IRON-MANGANESE RATIOS IN NUTRIENT SOLUTIONS IN RELATION TO THE CHLOROSIS OF SORGHUM PLANTS

by

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INTRODUCTION

Chlorosis, a reduction in the chlorophyll content of leaves which is attributed to deficiencies of one or more of several micronutrient elements, is a major problem in the growing of crops on western soils. Sorghums and shrubery of western and central Kansas suffer a great deal from chlorosis. The application of iron sprays to these chlorotic plants has given temporary relief.

Because sorghum is the major cash crop in western and southwestern Kansas, the demand for more information on the subject of chlorosis is very great. This demand has caused some research at the Kansas Agricultural Experiment Station to be devoted to gathering information on this subject.

Several factors have been given as contributors to iron chlorosis. Among these is the ratio of iron to manganese in the nutrient medium and in the plant sap.

Previous work at other stations has led some workers to believe that manganese tends to oxidize iron to the insoluble ferric form. They feel that if the proper ratios of iron to manganese are maintained, normal plants will be produced. Results of earlier work with soils at the Kansas station did not show these clearly defined ratios to be of importance.

It was the purpose of the present study to determine if iron-manganese ratios are important in producing normal plants. In order to make this study possible, sorghum plants were grown in a greenhouse in solution cultures in which the iron-manganese

ratios were varied.

REVIEW OF LITERATURE

Johnson (10), working with pineapple in Oahu, found that chlorotic plants occurred on soils high in manganese. After being able to correct the chlorosis by spraying the plants with a solution of ferrous sulphate, he concluded that the high manganese tended to depress the assimilation of iron. Observation caused Johnson to believe that the higher the content of manganese, the greater the symptoms of chlorosis. Ash analysis showed that plant tissues were high in manganese.

Chapman (3), working with forest seedlings in sand, found that the presence of manganese in leaves caused the solubility of iron to be lowered. He found that the injection of large quantities of manganese into the tree caused the soluble iron of the wood to diminish. Chapman concluded that manganese was converting iron from a soluble to an insoluble form.

Somers, Gilbert and Shive (19), studying the carbon dioxide given off by roots of soybeans grown in iron and manganese solutions, found that normal plants were produced only when the ratio of iron to manganese in the plant was between the narrow limits of 1.5 to 2.5. The highest yield of carbon dioxide was given off by the roots within this range. Above and below this optimum ratio pathogenic symptoms were produced which became worse the further the ratio got from the optimum. They found that ratios lower than the optimum produced chlorosis. Symptoms of iron deficiencies

were found to be the same as manganese toxicity. Ratios higher than the optimum caused plants to be necrotic. These investigators found that plants suffering from manganese deficiency had the same appearance as those plants getting too much iron. In the same experiment, normal plants were produced with unbelievably low and relatively high concentrations of both manganese and iron as long as the ratio was maintained between the narrow limits of 1.5 to 2.5.

Later work by Somers and Shive (20) revealed that chlorotic plants are higher in total iron than normal ones. Workers grew soybeans in glass jars which were fed by Shive and Robbins' modified continuous flow system. By this system the solution was completely changed every day. The plant material was analyzed for active and inactive iron. When the ratio of iron to manganese was kept between 1.5 and 2.5, the active iron was high. Below or above this ratio the iron was in the inactive state.

Work by Hopkins, Pagan and Silva in Puerto Rico (9) substantiated the importance of the ratio of iron to manganese in producing chlorosis. Puerto Rican soils were high in water soluble manganese which produced chlorotic pineapple plants. Spraying plants frequently with ferrous sulphate corrected this condition.

The above results caused the workers to conclude that the ratio of iron to manganese was more important than the concentration of either element. However, the narrow ratio discovered by Shive and co-workers was not found, but a much wider ratio prevailed. The Puerto Rico station workers were able to produce manganese

toxicity at low iron-manganese ratios which were regarded as being equivalent to iron deficiency. However, they were never able to get iron toxicity symptoms which were similar to manganese deficiency symptoms no matter how high the iron-manganese ratios were.

Analyses made by Hopkins, Pagan, and Silva showed that the ratio of iron to manganese in the substrata was very much the same as that in the plant. This caused them to believe that if the proper ratio of iron to manganese were present in the soil, the plant would contain the proper ratio. They concluded that the absorption rates of iron and manganese are determined largely by their concentration in the substrate.

Hopkins, Pagan and Silva also found that the soluble iron found in plant leaves was high when manganese was low and vice versa. These results led the investigators to conclude that the active iron in the leaves was being made inactive by the oxidizing potential of the manganese. The potential of manganese was known to be higher than that of iron. The plant has within itself a strong reducing system, but the presence of large amounts of manganese would tend to cause the iron present to be in the oxidized form.

Hopkins, Paga, and Silva agreed with Somers and Shive in the theory that excess manganese catalyzed the oxidation of ferrous iron to ferric iron in the plant. This was borne out by results of plants grown with different amounts of light. Plants grown in limited light developed chlorosis sooner and more intensely than

those grown in full light. These results assured workers that the oxidation potential of manganese was impaired by the plant's reducing activity. Limited light caused the reducing properties of the plant to be curtailed letting manganese oxidation take place.

The work with potatoes by Berger and Gerloff (2) at the University of Wisconsin did not result in the same conclusions. An attempt to prevent potato plants from developing manganese toxicity by introducing ferrous sulphate into the plant stem with a hypodermic needle was not successful. Necrosis developed as rapidly and was as severe in treated plants as it was in the untreated plants. It was also found that manganese toxicity symptoms were entirely different from the iron deficiency symptoms.

Morris and Pierre (11) of the Iowa Experiment Station working with lespedeza investigated the effects of calcium, phosphorus and iron on the tolerance of lespedeza to manganese toxicity in culture solutions. Plant analyses showed that lack of chlorosis in plants with high iron concentrations was not due to an increase in total iron in the plants but to a 50 per cent decrease in the manganese content of the plants. In no case did the workers find that the iron-manganese ratio of the culture solutions was a factor controlling plant growth; rather they found that the total amounts of these elements was of the greatest importance.

Later work by Morris and Pierre (12) with peanuts, soybeans and lespedeza resulted in their finding a great difference between manganese toxicity and iron deficiency. Investigators pointed out

that the total concentration of iron or manganese was much more important than the ratio of iron to manganese.

Olson and Carlson (13), working with Kansas soils, investigated the importance of the iron-manganese relations in neutral and alkaline soils of that state. Three groups of soils were studied: soils on which the less sensitive plants showed chlorosis, soils on which sensitive plants produced chlorosis and soils on which no plants showed symptoms of chlorosis. Iron-manganese ratios were significantly less in soils producing chlorosis in the less sensitive plants than in soils of the other two groups. The ratios of the other two soils did not differ significantly. A correlation between extractable iron and field observations existed in all three soils.

There was no significant difference in the easily reducible manganese content of the three soils and only partial significance in exchangeable manganese. This causes it to seem as if the difference in iron-manganese ratio is caused by the differences in extractable iron content of those soils.

