IRON AND MANGANESE IN RELATION TO PLANT GROWTH AND ITS IMPORTANCE IN PUERTO RICO

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In recent years attention has been focused on minor element deficiencies. Many such cases having great agricultural importance have been pointed out and remedies for their prevention have been put into practice. The matter of toxicity of the minor elements has not been emphasized to a great extent from the standpoint of practical plant culture except for certain limited cases. Further, the possibility of balance or antagonism between the minor elements themselves has been the subject of still less investigation. It is the purpose of this paper to point out that toxicity of manganese to plant growth occurs in Puerto Rico, and to attempt to make clear the mechanism by which iron antidotes this toxicity.

The toxicity of manganese to pineapple plants in Hawaii as reported by Kelley (20, 21, 22) and by Johnson (15, 16, 17, 19) might be cited as such a particular instance of toxicity just referred to. This results from the peculiarities of the soil there which develops under cultivation a high amount of soluble manganese. In fact, Gile (6, 7) in investigating chlorosis of pineapple plants on calcareous coastal plain soils in Puerto Rico found no excessive manganese and considered pineapple chlorosis on the Island to be of a different nature. In other words, it was caused by a high alkalinity bringing about a precipitation of iron and with it an iron deficiency. This may be true of these calcareous soils. Nevertheless it will be shown later in this paper that in the principal pineapple growing areas in Puerto Rico manganese toxicity chlorosis occurs on acid soils, and this chlorosis may be prevented by raising the pH with calcium carbonate.

It is of particular interest that while in Hawaii, Johnson (15, 16) found that spraying the plants with an iron sulphate solution counteracted the effect of manganese present in the soil, Gile (6) found that iron sulphate added to the soil had no effect and that while spraying the plants with this chemical would prevent chlorosis, the spraying had to be repeated every few months to keep the plants green. He concluded he was dealing with a different condition from that in Hawaii and that iron sprays were of no practical value.

The question as to whether the cause of this chlorosis on calcareous soils in Puerto Rico is fundamentally different from the other will be discussed later. However, it is now clear that in the principal pineapple growing area there exists at the present time a condition identical to that in Hawaii, i.e., high manganese and low iron on acid soils.

PRELIMINARY EXPERIMENTAL WORK WITH SOILS[1] AND PLANTS AND DISCUSSION OF THE PROBLEM

To show that the same condition exists in Puerto Rico as in Hawaii it is sufficient merely to point out the following facts resulting from our experiments and observations: 1—chemical analysis of certain soils revealed them to have a high amount of manganese soluble in distilled water varying from 20 to over 130 ppm of manganese and no detectable water soluble iron; 2—pineapple plants grown on these soils without receiving iron sprays show extreme chlorosis characteristic of manganese toxicity; 3—practical growers have found that iron sulphate spray is essential and

FIG. 1. Bean plants growing in soil from pineapple field containing a high concentration of soluble manganese. A—Check, with no additions. B—With calcium carbonate to pH 6.2. C—With calcium carbonate to pH 6.2 plus humate iron.

this is now a common practice; 4—the common bean when grown on these soils shows severe chlorosis of the first trifoliate leaves in from 6 to 10 days from planting and no further growth of the plant occurs; 5—when the manganese is immobilized by adjusting the pH of the soil to about 6.2 with calcium carbonate, chlorosis is prevented and if, in addition, iron in a soluble form (as humate iron) is used, normal growth of beans results (see figure 1); 6—even when chlorosis in pineapple plants is corrected by iron sulphate sprays, certain abnormalities occur that are undoubtedly due to manganese toxicity: there is a reduction in the size of the plant,

[1] The pineapple soils referred to here were obtained from the vicinity of Arecibo and Manatí. The soil type was determined by Dr. J. A. Bonnet of the Soils Department, to be "Bayamón Sandy Clay Loam."

the leaves are narrow and tend to develop more red pigment than normal; 7—plants of the variety "Red Spanish" grown from slips obtained from Cuba and which probably have a higher iron reserve are, in the first generation, much larger and greener and appear to be a different variety from those grown from Puerto Rican slips; 8—after about two generations

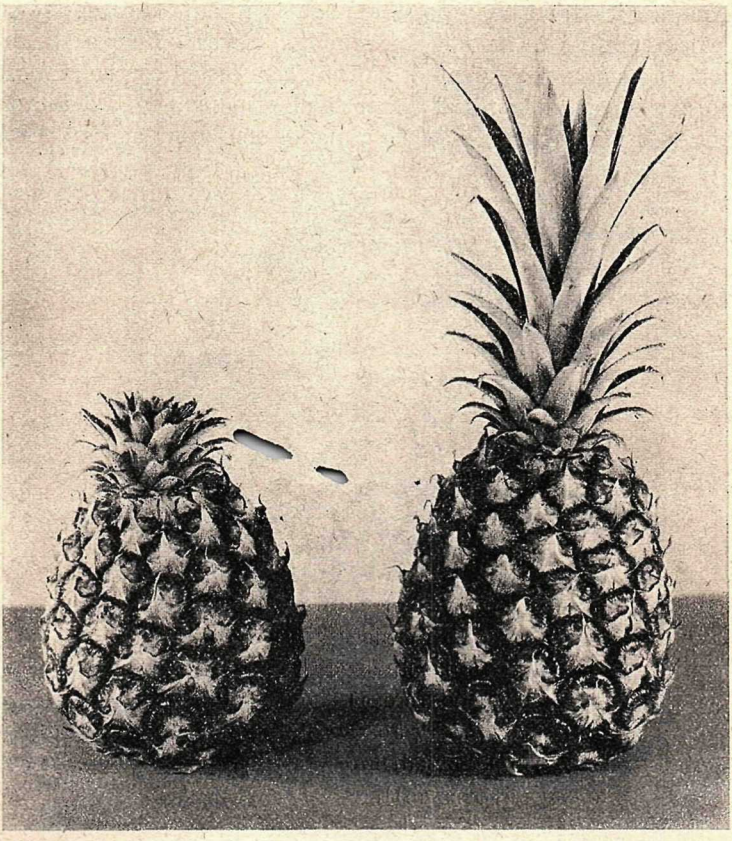

FIG. 2. Pineapple fruits showing—left—the "short top" symptoms resulting from high content of soluble manganese in the soil at high light intensity. Right, a normal fruit.

in Puerto Rico plants originally from Cuban slips revert to the type described above; 9—many fruits produced on these soils are affected with "short top"[2] (figure 2) which appears to be brought about by high soluble

[2] This was first called to our attention by Mr. John Raymer of the Palo Blanco Fruit Company, Arecibo, Puerto Rico, and has since been observed on fruit from other plantations.

manganese in combination with high light intensity; 10—ash analyses of pineapple fruits and other plants from these areas show such very high amounts of manganese that the carbon free ash when removed from the muffle furnace has the characteristic blue-green color due to a large content of manganese (figure 3).[3]

To what extent this soil condition exists in the West Indies, Central and South America it would be impossible to say without an extensive survey but it would appear to be not uncommon. Several Puerto Rican soils other than those used for pineapple culture were tested by the authors and found to have high amounts of water soluble manganese. Ash analyses of banana seed pieces from Guatemala made some years ago by the senior

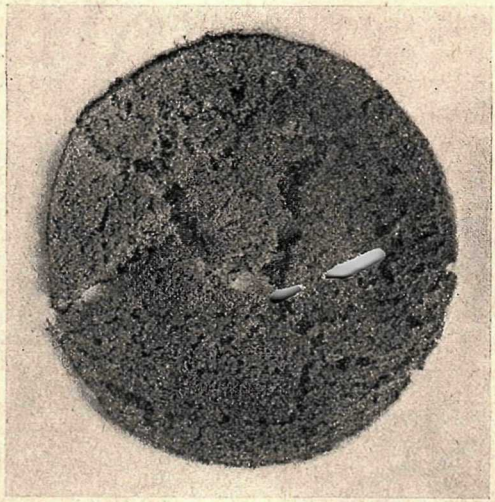

FIG. 3. Carbon free ash of pineapple fruit produced on soil with high content of soluble manganese. The "dark" color of the ash in the photograph is a blue-green color characteristic of plant ash with high manganese content.

author revealed high amounts of manganese. In continental United States a similar condition has been reported by Funchess (3). He found that certain soils in Alabama, when fertilized with either dried blood or ammonium sulphate, developed a high amount of soluble manganese very toxic to plants. One soil from Pennsylvania investigated by him showed

[3] Analyses of 4 fruits from iron sprayed plants and their tops (crown leaves) showed on the oven dry basis:

Fruit	Iron	75 to 200 ppm	Ave. 141 ppm Fe
	Manganese	175 to 520 ppm	Ave. 300 ppm Mn
Tops	Iron	100 to 178 ppm	Ave. 129 ppm Fe
	Manganese	250 to 1495 ppm	Ave. 871 ppm Mn

the same phenomenon. Toxic concentrations of soluble or "available" manganese in soils have also been reported from Kentucky (27, 1, 31), Connecticut (14), Indiana (2), New South Wales (8), Sweden (34), India (9), and probably occur in many other places.

This brings up the matter of fertilizer practice in relation to the problem. It was pointed out by one pineapple grower in Puerto Rico that he obtained much better growth of the plants on virgin soils. After these soils had been in use for several years the development of the plants was much poorer. This is undoubtedly due to the continued use of the ammonium sulphate which constitutes the usual form of nitrogen in the fertilizer used in pineapple culture. This results in a highly acid condition of the soil which brings manganese into solution. Certain pineapple soils investigated by us have shown pH values as low as 4.0 and 4.2, and one grower reported the low value of 3.8.

That the fertilizer is responsible for this high acidity is shown by the fact that when samples of soil are taken from the top two inches of the soil near the rows where fertilizer is applied a much lower pH and higher soluble manganese are obtained than when taken from a depth of 6 inches midway between banks. In one instance the first method of sampling showed a pH of 4.2 and water soluble manganese, 134 ppm; while the second method showed a pH of 5.5 and water soluble manganese, 65 ppm. The latter soil sample was much less toxic to the common bean, which was used as a test plant, than the former.

Some preliminary experiments on this soil condition will be described here. In these experiments beans were used as test plants since they show the effects of manganese toxicity in a short time while with pineapple plants the development of symptoms may require several months. At first an attempt was made to antidote the manganese toxicity by adding iron in a soluble form to the soil. A sample of a pineapple soil showing well over 100 ppm water soluble manganese was used for pot cultures. Check cultures without any additions showed severe chlorosis of the bean plants 9 or 10 days from planting. While the seed leaves were not distinctly chlorotic they were a somewhat lighter green than those of normal plants and developed characteristic minute necrotic lesions, dark brown in color, distributed over the entire leaf. Some of these lesions also appeared on the stems and leaf petioles. When, however, the first true or trifoliate leaves appeared they were extremely chlorotic and finally died. The plant, therefore, failed to develop further since the growing point was dead. This is shown in figure 1, culture "A." When humate iron was added to the same soil at the rate of 20 ppm Fe, the same symptoms occurred as in the check plants and it was concluded that iron in this amount was unable to balance the large amount of soluble manganese in the soil.

Next, it was decided to first immobilize the high amount of solulbe manganese according to the procedure of Funchess (3). This was done by neutralizing the soil acidity with calcium carbonate and raising the pH to 6.2. To another set of soil cultures both calcium carbonate and humate iron (20 ppm Fe) were added to the soil. The result is shown in figure 1. Without treatment, severe chlorosis followed by necrosis and death occurred as before described. Addition of calcium carbonate to pH 6.2 resulted in the correction of chlorosis and other symptoms of manganese toxicity, and much better growth took place. (Compare in figure 1 the first trifoliate leaves in culture "B" which are about normal size, with the chlorotic aborted ones in culture "A.") The further addition of soluble iron gave still better growth as will be seen in figure 1, culture "C," where the plants are much taller and the second trifoliate leaves have formed and developed to considerable size. This latter is important in showing that additional iron has a further effect on the growth even after the chlorotic condition has been corrected. The following measurements were made in connection with the experiment just described.

No.	Treatment	Ave. height of plants, 13 days	Ave. width of seed leaves 13 days	Ave. length of first trifoliate leaves 18 days	Ave. height of plants, 18 days	Total dry weight of tops, 43 days
		mm	*mm*	*mm*	*mm*	*gms*
1	None, check	69.5	28	11	96	0.61
2	$CaCO_3$ to pH 6.2	70.5	45	47	140	2.05
3	Like 2 but plus 20 ppm Fe as humate	85.0	53	57.5	200	2.60

To show the effect of calcium carbonate on both the pH and soluble manganese content of this acid pineapple soil, an experiment was made. Varying amounts of calcium carbonate were added to a given amount of the soil and thoroughly mixed. The moisture was adjusted to a content about optimum for plant growth. The pH was then determined with the glass electrode, the soluble manganese extracted with distilled water and its amount determined by the potassium periodate method. The results are shown graphically in figure 4. As the amount of calcium carbonate increases and the pH likewise increases, the amount of soluble manganese falls off rapidly until at pH 7.4 it is somewhat less than 20 ppm.

A word should be said at this point as to why raising the pH of a soil which shows no water soluble iron to begin with (and especially with calcium carbonate) should correct a condition which is apparently an iron deficiency chlorosis. It was formerly considered that most cases of this type are associated with high pH and high calcium carbonate. Iron defi-

ciency was not thought of in connection with acid soils. We offer the following explanation. For some reason when these soils become acid, manganese becomes very soluble but at the same time iron remains locked up in an insoluble form or at least does not go into solution to any great extent. The chlorosis in this case is due to manganese toxicity which will be shown later to be equivalent to iron deficiency or lack of balance between the two elements. When the soluble manganese is immobilized by raising the pH with calcium carbonate the chlorosis disappears.

Johnson (19) proposed to explain the action of manganese by its oxidizing effect in the soil. According to his idea, the iron is oxidized to the ferric

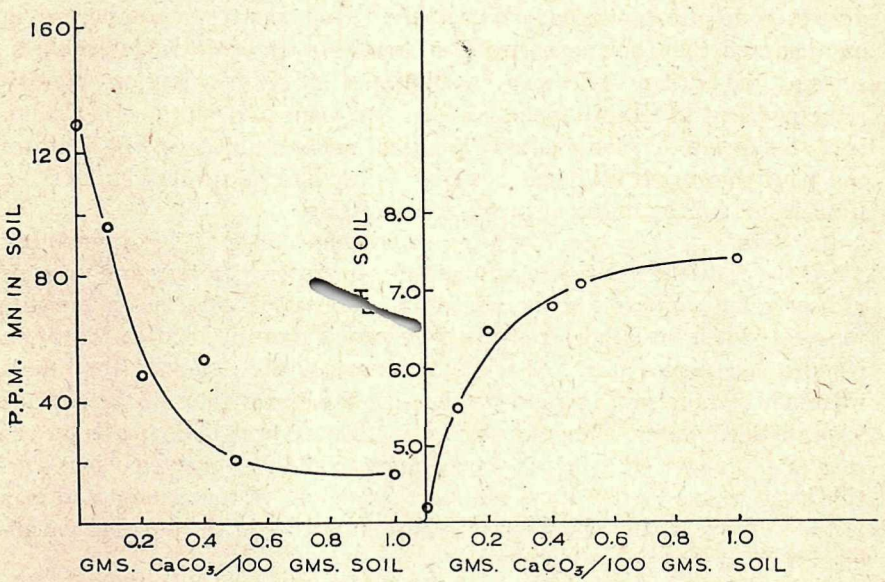

FIG. 4. Effect of calcium carbonate on the soluble manganese and the pH of acid pineapple soil.

condition in which form it is more readily precipitated and hence becomes unavailable to the plant. This hypothesis, however, would hardly apply to these soils of such a high acidity and would not explain why the mere addition of calcium carbonate, as shown above, will largely correct this condition. Furthermore, it would not explain the chlorosis in the solution culture experiments in our work in which the iron always remained in solution as humate iron, regardless of the amount of manganese present.

