 5 and 10 mg Mn/l showed the characteristic injury all over their foliage. Hence at the former higher temperature manganese had apparently been taken up by the plants without the induction of any visible signs of injury.

On March 21st a further comparative test was made with plants in von der Crone solution, provided with 10 mg Mn/l. 3 vessels were put in a warm compartment and 3 in a more moderately heated one. On April 8th all plants in the latter compartment were markedly injured. In the hot compartment plants in two of the vessels were quite healthy, but in the 3rd vessel the plants were injured. The foliage of all these plants was harvested and the content of manganese estimated. The following amounts were found:

at moderate	temperature	injured	2938 ppm
,, high	,,	injured	2635 ppm
,, ,,	,,	healthy	2635 ppm
,, ,,	,,	healthy	1496 ppm.

Hence at high temperature 8 plants had withstood without any visible injury, a content of manganese which under the usual temperature conditions of spring would have given rise to severe damage.

From May 2nd—25th beans were again grown in von der Crone solution in the presence of 2.5, 5 and 7.5 mg Mn/l in the heated compartment. None of these showed any signs of injury although the content of manganese in plants grown in the presence of 7.5 mg Mn was estimated as 1780 ppm. From June 22th to July 17th the behaviour of bean plants in von der Crone solution supplied with 0.5, 7.5 and 10 mg $MnSO_4$ was compared when cultivated either in the well ventilated warehouse or in the heated compartment.

The photograph (Fig. 8) of plants supplied with 7.5 mg/l shows the very marked difference. This experiment will be referred to later.

Although the exact conditions of temperature have not been studied it does appear that temperature may act strongly on the occurrence of visible injury, so that comparative investigations of susceptibility must be run under controlled conditions. This influence of temperature may account for the conflicting results I obtained in former years during periods of great heat in summer.

Other external conditions may also play a part. An influence of shading on the occurrence of manganese injury has been recorded by Mc Cool[15]). In greenhouse experiments a heavy shading prevented the occurrence of any visible injury in beans and tobacco. In beans, however, the shaded plants had a markedly lower content of manganese (1330 ppm) than the unshaded (2270 ppm). Hopkins *et al.* [10]) suggested that a greater exposure to light could bring about particular symptoms of manganese toxicity in pineapples. Shaded leaves, however, without any visible symptom had high contents of manganese similar to the affected ones in the light.

When plants are grown in solutions varying in composition the susceptibility to excess of manganese may vary greatly. This had been noted in former years, but the need of comparing the degree of susceptibility under identical external conditions has only recently been established, and the evidence gathered is somewhat scanty.

In solution Zinzadze pH5 *) supplied with 10 mg Mn/l in 3 vessels kept at moderate temperature quite healthy plants were produced, and only the seed leaves showed the minute, superficial purplish speckling which indicates an excess of manganese. Seed leaves were harvested separately from the remaining foliage; the former contained 1318 ppm Mn, the latter 734. The pH in this solution was measured as 4.8 and when checked with the initial culture solution all manganese was proved to be in solution.

A further instance of the influence of the composition of the culture medium is furnished by the following experiments, run from March 21th till April 8th. Two lots in triplicate, were set up, one with von der Crone solution, the other with the Zinzadze solution pH5, all supplied with 10 mg Mn/l. Whilst in the former solution all plants were markedly injured, the plants in the latter had remained healthy. In von der Crone solution the foliage contained 2938 ppm, in Zinzadze solution 591. In the latter medium all manganese when checked at the start proved to be in solution, whilst in the von der Crone medium only 6.3 mg Mn/l was in solution.

The difference in plant behaviour cannot be explained by a difference of solubility of manganese in the culture medium. The part played by antagonistic action of constituents of the culture solution will be discussed later.

d. Soil cultures.

The experiment, however, which furnishes the most convin-

*) NH_4NO_3 0.334 g; KNO_3 0.166 g; $Ca_3(PO_4)_2$ 0.7 g; KCl 0.616 g; $MgSO_4 . 7H_2O$ 0.5 g; $CaSO_4$ 0.5 g; $Fe_2(SO_4)_3$ 0.25 g. 1 l tapwater. Minor elements as usual.

Fig. 1.

Fig. 8.

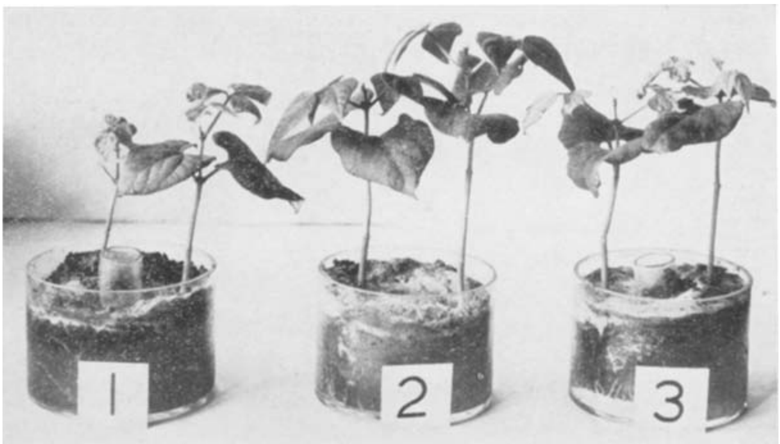


Fig. 9.

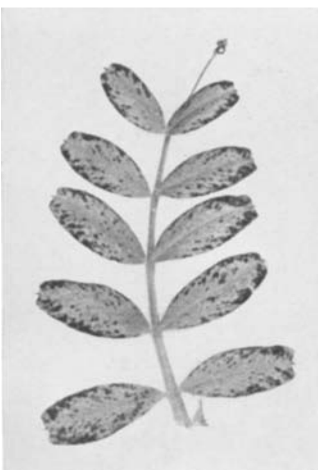


Fig. 11.

Fig. 1. Bean plant (Bruine boon) in an unlimed plot
(Photo Plantenziektenkundige Dienst Wageningen).

Fig. 8. Bean (Prinsesseboon), v. d. Crone solution, 7.5 mg Mn/l
Left moderate, and right high temperature.

Fig. 9. Bean (Prinsesseboon), 1. unlimed soil, 2. $CaCO_3$,
3. $CaCO_3$ + $MnSO_4$.

Fig. 11. *Vicia sativa*, unlimed plot.
(Photo Plantenziektenkundige Dienst Wageningen).

Fig. 12. *Vicia sativa*,
Unlimed plot and 60kg lime.

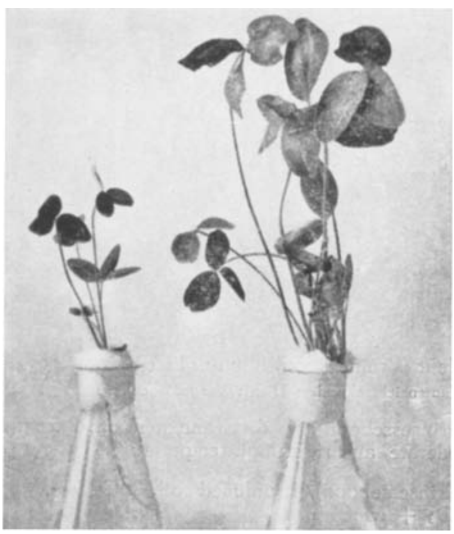


Fig. 16. Red clover,
Unlimed plot and 60kg lime.

Fig. 15. Lucerne,
Solution II,
0.5 mg Mn/l and 5 mg Mn/l.
(Photo J. Boekhart)

Fig. 18. Red clover,
v. d. Crone solution,
10 mg Mn/l and 0.5 mg Mn/l.