Pineapples grown in culture solutions at the University of Hawaii by Sideris and Young (17) yielded good results when the ratio of iron to manganese was maintained between 1 to 1 and 1 to 10. When the iron-manganese ratio was wider than 1 to 10, chlorosis developed. Investigation of iron and manganese contents of roots and leaves revealed that high amounts of manganese in roots tended to inhibit movement of iron from the roots to the leaves.

The work of Sideris and Young also showed that chlorophyll and iron in leaves correlated with iron supplied in nutrient solutions. The plant protein and chlorophyll content increased with greater amounts of iron and decreased with increased manganese. This led the workers to believe that iron was a component of the system of chlorophyll and chloroplast formation. Because of work by Granick (7), showing that protophycin 9 is a precursor of chlorophyll, the above investigators concluded that the presence of large amounts of manganese caused manganese to replace iron in the pyrrol ring of this formation. This caused the protophycin to become inactive and never form chlorophyll or chloroplasts.

Bennett (1), working with tomato plants in culture solutions, found that if he kept the concentration of iron stable and increased the concentration of manganese, green plants were produced when the ratio of manganese to iron was kept between 4 and 47. Ratios of 60 to 108 produced chlorotic plants. In the same investigation, the worker kept the concentration of manganese constant and altered the iron concentration. Ratios of manganese to iron from 17 to 27 produced green plants, while ratios from 36 to 64 produced chlorotic plants. These data caused Bennett to conclude that manganese produces chlorosis by depressing the absorption of iron because of an antagonism, existing between the two elements, which is apparently mutual. There were no data to indicate that manganese interfered with the utilization of iron in the leaf or that the ratio of manganese to iron in the tissue was related to the chlorosis produced.

EXPERIMENTAL METHODS

Cultural Methods

Westland milo was produced in ten different culture solutions as shown in Table 1. Each treatment had three plants and was replicated four times.

Because iron forms oxides quickly in the presence of oxygen, plants were grown in glass jars in which there was a constant flow of nutrient solution by the drip method. The drip was controlled so as to renew the culture solution at a constant rate. The apparatus used, which is shown in the picture in Plate I was similar to that described by Shive and Robbins (16).

Table 1. Iron and manganese contents of nutrient solutions used in studying the relationship of iron-manganese ratios to chlorosis in sorghums.

Treatment	Iron-manganese ratio of nutrient solution	Parts per million iron in solution	Parts per million manganese in solution
High Fe - high Mn	1	3.00	3.000
High Fe - medium Mn	6	3.00	0.500
High Fe - low Mn	600	3.00	0.005
Medium Fe - high Mn	0.166	0.50	3.000
Medium Fe - medium Mn	1	0.50	0.500
Medium Fe - low Mn	100	0.50	0.005
Low Fe - high Mn	0.013	0.04	3.000
Low Fe - medium Mn	0.08	0.04	0.500
Low Fe - low Mn	8	0.04	0.005
Shive's optimum ratio	2	3.00	1.500

Plants were started in pure quartz sane and transplanted to jars when the plants were two weeks old. Five-pound candy jars were used on which a wooden lid was fitted. Three holes

were drilled in the lid; an inch hole in which the plants were placed surrounded by non-absorbent cotton and two three-eighths inch holes. In one of these holes a siphon tube was placed to keep the solution in the jar at a constant height and in the other hole a small funnel was placed into which the drip was directed. A picture of the described setup can be found on page 10.

Three plants were planted in each jar with the nutrient solution at a level such that the roots were just in the solution. Had the solution been kept at a higher level, algal growth would have become excessive. The jars and the feeder tubes were painted with black paint to prevent algal growth. Non-absorbent cotton was packed in the hole to keep the plants upright.

The pH of the nutrient solution was maintained at 5 to 5.5 by additions of hydrochloric acid or ammonium hydroxide. A Beckman pH meter was used in making pH measurements.

Major plant nutrients were added in the same amounts to all treatments as shown in Table 2. The solutions were all made up in molar stock solutions and were added in measured amounts to the feeder jugs.

EXPLANATION OF PLATE I

Illustration of the setup used in the study of the relationship or iron-manganese ratios to chlorosis in sorghums. The carboys were used as feeder jugs and the black candy jars contained the nutrient solution in which the plants were grown. Glass siphon tubes carried the solution from the jugs to the funnels in the jars. The level of the nutrient solution in the jar was maintained by a siphon tube to the pint Mason jar.

PLATE I

Table 2. Major element composition of nutrient solutions used in all cultures.

Compound used	Parts per million of elements obtained in nutrient solutions					
	Potassium	Phosphorus	Nitrogen	Calcium	Magnesium	Sulfur
$NH_4 H_2 PO_4$		49.76	22.60			
KNO_3	75		26.95			
$Ca(NO_3)_2 2H_2O$			71.52	102.3		
$M_g SO_4 . 7H_2O$					47.40	62.40
Total	75	49.76	121.07	102.3	47.40	62.40

Minor elements were supplied in the same proportion as that suggested by Hoagland and Arnon (8) except that the manganese and iron were left out of the minor element stocks solution. The elements were made up in a stock solution in the proportions shown in Table 3. One milliliter of the stock solution was added per liter of culture solution.

Table 3. Composition of minor element stock solution used in making nutrient solutions.

Compound used	Grams of salt per liter	Parts per million of element in solution when 1 ml is diluted to 1 liter.	
$H_3 BO_3$	2.86	Boron	0.50
$ZnSO_4.7H_2O$	0.22	Zinc	0.05
$CuSO_4.5H_2O$	0.08	Copper	0.02
$H_3 MoO_4.H_2O$ 85%	0.09	Molybdenum	0.05

Iron was supplied in the form of ferric citrate and manganese

in the form of manganese dioxide. One hundred parts per million stock solutions were made up. Measured amounts of the stock solutions were added to the treatments in order to get the desired concentrations shown in Table 1.

The drip was controlled so as to keep the desired iron-manganese ratio for the plants at all times. This rate of flow was determined by checking the iron and manganese contents of the nutrient solutions in which the plants were growing. The flow necessary to maintain the desired ratio was fifty drops per minute. Table 4 represents the results of the flow study. After 24 hours the ratio of iron to manganese was less than one at flow rates of 20 and 40 drops per minute but was almost exactly one at rates of 50 and 55 drops per minute. This rate made a complete change of solution in the jars every twenty-four hours.

A comparison of the soluble iron in the feeder jug with that in the jars is shown in the graph appearing in Fig. 1 and the data in Table 5. The concentration of iron was less in the jars in which the plants were grown than in the feeder jug. The data for Fig. 1 and Table 5 were collected when the plants were four weeks old.

Manganese content of feeder jugs and jars are also shown in Table 5. The concentration in the feeder jug and the jars in which the plants were grown stayed about the same over a period of 24 hours.

The concentration of manganese did not appear to lessen the solubility of iron. The iron concentration compared well at all three levels of manganese when the flow of solution from the

Table 4. Effect of rate of flow of nutrient solution into culture jar on the soluble iron and manganese content of the solution in jars. Three parts per million iron were supplied to all jars.