In regard to iron our tests were made by extracting the soil with distilled water. The negative tests obtained for iron do not mean, of course, that no iron was available to plants. It seems, however, that the available

iron in these soils was relatively low since certain other Puerto Rican soils extracted and tested in the same way have given good positive reactions for iron.

It is, therefore, not always a low concentration of iron that brings about "iron deficiency chlorosis" but the ratio or balance between iron and manganese (or possibly some of the other heavy metals). In this connection, reference should be made to the important observation of Schappelle (29, 30) in his experiments on the effect of minor elements on pineapple plants. He found that if all the usual minor elements were present in the culture solution, normal green plants were produced. If, however, all the minor elements were omitted, green plants with luxuriant vegetative growth were also produced. While these latter plants were abnormal in another way, that they never produced flowers or fruits although "smoked" several times with acetylene gas in solution, they did not develop chlorosis although kept in culture for more than two years. If on the other hand, iron alone were omitted from the solution, marked chlorosis and death of the plants occurred although obviously they had as much iron as those from which all the minor elements were missing.

In one of our own water culture experiments (26-I) it was found that when the nutrient solution was made in tap water, chlorosis and necrosis of bean plants appeared sooner and were more severe, and the dry weights were less when no minor elements were added than in identical solutions made in distilled water. Analysis of these solutions showed that there was slightly more iron in those made with tap water than in those made with distilled water. The amount of manganese was also greater in the case of tap water. In fact, no manganese could be detected where distilled water was used. If we add to the amount of manganese and iron in the solution (that from the water[4] and impurities in the nutrient chemicals) the amount present in the seed, we have:

	ppm	
	Fe	Mn
Cultures made with distilled water	0.10	0.15
Cultures made with tap water	0.12	0.30

This is a case then where iron deficiency chlorosis is more severe with a higher concentration of iron. The apparent anomaly is due to the higher ratio of manganese to iron where tap water was used. Since the relative amounts of manganese and iron in the seed that are in an available form are unknown, this ratio may actually be higher than would appear from

[4] By analysis it was found that the tap water at the Experiment Station at Río Piedras contained 0.02 ppm iron as Fe and 0.1 ppm manganese as Mn.

the figures given above. When 0.5 ppm of manganese was added to cultures identical with the above, severe chlorosis appeared in both cases.

Since this work was completed, an excellent paper by Somers and Shive (33) has appeared in which they have also shown clearly for soybean plants that "iron deficiency chlorosis" is identical with manganese toxicity chlorosis. They also point out that pathological symptoms produced by excessive iron are identical with those produced when manganese is deficient.

The antidoting effect of iron on manganese toxicity had previously been reported in the literature. Among these reports may be mentioned the work of Tottingham and Beck (35) with wheat, Johnson (18) with rice, and Rippel (28) with barley. In all these cases chlorosis caused by manganese in solution cultures was prevented by the addition of iron.

In our investigations iron toxicity was not encountered, either in soil or in water cultures. This, we think, was due primarily to the fact that manganese, due to impurities in nutrient salts and the amounts in seed or seed pieces (pineapple slips), never was at a low enough concentration for iron to become toxic although concentrations as high as 20 and 30 ppm of iron were used.

There is also the possibility that iron toxicity may not have shown up because of: 1—a difference in the reactions of the plants used in this work (bean, tomato and pineapple) compared with the soybean plant used by Somers and Shive; 2—the greater amount of reduction of iron at the high light intensities prevailing in the tropics and; 3—the maintenance of a relatively low pH and the use of inorganic iron by Somers and Shive may have resulted in a higher iron-ion concentration (Hopkins 11) than was realized in the present work at a higher pH with humate iron. A slight indication of iron toxicity was previously noted by Hopkins (12, p. 28 and figure 2). This was in the growth of the common duckweed *Lemna minor*, in various combinations of iron and manganese. Growth with iron but without manganese was slower than where both elements were lacking. The author suggested that it might be due either to a greater proportion of reduced iron or to lack of antagonism.

Further, there is the possibility that in solutions of relatively low pH in which iron is added as a ferrous salt and the solutions frequently changed, the toxicity observed may be ferrous iron toxicity rather than just iron toxicity. The conditions maintained may tend to stabilize iron in the ferrous state.

The purpose in this investigation was, from the beginning, to concentrate mainly on amounts of these elements within their normal range as might be encountered in soil culture, rather than to use highly refined methods and purified chemicals such as are used to bring out deficiency symptoms and to demonstrate the necessity of minor elements. It was proposed to

determine if within these normal limits marked variation in yields could be obtained which would point the way to better crop production. Iron and manganese were chosen because their essential nature for the growth of green plants has been shown beyond doubt (12). In some unpublished work the senior author had also demonstrated for the unicellular alga *Chlorella* sp., that a very important reciprocal relationship between iron and manganese, similar to the above mentioned effect on higher plants, exists. Besides being necessary elements they are two of the most important minor elements from the standpoint of practical agriculture.

EXPERIMENTAL METHODS

Culture solution. The basic culture solution used in most of these experiments was according to the following formula:

Salt	Formula	
	pH 5.5	Gms./L
Potassium dihydrogen phosphate.....	KH_2PO_4	0.300
Calcium nitrate.....	$Ca(NO_3)_2 \cdot 4H_2O$	0.500
Magnesium sulphate.....	$MgSO_4 \cdot 7H_2O$	0.500
Ammonium sulphate.....	$(NH_4)_2SO_4$	0.100

Chemicals of CP grade without further purification and distilled water, rain water and tap water were used in making the solutions as indicated in the individual experiments. Iron was added in the form of potassium iron humate prepared according to the method of Horner, Burk and Hoover (13) and manganese as manganous sulphate.

Culture vessels. For some of the preliminary experiments and special tests, liter pyrex beakers were fitted with shields to exclude light from the solution and the tops were covered with paraffined cardboard in which holes were made for the plants. The plants were supported with pieces of absorbent cotton. The number of cultures varied in the different experiments and the solutions were changed once a week. For the larger and more exact experiments a series of 42, 20-liter pyrex jars were employed. These were buried in sand in two adjacent benches in the greenhouse to within about 2 inches of the top, the exposed part of which was painted black. The type of cover varied with the kind of plant used. Air from an air-pressure line was constantly bubbled through the solution for the purpose of stirring and aeration. Solutions were changed once a month. The jars were numbered at random so that treatments and replications were also at random. A partial view of this equipment is shown in figure 5.

One experiment (26-I) was carried out in ten subirrigation gravel beds, 6 x 4 x 1 feet deep, each made of sheet metal coated with asphalt paint. In each case the bed was connected with a solution storage tank and a centrifugal motor pump. The pumps were controlled by a time clock in

such a way that the nutrient solution was pumped 6 times a day (twice at night and 4 times in the daytime) to within about 1 inch of the gravel surface. The solution then drained back by gravity into the storage tank. The storage tanks had a capacity of 100 gallons, and 50 gallons of nutrient solution were used for each culture. Detailed description of the apparatus will not be given since it is very similar to that used by Withrow and Biebel (37) and others.

Observations and measurements. The cultures were examined frequently for chlorosis, necrosis, general vigor of the plants and any other condition

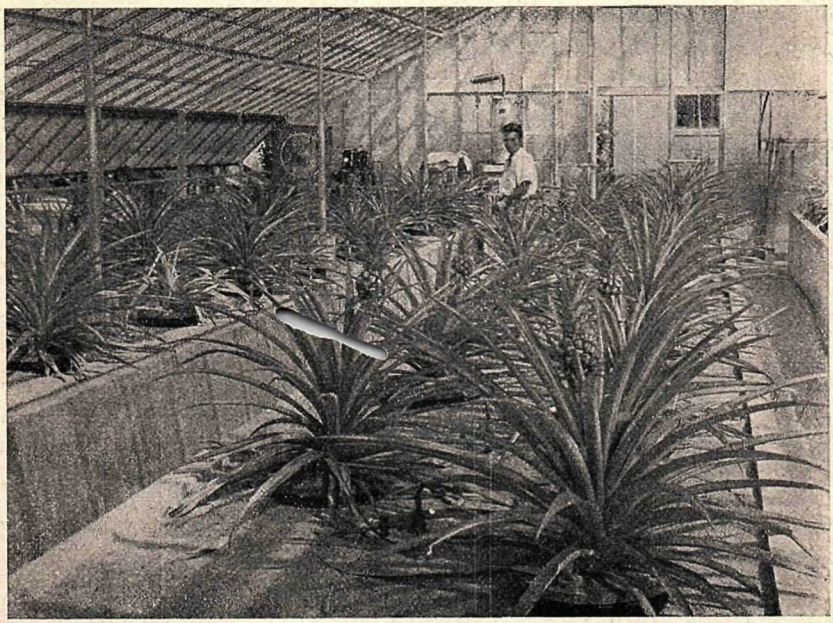

FIG. 5. Partial view of solution culture equipment used in these investigations. Photograph shows pineapple cultures.

noted. For these items a numerical estimation, usually on the basis of 10 or merely a plus and minus record, was made and since in the large experiments the treatments were at random, the estimation was made without knowing the given treatment. Records were kept of these observations so that, for example, in the case of chlorosis, not only the fact that it occurred but also the time of its appearance and its relative severity were noted. This, combined with the number of plants in a given treatment to show it, gave a rather good measure of the effect of the treatment.

The time of appearance and number of such things as first, second and third trifoliate leaves, tendrils, flower buds, flowers, fruits, etc., were also noted. Measurements of the heights of plants and lengths of the corre-

sponding leaflets for each plant were taken at intervals and finally the plants were harvested and the green and dry weights of tops and roots determined.

EXPERIMENTAL RESULTS

Experiment 26-A. Bean

Bean plants were grown in liter beakers with variable amounts of both manganese and iron merely for the purpose of observing the symptoms of manganese toxicity and the antidoting effect of iron. In the basic culture solution (p. 52) ammonium sulphate was used as the entire source of nitrogen and calcium was furnished by calcium chloride. Although the plants were grown for a period of only 10 days, the final pH of the solutions varied from 3.50 to 3.65.

The following diagram shows the set-up of the experiment and the relative amount of chlorosis after 3 days from planting.

Mn	Fe			
	3 ppm	6 ppm	9 ppm	27 ppm
ppm				
2	(4) 0	(5) 0	(6) 0	— — —
3	(7) 0	(8) 0	(9) 0	— — —
9	(10) 0	—	(11) 0	(12) 0
20	(1) ++++	(2) +++	(3) ++	— — —

Culture numbers are given in parenthesis.

This chlorosis which showed up very soon only appeared in solutions having 20 ppm manganese and decreased rapidly as the iron increased from 3 ppm to 9 ppm. At 7 days from planting, chlorosis was still very severe in culture number 1 and remained so until the tenth day when the experiment was terminated. On the other hand, chlorosis in cultures 2 and 3 was less at 7 days so that No. 2 showed only slight chlorosis and No. 3 very slight chlorosis. Both practically recovered from the chlorotic condition by the tenth day. None of the other cultures were chlorotic.

As stated above chlorosis was very severe after 3 days in culture 1. The cotyledons and stems were pale white on two of the seedlings and the seed leaves were chlorotic and stunted. On the other 2 seedlings the stems and cotyledons were partly chlorotic and the seed leaves which were about 1 inch wide showed a chlorotic pattern, white along the midrib and widening out at the tip. The chlorosis was pure white rather than yellow-

ish white. In this culture the roots were normal in appearance and well developed. The extreme chlorosis shown here is thought to be due to the high acidity resulting from the use of ammonium sulphate. In later experiments where calcium nitrate was used such marked symptoms were not found even at 20 ppm manganese and no added iron. The stems and cotyledons were not affected and the seed leaves while often pale green did not show definite chlorosis. However, they often showed minute brown necrotic spots which were always associated with high manganese and low iron.

The roots in all cultures of this experiment appeared to develop normally with good color and abundant fibrous secondary roots. The only abnormality was a slight browning of the root tips in practically all cultures. This was thought to be due to the high acidity in the medium after some growth had taken place. This trouble, however, was less severe in cultures with high manganese and low iron.

Dry weights of the tops and roots were taken at the end of the tenth day. They do not show a definite trend except in the low iron and the high manganese series. At the lowest iron concentration (3 ppm) there is a general decrease in the dry weight of the tops as the manganese increases and at the highest manganese concentrations there is an increase as the iron increases. The results are given below.

Mn	Fe			
	3 ppm	6 ppm	9 ppm	27 ppm
ppm				
2	(4) 193 47 240	(5) 180.5 41 221.5	(6) 174 42 216	—
3	(7) 170 45.5 215.5	(8) 181 39.5 220.5	(9) 159.5 38.5 198	—
9	(10) 154 44 198	—	(11) 237 62 299	(12) 212.5 61 273.5
20	(1) 165 47.5 212.5	(2) 202 53 255	(3) 256 68 324	—

Dry weights in milligrams, tops above, roots middle, totals below.

The above results show rather definitely the antidoting effect of iron on manganese toxicity and is in line with the effect shown in regard to chlorosis.

Several things are evident from this experiment. 1—Manganese at 20 ppm is injurious to the tops of bean plants at 3 ppm iron. This is shown both by severe chlorosis and decreased yield. 2—As the iron content is increased the injury becomes less and the dry weight increases. 3—Even with severe injury to the tops the roots appear to be normal. 4—Manganese injury to the tops is caused by an upset in the chlorophyll mechanism. 5—Iron in the form of potassium iron humate in concentrations up to 27 ppm is not injurious to bean plants with manganese at 9 ppm.

Experiment 26-B. Bean

This was also a preliminary experiment for the purpose of observing chlorosis. Twelve cultures all containing 20 ppm manganese were set up. The iron was varied from none to 30 ppm Fe. Ammonium sulphate was again used as the main source of nitrogen but the solutions were kept from becoming acid by subsequent additions of sodium nitrate. In this experiment there was no chlorosis of the cotyledons and stems but it showed up to a slight extent on some of the seed leaves. However, chlorosis was very severe on the first trifoliate leaves at low iron concentrations. Observations on chlorosis, 12 days after planting were as follows.

Culture Number	Iron	Chlorosis on seed leaves	Chlorosis on trifoliate leaves
	ppm		
1	0	slight	++
2	2	slight	+
3	4	slight	++
4	6	slight	++
5	8	slight on one plant	++
6	10	none	++
7	12	none	++++
8	14	none	+++
9	16	none	+
10	20	none	none
11	24	none	none
12	30	none	none

Culture No. 7 shows the most striking manganese chlorosis on the trifoliate leaves. However, these leaves were about $1\frac{1}{4}$ inches long, while in cultures 1 to 6 they were badly stunted and hence more affected by man-

ganese toxicity. It is clearly evident from this that manganese toxicity is counteracted by iron and that under the conditions of this experiment 20 ppm of manganese require about 16 ppm of iron to antidote its effect as far as chlorosis is concerned. After 15 days necrosis was observed to follow chlorosis in cultures with low amounts of iron. At this time culture 12 also showed slight but definite chlorosis. Whether this was a case of iron toxicity or was due to excessive amounts of both elements is uncertain. Cultures 10 and 11 did not show any evidence of chlorosis.