Fig. 17. Red clover,
Unlimed plot.

cing proof that manganese excess is the actual cause of the injury which occurs in the field is the following (L ö h n i s [13]). Bean plants were grown in glass dishes (N e u b a u e r dishes) provided with 400 g soil taken from plot I_0, giving rise to an affected crop (1). The soil in part of the dishes was mixed with 0.8 g $CaCO_3$ (2) whilst to half of the number of the latter 0.25 g $MnSO_4$ was also supplied (3). In the untreated soil (1) young bean plants showed symptoms similar to those noted in the field, in (2) normal healthy plants developed, whilst in (3) symptoms developed exactly similar to those in (1) (Fig. 9).

Here acidity as such has been eliminated as a possible causal agent and the injury is doubtless due to the manganese sulphate added.

This test may easily be carried out with large seeds. When seeds are small, however, it takes a longer interval of time before the plants have developed sufficiently to show symptoms of injury. In the meantime under more alkaline conditions the Mn^{++} may have been oxidised and as insoluble MnO_2 will no longer be available to plants.

3. SUSCEPTIBILITY OF OTHER CROPS

a. Literature.

The question of manganese toxicity has been studied in widely separated regions. The fact, that most of these investigations have been carried out simultaneously with the present one and that foreign literature has been unavailable during a long period, may explain that the facts reported in literature came under my notice for the most part a long time after their publication.

P a r b e r r y [20]) reports on "scald" in beans occurring on certain soils in New South Wales. The affected plants have a higher content of manganese, more especially those which are deficient in magnesium. As the symptoms are not described in detail, it is not certain that this phenomenon is identical with the above.

W a l l a c e, H e w i t t and N i c h o l a s [25]) and H e w i t t [6a]) induced the symptoms of manganese toxicity in runner beans and cauliflower by means of sand solution cultures. The symptoms described in beans correspond closely with those noted at Wageningen and personal discussion in 1947 corroborated this more fully.

H e w i t t [7]) [8]), aiming at a resolution of the factors in soil acidity by means of sand solution cultures has studied very thoroughly the effect of manganese excess upon a number of farm and market garden crops.

The description of the symptoms, where the same crops have been studied generally agrees closely with those I have noted.

Tobacco has been reported by Jacobson and Swanback[11]) to be susceptible to manganese excess which occurs in soils in Connecticut. Amounts recorded in damaged plants ranged between 5250–11670 ppm. Bortner[2]) reported the occurrence of similar injury in Kentucky.

Fried and Peech[5]) report that liming of the soil reduced the Mn content of the leaves of lucerne to $\frac{1}{8}$ of that found in the control. Added manganese proved distinctly toxic.

Peech and Bradfield[21]) point to the part manganese and aluminum may play in explaining the behaviour of lime-responsive crops.

Morris and Pierre[19]) have studied the minimum concentration of manganese which is necessary for injury in various Leguminosae in a given nutrient solution. Average yields and characteristic symptoms are described. The plants differed greatly in the amounts of manganese absorbed. At least two factors affect the tolerance of the plant, viz. the amount of manganese absorbed and the relative ability to endure large amounts within the plant.

C. R. Millikan[16]) describes in excess manganese cultures a characteristic type of leaf necrosis in flax (at concentrations in the solution of 25 ppm or more). Identical symptoms were also produced in highly acid soils by heavy applications of manganese sulphate. With excess manganese (25–150 ppm), the addition of molybdenum (5, 10 and 15 ppm) retarded the date of appearance and reduced the severity of lower leaf necrosis. This effect of Mo was confirmed in acid soils with heavy dressings of manganese sulphate.

In 1948[17]) he reported on an antagonism between manganese and molybdenum for peas, cabbage and tomatoes in solution cultures.

Sherman and Fujimoto[21a]) describe a disease of lettuce in Hawaï, termed "yellow leaf fringe", which could be controlled by treatment of the soil with mulch or lime. The manganese content of plants from untreated soil was 3800 ppm while in healthy plants it did not exceed 760.

Du Plessis[4]) suggests manganese excess as the cause of an injury of unknown origin in vine leaves.

Daji[3]) Manganese toxicity is suggested as a probable cause of the band disease of Areca palm.

b. Experimental results.

Neither potatoes (var. Eigenheimer) grown side by side with the beans nor strawberries, both crops grown for other purposes on these plots, had ever shown any signs of injury. Starting in 1947 various crops were grown in order to ascertain their comparative susceptibility. These crops were chosen from among those known to be limeresponsive or from those which were recorded as unsusceptible to manganese deficiency (greyspeck disease) such as *Vicia sativa.*

10. *Vicia sativa*, Unlimed plot. — 13. *Vicia sativa*, Soil culture, $CaCO_3$ and $MnSO_4$. — 14. Lucerne, Unlimed plot.

Vicia sativa.

Field test. When grown in 1947 vetch showed very striking differences in development in the various plots. In plots I_0 and $AS_{1 \text{ and } 2}$ it was a complete failure. In the highly limed plots the plants developed profusely. A definite pattern of injury of the leaves occurred, viz. a chlorosis of the young leaves and a very marked dark purplish discoloration along the margins of the fullgrown leaves. Sometimes small more orangered sunken spots occur in the leaf margins and the upper surface of the leaves may be speckled with minute dark spots. The whole plant remained small and spindly (Fig. 10, 11 and 12).

Purple margins may also be induced by potassium deficiency. Whilst excess manganese, however, induces this injury in the leaves of very young plants, the symptoms of potassium deficiency occur in more mature plants and on the lower leaves. These symptoms could be noted sometimes in large plants on the highly limed plots.

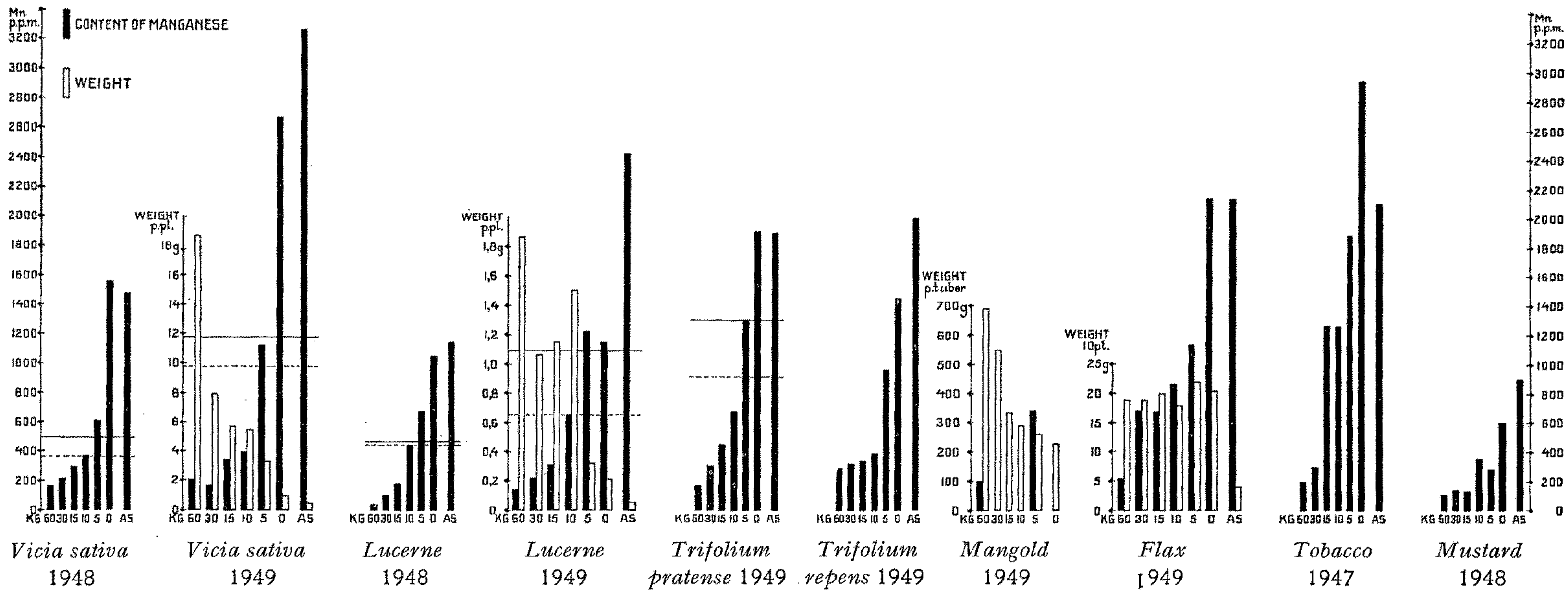
In 1948 June 11th young leaves were sampled in all plots. Gr. II gives the data for the manganese contents in Series I and AS_1. In 1949 on June 17th plants were cut at soil level (4 in each plot) and weighed. Fresh weight per plant and manganese content are given in Gr. II. A marked negative correlation can be noted. The minimum content of affected plants was 1117.