Rate of flow of nutrient solution in drops per min.	Parts per million iron in solution after indicated time in hours						Parts per million manganese in solution after indicated time in hours					
	1	2	3	8	17	24	1	2	3	8	17	24
20	3.00	3.04	2.91	3.00		1.90	3.03	3.00	3.06	2.98		3.06
40	3.06	3.00	3.00	2.63		2.30	3.00	2.96	3.05	2.90		2.90
50	3.08	3.02	2.91	3.12	3.13	3.08	2.96	3.02	3.04	3.00	3.10	3.00
55	3.10	3.00	2.94	2.94	3.09	3.15	2.94	2.96	3.03	3.06	3.00	2.94

Table 4. (cont'd)

Rate of flow of nutrient solution in drops per min.	Ratio of iron to manganese in solution after indicated time in hours						Desired ratio of iron to manganese
	1	2	3	8	17	24	
20	0.99	1.02	0.95	1.01		0.63	1
40	1.02	1.02	0.98	0.91		0.79	1
50	1.04	1.00	0.96	1.04	1.01	1.02	1
55	1.05	1.01	0.97	0.96	1.03	1.03	1

Table 5. Iron contents of feeder bottle and jars in which plants were growing over a twenty-four hour period. The nutrient solution was flowing from the feeder bottle at the rate of fifty drops per minute.

Time in hours	Parts per million			
	Iron in feeder bot.	Iron in jars	Manganese in feeder bot.	Manganese in jars
1	3.40	3.04	2.96	3.02
2	3.44	3.02	3.02	3.00
3	3.36	3.00	3.04	2.94
8	3.45	2.96	3.00	3.06
17	3.32	3.06	3.10	3.00
24	3.44	3.03	3.00	3.08

Table 6. Iron content of nutrient solutions with varying amounts of manganese. All solutions had three parts per million added.

Parts per million manganese in nutrient solution:	Parts per million iron in solution after indicated period of time in hours					
	1	2	3	8	17	24
3.100	3.04	3.02	3.00	2.96	3.06	3.03
0.550	2.90	2.90	2.94	2.98	3.00	2.92
0.005	3.08	3.00	3.00	3.10	3.05	3.05

feeder jug was kept at 50 drops per minute as is shown in Table 6.

Plants were started in the greenhouses of Kansas State College on May 14, 1949. All plants were grown in glass containers and fed through glass tubes in order to prevent iron contamination.

On May 23, 1949 it was necessary to put a shading compound on the greenhouse. The glass was replaced in the greenhouse during the early part of June. The roof was reshaded after this work was finished.

Plants used in chlorophyll analysis were harvested June 14,

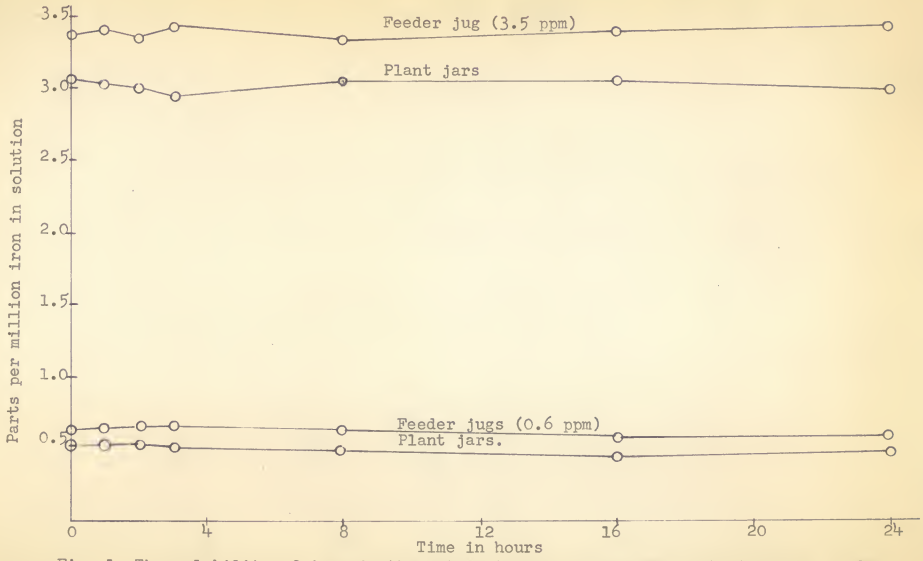


Fig. 1. The solubility of iron in the nutrient jars as compared to that in the feeder jug over a 24 hour period. The rate of flow was 50 drops per minute.

1950. The remainder of the plants were harvested on July 13, 1950. Half of the plant material was dried for analysis and the other half was used for plant sap extraction.

Solution and Plant Analysis

Chlorophyll was determined by the method described by Comor, Benner and Buteyn (5). A standard curve represented in Fig. 2, was made by determining the absolute chlorophyll content of an extract as described by Comer (4). The curve was constructed by Dr. R. V. Olson in some of his work.

Plants were removed from the jars when six weeks old and cut into small sections with a scissors. The cut material was placed in a pint jar to which a pinch of calcium carbonate and 50 ml of 85 per cent acetone were added. The mixture was blended in a Oster Mixer model No. 10 for five minutes. Tissue was allowed to stay in contact with the acetone in the dark for 30 minutes before filtering. The solution was filtered through a Buechner funnel and made up to 100 ml with 85 per cent acetone. Transmission was read on an Evelyn photometer using a No. 625 red filter.

Sap was extracted with a Carver Press using a 2¼ inch test cylinder. Plant material to be used for dry analysis was dried in an oven and passed through a Wiley mill.

Plant material and sap were digested as described by Piper (15). To 2 ml of sap or 1 gram of plant material, 7 ml of nitric acid, 2 ml of sulfuric acid, and 2 ml of perchloric acid were

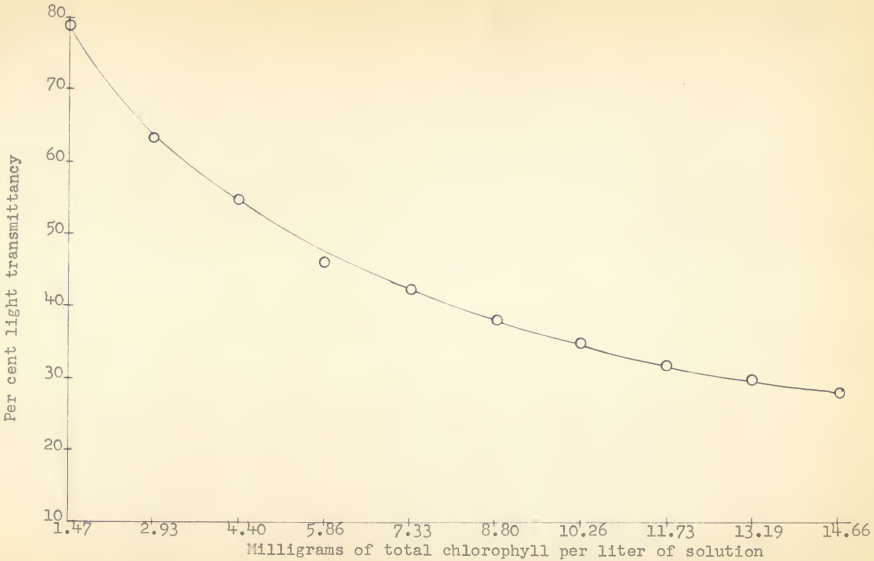


Fig. 2. Standard curve for use with an Evelyn photometer in determining milligrams chlorophyll per gram green plant tops. Air rest point of instrument was 68.75 with a 660 millimicron filter.

added. Digestion was carried out in a Kjeldahl flask over a low burning Bunsen burner. When dense brown fumes were evolved, the flask was removed and cooled for five minutes. Digestion was continued after cooling until white fumes appeared. Samples were again cooled and diluted with 20 ml of water after which the solution was boiled for two minutes, cooled and made up to a volume of 25 ml. Nutrient solutions were analyzed without any previous treatment.