Experiment 26-C. Bean

This was a preliminary experiment designed to find out if the humate fraction of the humate iron had any effect on manganese toxicity apart from the iron. Cultures were set up in the same manner as in the previous experiment, but instead of allowing the humate fraction to vary with the iron, as in previous tests, it was kept at a constant amount equivalent to that in the culture with the highest amount of iron (30 ppm). As before, all cultures had 20 ppm manganese.

Ten days after planting there was some evidence of chlorosis on the first trifoliate leaves in all cultures, which varied from very severe in culture 1 in which the leaves were reduced in size, to very slight in 10, 11 and 12. There was a uniform decrease in severity of chlorosis as the iron content increased. The plants in culture 1 were smaller, the seed leaves were smaller and definitely paler in color and the first trifoliate leaves were smaller, slower to open and, as before stated, very chlorotic. There was a marked contrast between culture 1 and culture 2 since in the latter the trifoliate leaves were open and measured from one to several centimeters across, while those of culture 1 were not yet fully open.

It was further found that with higher amounts of iron chlorosis which appeared in the early stages of growth would disappear often within as short a period as two days. This effect was observed later with tomato plants. Without frequent observations the symptoms could easily be missed in certain cultures. This was interpreted as indicating a relatively more rapid absorption of manganese than iron in the early stages. Later, when more iron was taken up, the toxic effect of manganese was antidoted. This experiment again showed the antidoting effect of iron on manganese, and further, that the humate fraction of the humate iron had no effect in correcting manganese toxicity.

An interesting phenomenon which will be discussed more fully later in this paper, was observed in this experiment at "zero" iron (culture 1). On bright sunny days the seed leaves of plants in this culture were oriented in such a way that the leaf surfaces were parallel to the sun's rays, while on

cloudy or rainy days these same leaves resumed their normal horizontal positions. This is apparently an adaptive mechanism which prevents severe injury at high light intensity. Culture 2, which differed from 1 in that it received 2 ppm iron, did not show this nor any of the other cultures in the experiment. The result was confirmed in a later experiment (26-F).

In explanation of the fact that no definite chlorosis of seed leaves appears in most of these experiments when the trifoliate leaves may show severe injury, it is suggested that the seed leaves may have a considerable portion of the reserve iron of the seed or that the reserve iron of the cotyledons is translocated mainly into the seed leaves and there antidotes to a large extent the manganese. With greater toxicity, of course, marked chlorosis appears on the stems, cotyledons and seed leaves which may be pure white.

Experiment 26-F. Bean

The purpose of this experiment, set up about 6 months later than No. 26-C, was to check on the matter of leaf orientation in the light, to check again the effect of the humate fraction apart from the iron and also to obtain more data on the antagonistic action of iron towards manganese. The set-up was as follows.

Culture Number	Iron	Manganese	Sol. 3-A	Sol. 3-B
	ppm	*ppm*	*ml per liter*	*ml per liter*
1	0	20	0	12
2	2	20	2	10
3	4	20	4	8
4	8	20	8	4
5	12	20	12	0

Solution of 3-A was a potassium iron humate solution while 3-B was the same humate preparation without iron. Hence, there was the same amount of humate in each culture.

On the fourth day after planting, although the light in the greenhouse was not very intense, quite a number of the seed leaves in each of the cultures 1 to 4 were elevated from the horizontal about 20 to 30 degrees. All seed leaves in culture 5 were horizontal. On the fifth day, which was partly cloudy, seed leaves in all cultures were horizontal. The same was true on the eighth day. On the tenth day two seed leaves in culture 1 were found to be vertical in a light of medium intensity while all other seed leaves were horizontal.

On the twelfth day a total of six seed leaves in culture 1 were vertical in the sunlight. The other two which did not respond to the light were somewhat wilted. Movement of these leaves was observed, during the

time of taking notes, from an angle of about 60 degrees from the horizontal to the vertical. This was caused by the sun coming from behind a cloud. Again, the seed leaves in all other cultures were horizontal. Later on a sunny morning all of the eight seed leaves of culture 1 were vertical and this time, as a cloud passed over the sun, they were seen to move from the vertical to an angle of about 60 degrees in one-half hour. With continued

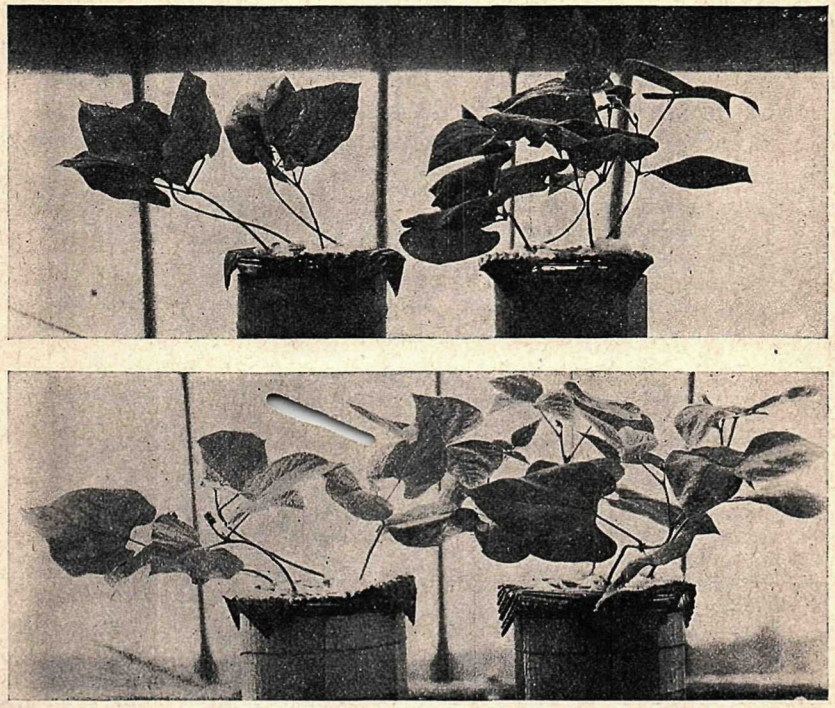

FIG. 6. Phototropism of seed leaves of bean plants caused by manganese toxicity. Above and left—20 ppm manganese and no iron; leaves are parallel to sun's rays. Right—20 ppm manganese plus 2 ppm iron; leaves are horizontal. Both cultures in sunlight. Below—The same 2 cultures after shading; the leaves are horizontal in both cultures.

cloudiness for another hour they all became perfectly horizontal. These movements were frequently observed and also produced at will by artificial shading. The effect is illustrated in figure 6. At the top are shown cultures 1 and 2 on a sunny day; the seed leaves of culture 1 are vertical while those of 2, which differs only in that it contains 2 ppm iron, are horizontal. The same cultures are shown below after they were artificially shaded for about 1 hour. The seed leaves are horizontal in both cultures.

As mentioned before, this phototropism is apparently a mechanism which prevents excessive injury to the leaves at high light intensity. In explanation of the fact that on the fourth day phototropic response was exhibited by seed leaves in all cultures except No. 5, it should be said that in cultures 2 to 4 and to some extent in 5 there was chlorosis in the early stages with subsequent recovery.

In regard to chlorosis in this experiment it was again most severe where no iron was added, and gradually decreased as the iron content of the solution increased. The same was true of necrosis and other symptoms of manganese toxicity. At 4 days the cotyledons of one plant in culture 1 showed chlorosis and some of the seed leaves in both cultures 1 and 2 had chlorotic patterns. Seed leaves in culture 5 at this time were a good green. The first trifoliate leaves in culture 1 were very small, did not fully open and finally became necrotic and died, while in the other cultures, although they showed chlorosis which varied inversely with the amount of iron, this disappeared about 14 days from planting. The effect of treatment on development is very well shown in figure 6 where it will be seen that both the first and second trifoliate leaves have formed and opened in the plants of culture 2 while in culture 1 only seed leaves are in evidence. The following counts and measurements were made of the plants in this experiment.

Culture number	Iron	Ave. width seed leaves 10 days	Ave. length 1st trifoliate leaves 17 days	No. 3rd trifoliate leaves opened 17 days	Oven dry wts. per plant, gms. 21 days		
					Top	Root	Total
	ppm	*mm*	*mm*				
1	0	63	20*	0	0.317	0.090	0.407
2	2	72	90	1	0.740	0.265	1.005
3	4	77	91	2	0.959	0.284	1.243
4	8	71	97	3	0.915	0.245	1.160
5	12	70	100	4	1.110	0.262	1.372

* Leaves dead.

In the above experiment the antagonistic action of iron towards manganese is shown by variation in: (1) chlorosis of seed leaves; (2) chlorosis and necrosis of the first trifoliate leaves; (3) rate of development and size of the trifoliate leaves; (4) size of the seed leaves and dry weights of tops and roots of the plants. It is also shown in a general way by their phototropic response. In regard to culture 3, which is out of line in some respects, it should be noted that only two of the 4 plants developed in this culture and hence there was less competition.

Experiment 26-G. Bean

This time calcium nitrate was used as the main source of nitrogen although some ammonium sulphate was used for the purpose of physiological buffering. Iron and manganese concentrations were varied simultaneously. The cultures were placed at random on the greenhouse bench. Five days after planting, the seed leaves which opened out were carefully measured but showed very little difference in size. At nine days, when the first trifoliate leaves were opened in all cultures but the check, they showed marked correlation of chlorosis with the treatment. In the table below, the plan of the experiment is shown and the relative amount of chlorosis at nine days is indicated by plus and minus signs.

Mn	Fe			
	2	5	10	20
ppm				
2	−	−	−	−
5	++	+	−	−
10	+++	++	+	+
20	++++	+++	−	++

"Check" with the same n[illegible]ro elements but *no Fe or Mn*. First trifoliate leaves not yet open.

The above shows clearly the interrelationship of iron and manganese in bean plants. With a high ratio of iron to manganese there is very little chlorosis and the reverse is true with high manganese and low iron. If a diagonal is drawn from the upper left to the lower right of the diagram, only one plus sign appears above the diagonal while 19 appear below. Plants in the check culture without either iron or manganese added showed the severest symptoms of toxicity. The first trifoliate leaves of these plants did not open until much later than those in the other cultures and they were very small and completely chlorotic. This was later found to be due to manganese in the tap water used in these cultures (see Experiment 26-I).

Measurements of the length of the central leaflet of each trifoliate leaf were made on the ninth and on the eleventh day. The averages for each of two categories and the check are as follows:

	9 day	11 day
	mm	*mm*
High iron-low manganese (upper right)	51.2	85.0
High manganese-low iron (lower left)	44.1	72.7
Check plants	0.0	23.0

Dry weight determinations of the tops and roots made after 31 days growth are given below.

Mn	Fe				
	2 ppm	5 ppm	10 ppm	20 ppm	"Check"
ppm					
2	4.77	4.09	4.58	4.86	2.01
	2.32	1.97	1.76	1.56	1.34
	7.09	6.06	6.34	6.42	3.35
5	3.60	3.65	4.70	4.58	
	1.64	1.67	1.69	1.87	
	4.94	5.32	6.39	6.46	
10	3.96	4.68	4.36	4.89	
	1.54	1.84	2.20	1.58	
	5.50	6.52	6.56	6.47	
20	3.36	4.16	4.25	4.75	
	1.42	1.70	1.50	2.00	
	4.78	5.86	5.75	6.71	

Note. Weights are in grams for 4 plants in each culture. In each square the dry weight of tops is at the top, roots in the middle and the total below.

Although the differences in the dry weights are not large, there are definite trends. At 5, 10 and 20 ppm manganese the dry weight increases as the iron increases, and at 2 ppm iron they decrease as the manganese increases. These yields, in connection with the previous observations on chlorosis and the development of the trifoliate leaves, show very well the antagonistic effect of iron on manganese and bring out the fact, as did the other experiments, that the two elements cannot be considered apart from each other in judging their physiological action. For example, a very slight amount of manganese may be much more toxic than 10 or 20 ppm when not balanced by sufficient iron. This is brought out by the check plants where the concentrations of manganese and iron were 0.30 and 0.12 ppm respectively.

An observation made in the course of this experiment may have some practical importance. Leaves showing manganese chlorosis were placed between blotters to dry. Several days later it was noted that the original light colored areas had turned very dark, almost black. This was apparently due to rapid oxidase action in the presence of reatively large amounts of manganese. This is similar to the observation reported by Kelley (20) for pineapple leaf tissue, and may also be related to "black tobacco" reported from Connecticut by Le Compte (23) as occurring in tobacco

plants with higher than the average manganese and iron contents. The leaves from this tobacco cure very dark brown with a blue-gray or purple-gray hue.

Experiment 26-H. Bean

The purpose of this experiment was to study the antagonistic action of iron on manganese, this time on a larger scale. A greater number of treatments, larger culture vessels and a larger number of plants per culture were used. The 20-liter pyrex culture jars described on page 52 were used. Nineteen liters of culture solution were placed in each jar and the solutions were constantly aerated. Seven bean seedlings were originally planted, and after several days when a few abnormal ones were eliminated, the number was reduced to six normal seedlings in each culture. The solution, as regards the macro elements, was according to the formula given on page 52. The solutions were made with tap water. There were seven different concentratons of iron, and for each concentration of iron six concentrations of manganese. Thus a total of 42 cultures resulted which were randomized as before described. The following diagram gives the details of the set-up.

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
	Culture Numbers						
ppm							
0	1	2	3	4	5	6	7
1	8	9	10	11	12	13	14
2	15	16	17	18	19	20	21
5	22	23	24	25	26	27	28
10	29	30	31	32	33	34	35
15	36	37	38	39	40	41	42

Various observations, measurements and estimations of general development were made during the course of the experiments and finally determinations of fresh and dry weights of the plants were carried out. In tables 1 to 10, arranged similarly to the above, these are given. As will be seen, there is a consistent advantage from the standpoint of the plant, for each item, as the iron content increases from 0 to 15 ppm, and a corresponding disadvantage as the manganese is increased. The relative effect of each of the two factors (iron and manganese) is dependent on the concentration of the other.