Solution cultures. The symptoms caused by manganese excess were exactly reproduced in solution cultures. In von der Crone solution a dose as low as 2.5 mg Mn/l induced slight injury. The leaves of such plants contained 1021 ppm. 5 mg Mn/l induced marked injury and in the leaves a content of 1524 ppm was estimated.

Soil cultures also such as described on p. 201 furnished very clear-cut results. The injury in plants in the untreated soil agreed very closely with that in the soil treated with $CaCO_3$ and $MnSO_4$ (Fig. 13).

Medicago sativa.

Field test. Lucerne, known as a lime-responsive crop was grown in 1948 and 1949. A very marked delay in development in the more acid plots was noted. In this stage definite signs of injury occur in the leaves: for instance the leaflets are brightly yellow often with brown necrotic spots, and a narrow yellow margin extends towards the base. In older leaflets a browning and



Gr. II. Content of manganese in the foliage of various crop plants from the plots of Series I and AS_1.

——————— Lowest level of affected plants. - - - - - - - - - Highest level of healthy plants.

scorching of the margins occur (Fig. 14). Sometimes sunken discoloured spots occur in the marginal zone of the leaflets, reminiscent of symptoms of potassium deficiency. But here again these spots occur on the younger leaves of young plants. Many plants die off. Those that can stand the initial damage catch up with healthy plants so that when mature they show hardly any signs of injury. The symptoms described by Hewitt [8]) correspond closely with these.

The manganese of young leaves was estimated in 1948 and 1949. On June 20th 1949 representative samples of young plants were collected and weighed. In Gr. II the data are presented; a high content of manganese is correlated with low weight. The minimum content of manganese in affected plants was at a much higher level (1083) in 1949 than in 1948 (477).

Solution cultures. Here again it was possible to reproduce the field symptoms. 2.5 mg Mn/l in von der Crone solution induced slight injury, in solution II the marginal discoloration occurred in the first leaves only. When 5 mg Mn/l was supplied the leaves of the plants in both solutions were much smaller and very chlorotic (Fig. 15). The content of manganese of these plants was estimated and corresponded with the degree of visible injury.

Culture solution	mg/Mn/l	Mn ppm
von der Crone	2.5	1022
,,	5	2243
Solution II	2.5	588
,,	5	2115

Trifolium pratense.

Field test. Red clover was only grown in 1949. As the spring was exceptionally dry, the germination was very irregular and a valid comparison of the development in the various plots could not be made. Young plants (June 10th) in the acid plots were small (Fig. 16) and the leaflets showed narrow greenish chlorotic margins. In course of time the plants recovered in all but the AS plots which bore hardly any plants. In the most acid plots the outer (oldest) leaves of the mature plants sometimes still showed chlorotic margins with a dark necrotic outer edge, and indeed the chlorotic areas might extend up to the main rib and the leaf area might be crinkled (Fig. 17). Such plants, however, flowered normally and quite normal younger leaves developed.

The symptoms correspond with those described by Hewitt [7]).

Samples were taken at a time when the differences in development were still conspicuous. In Gr. II the very marked influence of the degree of liming on the content of manganese can be seen.

Solution cultures. In von der Crone solution supplied with 5 mg Mn/l set up on May 23rd plants showed on June 8th small necrotic light green spots in the marginal zone. These plants were somewhat smaller than the controls. Later these plants became quite healthy. Plants supplied with 10 mg Mn/l were quite small on June 8th and the leaves bore many chlorotic and necrotic spots. Fig. 18 taken June 22nd shows them still lagging far behind the controls in development, although the younger leaves were healthy and of the same size as those of the controls. On July 9th they had caught up with the latter.

In Solution II the same amounts of $MnSo_4$ affected the plants in a lesser degree. A second series of solution II, set up in triplicate (July 12th—Sept. 9th) with amounts of 0.5, 10 and 15 mg Mn/l, showed on August 5th chlorotic margins in the presence of either 10 and 15 mg Mn/l. On September 9th the development in the 10 mg Mn and in two of the 15 mg Mn vessels was profuse, but in one of the latter it was weak. Controls and plants in a 10 mg Mn vessel had been infested by grasshoppers. The following contents of manganese were recorded:

mg Mn/l	number of plants	fresh weight per plant g	Mn ppm
10	4	9.5	1318
10	4	9.1	1253
15	3	11	1631
15	3	11.3	
15	3	3.7	2208

Hence more mature plants can stand a high content of manganese without damage. Both field trials and solution cultures show that older plants are markedly more tolerant for manganese than young plants.

Trifolium repens.

Field test. White clover was only grown in 1949 and here again weather conditions acted very unfavorably on germination. Young plants in the acid plots lagged greatly in development; actual symptoms of injury, however, were not recorded. Later these plants caught up with the others and finally plants in the acid plots could not be distinguished from the initially healthy ones. Samples taken on August 8th in the field show the influence of the liming on the manganese content (Gr. II).

S o l u t i o n s C u l t u r e s . In v o n d e r C r o n e solution (July 15th—August 16th) very young plants did show in the presence of 2.5 and 5 mg Mn/l some minute necrotic spots, but at an early date these could no longer be seen. Plants supplied with 5 mg Mn/l, when young, produced small leaves and lagged in development. Fig. 19 taken on August 16th, however, shows that by then the latter plants had started to grow better, the young leaves were then of a same size as those of the control plants. The content of manganese in ppm, in the foliage of these plants was 813 as against 164 in the controls.

In an experiment in solution II (August 12th—October 4th) the final difference between cultures supplied with 5 and 0.5 mg Mn/l was very slight; in the presence of 10 mg Mn/l foliage and roots were smaller (Fig. 20). The content of manganese in the foliage of these plants was 160 (0.5 mg), 726 (5 mg) and 1140 (10 mg) ppm.

M a n g o l d.