Iron was determined using the orthophenanthroline method (6). When ferrous iron was determined, 2 ml of a 0.1 per cent solution of orthophenanthroline was added to 10 ml of the solution to be analyzed. The ferrous plus ferric iron was evaluated by further adding 2 ml of a one per cent solution of hydroxylamine hydrochloride. The color reaction was found to follow Beer's law in an Evelyn Photometer using a Corning No. 515 filter. The concentration of iron in the solution analyzed was obtained by getting the L Value (2 minus the $\log_{10}$ of light transmittance for the transmittancy of the sample) and multiplying by the constant 3.3. The constant 3.3 resulted from the slope of the line obtained by plotting the L value against known iron concentrations.

Manganese was determined colorimetrically as suggested by Peech, Dean and Reed (14). To 10 ml of the solution to be analyzed 5 ml of 85 per cent phosphoric acid solution and 0.3 grams of sodium periodate were added. The solution was boiled until full color developed and has then diluted to a volume of 50 ml with 5 per cent phosphoric acid. Transmittancy of the extract was read

on an Evelyn Photometer using a Corning No. 515 filter. Concentrations of manganese were read from a standard curve made by measuring the light transmittancies for solutions containing known concentrations of manganese. The standardization curve obtained can be found in Fig. 3.

Analysis of variance as described by Snedecor (18) was applied to all results.

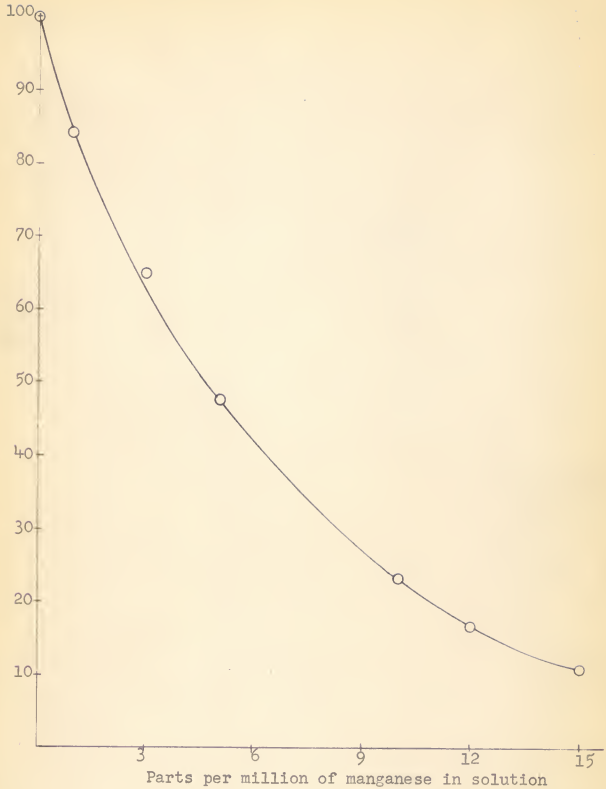


Fig. 3. Standard curve for use with an Evelyn photometer in determining parts per million manganese in solution. Air rest point of instrument is 69.0 with a 515 millimicron filter.

EXPERIMENTAL RESULTS

Yields

The total weights of all green plant tops are shown in Table 7. Results of analysis of variance of the data are shown in Table 8. The interaction between iron and manganese in the treatments was highly significant. The effect of iron on the yield of plant material was significant at the one per cent level while the effect of manganese was not significant. The effect of manganese treatments on plant material produced was not significant for all treatments but because of a highly significant interaction a significant difference between means did exist in the medium iron treatment.

Plants receiving no iron produced little plant material regardless of the amount of manganese supplied. In the group receiving a medium treatment of iron, the high manganese cultures produced significantly less plant material than the medium and low manganese cultures. The amount of plant material produced by the high iron treatment was independent of the manganese content of the nutrient solutions. The plants growing in a solution having the optimum ratio of iron to manganese as suggested by Somers and Shive (20) did not produce significantly more plant material than any other high iron treatment.

The total green plant material produced at different iron and manganese levels appears in a graph in Fig. 5. It is apparent from these data that iron-manganese ratios have no effect on

Table 7. Total green weight of sorghum plants grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nutrient solution	Weight of plants in grams per culture				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	82.40	65.30	88.30	91.30	81.83
High Fe - medium Mn	6	78.20	94.30	64.10	96.40	84.25
High Fe - low Mn	600	82.80	69.40	80.00	99.10	82.82
Medium Fe - high Mn	0.166	2.60	2.10	9.60	1.10	3.85
Medium Fe - medium Mn	1	98.20	64.00	115.70	66.50	86.10
Medium Fe - low Mn	100	34.90	31.50	81.70	63.20	52.83
Low Fe - high Mn	0.013	2.60	2.40	2.10	1.70	2.20
Low Fe - medium Mn	0.08	0.39	0.26	0.31	0.44	0.34
Low Fe - low Mn	8	0.26	0.39	0.48	0.37	0.39
Shive's optimum ratio	2	97.30	78.80	79.29	86.60	85.50

*Difference significant at the 1 per cent level. Least significant difference at the 1 per cent level is 25.37, at 5 per cent level 20.50.

Table 8. Analysis of variance values obtained using yield data for green tops of sorghum plants. Plants were grown in nutrient solutions to which different amounts of iron and manganese had been added.

Factor	Variance	Calculated F. value	F value needed for significances 5% level	1% level
Manganese treatment	2665	0.97	6.94	18.00
Iron treatment	20229	7.39*	6.94	18.00
Manganese x iron (interaction)	2743	27.0**	2.73	4.11
Jars treated alike (error)	168			

*Difference significant at 5 per cent level.
**Difference significant at 1 per cent level.

sorghum yields at high iron levels. At medium iron levels the ratio has significant effect. In this case, a ratio of 1 to 1 is superior to a ratio of 100 to 1 and far superior to a ratio of 0.166 to 1. At low iron levels yields are extremely poor regardless of the iron-manganese ratio employed.