In Experiment 26-H, as shown by the data in tables 1 to 11, there was excellent growth in those cultures with a high iron concentration in relation to the manganese, i.e., in cultures represented in the upper and right area of the above diagrams (tables). From the standpoint of the appearance

of the plants, the best culture was number five, with no added manganese and 5 ppm iron. At 13 days it was rated as by far the best, with deep green

TABLE 1

Effect of iron and manganese on chlorosis after eight days. Experiment 26-H

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0	++++	+++					
1	++++	++++	++++	++++	+++	+	
2	++++	+++	+++	++++	+++	++	++
5	++++	+++++	+++	++++	++++	++++	++
10	++++	++++	++++	++++	++++	+++	++++
15	++++	++++	++++	+++++	++	+++	+++

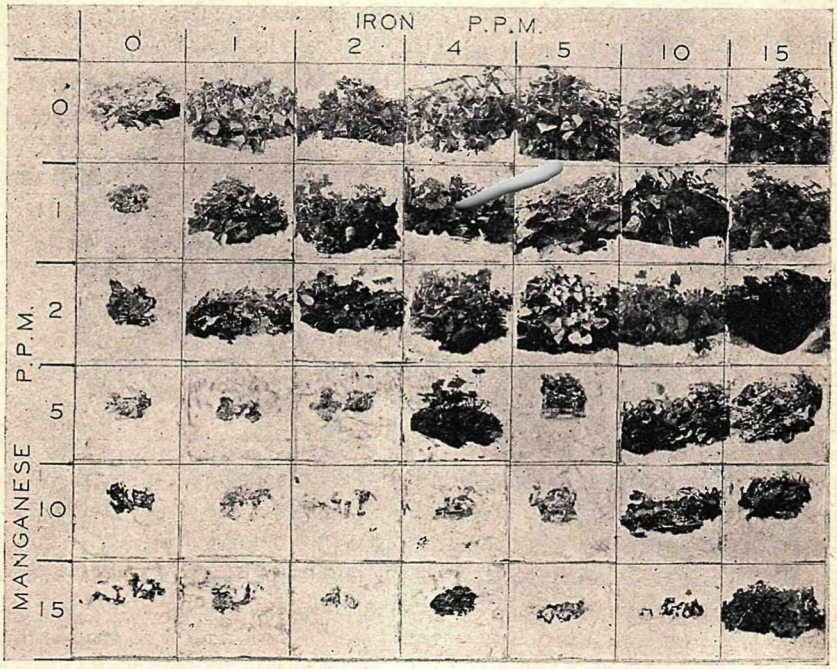

FIG. 7. Effect of the simultaneous variation of manganese and iron on the growth of bean plants. Experiment 26-H, 20 days after planting. Photographs of cultures are arranged according to treatment and extraneous background has been blocked out. The purpose is to show the general effect of treatment on growth rather than detail.

foliage and the third set of trifoliate leaves beginning to open. At 16 days the growth of the six plants in this culture was so luxuriant as to

more than cover the top of the culture jar. It was also the first culture in which flowers opened, but was only fourth in respect to the total amount of dry matter produced (table 11). A discussion of the various items in regard to the plants follows.

Chlorosis. From table 1 it is seen that at 8 days a large number of the cultures showed more or less chlorosis. Only 5 cultures at zero manganese and higher amounts of iron showed none. However, shortly thereafter many of the treatments with high iron and low manganese recovered from this initial chlorosis so that at 16 days very few plants in the high-iron low-

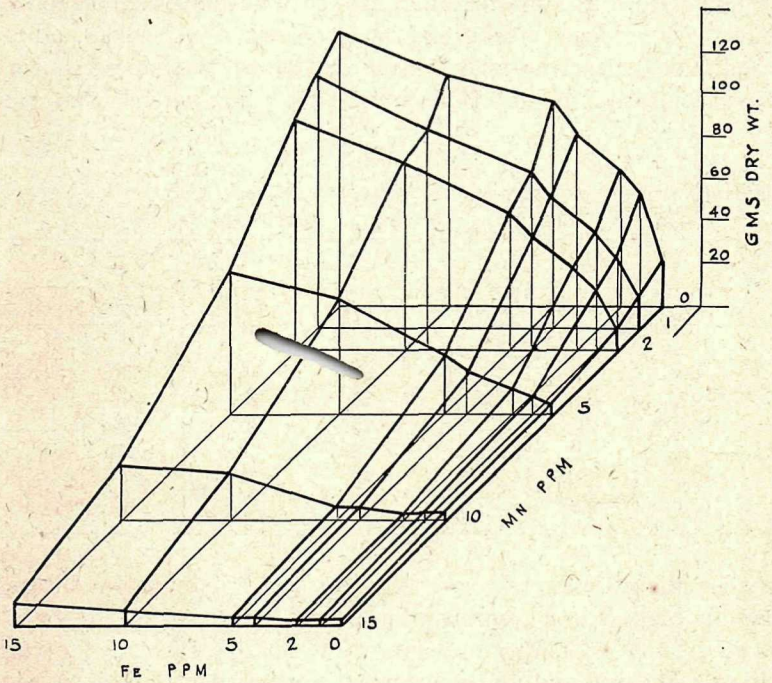

FIG. 8. Effect of simultaneous variation of manganese and iron on the total dry weights of bean plants, represented as a three-dimensional figure. Experiment 26-H.

manganese group (above a diagonal drawn from the upper left to the lower right of the diagram, figure 8) showed no chlorosis, while practically all those below the diagonal were still chlorotic. This was taken to mean, in the first case, that in the early stages of growth manganese was absorbed faster than iron, and later, when more iron was absorbed, the toxic effect of manganese was antidoted and chlorosis disappeared. On the other hand, in the second case, enough iron was not absorbed to antidote the manganese because of an insufficiency of the former and an abundance of the latter in the substrate. In the first case the injury was slight and the condition still reversible, but in the second case it was severe, irreversible,

and followed by necrosis of the tissue and final death of the plant. The general trends are: (1) the higher the iron the less chlorosis; (2) the higher the manganese the more chlorosis; and (3) it requires more manganese to bring about chlorosis as the iron concentration increases. The effect shown by culture 1 without either iron or manganese added has already been explained; the solution contained manganese impurities in small amounts from the tap water and from the ordinary CP chemicals in such amounts that the iron impurities were insufficient for proper balance. Therefore, these plants were severely affected. When iron alone is added at 1 ppm (culture 2) almost normal growth with only slight chlorosis is obtained. No evidence of iron toxicity as described by Somers and Shive (33) was indicated at the highest iron and lowest manganese concentrations used (culture 7) by any type of chlorosis. It is possible that enough

TABLE 2

Effect of iron and manganese on the length of central leaflet of first trifoliate leaf. Lengths in millimeters. Eight days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0	29	34	36.5	35	35	29	37
1	17	22	26	26	25	30	35
2	11	21	29	25	30	26	32
5	16	20	22	18.5	22.5	25	24
10	17	18.5	13	17	17.8	18	18
15	10	10	14.5	10	21	10	18

manganese impurities were present in the "zero" manganese cultures to balance the highest iron concentration used.

Necrosis. This is another measure of the relative effect of the various combinations of iron and manganese in this experiment, and is, of course, closely related to chlorosis as above mentioned. One type of necrosis is that shown on the seed leaves of bean plants, and consists of minute brown spots usually not more than a millimeter in diameter scattered over the leaf and often on the leaf petioles. Time did not permit a careful study of this, but it is apparently a definite symptom of manganese toxicity which occurs when the plants are quite severely affected. This is brought out in table 3 where it was recorded only in cultures with the highest manganese and the lowest iron concentrations. Why manganese injury takes this particular form and only on the seed leaves, is difficult to answer without further study. Only in a few cases, such as were found in some of the preliminary experiments, were chlorotic patterns followed by irregular

necrotic areas seen on seed leaves. It is suggested that it may be related to a particular kind of distribution of the iron which is translocated from the cotyledons into these leaves.

TABLE 3

Effect of iron and manganese on the number of cultures showing the formation of minute brown necrotic lesions on seed leaves. Nine days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0							
1							
2	+						
5	+	+					
10	+	+	+	+			
15	+	+	+	+			+

TABLE 4

Effect of iron and manganese on the number of first trifoliate leaves showing [illegible] necrosis. Nine days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0	+						
1	+++	+	++++	+++			
2	++	++++	++	++			
5	+ ++++	+++	+++	+	+ ++++	++ ++++	+++
10	+ ++++	+++	++++	++ ++++	+++	+	+++
15	++ ++++	+ ++++	+++	++++	++ ++++		+++

The number of first trifoliate leaves in each culture which showed any necrosis at 9 days is given in table 4. Here again, as for chlorosis, the greatest proportion was in the cultures with high manganese and low iron. The necrosis was characterized by irregular shaped brown areas on the trifoliate leaves. In some cases the entire leaf was killed preventing any

further development of the plant, while in others it was slight and the plant continued to grow. The general trends are the same as those just discussed for chlorosis.

Leaf size and general development of plants. A numerical expression of the general development of the plants was obtained by measuring the length of the central leaflet of each first trifoliate leaf from the base of the pedicel to the tip. The figures which are shown in table 2 are each the average length of the 6 leaflets for the corresponding culture. An average length of about 10 mm indicates that the leaf had formed but had not yet opened. While there is some variation it is obvious that the lengths of

TABLE 5

Effect of iron and manganese on susceptibility of the seed leaves to sunscald. Eleven days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm 0							+
1	+						
2							
5							
10	++++	+++	++ ++++	+	+++		
15	++	+ ++++ ++++	++ ++++ ++++			++++	

these leaflets vary directly with the iron concentration and inversely with the manganese concentration.[5]

At 12 days a count was made of the number of second trifoliate open leaves (table 6). The largest number was opened in cultures with high iron and low manganese. Likewise, at 22 days the number of cultures in which tendrils had formed were recorded (table 9). These were all in the upper right half of the diagram. Table 10 gives a record of the number of flowers per culture, and here practically all are in the upper right part of

[5] Analysis of variance of the data from table 2 gave the following results:

	Degrees of freedom	*"F" value Snedecor (32)*	
Variance between iron.....	6	5.02	highly significant
Variance between manganese.....	5	28.55	highly significant

TABLE 6

Effect of iron and manganese on the number of second trifoliate open leaves at 12 days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		++ ++++	++ ++++	++ ++++	++ ++++	++ ++++	++ ++++
1	++	+++	+ ++++	+++	+ ++++	++ ++++	++ ++++
2	++	++++	+ ++++	++++	++ ++++	++ ++++	++ ++++
5			++ ++++	++ ++++	+++	++ ++++	++ ++++
10						+++	+++
15							++++

TABLE 7

Effect of iron and manganese on the general development and vigor of the plants at 16 days. Evaluated on the basis of 10 = the best

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		++++ ++++	++++ ++++	++ ++++	++ ++++ ++++	+ ++++ ++++	+ ++++ ++++
1		++	+ ++++	++++	++++	++++ ++++	++++ ++++
2	+	+++	++	++++	++++	+++ ++++	++ ++++
5			++	++		++++	++++
10						+	+
15			+			+	++

the table. It is not intended to imply a specific effect of the treatments on reproduction, but merely to assume the effect to be the result of treatments on the general development of the plants. Table 7 gives an estimate of

the general development and vigor of the plants at 16 days from planting. At 20 days from planting each culture was photographed with the camera always placed at the same distance from the culture. The photographs obtained were arranged according to the diagram of this experiment, and after blocking out extraneous background with white ink, the whole was rephotographed to a smaller size. The result is presented in figure 7 which shows very strikingly the general effect of the treatments on the growth of bean plants. In the lower left portion of the figure the small and extremely chlorotic plants scarcely show up against the white background.

Sunscald. Due to somewhat excessive temperature over a week-end when the greenhouse was closed up, there was some injury from sunscald. This was not severe and occurred only on the seed leaves. As will be seen in table 5, this was present mostly at the two highest manganese concentrations and decreased as the iron content of the substrate increased. While an incidental observation, it is important in showing that iron exerts a protective action against high temperature just as it does against intense light. A further observation in regard to sunscald will be given in connection with Experiment 26-I.

Dry weights of plants. The plants were harvested after 44 days from planting. At this time many bean pods had developed to a good size in the high-iron low-manganese cultures. The dry weights of the tops, roots and their totals are given in table 11. In all cases there is an increase with increasing iron and a decrease with increasing manganese, and the cultures represented in the upper right part of the diagram are seen to have produced by far a greater total dry weight than those in the lower left. The ratio is more than 6 to 1. For the tops alone it is almost 7 to 1 and for the roots about 5 to 1. Because of the fact that the treatments were not replicated and, therefore, considerable variation might be assumed, we feel that too much weight should not be assigned to minor deviations from the general trends.

The data for the total dry weights are shown graphically in the form of a solid figure (figure 8) where the iron and manganese concentrations are given along two sides of the base and the dry weights represented by vertical distances from the base. The values used to construct this figure were obtained from smoothed curves of the actual data. From this graph the simultaneous effect of iron and manganese on growth can be clearly seen. If the total dry weights at each iron concentration (disregarding variation in manganese) are averaged, and the same is done for manganese, the two graphs shown in figure 9 can be constructed. This gives an overall picture of the effect of the two elements.

A preliminary statistical analysis of the data for total dry weights was made using Student's method and pairing first the values for the various iron concentrations in each case with "zero" iron at all the different man-

ganese concentrations. The odds of significance that the growth was greater, increased as the iron concentration increased. High significance was found at 4, 5, 10, and 15 ppm iron. The same was true for manganese: significant decreases in growth were found at 2, 5, 10 and 15 ppm manganese over "zero" manganese. The odds increased as the concentration of manganese increased. Later the data as a whole were tested by Fishers' method of analysis of variance and found to be highly significant.[6]

The ratio of dry weight of tops to the dry weight of roots was calculated for each culture, and while the general trend is the same as for the absolute

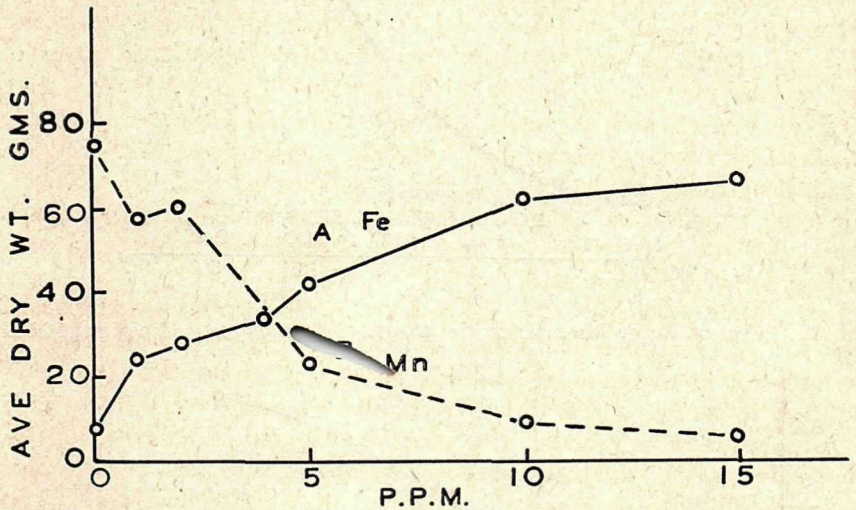

FIG. 9. Effect of manganese and iron on the growth of bean plants. Experiment 26-H. A—Average total dry weights at various iron concentrations disregarding variation in manganese. B—The same for various manganese concentrations disregarding variation in iron.

weights, the variation is such as to cast some doubt on the significance of this. It suggests, however, that iron and manganese have a relatively greater effect on the tops than on the roots.

[6] The calculation was made by Mr. K. W. Loucks of the Florida Citrus Experiment Station with the following results:

Dry weights of Iron	Iron		Manganese	
	Degrees of Freedom	"F" value	Degrees of Freedom	"F" value
Tops and roots	6	6.39	5	12.82
Tops	6	6.02	5	12.50
Roots	6	5.01	5	8.60

All "F" values are highly significant. (Snedecor, 32.)