F i e l d t e s t. Mangolds (var. G r o e n k r a a g) were grown in 1949. On June 29th the young plants in the acid plots were markedly depressed in growth (Fig. 21). During further development no visual signs of injury were noted, the depression of growth was, however, maintained. In the AS plots the crop failed almost completely. When in the early days of November roots were dug their weight corresponded with the foliar development. In Gr II the weight of the roots and the manganese content of the foliage, as far as this has been estimated, is recorded for series I and AS_1.

The contents of Mn increase inversely with the degree of liming, but do not nearly reach the high values recorded in the other crops. The maximum found in all the plots was 947. Although doubtless some adverse action is exercised in the more acid plots, it remains to be proved that excess of manganese is the actual cause.

H e w i t t [7]) reports that sugar beet is remarkably tolerant of high concentrations of manganese in spite of its liability to fail on moderately acid soils.

S o l u t i o n c u l t u r e s. Plants in v o n d e r C r o n e solution in the presence of 5 mg Mn/l, which induced a content of 1424 ppm in the foliage, actually exceeded in fresh weight of the foliage

(84 gr 12 pl.) the controls the fresh weight of which was 77 g (12 pl.), whilst containing 249 Mm ppm. In the presence of 10 mg Mn/l a slight depression in growth could be observed, viz. 59 g (in 10 plants, two plants having succombed in the course of the experiments). These ten plants contained 1650 ppm. As this growth depression together with a high content of manganese was so much slighter than the depression noted in the field along with a much lower content, the latter could not presumably be ascribed to manganese toxicity. This experiment will be referred to in a later paragraph. These cultures showed in addition an unfamiliar symptom. In the presence of 5 and 10 mg Mn/l some of the leaves were of an uniform yellowish or nearly white shade, whilst after the supply of 15 mg Mn/l all of the leaves were narrow and white. When areas of such discolored leaves were painted over with $\frac{1}{4}\%$ $FeSO_4$ solution they became a bright green after two days. Thus an iron deficiency was confirmed. This phenomenon will be discussed later.

Hewitt[7]) mentions that mangold cultures, which have been provided with a large amount of manganese, produced young leaves abnormally narrow, with paling margins, followed by a yellow chlorosis, probably due to iron deficiency.

In solution II 10 and 15 mg Mn/l produced no such symptoms, only a slight depression in growth.

Flax.

Field test. Flax (Var. Concurrent) grown in 1949 showed no difference in development or any sign of injury in any of the unlimed plots. Only in the AS plots the plants were very small and the lower leaves yellow with dry brown tips (Fig. 22). On June 17th 10 representative stems cut at the level of the cotyledons were gathered from each plot and the fresh weight determined. In Gr. II the weights of plants and the manganese content of the leaves are recorded. Whilst it shows clearly that the increase in manganese content runs the familiar course, reaching maximally 2137 ppm, no correlation with the yield of young plants could be ascertained. Only in the AS plots could a very marked growth depression along with a high content of manganese (2134 ppm) be noted. Hence field experiments point to a marked tolerance.

Hewitt[7]) too did not observe any action of manganese excess. This is however not in accordance with data furnished by Millikan[16]), who claims to have induced a "lower leaf scorch" by excess

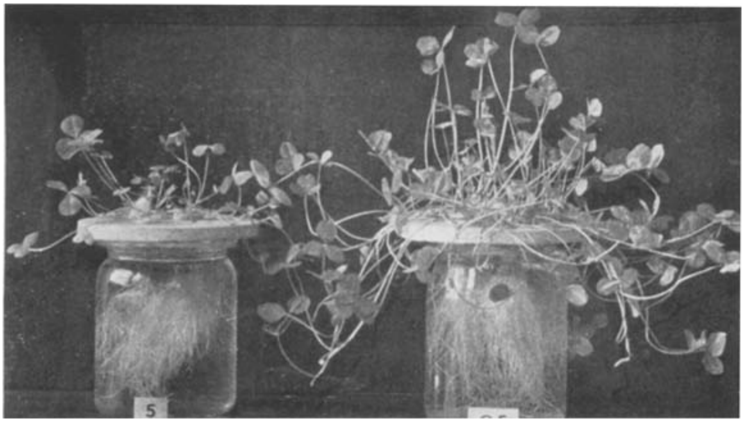


Fig. 19. *Trifolium repens,*
v. d. Crone solution,
5 mg Mn/l and 0.5 mg Mn/l.

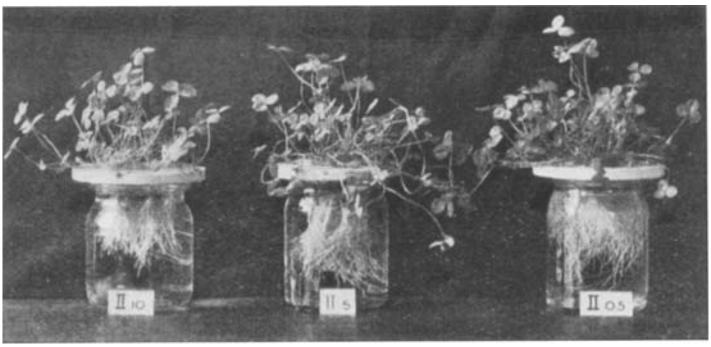


Fig. 20. *Trifolium repens,*
Solution II,
10 mg Mn/l, 5 mg Mn/l and 0,5 mg Mn/l.

Fig. 21. Mangold,
Unlimed plot and 60 kg lime.

Fig. 22. Flax,
AS plot and 60 kg lime.

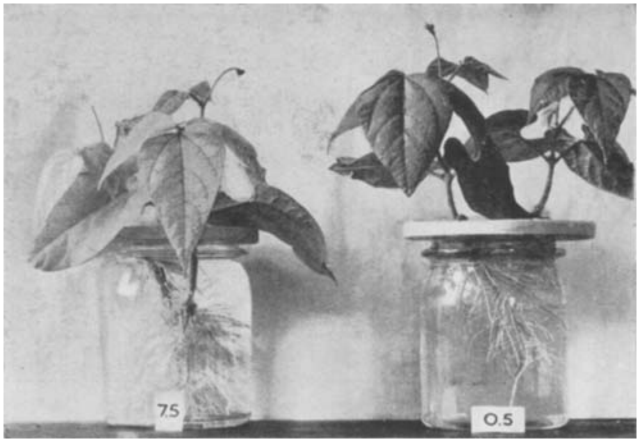


Fig. 24. Bean (Prinsesseboon),
7.5 mg Mn/l, showing iron deficiency, and 0.5 mg Mn/l.

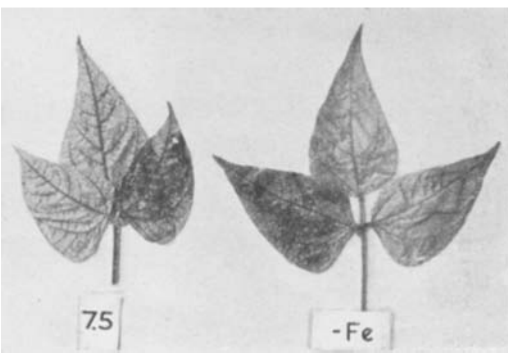


Fig. 25. Bean (Prinsesseboon),
7.5 mg Mn/I and iron omitted,
both treated with $FeSO_4$.