Chlorophyll Content of Leaves

Chlorosis developed in plants after varied lengths of time. The plants receiving the low iron treatment became yellow five days after being transplanted, turned white after eleven days in the jars and died six weeks after replanting. Plants receiving the medium treatment of iron and the heavy treatment of manganese showed the first symptoms of chlorosis ten days after being moved to the jars. Chlorosis developed in the medium iron and medium manganese treatment after twenty-eight days. The plants getting

the medium iron and low manganese treatment failed to develop chlorosis.

Plants receiving the high iron treatment never did show chlorosis regardless of the manganese treatment. No chlorosis was observed in the plants treated with Shive's optimum ratio treatment.

Table 9 gives the milligrams of chlorophyll produced per gram of green tops. A graph showing chlorophyll produced at different iron and manganese contents appears in Fig. 4. Analysis of variance results are shown in Table 10. The interaction between iron and manganese was significant at the 1 per cent level. Effect of manganese treatment on chlorophyll produced was not significant but iron was significant at the 1 per cent level.

Although the effect of manganese treatments on chlorophyll produced was not significant for all treatments, a significant difference between means did exist in the medium iron treatment. This significance took place because of the significant interaction.

Plants receiving no iron produced little chlorophyll irrespective of the manganese amounts supplied. The high iron treatment produced high amounts of chlorophyll in all three manganese treatments. The plant chlorophyll in the medium iron treatment and high manganese was less than half that in the medium and low treatment. The highest amount at the medium iron level was in the medium manganese treatment.

Table 9. Chlorophyll contents of sorghum tops from plants grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nutrient solution	Milligrams of chlorophyll per gram of fresh tops				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	1.592	1.546	1.674	1.466	1.570
High Fe - medium Mn	6	1.546	1.144	1.531	1.385	1.402
High Fe - low Mn	600	1.485	1.290	1.419	1.480	1.414
Medium Fe - high Mn	0.166	0.293	0.234	0.557	0.147	0.308
Medium Fe - medium Mn	1	0.989	0.601	1.312	0.447	0.837
Medium Fe - low Mn	100	0.747	0.817	0.674	0.535	0.693
Low Fe - high Mn	0.013	0.154	0.166	0.147	0.198	0.166
Low Fe - medium Mn	0.08	0.201	0.198	0.392	0.261	0.263
Low Fe - low Mn	8	0.166	0.123	0.179	0.243	0.178
Shive's optimum ratio	2	1.123	1.144	1.230	1.466	1.241

*Difference significant at the 1 per cent level. Least significant difference at the 1 per cent level is 0.27, at 5 per cent level 0.20.

Table 10. Analysis of variance values obtained using data for chlorophyll content of sorghum plants. Plants were grown in nutrient solutions to which different amounts of iron and manganese had been added.

Factor	Variance	Calculated F value	F value needed for significance 5% level	1% level
Manganese treatment	0.125	0.21	6.94	18.00
Iron treatment	4.753	20.60**	6.94	18.00
Manganese x iron (interaction)	0.230	8.51**	2.73	4.11
Jars treated alike (error)	0.027			

**Difference significant at 1 per cent level.

The chlorophyll content produced by plants growing at different iron and manganese levels appears in a graph in Fig. 4. The curves of Figs. 4 and 5 compare favorably. This indicates that iron-manganese ratios are not an important factor in chlorophyll production at high iron concentrations. At low concentrations of iron little chlorophyll was produced no matter what the iron-manganese ratio is. The data indicate that iron-manganese ratios are significant at the medium iron level. The ratio of 1 to 1 produces more chlorophyll than the ratio of 100 to 1. The ratio 0.166 to 1 produced the least chlorophyll of all ratios in the medium iron group.

The treatment getting medium iron and low manganese showed no chlorosis during the experiment. In spite of this the treatment produced less plant material and chlorophyll. The plants in this treatment had a streaked appearance after three weeks in the jars. This condition remained until the end of the

EXPLANATION OF PLATE II

Illustration of the medium iron treatment used in the greenhouse study of the relationship of the ratio of iron and manganese as a factor in the occurrence of chlorosis in sorghums. Jar No. 1 is the medium iron-high manganese treatment; jar No. 2 is the medium iron-medium manganese treatment and jar No. 3 is the medium iron-low manganese treatment. Picture was taken when plants were four weeks old.

PLATE II

EXPLANATION OF PLATE III

Illustration of the low iron treatment used in the greenhouse study of the relationship of iron-manganese ratio as a factor in the occurrence of chlorosis in sorghums. Jar No. 1 is the no iron-high manganese treatment. Jar No. 2 is the no iron-medium manganese treatment, and Jar No. 3 is the no iron-no manganese treatment. Plants were four weeks old when the picture was taken.

PLATE III

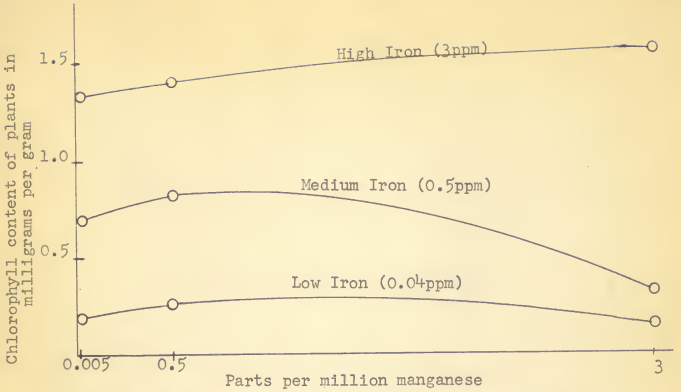


Fig. 4. Milligrams of chlorophyll produced by plants growing in nutrient solutions to which different amounts of iron and manganese had been added.

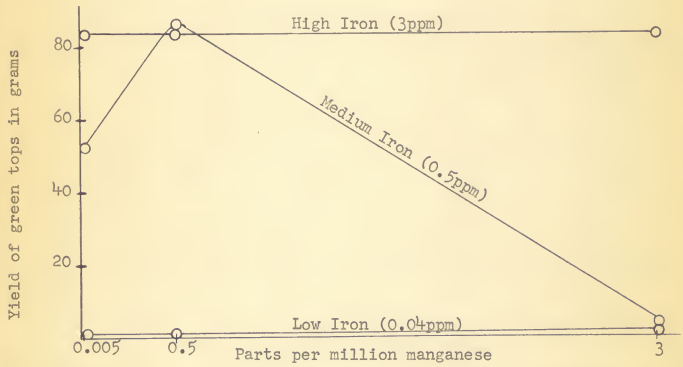
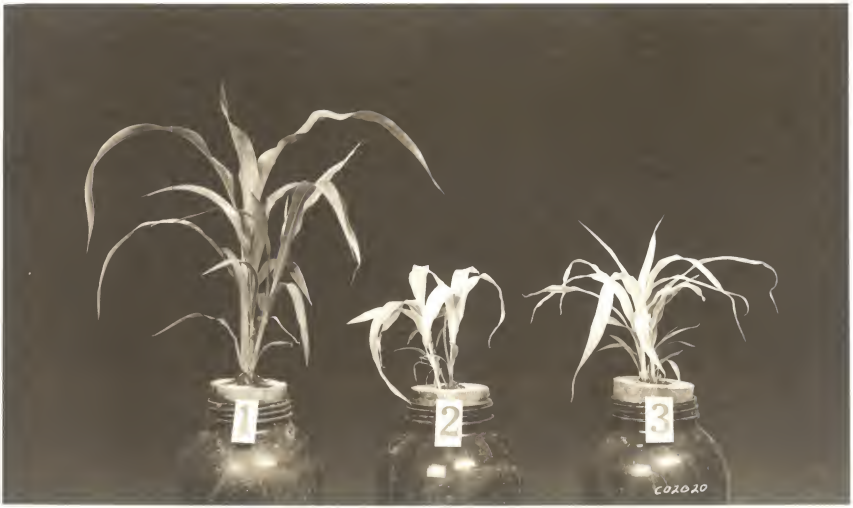


Fig. 5. Grams of green plant tops produced at different concentrations of iron and manganese used in the study of the importance of iron-manganese ratios of a factor in the occurrence of chlorosis in sorghums.