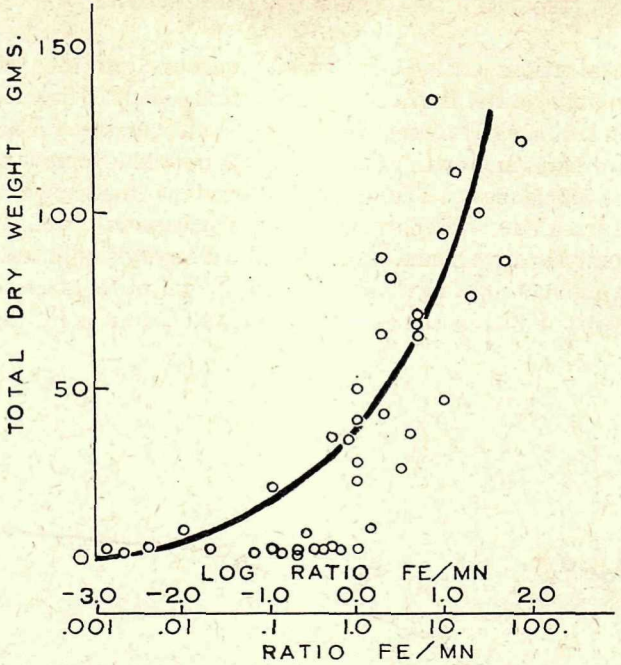

FIG. 10. Effect of the Fe/Mn ratio on the growth of bean plants. Experiment 26-H

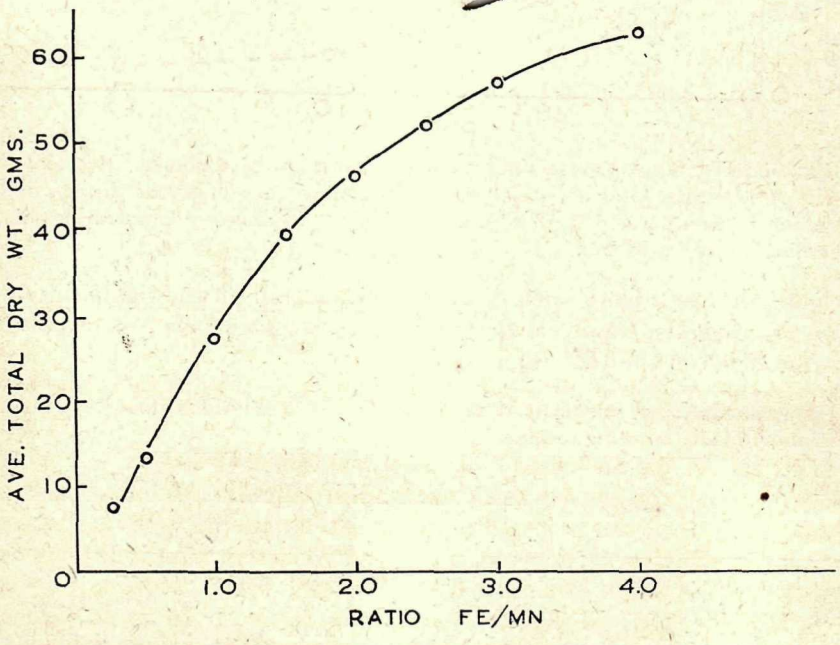

FIG. 11. Average total dry weight for each Fe/Mn ratio plotted against the ratio. Experiment 26-H

The ratio iron-manganese. If the actual values for total dry weight are plotted against the ratio Fe/Mn expressed in logarithmic form so as to include the wide range of ratios in one graph, there is much variation of the points, from a smooth curve, as shown in figure 10. There is no doubt, however, of a general increase in total dry weight with an increase in the Fe/Mn ratio from 0.001 to 100. However, the variation mentioned suggests that another factor, namely, the total concentration of the two ele-

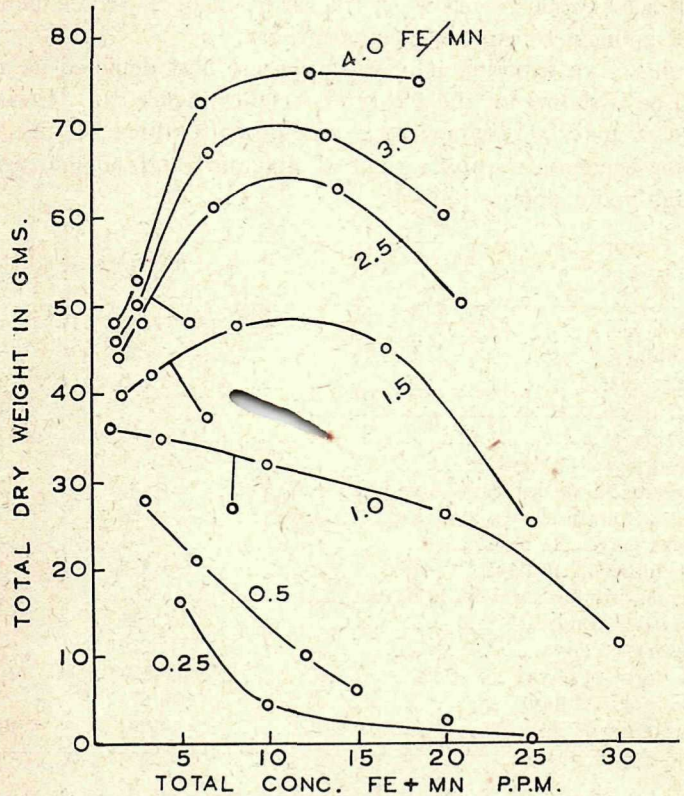

FIG. 12. Effect on bean plants of total concentration of iron plus manganese at various Fe/Mn ratios. Experiment 26-H.

ments, may also be involved. This idea was tested further by arranging values from smoothed curves in various groups, each with a different Fe/Mn ratio, from 0.25 to 4.0, but within each group a variable total amount of iron plus manganese. The average values for these groups plot out as a smooth curve showing a marked increase in dry weight with increase in the ratio (figure 11). Within any given group, however, that is, at a given ratio, the dry weight varies in a rather consistent manner with the combined amount of iron and manganese (figure 12). At the ratios Fe/Mn

0.25 and 0.5 there is a rapid decrease as the total concentrations of these elements increase. At a ratio of 1.0 the decrease is more gradual, while at the higher ratios the dry weight passes through a maximum and then declines, except at 4.0, where there is an increase and no decrease. Therefore, we conclude that under the conditions of this experiment the ratio of iron to manganese is not the only factor involved. The total amount of iron plus manganese is also important. Both are factors in growth, and under certain conditions, even when the ratio is kept the same, increasing amounts of manganese exert a greater toxicity.

The results of an experiment of this type are best depicted as a solid figure such as is shown for the total dry weights (figure 8). However, a brief summary may be obtained by averaging or totalling the results for high-iron low-manganese (upper right of diagram) and comparing with low-iron high-manganese as follows:

Item	High-iron low-manganese	Low-iron high-manganese
Chlorosis, 8 days	32	59
Chlorosis, 16 days	57	183
Chlorosis, 22 days	8	21
Chlorosis, 27 days	4	18
Chlorosis, 34 days	5	17
Necrosis, seed leaves, 9 days	1	11
Necrosis, first trifoliate leaves, 9 days	30	70
Length, first trifoliate leaves, 8 days	28	17
Sunscald, seed leaves, 11 days	1	44
General development, 16 days	115	13
Number second trifoliate leaves, 12 days	107	28
Number tendrils, 22 days	11	0
Number flowers, 36 days	279	5
Dry weight, tops, 44 days	1124	166
Dry weight, roots, 44 days	274	61
Total dry weights, 44 days	1397	228

Conclusions. For all of the above expressions of development and growth, striking correlation with treatment is shown. The toxic action of manganese and its antagonism by iron are clearly brought out. Roots are relatively less affected by treatment than are the tops. This points to the possibility that the chlorophyll mechanism or photosynthesis, or both, are involved, directly or indirectly. Under the conditions of this experiment mutual antagonism is not evident, only the antidoting effect of iron on manganese. There is a general correlation of the Fe/Mn ratio with growth, but the results also indicate that the total concentration of these elements is also important. The experiment suggests that both a

high ratio of iron to manganese and a relatively high concentration of iron are desirable for good growth of green plants.

Experiment 26-I. Bean

This experiment was set up in two parts: A, to test again the effect of the simultaneous variation of iron and manganese, and B, to compare the effect of the use of tap water in the preparation of the culture solution with

TABLE 8

Effect of iron and manganese on chlorosis at 16 days. Evaluated on the basis of 10 = the most chlorotic

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0	++ ++++ ++++	+		+			
1	++ ++++ ++++	++++ ++++	++	++++	++++		
2	++ ++++ ++++	++ ++++	+ ++++	++	++	+	+
5	++ ++++ ++++	++ ++++ ++++	+ ++++	+++	++ ++++ ++++	++	+++
10	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	+ ++++ ++++	+++ ++++	+ ++++ ++++
15	+ ++++ ++++	+ ++++ ++++	+ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++++ ++++

that of distilled water. Liter beakers were used as culture vessels. The treatments are shown in table 12 which also gives the result of observations on chlorosis and necrosis 18 days after planting. The oven dry weights are shown in table 13.

Part "A" of this experiment shows the same effect as previous tests as regards chlorosis and necrosis. It is seen from the results obtained in Part "B" that the culture solution made with tap water gave a markedly

lower yield than that made with distilled water when neither iron nor manganese was added. This is caused by the fact, previously discussed, that tap water contained 0.1 ppm manganese and only 0.02 ppm iron as impurities (see discussion on pages 50 and 51). The addition of 0.5 ppm manganese accentuated the condition in the case of tap water causing necrosis as well as chlorosis and reducing markedly the dry weight. In the case of distilled water a toxic condition is brought about by this amount

TABLE 9

Effect of iron and manganese on the number of cultures in which tendrils had formed at 22 days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		+	+	+	+	+	+
1			+		+	+	+
2							+
5							
10							
15							

TABLE 10

Effect of iron and manganese on the number of flowers per culture at 36 days

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		13	6		65*	38	32
1		1	11		2	17	22
2			2		4	14	34
5				2		3	7
10						2	
15							

* In this culture (No. 5) many bean pods had formed at this time.

of manganese. When iron is added at the rate of 5 ppm normal growth results in both cases either without manganese or with 0.5 ppm.

Experiment 26-K. Tomato

This experiment is recorded in this order, since it constitutes a repetition of part "B" of the previous one (26-I), only using tomatoes in place of beans. The purpose was to test the sensitivity of tomato plants to man-

ganese impurities in the tap water just as was done with beans. At the time it was set up, observations on Experiment 26-J had already shown that tomato seedlings were more sensitive (or reacted sooner) to manganese toxicity than beans. This was probably due to a smaller iron reserve in the seed. The variety "Marglobe" was used and one seedling was placed in each culture. Liter beakers were employed as culture vessels. Observa-

TABLE 11

Effect of iron and manganese on yield. Oven-dry weights per culture (6 plants) at 44 days, in grams

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
				Tops			
ppm							
0	13.0	59.2	38.1	66.0	80.7	76.7	95.7
1	2.6	22.6	55.5	27.6	54.3	77.0	90.5
2	6.7	30.0	36.5	30.0	65.5	55.5	106.2
5	2.6	2.2	2.6	24.0	2.2	77.0	19.0
10	1.0	2.5	1.1	2.1	3.0	22.0	7.0
15	2.6	1.9	1.3	5.0	1.8	2.1	18.0
				Roots			
0	8.4	11.8	9.6	11.7	19.3	9.5	25.8
1	0.8	5.5	10.6	9.0	10.4	17.5	21.5
2	3.1	6.3	13.5	13.6	15.7	12.5	28.2
5	1.1	0.6	0.8	11.3	0.9	11.8	8.5
10	0.9	1.3	0.4	0.8	1.2	18.5	2.2
15	0.8	0.4	0.4	2.4	0.4	0.4	5.2
				Totals			
0	21.4	71.0	47.7	77.7	100.0	86.2	121.5
1	3.4	28.2	66.1	36.6	64.7	94.5	112.0
2	9.8	36.3	50.0	43.6	81.2	68.0	134.4
5	3.8	2.7	3.4	35.3	3.1	88.8	27.5
10	1.9	3.8	1.5	2.9	4.2	40.5	9.2
15	3.4	1.4	1.7	7.4	3.2	2.5	23.2

tions on chlorosis are given for 6, 10 and 17 days in table 14. Just as in the case of bean plants, when tap water was used to prepare the culture solution and no iron or manganese was added the tomato plants showed severe chlorosis. The addition of iron counteracted this condition while the addition of small amounts of manganese intensified it. In the corresponding cultures in which distilled water was used toxicity was less severe.

TABLE 12

Chlorosis and necrosis in bean plants after 18 days. Experiment 26-I

Part "A"					Part "B"				
Mn	Fe				Cult. No.	Kind water	ppm		Chlorosis necrosis
	2 ppm	5 ppm	10 ppm	20 ppm			Fe	Mn	
ppm									
2	−−	−−	−−	−−	1	D.W.	0	0	−−
5	++	−−	−−	−−	2	T.W.	0	0	+−
10	++	+−	++	−−	3	D.W.	5	0	−−
20	++	++	++	+−	4	D.W.	0	½	++
					5	D.W.	5	½	−−
					6	T.W.	5	0	−−
					7	T.W.	0	½	++
					8	T.W.	5	½	−−

One "plus" sign indicates chlorosis. Two "plus" signs indicate both chlorosis and necrosis.

TABLE 13

*Oven-dry weights after 31 days. Experiment 26-I. Part "B"**

Cult. no.	Kind water	ppm		Dry weights		Total
		Fe	Mn	Tops	Roots	
				grams	*grams*	
1	D.W.	0	0	3.6	2.5	6.1
2	T.W.	0	0	3.2	1.6	4.8
3	D.W.	5	0	4.2	2.1	6.3
4	D.W.	0	½	1.7	1.7	3.4
5	D.W.	5	½	5.5	1.6	7.1
6	T.W.	5	0	5.5	3.5	9.0
7	T.W.	0	½	2.6	1.7	4.3
8	T.W.	5	½	5.7	2.7	8.4

* Due to heavy production of root nodules in a number of the cultures of Part "A" the dry weights for that part are not given in full. At 20 ppm Mn the dry weights were:

	Fe ppm			
	2	5	10	20
Dry weight tops (Grams).....	1.2	3.0	2.9	5.4
Roots.....	0.9	4.1	2.6	3.5
Total.....	2.1	7.1	5.5	8.9

In both cases tomato plants were more severely affected than bean plants. This was particularly shown by plants in solutions prepared with distilled water and lacking both iron and manganese. They developed chlorosis in 14 days from planting while the bean plants in the corresponding cultures in Experiment 26-I remained green for about 20 days. While tomato plants may be actually more sensitive to manganese toxicity it is thought that a logical explanation of this is that the smaller reserves of iron in the smaller tomato seed are insufficient to balance the manganese impurities.

TABLE 14

Effect of small traces of manganese in the tap water used in preparing culture solution on chlorosis in tomato plants. Experiment 26-K

Cult. No.	Nutrient Solution Prepared with:	ppm		Chlorosis		
		Fe	Mn	Six days	Ten days	Seventeen days
1	Distilled water	0	0	−	−	++
2	Tap water	0	0	+	++	+++
3	Distilled water	4	0	−	−	−
4	Distilled water	0	0.4	+	++	+++
5	Distilled water	4	0.4	−	−	−
6	Tap water	4	0	−	−	−
7	Tap water	0	0.4	++	+++	++++*
8	Tap water	4	0.4	−	−	−

* Plant was dead at this time.