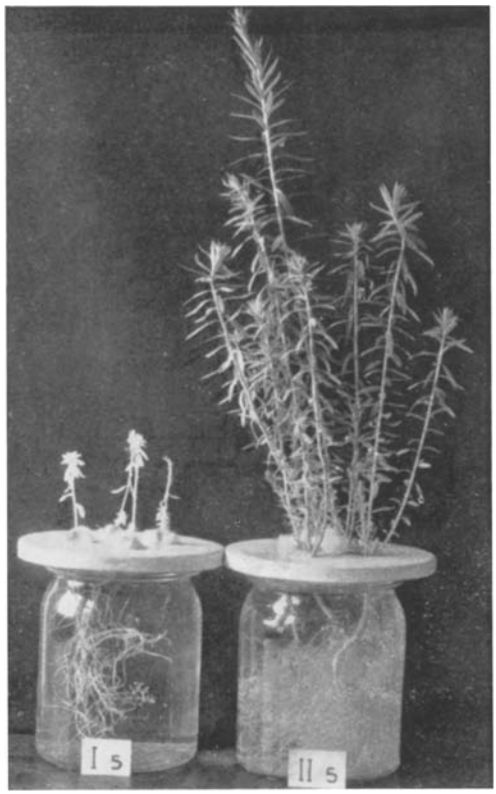


Fig. 23. Flax,
v. d. Crone solution (I) and solution II,
5 mg Mn/l.

of manganese, which could be reduced by application of molybdenum. It has not been ascertained, whether the field symptoms observed in AS plots, and which might be described as "lower-leaf scorch" do respond to a supply of molybdenum.

Solution cultures. In von der Crone solution with 0.5 mg Mn/l very healthy plants were grown. A supply with 0.5 or 1.0 mg sodium molybdate did not influence the development. In the presence of 5 and 10 mg Mn/l with or without molybdenum the plants remainded very short and the foliage was a very pale green or quite white. Painting these leaves with either $\frac{1}{4}\%$ $FeSO_4$ or 0.0025% $Na_2MoO_4.2H_2O$ was ineffective. The contents of the leaves were 1211 (5 mg Mn/l) and 1261 (10 mg Mn/l). These symptoms did not correspond with those noted in AS plots or those described by Millikan as "lower-leaf scorch".

In solution II with 5 mg Mn/l the largest plants grew (Fig. 23). The manganese content of these plants was 940 ppm. Even in 10 mg Mn/l one plant reached a height of 17 cm (content 1232 ppm), but 4 were much smaller and strongly chlorotic (content 1816 ppm). Here again was a supply of molybdenum ineffective.

Since these results with flax are conflicting, the behaviour in solution cultures needs further investigation.

Potatoes.

Field test. In potatoes (Var. Rode Eigenheimer) in none of the plots could any injury be noted. In 1946 young leaves were sampled in some of the plots. The highest values recorded were in Plot II_0 1620, III_0 1717, AS_1 2225, AS_2 1771.

Potatoes which are known to thrive well on moderately acid soils appear to tolerate fairly high contents of manganese without injury. Hewitt[7]), however, describes a characteristic injury induced by a moderate level of manganese, stem specklings or lesions being the most frequent symptoms. It may be conceived that the tolerance might vary in different varieties and further investigation is planned.

Tobacco.

Field test. When grown in 1947 no symptoms of injury could be noted. A close correlation, however, could be established between the manganese content of the young leaves and the amount of liming of the plot (Gr. II). High contents of manganese were recorded (max. 2936 ppm), so that tobacco appeared to be highly tolerant. The amounts recorded by Jacobson and

Swanback in affected plants, which varied between 5250 and 11670 ppm, greatly exceed the amounts recorded here.

Mustard.

Field test. In 1948 mustard was grown. In none of the plots did any difference in growth occur and all plants were unaffected. Samples of young leaves were taken on June 9th before flowering began. In Gr. II the contents of manganese are recorded. Although the amounts of manganese increase along with a decrease in liming (max. 598 ppm, in AS 901) all amounts remain very much below the level reached by other crops. This insensitiveness of mustard is doubtless due to a low intake of manganese. The behaviour of mustard in solution cultures and the level tolerated therein has not been tested. Hewitt [7]) reports the occurrence of symptoms of injury in mustard.

Oats.

In acid plots magnesium may be washed out from the soil so causing disorder in crop plants. As oats are most susceptible to magnesium deficiency, they were grown in 1944 in some of the plots in order to establish whether symptoms of magnesium deficiency might occur. In none of the plots were the oats affected. The amounts of manganese recorded in the young leaves were:

kg lime	0	60
Series I	314 ppm	87.2 ppm
,, II	301 ppm	—
,, III	370 ppm	89 ppm

The amounts of manganese absorbed by oats are much below the level of other plants. This may explain their susceptibility to manganese deficiency in more alkaline soils. (Steenbjerg [24]).

Strawberry.

In none of the plots did any of the plants appear affected. Plants seemed to thrive even better in more acid soils. In 1946 some samples were analyzed; the highest amounts found were in the three unlimed plots, viz. 1264, 1641 and 1220 and in an AS plot 1328.

Peas.

Field test. The very dry spring 1949 made the growth of peas in the experimental plots a failure.

Solution cultures. In von der Crone solution peas throve well in the presence of 10 and 15 mg Mn/l; i.e. amounts that would severely affect other crop plants. All plants were harvested at the budding stage. The amounts of manganese in the foliage were:

in the presence of 10 mg Mn/l 783 and 837
,, ,, ,, ,, 15 ,, ,, 897 ,, 1282.

Peas had apparently taken up little manganese under these conditions and a susceptibility is hardly likely.

The field test is to be repeated.

Broad beans.

Field test. The field crop in 1949 did not yield any clear-cut results as a severe infestation by black aphis resulted in a very irregular stand.

Solution cultures. An experiment in von der Crone solution gave the following results:

mg Mn/l	5		10		15	
Fresh weight g	63	(7 pl.)	65	(7 pl.)	74	(8 pl.)
Mn ppm in yield of vessel	787	641	1489	1766	1645	1795

Plants throve uniformly well in all the vessels and indeed the fresh weight of plants grown in the presence of 15 mg Mn/l was highest. The fact that a content of 1795 ppm did not affect the plants, points to tolerance.

Hewitt [7] [8]) considers broad beans unsusceptible.

Lettuce.

Sherman and Fujimoto [21a]) claim that injury of lettuce consisting in a yellowing of the leaves ("yellow leaf fringe") is due to manganese excess. Since in the culture of lettuce an injury known as "tipburn" (Andersen [1])) (in the Netherlands called "randjes") is known, the cause of which has been uncertain up to now, I prepared some solution cultures (1 plant per vessel) in order to get acquainted with such damage as manganese excess might induce. The lettuce throve very well in these culture solutions and headed normally.

In the variety „Wonder van Voorburg" 12.5 mg Mn/l in von der Crone solution induced a content in the foliage of 2023 ppm, in solution II 25 mg Mn/l 1766. The only slight injury that could be noted consisted of a paling of the outer margins along with a darkening of the veins, more or less reminiscent of bacterial injury. The pattern of "tipburn" injury was not reproduced. Nor was this the case in the variety "New York" in solution II, where a dose of 12.5 mg Mn/l induced a content of 1638 in the foliage and only a slight paling of the margins. Even in the presence of 25 mg Mn/l plants were hardly affected.

Hence it seems unlikely that manganese excess plays a part in the causation of "tipburn".

Hewitt [8]) describes a manganese induced injury in which the foliage is pale and dull yellow around the leaf margin.

Vine.

Du Plessis [4]) suggests that a mottling of vine leaves may be due to manganese excess. This led me to try to determine whether manganese excess might affect the variety "Black Alicante" in the same manner.

Cuttings were made to root and grow in large flower pots filled with soil taken from the plots I_0, I_{60}, AS_1 and Ch_1. The plants were grown from April 14th–Sept. 3rd in a greenhouse. In none of the plants could any injury in the foliage be noted. The leaves were gathered and the following data were recorded.