EXPLANATION OF PLATE IV

Illustration of the high manganese treatment used in the greenhouse study of the relationship of the ratio of iron to manganese as a factor in the occurrence of chlorosis in sorghums. Jar No. 1 is the high iron-high manganese treatment; Jar No. 2 is the medium iron-high manganese treatment, and Jar No. 3 is the low iron-high manganese treatment. Pictures were taken when plants were four weeks old.

PLATE IV



experiment and may have caused the difference in chlorophyll and plant material produced.

Iron Content of Plant Material

Because of the limited growth of those plants receiving the no iron treatment, there was not sufficient sample of dried tops or sap for iron or manganese analysis.

Values found for the iron content of plant sap for the medium and high iron levels are represented in Table 11. Analysis of variance results are shown in Table 12. The interaction between iron and manganese was highly significant. Neither the iron or manganese treatment was significant. It can be seen from Table 11 that the iron content of the plant sap was independent of iron-manganese ratios in the nutrient medium.

The graph in Fig. 6 shows the iron content of the plant sap. At the high iron levels the 0.5 parts per million manganese suppressed the accumulation of iron in the plant sap as compared to the 0.05 or 3 parts per million manganese treatments.

The plants receiving the medium concentration of iron did not differ significantly in the amount of iron in the plant sap regardless of the manganese treatment.

Results of iron analyses of dried plant tops can be found in Table 13. Analysis of variance data appears in Table 14. The interaction between iron and manganese effects on the amount of iron in dried plant tops was significant at the 5 per cent level. Neither the iron nor manganese treatments were significant.

Table 11. Iron contents of sap from sorghum plants grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nut. solution	Parts per million iron in sap				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	3.68	3.50	2.92	2.56	3.16
High Fe - medium Mn	6	1.21	0.65	1.19	1.58	1.16
High Fe - low Mn	600	2.45	1.45	2.18	2.45	1.88
Medium Fe - high Mn	0.166	1.76	1.18	0.93	0.88	1.19
Medium Fe - medium Mn	1	1.04	1.18	1.19	1.31	1.18
Medium Fe - low Mn	100	0.73	1.59	0.58	0.77	0.92
Shive's optimum ratio	2	1.55	1.45	1.19	1.16	1.34

*Least significant difference at 1 per cent level is 1.93; at 5 per cent level 1.43.

Table 12. Analysis of variance values obtained using iron contents of sorghum plant saps.

Factor	Variance	Calculated F value	F value needed for significance 5% level	1% level
Manganese treatment	2.63	1.21	19.00	99.00
Iron treatment	6.19	2.85	18.51	98.49
Manganese x iron (interaction)	2.17	27.8**	3.55	6.01
Jars treated alike (error)	0.078			

EXPLANATION OF PLATE V

Illustration of the high iron treatment used in the greenhouse study of the relationship of the ratio of iron to manganese as a factor in the occurrence of chlorosis in sorghums. Jar No. 1 is the high iron-high manganese treatment; Jar No. 2 is the high iron-medium manganese treatment, and Jar No. 3 is the high iron-low manganese treatments. Picture was taken when plants were four weeks old.

PLATE V

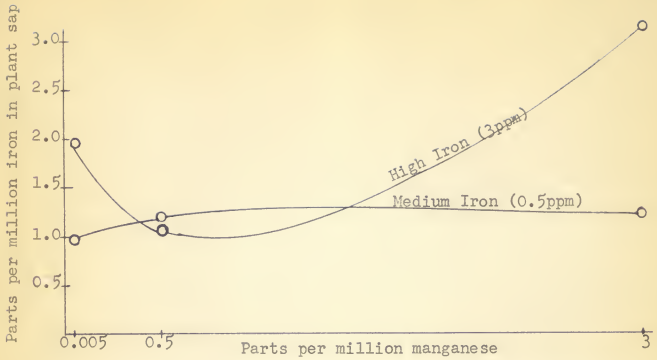


Fig. 6 Iron content of plant sap from sorghum plants growing in nutrient solutions to which different concentrations of iron and manganese had been added.

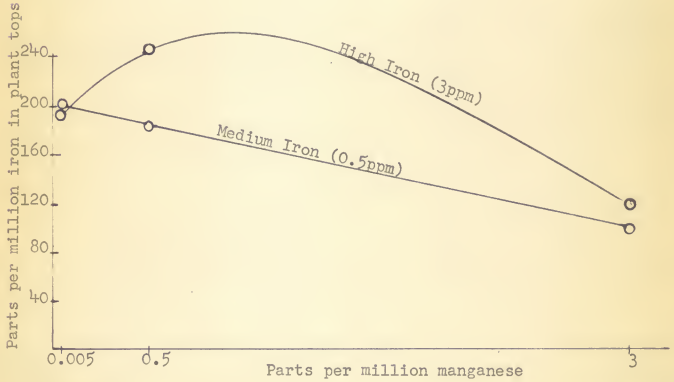


Fig. 7. Iron content of dried plant tops from sorghum plants growing in nutrient solution to which different concentration of iron and manganese had been added.

Table 13. Iron contents of dried sorghum tops grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nutrient solution	Parts per million iron in dried tops				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	126	122	111	115	119
High Fe - medium Mn	6	300	207	232	255	249
High Fe - low Mn	600	196	190	215	173	194
Medium Fe - high Mn	0.166	111	93	115	81	100
Medium Fe - medium Mn	1	200	192	157	177	181
Medium Fe - low Mn	100	233	190	231	146	200
Shive's optimum ratio	2	147	160	270	127	176

*Least significant difference at the 1 per cent level is 53.14; at 5 per cent level 38.89.

Table 14. Analysis of variance values obtained by using iron contents of dried sorghum plant tops.

Factor	Variance	Calculated F value	F value needed for significance	
			5% level	1% level
Manganese treatment	21749	6.70	19.00	99.00
Iron treatment	2726	0.85	18.51	98.49
Manganese x iron (interaction)	3194	4.65*	3.55	6.01
Jars treated alike (error)	686			

*Significant at 5 per cent level.