Experiment 26-J. Tomato

This set-up was like that of Experiment 26-H with the following exceptions: (1) tomato plants were used, one to each of the 42 large culture vessels; (2) concentrations of manganese up to 1 ppm only were employed; (3) each series at the three manganese concentrations were in duplicate; and (4) a "zero" iron concentration was not used.

Tomato seeds of the variety "Marglobe" were shown in quartz sand which had been previously sterilized with hot water and then watered with a nutrient solution (macro elements only). The plants were about 2 inches in height when they were transferred to the cultures. The solution described on page 52 was employed and the cultures were randomized as before.

In the early stages practically all plants developed some degree of chlorosis, and at 6 days many of them showed definite patterns on the first pair of true leaves while the cotyledons in every case were entirely green. This is undoubtedly due to iron reserves in the cotyledons. A series of

observations and records were made of the extent of chlorosis, and while marked differences were found between the high iron cultures as contrasted with the high manganese cultures, such a definite conformity to treatment in the early stages was not found as was true for beans. However, at 23 days after planting and thereafter plants at high iron concentrations had recovered from the initial chlorosis while the others did not. Many of

TABLE 15

Chlorosis and necrosis of tomato plants at 26 days. + = Chlorosis, D = Dead plants or those that will die. Experiment 26-J

Mn	Fe						
	½ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
ppm							
0	+	+	−	−	−	−	−
0	+	+	+ D	−	−	−	−
½	+ D	+ D	−	−	−	−	−
½	+	+ D	+	−	+	+	−
1	+ D	+	+ D	+	+ D	+	−
1	+ D	+ D	+ D	+ D	+	−	−

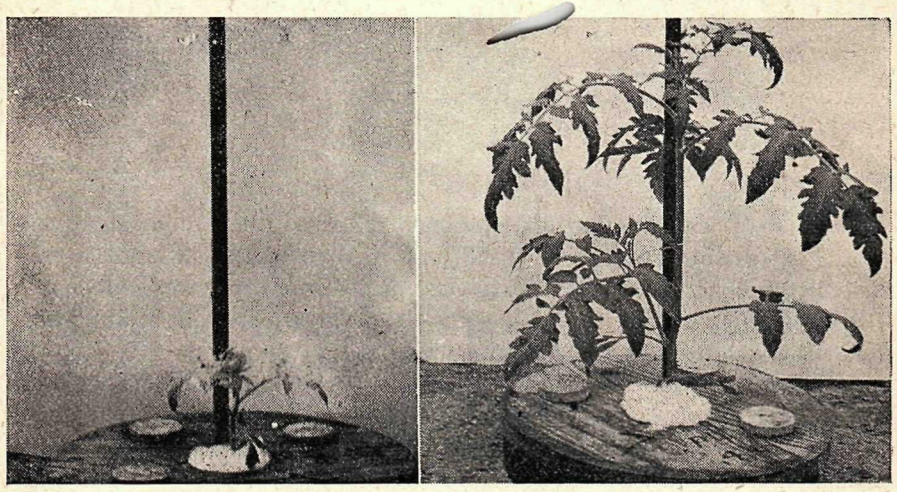

FIG. 13. Antidoting effect of iron on manganese toxicity in tomatoes. Left—One ppm manganese and 4 ppm iron. Right—One ppm manganese and 8 ppm iron.

the latter showed various degrees of necrosis and quite a few were dead 23 days after planting. This is brought out in table 15 and also in figure 13 which is a photograph of two plants, both of which received 1 ppm manganese but different amounts of iron.

After 30 days the height of each plant from the base of the stem to the point where the topmost leaves originated was measured. These measure-

ments which are presented in table 16 show with some minor variations an excellent correlation with the iron manganese treatments. The general effect is also brought out in figure 14 where the values are plotted as a solid

TABLE 16

Height of tomato plants in inches at 39 days. Experiment 26-J

Mn	Fe						
	½ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
ppm							
0	3.1	3.4	7.7	8.1	9.1	9.1	15.4
0	4.5	2.4	6.1	5.1	6.6	9.5	—
½	3.5	4.0	—	5.4	9.2	5.5	6.1
½	2.6	2.6	2.2	7.4	4.6	8.0	9.1
1	2.9	2.0	2.5	3.1	1.7	4.5	2.5
1	2.6	2.6	1.2	2.1	2.9	3.9	6.2

TABLE 17

Number of flower buds and flowers at 44 days. Experiment 26-J

Mn	Fe						
	½ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
ppm							
0	0	0	6	8	13	10	10
0	4	0	6	4	6	9	—
½	0	0	0	4	5	4	2
½	0	0	0	0	4	7	6
1	0	0	0	0	0	6	6
1	0	0	0	0	0	0	0

TABLE 18

Number of tomato fruits at 64 days. Experiment 26-J

Mn	Fe						
	½ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
ppm							
0	0	0	5	6	6	5	10
0	0	0	3	2	5	6	—
½	0	0	—	1	3	4	4
½	0	0	0	5	2	7	0
1	0	0	0	0	0	2	6
1	0	0	0	0	0	2	2

figure. The same sort of relationship, as shown for the dry weights of bean plants, (figure 8) is also evident here.

A count of the number of flower buds and flowers at 44 days is given in table 17 and the number of fruits at 64 days in table 18. These also show a

marked correlation with treatment. A summary of the various observations follows in which the 21 cultures at high iron and low manganese are compared with the 21 at high manganese and low iron, using totals or averages.

Summary. Experiment 26-J. Tomato:

Item	High-iron Low-manganese	Low-iron High-manganese
Chlorosis, 5 days	89	143
Chlorosis, 12 days	83	145
Chlorosis, 14 days	76	150
Chlorosis, 16 days	73	156
Chlorosis, 20 days	86	186
Chlorosis, 26 days	5	18
Chlorosis, 34 days	4	19
Necrosis, 36 days	1	10
Height of plants, 23 days, average in inches	4.68	2.81
Height of plants, 30 days, average in inches	7.41	2.85
Number flower buds 44 days	93	4
Number flowers open 44 days	20	0
Number fruits, 64 days	77	4

Dry weights of the plants were not determined but it is obvious that sufficient data were obtained to show clearly the relative effect of the two elements. Aside from the greater sensitivity of the tomato plants to manganese toxicity, the same relationship is evident that was found for bean plants.

Experiment 26-I. Bean

Bean plants were grown on a larger scale using the gravel subirrigation culture equipment described under "Experimental Method." The culture solution of the macro elements which was made in 50-gallon lots had the following composition:

Salt	Grams per liter
KH_2PO_4	0.16
$Ca(NO_3)2 \cdot 4H_2O$	0.26
$MgSO_4 \cdot 7H_2O$	0.26
$(NH_4)_2SO_4$	0.048

Iron was added as potassium iron humate and manganese as the sulphate. The plan of the experiment was as shown in the following diagram:

Mn	Fe				
	0	2	4	5	6
	Gravel bed numbers				
ppm					
0	1	2	3	4	5
1	6	7	8	9	10

Four rows of beans of 12 "hills" each were planted 1 foot apart with the side rows 6 inches from the sides of the bed. This gave a total of 48 hills in each of the 10 gravel beds. The seeds were planted at a depth of 1 inch or about at the upper limit of the culture solution at the end of the pumping period. The plants made excellent growth in all ten cultures

TABLE 19

Lengths of the first trifoliate leaves in row two in each bud in centimeters. Nine days. Experiment 26-I

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	3.06 ±.075	2.52 ±.063	2.71 ±.11	2.94 ±.061	3.85 ±.096
1	4.37 ±.090	3.85 ±.104	4.46 ±.100	3.74 ±.189	3.62 ±.140

and up to the eleventh day there were no obvious differences. No chlorosis was observed throughout the experiment.

Unfortunately, possibly due to slight admixture of limestone particles with the igneous rock gravel used in the beds, the pH value of the solutions in all cases rose from 7.1 to 7.4 by the eleventh day. Because of this, the iron was precipitated, or absorbed, to a large extent, on the calcium humate that formed. Therefore, the concentrations in respect to iron in the various tanks were rather uncertain. In spite of this drawback from the experimental standpoint the results are presented because, first, some rather significant facts are brought out, and second, because the plants made such excellent growth that the experiment may be of interest for that reason. There are also significant differences in yield and in other respects between plants that showed no differences in appearance such as chlorosis, etc. This should be of practical importance. The various counts and measurements are given in tables 19 to 23.

At 9 days there is an increase in the size of trifoliate leaves with increasing iron from 2 ppm to 6 ppm (table 19) at zero manganese, while at 1 ppm

TABLE 20

Percentage of second trifoliate open leaves, eleven days. Total number of plants per bed counted varied from 85 to 96. Experiment 26-I

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	30.1	24.2	52.2	46.7	39.8
1	59.8	47.9	60.6	58.9	58.2

TABLE 21

Height of plants in centimeters. Eleven days. Experiment 26-I

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	9.20 ±.63	9.38 ±.70	7.94 ±.56	8.28 ±.62	8.08 ±.55
1	8.24 ±.52	8.03 ±.41	9.08 ±.61	7.82 ±.63	8.32 ±.51

TABLE 22

Number of leaves per bed showing sunscald. Eighteen days. Experiment 26-I

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	6	11	9	37	13
1	69	57	34	14	17

TABLE 23

Total dry weight of plants per bed in grams. Thirty-one days. Experiment 26-I

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	262	276	276	277	282
1	209	206	250	232	225

manganese there is no definite trend with change in the iron concentration. The leaves, however, were significantly larger where 1 ppm manganese

was used. The percentage of second trifoliate open leaves at 11 days (table 20) showed no definite relation to the iron concentration at either manganese concentration but again there was a significant increase as the manganese was increased from zero to 1 ppm. Both of these measurements indicate a more rapid growth at 1 ppm manganese in the early stages although measurements of heights of the plants showed no significant difference.

Later, evidence of manganese injury was shown by susceptibility to sunscald at 18 days. The degree of sunscald was not severe and it did not appear to affect the luxuriant growth of the plants. Careful counts of the total number of leaves affected in each bed revealed that practically all the sunscald occurred in the cultures to which manganese had been added where it was inversely proportional to the iron concentration. The slight amounts at zero manganese showed no differences as the iron varied. (See table 22 and figure 15.)

Examination of the results of the determinations of dry weights of plants as given in table 23 show that while there was no difference in the appearance of the plants there was a significant reduction in growth due to the addition of 1 ppm manganese. Statistical analysis by the pairing method gave odds of 768 to 1 that the difference is not due to chance. The increase in yield when manganese is omitted is about 22 per cent. Although as seen from the data there was a general increase in dry weight with increase in the iron the trend is somewhat irregular. The fact that there was not a more marked relationship is due no doubt to the high pH and iron precipitation as previously noted.

Experiment 15-5. Pineapple

Forty-two cultures were prepared in the large culture jars. The variety "Smooth Cayenne" was used. One-half of these cultures were devoted to a study of other minor elements and will not be considered here. All cultures, however, were randomized according to the previous scheme. Each treatment was in triplicate as shown below.

Culture numbers	Parts per million		Notes
	Iron	Manganese	
1, 2, 3	0	0	No minor elements added.
4, 5, 6	5	0	Iron only, added as $FeSO_4$
7, 8, 9	0	2	Manganese only, added as $MnSO_4$
28, 29, 30	0	5	Manganese as $MnSO_4$, iron as potassium iron humate, copper 2 ppm and boron zinc and aluminum $\frac{1}{2}$ ppm each.
31, 32, 33	1	5	
34, 35, 36	3	5	
37, 38, 39	5	5	
40, 41, 42	10	5	

Culture solution

	gms/liter
KH_2PO_4	0.132
$MgSO_4 \cdot 7H_2O$	0.410
$Ca(NO_3)2 \cdot 4H_2O$	0.472
NH_4NO_3	0.126
K_2SO_4	0.166

Distilled water was used. pH of solution 4.5.

Uniform healthy slips from "Smooth Cayenne" plants were used and no replanting was necessary. The roots showed active growth 8 days after

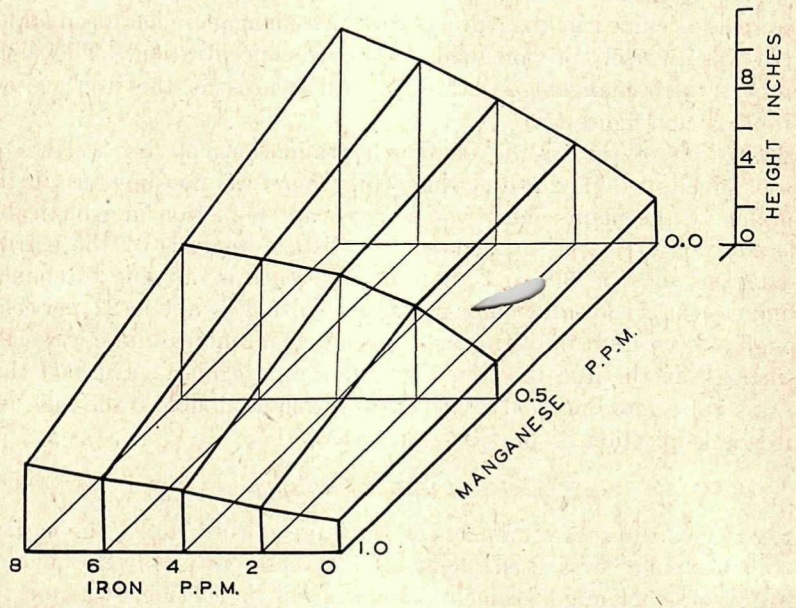

FIG. 14. Effect of iron and manganese on the growth of tomato plants. Experiment 26-J

planting and the plants all seemed to develop normally up to the 88 day. At this time slight but definite chlorosis was noted in cultures 28, 29 and 30 with 5 ppm manganese and no added iron. These cultures were severely affected by chlorosis at 97 days which was noted to increase in severity at 101 days. A photograph taken at 102 days (figure 16) shows culture 29 with no iron compared with culture 31 which in addition to the manganese had 1 ppm iron as potassium iron humate. The three cultures 28, 29 and 30 showed very clearly a variation in the time of appearance of manganese toxicity symptoms. Number 29 was the first to show them, then No. 30 and finally No. 28. This we consider to be due principally to

variation in the original slips in regard to the amounts of iron and manganese they contained. Later, necrosis showed up in the same order. The weights of the plants at 149 days also brings this out (No. 29, 518 gms; No. 30, 970 gms; No. 28, 1108 gms).