Soil from plot	Mn ppm in leaves per pot		
I_0	395	482	
I_{60}	273	182	
AS_1	477		
Ch_1	224	214	157

In all the foliage the content of manganese was low, so that any risk of injury due to manganese excess was hardly probable.

In addition, cuttings were grown in von der Crone solution, one cutting per vessel, all having been rooted in the presence of 0.5 mg Mn/l. The amounts recorded in the leaves were:

Mg Mn/l	0.5	12.5	20	50
Mn ppm	222	388	553	342
per cutting		620		865

The foliage of all the cuttings was unaffected and here again the content in the presence of much manganese was low. Hence these experiments do not point to the probability that the injury is in any way due to manganese excess.

As the cuttings have not been grown into adult vines these data are not considered to furnish a definite answer.

c. Discussion.

When the data gathered from all the plots (partly presented in Gr. I and II) are compared, it appears that a tolerance for manganese may be due:

1. to a weak absorption of manganese, preventing any risk of injury through an excess. This could be noted in oats, mustard, mangold and perhaps white clover and vine.

2. To a strong tolerance within the plant, such as appears to be true for tobacco, flax, strawberry, potatoe and probably broad bean. No investigation has been made of the way in which this excess of manganese is distributed within the plant.

The lowest manganese content which appeared to induce visible injury and the highest content still allowing a healthy development of the various young crops are recorded in Table II and are indicated by horizontal lines in Gr. I and II. The actual threshold value will occur between these levels. Whilst for beans the upper level was as already shown, very constant in successive years (Gr. I), the levels for vetch and lucerne were much lower in 1948 than in 1949. For these crops no definite value could be decided upon. For the other crops data are available for one year only.

TABLE II

Lowest content of manganese in young affected plants and highest in young healthy plants

Crop plant	Content of manganese 1 : 1 000,000 dry weight in							
	1946		1947		1948		1949	
	aff.	healthy	aff.	healthy	aff.	healthy	aff.	healthy
Bean (Prinsesse boon)	1210	536	1211	642	1167	670	1104	904
Bean (Bruine boon)			1589	1142	1092	1036	922	855
Vetch					496	363	1177	975
Lucerne					477	452	1083	648
Red Clover							1300	910

The maxima reached in all the liming series by the various crop plants are recorded in Table III. When the content in the AS plots exceeded this amount, this figure is added in parenthesis. Since in the later years all crops in these plots were somewhat depressed in growth, even when they showed no visible signs of injury, it is possible that other factors acting together with manganese excess may have affected the crops; hence these figures are less valid than the others.

TABLE III

Maximum content of manganese in young plants in the series of limed plots (AS plots in parenthesis)

Crop plant	Content of manganese 1 : 1 000.000 dry weight in				
	1944	1946	1947	1948	1949
Bean Prinsesseboon		**3510**	**3665**	2702 (4216)	1709
Boon Bruine boon	**2052**	2290 (2981)	2670	**3047**	2236 (2535)
Vetch			2709	1552	**2678** (3276)
Lucerne				1047 (1152)	1225 (2429)
Red clover					1905
Tobacco			2936		
Potato Eigenheimer	896	1717 (2225)			
Flax					2137
Mangold					947
Mustard				598 (901)	
Strawberry		1641			
Oats	370				

When the data for the various crops are compared, it appears that the highest manganese content occurred in *Phaseolus* in four of the years. Presumably the great susceptibility of this species may be connected with the ease by which manganese is absorbed.

When the data furnished by the solution cultures are compared, it is seen that the tolerance for manganese of the various plants was generally less in von der Crone solution than in solution II. No definite conclusion can be drawn, however, since the experimental data are not sufficient in number and temperature conditions may have played a part because comparative experiments were not always run simultaneously.

When the contents of plants grown in von der Crone solution in the presence of a similar supply of $MnSO_4$ are compared the amounts may vary greatly (Table IV).

TABLE IV

Manganese content of foliage in solution cultures		
Mg Mn/l	5 mg	10 mg
Lucerne	2243 A	—
Vetch	1524 A	—
Mangold	1424	1650
Flax	1261	—
White clover	813	—
Broad beans	787, 641	1489, 1766
Pea	392	783, 837
French bean	—	2938 A
Lettuce	—	2036 SA

A = affected; SA = slightly affected.

These data corroborate the results of field experiments in as much as tolerant plants may be recognized as such by means of solution cultures. They do not allow a conclusion as to the actual cause of this tolerance in the field, when, as in the case of broad bean, both a low uptake in the presence of 5 mg and a fairly high one in the presence of 10 mg occurs.

4. THE ANTAGONISTICS OF MANGANESE

Constituents of the culture solution may influence the amount of manganese actually absorbed.

a. Literature.

Parberry[20]) noted an increased absorption of manganese, when magnesium was deficient. Hewitt[7]) has described reduced toxicity of manganese in the presence of calcium. Morris and Pierre[18]) failed to corroborate these results for *Lespedeza*. Millikan[16]) described an antagonistic action of molybdenum on manganese toxity in flax and (1948) in pea, cabbage and potato.

The antagonistic action of iron has, however, been studied more closely but with conflicting conclusions.

The first instance of injury caused by excess of magnanese in the soil was reported in Hawaï, where pineapples suffered injury on manganiferous soils.

Johnson[11a]) proved that excess of manganese made iron unavailable. He ascribed the effect to the oxidising action of MnO_2, the main form in which manganese occurs in Hawaian soil. The identity of the manganese induced injury with iron deficiency for pineapples has been well substantiated.

Somers and Shive[23]) in an extensive study of soy beans in streaming solution cultures were able to establish a distinct reciprocal antagonism between iron and manganese. They arrived at the conclusion that it is the ratio of soluble Fe/Mn in the plant (optimum about 2) which determines the occurrence of the injury, not the absolute level of either element. In their opinion the symptoms caused by high soluble manganese and low iron are identical with those due to iron deficiency and conversely, low manganese will induce symptoms of iron excess. The manganese induced injury as described by them is, however, not identical with the injury Morris and Pierre [19]) induced in soy beans by excess of manganese. The latter scientists noted a clear difference between manganese-induced and iron-deficiency symptoms in soy beans.

Hopkins *et al* [10]). showed clearly by means of experiments with beans that iron could act as an antidote to manganese toxicity. Normal growth was observed in media containing high Fe/Mn ratios and no symptoms due to excess of iron could be found. Since these workers did not show whether iron deficiency could lead to symptoms similar to those produced in high manganese cultures their conclusion as to its induction in the latter cultures is not convincing.

Hewitt [7]) reported that manganese-induced injury in beans does not respond to a "painting" with ferric citrate. In [9]) he furnished arguments against the existence of a reciprocal action of iron and manganese.

Morris and Pierre [18]) working with *Lespedeza* established that the iron-manganese ratio in solutions was not a primary factor in the growth of the plant. Good growth was obtained over a wide range of iron-manganese ratios. The beneficial effect of iron in reducing manganese toxicity was due to a decrease in the manganese absorbed by the plant rather than to an increase in iron absorption. Iron deficiency symptoms were clearly different from those induced by manganese excess.

b. Experimental results.

Whilst the various investigators arrive at similar results in one respect, viz the antidotic effect of iron on the toxicity of manganese, in other respects their conclusions are conflicting. Neither the toxic affect of large doses of iron nor the identity of the manganese-induced injury with iron deficiency as claimed by Somers and Shive could be corroborated by other investigations.