Figure 7 shows the iron content of dried plant tops at different iron and manganese levels. From the graph it is apparent that no significant difference exists in the iron treatment when high or low manganese was added. The medium manganese treatment does show a significant difference between the high and medium iron treatment.

The interaction caused the differences in manganese concentration at different levels of iron to not be statistically significant. The small number of degrees of freedom also tended to keep this difference from showing up in the analysis of variance. For this case the ratio of iron to manganese apparently does not have an effect on the iron content of dried tops. The concentration of manganese appears to have an effect by causing the iron content of dried tops of the medium iron treatment to vary indirectly with manganese. The high iron treatment does not react in this way.

Manganese Content of Plant Material

Manganese contents found in the plant sap appear in Table 15. The results of analysis of variance, presented in Table 16, showed an interaction between iron and manganese to exist which was significant at the 5 per cent level. The effect of the iron and manganese treatments was not significant. However, the graph in Fig. 8 shows added manganese does have an effect on the manganese found in plant sap.

Table 15. Manganese contents of sap from sorghum plants grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nutrient solutions	Parts per million manganese in sap				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	6.25	7.50	11.25	7.50	8.37
High Fe - medium Mn	6	2.63	4.25	7.35	5.13	4.84
High Fe - low Mn	600	0.39	0.97	0.65	0.23	0.56
Medium Fe - high Mn	0.166	18.72	9.45	11.35	8.95	12.12
Medium Fe - medium Mn	1	2.63	1.98	3.59	3.12	2.83
Medium Fe - low Mn	100	1.20	0.38	0.25	0.13	0.49
Shive's optimum ratio	2	5.96	4.99	4.69	3.60	4.81

*Least significant difference at 1 per cent level is 4.26; at 5 per cent level 3.12.

Table 16. Analysis of variance values obtained using manganese contents of sap from sorghum plants. Plants were grown in nutrient solutions to which different amounts of iron and manganese had been added.

Factor	Variance	Calculated F value	F value needed for significance 5% level	1% level
Manganese treatment	378.42	17.76	19.00	99.00
Iron treatment	1.33	0.06	18.51	98.49
Manganese x iron (interaction)	21.30	4.47*	3.55	6.01
Jars within treatments (error)	4.77			

The manganese contents of dried plant tops are shown in Table 17. The analysis of variance data from these results shown in Table 18 reveal that the interaction between iron and manganese is not significant. The effect or iron treatments is not significant while the manganese treatment effect is highly significant.

The graph in Fig. 9 bears these findings out. It can be concluded from the data of this experiment that the manganese content of total plant material varies directly with the manganese concentrate of the nutrient solution. The ratios of iron to manganese have no effect. The concentration of iron does not seem to be an important factor in the manganese content of plant material.

Ratios of Iron to Manganese in Plant Material

Analysis of plant material revealed that a correlation did exist between the ratios of iron to manganese in the substrata as compared to that in plant material. The correlation was better in

Table 17. Manganese contents of dried sorghum tops grown in nutrient solutions having different iron and manganese contents.

Treatment	Iron-manganese ratios of nutrient solutions	Parts per million manganese in dried tops				
		Rep. A	Rep. B	Rep. C	Rep. D	Mean*
High Fe - high Mn	1	101.0	106.4	112.3	118.8	109.6
High Fe - medium Mn	6	37.5	26.3	28.8	7.5	25.0
High Fe - low Mn	600	3.4	2.8	3.9	1.6	2.9
Medium Fe - high Mn	0.166	104.8	101.9	96.3	116.8	104.9
Medium Fe - medium Mn	1	22.5	26.3	30.0	30.5	27.3
Medium Fe - low Mn	100	2.5	2.9	3.4	1.9	2.7
Shive's optimum ratio	2	28.7	15.0	65.0	51.3	40.0

*Least significant difference at 1 per cent level is 40.49; at 5 per cent level 28.6.

Table 18. Analysis of variance values obtained using manganese contents of dried tops of sorghum plants. Plants were grown in nutrient solutions to which different amounts of iron and manganese had been added.

Factor	Variance	Calculated F value	F value needed for significance 5% level	1% level
Manganese treatment	48106	112.13*	3.55	6.01
Iron treatment	4	0.15	4.41	8.28
Manganese x iron (interaction)	26	0.06	3.55	6.01
Jars within treatment (error)	429			

*Significant at the 1 per cent level.

dried plant tops than in sap. Table 19 shows the ratios found in dried plant tops and sap as compared to that in the substrata. The correlation in no case was as good as that reported by Hopkins (9).

Table 19. Ratios of iron to manganese in sorghum plants grown in nutrient solutions with different iron to manganese ratios.

Treatment	Ratio of iron to manganese in nutrient solution	Mean ratio of iron to manganese	
		In plant sap	In dried tops
High Fe - high Mn	1	0.414	1.080
High Fe - medium Mn	6	0.237	14.477
High Fe - low Mn	600	5.554	72.239
Medium Fe - high Mn	0.166	0.099	0.991
Medium Fe - medium Mn	1	0.436	6.807
Medium Fe - low Mn	100	3.328	16.081
Shive's optimum ratio	2	0.298	5.571

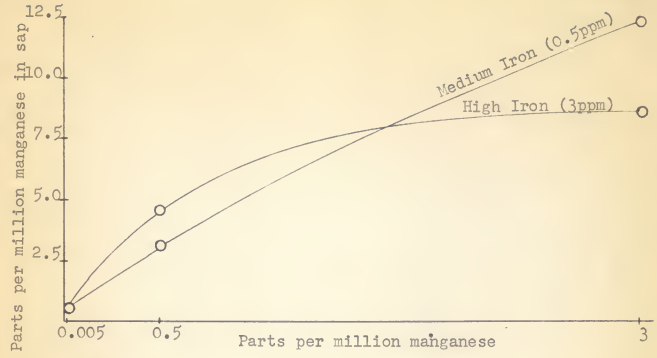


Fig. 8. Manganese content of sap from sorghum plants growing in nutrient solutions to which different concentrations of iron and manganese had been added.

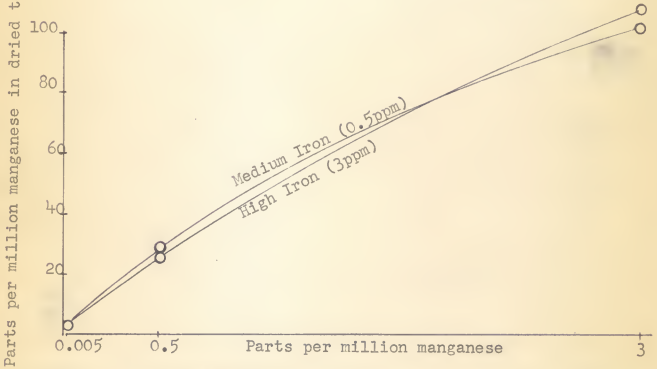


Fig. 9. Manganese content of dried plant tops from sorghum plants growing in nutrient solutions to which different amounts of iron and manganese have been added.