Meanwhile one of the three plants with 2 ppm manganese and no iron (No. 9) began to show chlorosis (94 days) and at 109 days No. 8 showed slight but definite chlorosis while No. 7, the third plant having the same treatment, showed no definite chlorosis at 143 days although the leaves

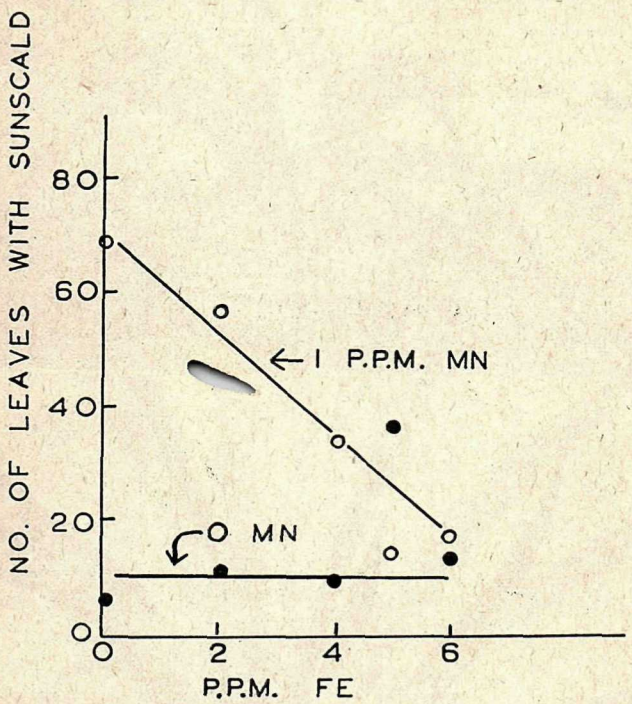

FIG. 15. Effect of iron and manganese on the susceptibility of bean plants to sunscald. Experiment 26-I

were not deep green in color. Here again the most severely affected plant was the first to develop necrosis. The plant had the lowest weight for this treatment (1218 gms). It had, however, a greater weight than culture No. 28 above.

The next treatment to show chlorosis in order of time and severity was that where all the minor elements were omitted: first, No. 2 showed slight chlorosis at 101 days and then No. 3 at 132 days. Necrosis was not observed in the case of any of these three cultures. All other cultures where iron was supplied developed no definite chlorosis although there was

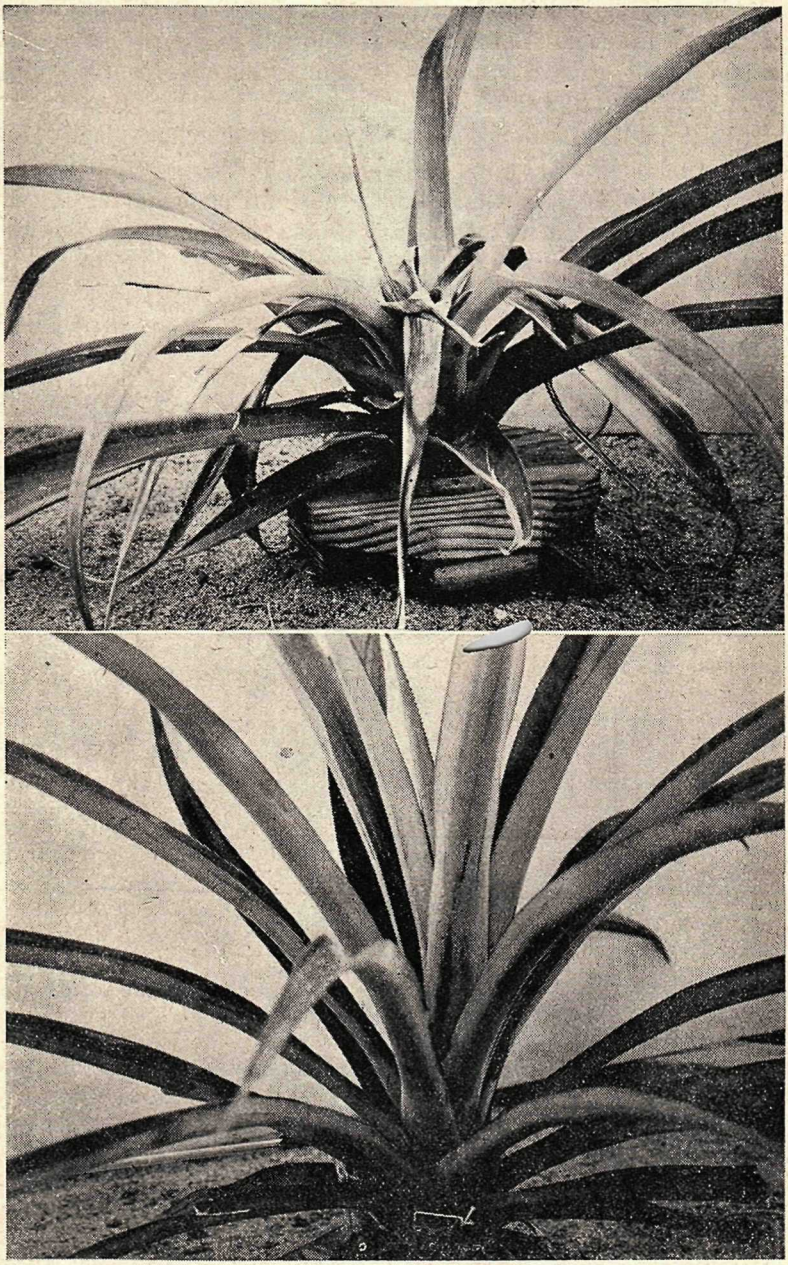

FIG. 16. Antidoting effect of iron on manganese toxicity in pineapples. Above—Five ppm manganese and no iron. Below—Five ppm manganese and 1 ppm iron. Experiment 15-5.

some difference in the greenness of the leaves. Cultures 4, 5 and 6 which received 5 ppm iron in the inorganic form had the deepest green color. These plants, however, were definitely smaller with narrower leaves than those in treatments with certain combinations of iron and manganese.

These preliminary remarks are made to indicate the natural variation that occurs in the slips used for planting and to point out a possible explanation for this variation. They also indicate a method of evaluating the relative severity of manganese toxicity. Numerical values can be obtained by giving weight to: (1) the severity of chlorosis; (2) the number of plants affected; (3) time of the first appearance of chlorosis, and (4) the time of appearance and severity of necrosis. This has been done, and in lieu of giving detailed records of the many observations made, the weighted values for records up to 143 days are given below.

Treatment	Manganese toxicity weighted value
No iron, no manganese	4.5
Iron only, 5 ppm as $FeSO_4$	0.0
Manganese only, 2 ppm as $MnSO_4$	16.5
Manganese 5 ppm, no iron	47.5
Manganese 5 ppm, iron 1 ppm as humate	0.0
Manganese 5 ppm, iron 3 ppm as humate	0.0
Manganese 5 ppm, iron 5 ppm as humate	0.0
Manganese 5 ppm, iron 10 ppm as humate	0.0

The fact that some degree of manganese toxicity is evident where both iron and manganese are omitted, is brought about as previously discussed by manganese impurities. This differs from the result obtained by Schappelle (29, 30) where he found no chlorosis when all the minor elements were omitted from the solution. While there is some possibility that the difference may be varietal since he used "Red Spanish" pineapples, it appears more probable from the above discussion that a difference in the amounts of iron and manganese in the slips used will better explain the difference in the appearance of symptoms. It is seen also that as the manganese concentration increases to 5 ppm the severity of toxicity increases in the absence of iron. Iron in a concentration of 1 ppm is sufficient to antagonize this toxicity at least as far as visible symptoms are concerned.

After the plants had grown for a period of 149 days they were carefully removed from the cultures, and after blotting most of the free water from the roots they were weighed. The plant in each case was then quickly returned to its culture vessel. The results of the weighings are given in table 24. While there is a high probable error for the mean of any given treatment several points seem clear. Pineapple plants may be affected

to quite a degree by manganese toxicity and still show a greater fresh weight than others which show no toxicity symptoms. Compare treatments 1 and 3 with 2. Only when the toxicity is extremely severe is the fresh weight reduced. While iron at 1 ppm prevents chlorosis and death of the plants, at 5 ppm manganese the fresh weights are not significantly different. Increase in the iron content to 3 ppm and higher at this same manganese concentration brings about a significant increase in the fresh weight over zero and 1 ppm iron. The data given in table 24, therefore, should be studied in conjunction with the toxicity symptoms previously noted. The average height of the plants, also shown in table 24, are similar to the fresh weights in their relation to treatment.

The first fruiting occurred in culture No. 40 at 5 ppm manganese and 10 ppm iron. The fruiting stalk appeared at 210 days; the fruit developed

TABLE 24

Fresh weights of pineapple plants after 149 days. Average height of plants, 215 days

No.	Treatment	Fresh weight in grams				Average height
		A	B	C	Ave.	
						inches
1	No Fe, no Mn	1592	1306	1315	1397	20.9
2	Fe, 5 ppm	998	1060	691	983	21.6
3	Mn, 2 ppm	1275	1718	1218	1409	23.2
4	Mn, 5 ppm	1108	518	970	863	14.5
5	Mn, 5 ppm; Fe, 1 ppm	930	714	842	826	23.7
6	Mn, 5 ppm; Fe, 3 ppm	1244	776	1633	1216	23.8
7	Mn, 5 ppm; Fe, 5 ppm	1134	1043	1050	1076	24.9
8	Mn, 5 ppm; Fe, 10 ppm	1438	706	1156	1107	25.2

normally and was harvested about $10\frac{1}{2}$ months from planting when it was beginning to turn color near the base. Records were made in each case of the time of flowering and the time for maturity of the fruit. As the fruits matured they were harvested, weighed, measured and the juice analyzed. These data are presented in table 25.

At 5 ppm manganese it will be seen that the plants required the longest time to flower and mature fruit, in fact, one plant never flowered. As the iron content increased the time to flower and mature fruit became less. In this respect cultures without minor elements, those with iron alone, those with manganese alone at 2 ppm, and those with all the minor elements did not appear to differ significantly from each other. However, they required a longer time for flowering than those at the higher iron concentrations in the iron-manganese series. Likewise, at 5 ppm manganese, the total weight of the fruit (including the crown) was lowest and increased with the iron content. This is shown graphically in figure 17.

TABLE 25

Effect of iron and manganese on the production of pineapple fruits. Experiment 15-5

Culture numbers	Treatment	Days to flower, average	Days to mature fruit, average	Weight fruit-crown, average	Weight crown average	Total fruit & crown, average	Average diameter	Average height	Juice analysis		
									Invert sugar, 100 cc.	Total sugar, 100 cc.	Brix
				grams	*grams*	*grams*	*inches*	*inches*	*grams*	*grams*	*degrees*
1, 2, 3	—M.E.	424	536	965	395	1360	4.6	5.0	3.30	13.86	15.7
4, 5, 6	Fe 5**	423	534	1088	274	1362	5.1	4.9	3.10	11.26	13.2
7, 8, 9	Mn 2**	423	539	1057	261	1318	4.8	4.7	3.65	11.09	14.7
22, 23, 24	M.E.	411	533	1002	199	1201	4.6	5.1	4.86	13.28	14.8
28, 29, 30	Mn 5**	456*	675*	430	238	668	4.4	3.9	2.93	11.30	13.9
31, 32, 33	Mn 5 Fe 1**	415	525	1057	228	1285	4.6	5.5	5.72	10.55	12.6
34, 35, 36	Mn 5 Fe 3**	406	509	1064	319	1383	4.7	5.3	3.40	9.47	14.0
37, 38, 39	Mn 5 Fe 5**	406	514	1123	288	1411	5.2	6.2	3.66	11.80	13.9
40, 41, 42	Mn 5 Fe 10**	340	447	1078	376	1454	5.1	5.1	3.84	13.14	14.3

* Two fruits.

** Number following symbol for element indicates concentration in ppm.

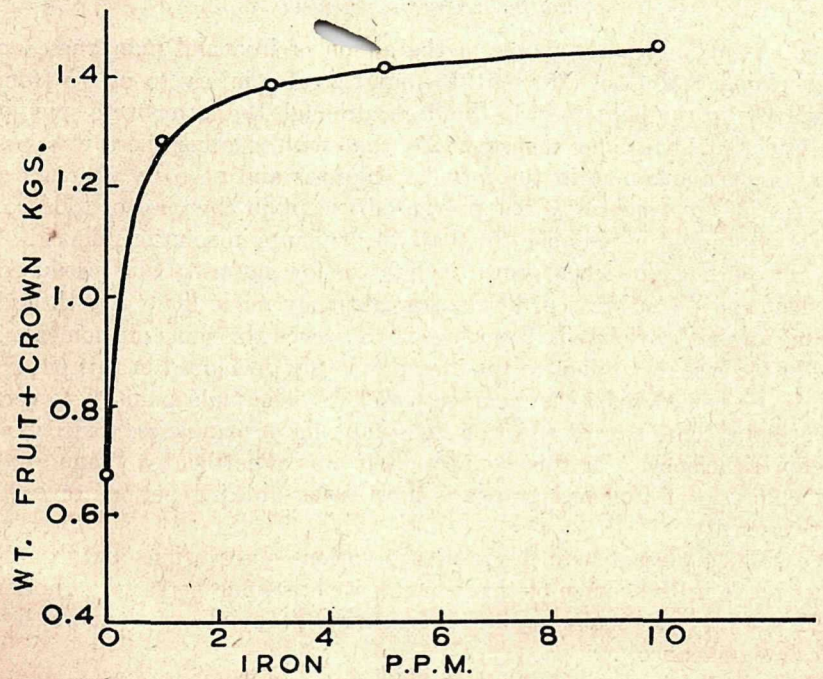

FIG. 17. Effect of iron on the production of pineapple fruits at 5 ppm manganese. Experiment 15-5

The large increase was from zero iron to 1 ppm iron. Without any minor elements the high proportion of the weight of crown leaves to that of fruit suggests a tendency towards a vegetative condition similar to that found by Schappelle (29, 30). The size of the fruit showed practically the same relationship to treatments as the weights. No particular correlation between the sugar content of the juice and the iron-manganese ratio is evident.

This experiment with pineapple plants shows the same antagonistic effect of iron on manganese as was shown by the experiments with beans and tomatoes. Certain differences due to the type of plant, the size of the seed piece, etc., are evident, but the antidoting action of iron is basically the same. The difference in time of appearance of the symptoms of manganese toxicity is of interest. Whereas with tomatoes and beans it is a matter of days, with pineapples it requires several months for chlorosis to show up. The effect of only 1 ppm iron in antidoting 5 ppm manganese as regards chlorosis, necrosis, height of the plants, and weights of fruit produced, is very striking.

Experiments on the effect of light in relation to iron and manganese and the oxidation potential

Light is an important factor in the action of iron and manganese on green plants. McCool (25) in 1913 found greater injury to plants from manganese in the light than in the dark although the latter were spindly and etiolated. He later reported (26) that with shading there was less injury from manganese in the form of chlorosis and necrosis although a decrease in dry weight was not prevented except in the case of tobacco. It was also noted by Gericke (5) that wheat plants in solutions devoid of iron remained green when grown in light of low intensity and produced excellent growth whereas in bright sunlight they made little growth and became markedly etiolated. Loewing (24) observed this same phenomenon. This latter case, according to the idea previously developed in this paper, can also be considered as manganese toxicity. The main point, however, is that light exerts a great effect on the symptoms of manganese toxicity or on iron deficiency. In this connection it was reported by Wann (36) that chlorosis of Concord grapes is most severe during periods of high light intensity.

We wish to present here the results of various observations and experiments made in the course of this work as well as some heretofore unpublished work by the senior author and attempt to point out their physiological significance.