When I started these investigations in 1943 I set up some observational solution cultures with beans, omitting iron. By this means a chlorosis all over the leaves was induced, which gradually turned a paperwhite, the veins only remaining green. This injury differed sharply from the injury induced by manganese excess.

Incidentally, however, I made observations which may throw some light on the existing controversy.

Beans grown in a greenhouse in summer (June 22th–July 21th 1949) in von der Crone solution provided with 7.5 and 10 mg Mn/l showed symptoms differing from those with which I was familiar; the leaves were of a much more even white than usual. Areas of these leaves were painted with a $\frac{1}{4}\%$ $FeSO_4$ solution and after two days these areas had responded with a bright green colour. Apparently manganese excess had here induced an injury, differing from the usual one and identical with the iron deficiency injury noted by Somer and Shive.

On August 8th a fresh series of bean plants was set up in von der Crone solution with varying amounts of $MnSO_4$ in an unheated warehouse. When the first leaves developed a period of great heat set in and again in several vessels with 5–15 mg Mn/l the iron deficiency symptoms occurred (Fig. 24) which could be cured by means of $FeSO_4$ (Fig. 25). In some plants both types of injury could be noted.

These are merely preliminary observations and the actual conditions causing this phenomenon have not yet been worked out. Perhaps in former years it may have occurred sporadically in bean cultures during periods of great heat, but was then overlooked.

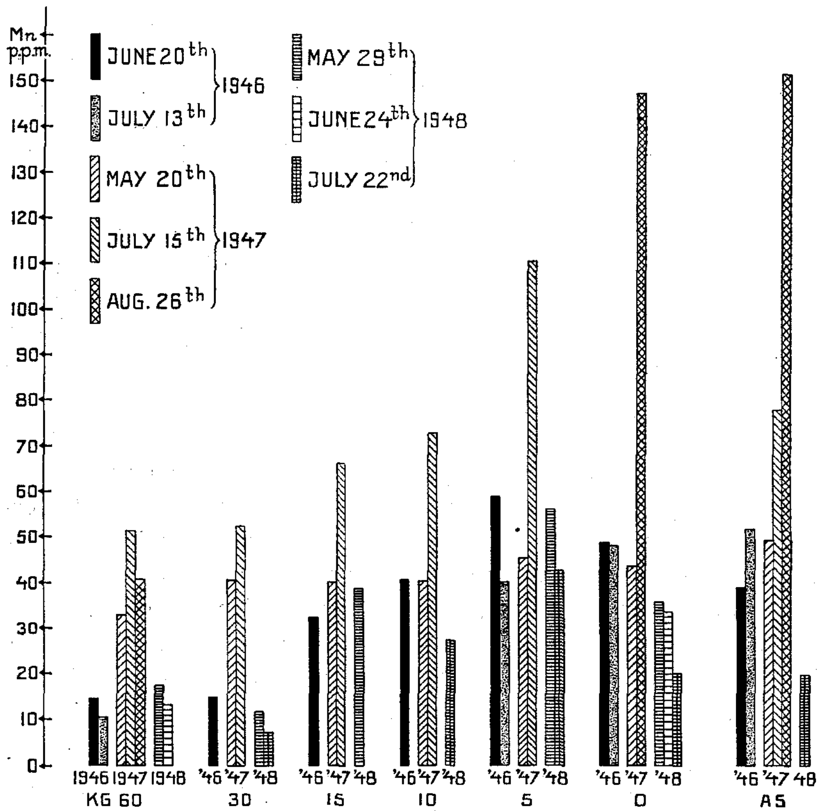
In the experimental field, however, where plants are mature before the hot weather begins, it has never been observed. It is possible that the fact that research on plant nutrition is usually carried out under greenhouse conditions may have caused the existing controversy.

As has already been mentioned symptoms of iron deficiency have been induced in mangold cultures by manganese excess. Since these plants were grown side by side with the beans in the experiment of August 8th–September 7th external conditions might have played their part here as well.

5. MANGANESE IN THE SOIL.

Another question which requires discussion is the effect of soil variation upon the availability of manganese. It is well known that the pH of the soil and its oxido-reduction status determine the form in which manganese will occur. Manganese may exist in varying degrees of oxidation; in neutral or alkaline soils the soluble bivalent manganous salts undergo rapid oxidation to form the insoluble MnO_2 and other higher oxides. Doubtless microorganisms also play a part in this proces.

The injury from which plants are found to suffer in acid soils may be due to various causes. In the Netherlands the main cause has



Gr. III. Exchangeable manganese estimated in soil from the plots in Series I and AS_1.

been recognized as magnesium deficiency (Smit and Mulder 1942). Nevertheless, in some soils excess of manganese appears to cause damage to susceptible crops. The question is therefore posed as to, whether it might be possible to measure the manganese available to the plant directly in the soil. The very extensive literature on this subject, which concern chiefly the methods suitable for determining manganese deficiency in soil, will be passed over. A simple method has been applied by Heintze [6]) in Rothamsted to the estimation of excess of manganese and by it I have estimated the exchangeable manganese in soil.

5 g soil taken up in 100 ml ½ molar $Ca(NO_3)_2$ solution is shaken for one hour and then filtered. In the filtrate manganese is estimated. The soil tested has never been stored for more than 3 days.

When the investigation of the soil was started in 1946 a correlation seemed to exist. When, however, soil samples were gathered under greatly varying weather conditions in later years, amounts estimated in a same plot appeared to vary widely. In Gr. III the amounts estimated in three successive summers in plots I_0, I_{60} and AS_1 show striking differences. For instance in 1947, in an exceedingly dry summer, the amounts were very high whilst in the very rainy summer of 1948 low amounts were recorded, because the manganese fraction estimated in the filtrate had apparently been washed out. In Table V the contents of exchangeable manganese in limed and

TABLE V

Amounts of exchangeable manganese in soil compared with the content of manganese in the bean plants in ppm.

Plot	I_0 kg				I_{60} kg				AS			
	Soil filtrate		Plant foliage		Soil filtrate		Plant foliage		Soil filtrate		Plant foliage	
Year	Mn	date	Mn	date	Mn	date	Mn	date	Mn	date	Mn	date
1946	49	20/6	3510	27/6	14.6	20/6	277	27/6	39	20/6	2840	27/6
1947	13	20/5	3081	6/6	33.3	20/5	518	6/6	49.6	29/5	3394	6/6
					51.4	15/7	84	20/7	78	15/6	2702	9/7
1948	36.3	29/5	—	—	17.7	29/5						
	34	24/6	2442	16/6	13.5	24/6	113	16/6	19.9	24/6	4216	16/6

unlimed plots are recorded along with the contents of the bean plants grown on them.

Whilst in 1947 in plot I_{60} the exchangeable manganese exceeds the quantity in I_0 1946, the plants in the former plot were quite healthy and even contained the lowest amount of manganese recorded.

For a reliable detection of an injurious amount of available manganese in the soil this method proves of small value.

A culture test in soil, such as has been described on page 200 with "Prinsesseboon" as an indicator plant, will furnish a more reliable answer: within a few weeks young plants cultivated in a soil sample will, whenever available manganese is present in excess, present the characteristic pattern of injury.

Testing acid soils by these means might prove of practical importance and might help to disentangle the intricate complex termed as "soil acidity".