DISCUSSION AND CONCLUSIONS

The following conclusions can be drawn from the study of the importance of the ratio of iron to manganese in the nutrient medium as a factor in the occurrence of chlorosis in sorghum plants.

Soluble iron could only be sustained if a continuous flow of nutrient solutions was supplied to the jars in which plants were growing. The rate necessary to accomplish this was 50 drops per minute. At this rate the nutrient solution of the jars was completely changed every 24 hours. This rate of flow was twice that suggested by Somers, Gilbert and Shive (19) in their work.

Treatments receiving high concentrations of iron produced a large amount of plant material and chlorophyll irrespective of manganese treatments. At low iron concentrations poor plant growth and low chlorophyll contents resulted for all treatments. At the medium iron level the plant material and chlorophyll produced depended on the iron-manganese ratio. For this experiment in the medium iron level the ratio of 1 to 1 produced the most plant material and chlorophyll but ratios less than 1 produced severe chlorosis.

Ratios from 1 to 600 produced about the same amount of plant material and chlorophyll in this experiment. These ratios do not compare with Somers, Gilbert and Shive's (19) optimum ratio of 1.5 to 2.5 but does agree with Sideris and Young's (17) results.

The confusing results obtained by different workers studying

the ratios of iron to manganese and the occurrence of chlorosis, may be caused by workers using different concentrations of iron in making their study. If high concentrations of iron are used chlorosis may not develop unless very high concentrations of manganese are added.

Results of this experiment indicate that plants will not survive in concentrations of iron as low as 0.04 parts per million regardless of iron-manganese ratios. These data conflict with Somers, Gilbert and Shive's results in which they report normal plants growing at this iron concentration with the proper iron-manganese ratio.

Results of iron analyses of plant sap suggest that soluble iron cannot be used to predict chlorophyll formation. The high iron-medium manganese treatment produced considerably less iron in the sap than the other high iron treatments but produced about the same chlorophyll and growth as the other treatments.

There are no consistent data from this experiment that would indicate the concentration of iron in nutrient solutions or the ratio of iron to manganese has any effect on iron contents of plant sap. The reciprocal relations of iron and manganese in plant sap reported by Somers and Shive (20) did not exist in this experiment.

The iron content of dried plant tops showed that there was no relation between iron-manganese ratios and iron in dried tops. At the medium iron range the iron found in tops varied directly with the manganese added. These results compare with those of

Bennett (1). Apparently an antagonism exists between iron and manganese such that increased concentrations of manganese caused a decrease in the absorption of iron.

The manganese contents of plant material varied directly with that supplied in the nutrient solution. In spite of large amounts of manganese in the plant material, iron remained in the plant sap in large amounts in both the high and medium iron treatment. This would suggest that manganese is not oxidizing the ferrous iron to the ferric form as suggested by Somers, Gilbert and Shive (19).

There was a good correlation between the iron-manganese ratio in the substrata as compared to that in the plant sap and in dried plant tops. This correlation compares to that found by Hopkins, Pagan and Silva (9) in their work. It would seem that if the proper ratio of iron to manganese existed in the nutrient solution or in the soil this ratio would exist in the plant material.

SUMMARY

The object of this experiment was to evaluate the importance of the ratio of iron to manganese in the nutrient medium as a factor in the occurrence of chlorosis in sorghum plants.

Three Westland sorghum plants were grown in each of forty candy jars to which nutrient solutions containing varying amounts of iron and manganese were added. Iron levels of 0.04, 0.5 and 3 parts per million were included. At each level 0.005, 0.5 and 3 parts per million of manganese were supplied. This gave ratios of iron to manganese from 0.01 to 600. All treatments were repeated four times.

Because iron forms oxides quickly, a continuous flow of nutrient solution was supplied the plants in the jars by the drip method.

The major and minor plant nutrients were added in the same amounts to all treatments. Iron was added in the form of ferric citrate and manganese in the form of manganese dioxide.

A study was made of the flow of nutrient solution necessary to maintain the desired iron-manganese ratio in the solution. This study included laboratory analyses of nutrient solutions for iron and manganese. Results revealed that 50 drops a minute from the feeder jug to the culture jar were necessary to maintain the desired iron contents.

When the plants were three weeks old, one plant was harvested for chlorophyll analysis. After growing in the jars for nine weeks, the remaining two plants were harvested. One plant was dried for

analysis and the other was used for extraction of plant sap.

An analysis of variance of the total green weights of tops showed that the interaction between iron and manganese was highly significant. The effect of iron was also significant while the effect of manganese was not.

At high and low iron concentrations iron-manganese ratios were not important in top growth. However, at medium concentrations of iron the ratios became important. The best ratio of iron to manganese for top growth at medium iron concentrations was about 1 to 1.

Chlorophyll contents were determined colorimetrically. Results disclosed that an interaction between iron and manganese was significant. Iron treatments were significant but manganese treatments were not. Little chlorophyll was present in any of the low iron treatments regardless of the manganese present. At high iron levels large amounts of chlorophyll resulted irrespective of the ratio of iron to manganese. However, the ratios became important at the medium iron level. The ratio of 1 to 1 was the best for chlorophyll production at the medium iron concentration.

Dried tops and sap were digested by the perchloric acid method. Iron determinations of digested dried tops and sap were carried out colorimetrically by the orthophenanthroline method and manganese was determined colorimetrically by the permanganate method.

Iron analyses of plant sap revealed that the iron content of plant sap was not in any way dependent on the iron-manganese ratios in the nutrient medium. At high iron concentrations the

presence of a medium concentration of manganese caused the accumulation of iron in plant sap to be lessened in comparison with low or high manganese rates.

Results of iron analyses of dried plant tops showed that at the medium iron range the iron in dried plant varied indirectly with the manganese in the nutrient solution. At high iron levels a linear relationship of this nature did not exist. Concentrations of iron or iron-manganese ratios do not appear to have a consistent influence on the total iron content of sorghum tops.

Analyses showed that the manganese content of plant sap and dried plant tops varied directly with the manganese concentrations of the nutrient solutions. Iron concentrations or iron-manganese ratios had no effect on the manganese contents.

The ratios of iron to manganese in the plant sap and in the dried tops followed that supplied the plants in the nutrient solution. The correlation in dried tops was better than that in plant sap. In neither case was the ratio in the plant material the same as in the nutrient solution.

From the greenhouse study of the importance of the ratio of iron to manganese as a factor in the occurrence of chlorosis the following conclusions can be made.

1. A certain iron concentration is necessary for normal plant growth regardless of the ratio of iron to manganese.

2. At levels of medium iron (0.5 parts per million) excessively high or low amounts of manganese reduce growth and produce chlorosis.

3. There is no reciprocal effect of iron and manganese on

plant growth. High iron-manganese ratios do not affect growth, chlorophyll formation, or iron or manganese absorption.

4. Manganese has no consistent effect on the soluble iron in plant sap and iron has no effect on manganese absorption or on the manganese in the plant sap.

5. The reduced growth and chlorophyll formation in the medium iron level by high amounts of manganese cannot be explained on the basis of reduced iron absorption nor as a reduction in the soluble iron in the plant sap.

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