"Short top" of pineapple fruits. That this symptom (see figure 2) is one of manganese toxicity is probable although the evidence is somewhat

circumstantial. It was observed to be very prevalent in Puerto Rico on soils with a high content of soluble manganese. On one plantation the following extraordinary condition was noted. In fields where the two row banks ran east to west "short top" was found almost exclusively in the south rows. A careful count showed a ratio of 25 to 2 of such fruits in the south row as compared with the north. In fields where the banks ran from north to south, short top fruits were evenly distributed throughout the field. Further, in one field with a high percentage of "short top" fruits none were found in one small area shaded by trees. On the contrary, the fruit in this area while somewhat smaller in size had abnormally long tops. A large number of leaves collected from "short top" plants showed no difference in their greenness from those from normal top plants. Chemical analyses of "short top" and normal fruit showed both to have a very high manganese content with no significant difference between the two. It was concluded that greater exposure to light brought out this particular symptom of manganese toxicity.

Phototropism. This is another effect of light in combination with manganese toxicity which can be looked on as manganese induced phototropism.[7] This interesting and striking phenomenon occurs in the seed leaves of bean plants grown in solutions with 20 ppm manganese and no added iron. (See figure 6.) As was described in Experiments 26-C and 26-F, in bright sunlight the seed leaves orient themselves so as to be parallel to the incident light. On cloudy days and at night they are horizontal. At the same manganese concentration 2 ppm of iron will prevent this phototropic movement.

Sunscald. In experiments reported in this paper it will be recalled that, where sunscald occurred, it was definitely associated with high manganese and low iron. Whether this is primarily a light or heat effect is uncertain. It is, however, a distinctly different condition than that of necrosis on plants at a toxic manganese concentration and not subjected to such extreme conditions of light and temperature.

Photoreduction of iron as influenced by manganese.[8] The tests to be described here were made *in vitro* with solutions but it is thought they may help to elucidate what may take place in the leaves of green plants. In the course of some experiments on plant nutrition a marked change in color was noted in one of two of the stock solutions: "A," which contained

[7] A similar movement of the leaves of *Gluicidia sepium* in direct sunlight was described by Gates (4) and designated by him as "xerofotic" from the fact that it is due to differential turgidity in the pulvini caused by greater drying effect on the upper side under one-sided illumination. His observations, however, were made on apparently normal plants and no relation to nutritional factors was suggested.

[8] These observations and tests were made by the senior author at Cornell University and not previously published.

0.1 gm iron and 4 gms of sodium citrate and "B," which contained in addition to that in "A," 0.01 gm manganese. After these had stood in 100 cc volumetric glass stoppered flasks in a north window of the laboratory it was found that in "A" the greenish yellow color of the ferric citrate had disappeared. Solution "B," however, still retained the yellow color. This indicated reduction in "A" and not (or only partial reduction) in "B." On removing the stopper from "A" and agitating the solution the iron was reoxidized to the ferric condition with return of the yellow color.

This effect was investigated further in a series of test-tube experiments in which it was found that ferric citrate solutions like the above could be reduced to colorless in 5 to 10 minutes in direct sunlight (through window glass plus the glass of the containers for the solutions) and quickly reoxidized again by agitating them or bubbling air through the solutions. Methylene blue was used as an oxidation reduction indicator giving very striking color changes as the iron was reduced or oxidized. It was further found, as suggested by the above observation, that manganese had a retarding effect on the reduction of iron by light. Manganese also accelerated the reoxidation in the dark.

By placing a layer of oil above the solution to exclude atmospheric oxygen and exposing it to direct sunlight the solution could be reduced and prevented from reoxidizing in the dark. After a long time with slow diffusion of oxygen through the oil layer, slight oxidation would be noted at the top of the solution. Tests for ferric and ferrous ions were made by carrying out the reactions under oil. Solutions reduced in direct sunlight for 10 to 15 minutes gave no test for ferric ions with KSCN but a strong test for ferrous ions with $K_3Fe(CN)_6$. The reverse was true for solutions kept in the dark and in contact with air. As a further demonstration a photoelectric cell was prepared by connecting two solutions of iron citrate, one in the light and the other in the dark by means of an agar-KCl bridge and measuring its E.M.F.

The above experiments show: (1) that the oxidation-reduction of iron citrate (and probably other organic forms of iron) is reversible; (2) that light catalyzes the reduction of iron; (3) that manganese catalyzes the oxidation of iron; and (4) that the access of atmospheric oxygen is a factor in the rate of reduction or oxidation of iron.

Discussion of the effect of light. In applying these results to the leaves of green plants it is suggested that the state of oxidation of iron in leaves is by no means static but very changeable, depending on the light intensity, the permeability of the leaf tissues to light, the amount of manganese, the permeability of the tissues to oxygen and carbon dioxide, and the rate of photosynthesis and respiration. The latter processes would affect the composition of the intercellular atmosphere and thus affect the course of

oxidation and reduction. The organic composition of the leaf and its pH may be important factors in determining the solubility and ionization of the iron (Hopkins 11) and hence the rate of oxidation. The problem, because of the number of interrelated and, in nature, unpredictable factors is complex. Nevertheless, light appears to be of great importance in influencing the effect of iron and manganese on plant growth, and that its main rôle consists of its control of the oxidation potential.

Much more experimental work is needed to make clear the effect of the above factors, their interrelation, and mode of action, but for the present we suggest the following as a tentative hypothesis. Just as for factors such as pH and temperature, there is an optimum range of oxidation-reduction potential for the growth of green plants. Beyond these limits, either at a higher or lower potential, injury results. When manganese is present in high amounts in relation to iron the oxidation potential is above the optimum range and chlorosis followed by necrosis results, that is, symptoms of manganese toxicity (iron deficiency). Because of the lack of iron no system is present by which reduction can take place in the light and the oxidation process may even be accelerated under these conditions by light. This would explain previous results that manganese toxicity (iron deficiency) injury is greater in more intense light.

If cobalt is used in the absence of iron and the oxidation potential raised to a still higher level, as was done by Somers and Shive (33), toxicity is more severe than with manganese. With moderate amounts of iron and manganese in a ratio of, say, 2 to 1 or 10 to 1 an average optimum value or optimum range, depending somewhat on environmental conditions, is obtained and normal growth occurs. Lastly, if very low manganese and high iron concentrations are used the average potential falls below the optimum range and iron toxicity (manganese deficiency) results. In other words, without sufficient manganese there is no catalyst to oxidize the iron to the ferric condition. One, therefore, might expect the symptoms to be different from those of manganese toxicity as Somers and Shive found to be the case.

The above is based on the premise that iron, manganese and light are the principal factors in controlling the oxidation potential in the plant cell. However that may be, the experimental indications are that they do exercise a strong effect in this regard. This is an extension of the mechanism previously suggested by Hopkins (10) that manganese functions physiologically by its action on the state of oxidation of iron.[9]

[9] Other unpublished experiments by the senior author have shown that very little oxidation of hydroquinone takes place in the presence of iron without manganese or in the presence of manganese without iron but in the presence of both elements rapid oxidation occurs.

DISCUSSION

It is clear from the data presented in this article that manganese toxicity is a problem in certain sections of Puerto Rico due to the development of toxic concentrations of soluble manganese in the soil. It is also true that this condition may occur in many other agricultural areas. The relation of iron in antagonizing or antidoting the effect of manganese has been shown to be of great value, and it is obvious that the manganese-iron relationship is not merely of laboratory interest but a practical matter. One thing that should be emphasized is that the balance between these elements should be carefully considered in what might be called the normal range. That is, manganese toxicity as exhibited by chlorosis and necrosis should, of course, be corrected, but a further study should be made to find the proper balance for highest crop yields.

Not forgetting that manganese is a necessary element for the growth of green plants (12) and is absolutely required in small amounts, it is of great practical value to know at what concentrations and under what conditions it becomes injurious. The factors that influence its toxicity should be known in order that the condition can be corrected.

Among these factors the relative concentration of iron and light intensity probably have the greatest influence. Attention in this work was directed to the iron factor, since it was more readily controlled than light. The antidoting effect of iron has been repeatedly shown both in soil and water culture tests. Where the concentrations of the two elements have been varied at the same time, a rather complete picture of their action is brought out and the relationship depicted graphically by 3-dimensional figures. This has shown that the effect produced on the plant is not due to one of the elements alone but by their interaction. Even at relatively low manganese concentrations marked toxicity may occur with insufficient iron and not at higher manganese concentrations with sufficient iron. This gives rise to an interesting anomaly since so-called "iron deficiency" symptoms can occur at a relatively high iron concentration and not at a lower one. As has been pointed out before this is due to the fact that iron at the higher concentration may be insufficient to balance the manganese, while at the lower concentration it may suffice. The matter becomes clear when iron deficiency is regarded as equivalent to manganese toxicity.

In attempting to elucidate the mechanism by which iron acts in antidoting manganese it can be postulated that its effectiveness depends on its being a reversible oxidation-reduction system which in the presence of certain proportions of manganese varies in a normal range of potential which green plants can withstand without injury. Within this range of

potential, iron can act as an oxygen donator or acceptor in carrying on the metabolic oxidations and reductions of the cell.[10]

Manganese acts as a catalyst in oxidizing iron and thus shifting the potential to a higher level. This is counterbalanced under normal conditions by the effect of light in reducing the iron and lowering the potential. With high manganese and low iron the manganese maintains too high a potential for normal growth and in the presence of insufficient iron light tends to intensify the toxic effect. Under such conditions the addition of iron may be looked on as a protective measure against high light intensity.[11] On the other hand, at high iron concentrations and low manganese, light reduces the iron and maintains a potential below the normal range. This causes iron toxicity. Iron toxicity was not found in the experiments reported here probably because in all cases the amount of manganese (from impurities) was sufficient to balance the highest amounts of iron used. It is hardly probable that iron toxicity is of any practical importance in Puerto Rico where iron is usually a limiting factor.

Important modifying effects on the action of iron and manganese taken up into the leaves of green plants are brought about by other factors such as pH of the cell sap, PO_4 ion concentration, organic composition of the cell sap especially in respect to organic hydroxy acids or their salts, conditions more or less favorable for adsorption and the amounts of readily oxidized or reduced organic substances. These factors are practically all interrelated in their effect on the solubility, ionization and states of oxidation of iron. In other words they have a marked effect on the concentration of *physiologically active iron*.

Several of these factors are also concerned with the availability of iron and manganese in the substrata in which the plants grow. This is well known. Iron, for instance, may be precipitated at a high pH, and the effect is more pronounced at a higher PO_4 ion concentration. Iron may become unavailable through adsorption, and this in turn is markedly affected by the pH, etc. Iron in organic combination is more soluble even at relatively high pH values but may be removed from such combination by adsorption.

[10] It is not intended here to place the whole matter of metabolism on such a simple basis as just outlined but merely to point out that an inorganic mechanism exists which can exert a strong effect in controlling the oxidation-reduction potential and the course of oxidations and reductions in the green plant.

[11] In this connection it would be of interest to know if such crops as coffee and vanilla, which usually require shade, could be grown successfully in strong light if furnished with a more abundant supply of iron. Hernández (The Journal of Agriculture, Univ. of Puerto Rico, **27**: 27 1943) has shown that vanilla plants grown in full sunlight exhibit marked symptoms of toxicity and chlorosis.

In regard to the prevention of manganese toxicity where it occurs in Puerto Rico, final recommendations cannot be made until the results of field tests are available. However, several suggestions based on the findings presented in this paper can be offered. On acid soil with a high content of soluble manganese it is suggested that the manganese be immobilized by careful adjustment of the soil with finely ground limestone to pH 6.0. At least part of the fertilizer nitrogen should be in the form of nitrate to prevent the development of high acidity caused by the use of ammonium sulphate. The organic content of the soil should be increased to make the iron more available. In the case of pineapple culture iron sulphate sprays should be continued until the soil condition has been corrected.

While human and animal nutrition are beyond the scope of this article, the high manganese content of pineapple fruits analyzed in this work (see footnote page 46) suggests that both feed and foodstuffs produced under these conditions may contain excessive amounts of manganese. It is entirely possible that serious deleterious effects might result from long continued intake of relatively large quantities of this element. Another phase of this is the relation of manganese to vitamins. It acts as a catalyst in the acceleration of oxidations which are responsible for the destruction of the vitamins especially B_1 and C. As was also shown in this work by analysis of the tap water, that the water supplies in certain areas may contain relatively large amounts of manganese and be deficient in iron. Thus there is the possibility of toxicity due to excess manganese and lack of iron and also insufficiency of vitamins.

SUMMARY

Investigation of pineapple soils and pineapple plants growing on them have shown that severe conditions of manganese toxicity exist in Puerto Rico. While these soils do not have excessive amounts of total manganese, chemical analyses have revealed as high as 130 ppm of water soluble manganese and no water soluble iron. It is necessary to spray pineapple plants growing on these soils with iron sulphate solution to prevent severe chlorosis and death of the plants. This has become a common practice in Puerto Rico. Even when chlorosis is prevented with iron sprays large amounts of manganese are taken up as shown by analyses of the fruits and other peculiarities of the plants developed which appear to be associated with manganese toxicity. One of these known as "short top" shows an interesting relation to light intensity.

One cause of the development of high amounts of soluble manganese in the soil is the continued use of ammonium sulphate whereby the pH of the soil is often lowered to 4.0 or less. By careful adjustment of the

pH of the soil to 6.2 the manganese was immobilized, or made insoluble, to such an extent that the available iron was sufficient to antidote its toxicity as regards chlorosis. The addition of iron in organic combination was found to improve the condition still further.

A rapid method of detecting conditions in soils leading to manganese toxicity was used. This was done by growing common bean plants in the soil in question. Within a period of 10 days, when the first trifoliate leaves opened, symptoms of chlorosis would appear and the approximate severity of the condition determined.

To study carefully the interaction of iron and manganese on the growth of plants extensive water cultures and subirrigation gravel culture experiments were carried out with beans, tomatoes and pineapples. The concentrations of these elements were usually varied simultaneously, iron being used in the form of potassium iron humate. Tomato plants were found to be more sensitive than beans to manganese toxicity and beans more sensitive than pineapples because of variation in the iron reserve in the seed or seed piece. For the same reason considerable variation in this respect was encountered in pineapple plants grown from different slips.

The relationship between iron and manganese in regard to growth is best shown by diagrammatic tables and 3-dimensional figures but the results can be expressed briefly as follows: Chlorosis, necrosis, sunscald and decreased growth of the plants were strikingly associated with low iron and high manganese, while such things as increased size and weight of the plants, earliness of appearance of trifoliate leaves, tendrils, flowers, and fruits, and the rate of recovery from chlorosis were markedly associated with low manganese and high iron.

The ratios of dry weights of tops to roots indicate that the tops were affected more by variation in the iron-manganese relationship than the roots. In general the Fe/Mn ratio was found to be the controlling factor in growth, but for each given ratio growth varied with the total concentration of iron plus manganese.

An interesting effect of manganese on phototropic movements of seed leaves of bean plants was studied. At 20 ppm manganese, 2 ppm iron were sufficient to prevent these movements. This and other phenomena in respect to light have led to the idea that iron acts as a protective agent against light and also that the interaction of the 3 factors: iron, manganese and light, are important determinants controlling the oxidation potential of green plants. In proper balance a normal range of the oxidation potential results. When not in proper balance a too high or too low a range of potential for plant tissues occurs and toxicity appears.

Tentative recommendations are given for preventing manganese toxicity

and it is further suggested that much may be gained in the way of increased crop yields by careful adjustment of the iron-manganese balance within the normal range where symptoms of toxicity are not apparent.

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