I am indebted to Professor Dr. J. Smit for granting me the opportunity of carrying out this investigation in the Laboratorium voor Microbiologie, to Dr. K. T. W i e r i n g a for good advice and interest in the problem, to the Technical staff of the Laboratory for their helpfulness, to Professor E. C. W a s s i n k for the opportunity of cultivating plants in the greenhouse of the Laboratorium voor Plantenphysiologisch Onderzoek and to the Organisation T.N.O. (Applied Scientific Research) for providing me with a warehouse.

SUMMARY

1. In unlimed or sparsely limed experimental plots *Phaseolus vulgaris* appeared seriously affected. Young leaves showed a characteristic pattern of injury and might completely fail. Less injured plants might recover. These symptoms could be reproduced in solution cultures supplied with excess of $MnSO_4$.

2. During several years the manganese content of beans growing in these plots has been estimated. In healthy young leaves the value ranged from 40–940 Mn ppm, in affected leaves from 1104–4261. The lowest level of injured leaves varied in 1946–1949 between 1211–1104 ppm.

3. In solution cultures (v o n d e r C r o n e) 5 mg Mn/l induced the symptoms. At high temperature bean plants appeared to tolerate a high content in the foliage without visible injury.

4. The composition of the culture solution acts greatly on the amount of manganese taken up by the plant. A difference in the proportion of the manganese supplied which remains in solution cannot explain this action.

5. Strict proof of the injury being due to manganese excess was furnished by the similarity of symptoms induced in beans, when cultured in a vessel with "sick" soil and in the same soil provided with $CaCO_3$ and $MnSO_4$. Acidity was here eliminated as causal agent.

6. In experimental plots *Vicia sativa* and *Medicago sativa* appeared very susceptible and showed characteristic signs of injury. *Trifolium pratense* showed slight symptoms and a final recovery. *Trifolium repens* merely lagged in initial development. Solution cultures corroborated the field tests.

7. Mangold was depressed in growth in more acid plots. Solution cultures did not corroborate the field tests.

8. Flax, potatoes, tobacco, mustard, oats and strawberry appeared tolerant in the field tests.

9. Peas, *Vicia faba* and vine appeared insensitive in solution cultures. Lettuce showed a slight injury in the presence of a large dose of $MnSO_4$.

10. When the contents of manganese in the foliage are compared, tolerance for manganese appears either to be due to a weak absorption or to a strong tolerance within the plant. The high sensitiveness of beans may be due to a very strong absorption.

11. Manganese induced symptoms of beans in the field are not identical with those induced by iron deficiency.

In solution cultures, however, at high temperature manganese excess was seen to induce iron deficiency.

12. The amount of exchangeable manganese in soil appeared to vary widely in plots investigated over a number of years. No correlation was found between these values and the content of manganese in the foliage of beans.

13. As a soil test for manganese excess *Phaseolus vulgaris* may prove of value as a test organism.

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REFERENCES

1) Andersen, E. M., Tipburn of lettuce, Effect of maturity, air and soil temperatures and soil moisture tension. Corn. Un. Agr. Exp. St. Ithaca N.Y. Bull. 829 (1946).

2) Bortner, C. E., Toxicity of manganese to turkish tobacco in acid Kentucky soils. Soil Sci. **39**, 15–33 (1935).

3) Daji, J. A., Manganese toxicity as a probable cause of the band disease of *Areca* palm. Curr. Sc. **17**, 259–260 (1948); R.A. M.**28**, 124 (1949).

4) Du Plessis, S. J., Wingerdsiektes in Suid-Afrika Un. of Stellenbosch 1948, 221 p.

5) Fried, M. and Peech, M., The comparative effects of lime and gypsum upon plants grown in acid soils. J. Am. Soc. Agron. **38**, 614–623 (1946).

6) Heintze, S. G., Readily soluble manganese of soils and marsh spot of peas. J. Agr. Sci. **28**, 175–185 (1938).

6*a*) Hewitt, E. J., The resolution of the factors in soil acidity. Progr. Rep. I. Ann. Rep. Long Ashton Res. Sta. 1945, 51–59.

7) Hewitt, E. J., The resolution of the factors in soil acidity. Some effects of manganese toxicity. Ann. Rep. Long Ashton Res. Sta. 1946, 50–61.

8) Hewitt, E. J., Resolution of the factor in soil acidity. The relative effects of aluminum and manganese toxicities in farm and market garden crops. Ann. Rep. Long Ashton Res. Sta. 1948, 58–65.

9) Hewitt, E. J., Experiments on iron metabolism in plants. Some effects of metal-induced iron deficiency. Ann. Rep. Lang Ashton Res. Sta., 1948, 66–80.

10) Hopkins, E. F., Pagán, V. and Rumurez Silva, F. J., Iron and manganese in relation to plant growth and its importance in Puerto Rico. J. Agric. Un. Puerto Rico, **28**, 43–99 (1944).

11) Jacobson, H. G. M. and Swanback, T. R., Manganese content of certain Connecticut soils and its relation to the growth of tobacco. J. Am. Soc. Agron. **24**, 327–245 (1932).

11*a*) Johnson, M. O., Manganese chlorosis of pineapples: Its cause and Control. Hawaii Sta. Bul. 52 (1924).

12) Löhnis, M. P., Een voedingsziekte in bonen (*Phaseolus*) (Summ. A nutritional disease in beans. Preliminary Rep.). Tijdschr. Plantenziekten **2**, 157–160 (1946).

13) Löhnis, M. P., Injury through excess of manganese. Trace elements in plant physiology. Lotsya. **3**, 63–76 (1950).

14) Marshall, H., Abstracts J. Chem. Soc. **80** II 350 (1901).

15) McCool, M. M., Effect of light intensity on the manganese content of plants. Boyce Thompson Inst. Contr. **7**, 427–437 (1935).

16) Millikan, C. R., Effect of molybdenum on the severity of toxicity symptoms in flax induced by an excess of either manganese, zinc, copper, nickel or cobalt in the nutrient solution. J. Australian Inst. Agr. Sci. **13**, 180–186 (1947).

17) Millikan, C. R., Antagonism between molybdenum and certain heavy metals in plant nutrition. Nature **161**, 528 (1948).
18) Morris, H. D. and Pierre, W. H., The effects of calcium, phosphorus and iron on the tolerance of *Lespedeza* to manganese toxicity in culture solutions. Soil. Sci. Soc. Am. Proc. **12**, 382–386 (1948).
19) Morris, H. D. and Pierre, W. H., Minimum concentration of manganese necessary for injury to various Legumes in culture solution. Agron J. **41**, 107–113 (1949).
20) Parberry, H. N., The excessive uptake of manganese by beans showing scald and magnesium deficiency, Its regulation by liming. Agr. Gaz. N. S. Wales **54**, 54 (1943).
21) Peech, M. and Bradfield, R., Chemical methods for estimating lime needs of soils. Soil Sci. **65**, 35 (1948).
21*a*) Sherman, G. D. and Fujimoto, C. K., The effect of the use of lime, soil fumigants and mulch on the solubility of manganese in Hawaiian soils. Proc. Amer. Soc. Soil Sci. 11. 206 (1946).
22) Smit, J. and Mulder, E. G., Magnesium deficiency as the cause of injury in cereals. Mededeel. Landbouwhogeschool, Wageningen, **46**, 3 (1942).
23) Somers, S. I. and Shive, J. W., The iron manganese relation in plant metabolism. Plant Physiol., **17**, 582–602 (1942).
24) Steenbjerg, F., The manganese content of Danish soils. I Exchangeable manganese, Tids. Planteavl **39**, 401–436 (1933).
25) Wallace, T., Hewitt, E. J. and Nicholas, D. J. D., Determination of factors injurious to plants in acid soils. Nature **156**, 778–782 (1945).