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THE EFFECT OF BORON AND MANGANESE ON THE GROWTH OF TOBACCO PLANTS

by

T. R. Swanback

Thesis, presented to the Faculty of the Graduate School of Massachusetts Agricultural College as partial fullfilment of the requirements for the degree of Master of Science.

May 1928

INTRODUCTION

It is common knowledge that there are ten elements (C, H, O, N, S, P, K, Mg, Fe, Ca) considered essential for plant growth. Recent studies, however, indicate that a few other may be included in the list. Among these elements, boron and manganese have attracted the greatest attention in the field of plant nutrition. Many phases of this line of work may yet have to be investigated, before the two elements will definitely--if ever--enter the list of "essentials".

During the war, when this country was thrown upon its native resources of potash, it was found that the domestic supply contained borax to such an extent as to cause injury to plant growth. Many experiments were made at that time in

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Acknowledgement:

The writer is indebted to Dr. A. B. Beaumont, Mass. Agr. College, for suggesting this work, as well as for helpful suggestions and for supervision of the work; to Drs. P. J. Anderson and N. T. Nelson at the Tobacco Experiment Station, Windsor, Conn. for helpful suggestions. Special credit is due to Director W. L. Slate of Conn. Agr. Exp. Station for rendering it possible for the investigator to carry out the work at the Windsor Station.

different parts of the country to find out how much borax in the fertilizers could be allowed and still avoid the harmful effects of the impurity. Incidentally these investigations added to the knowledge of the influences of boron on plant growth.

The interest in the question of manganese as a plant nutrient may be traced further back than that of boron. The progress of the manganese study, however, has not been so rapid because of contradictory results.

In previous work the writer (3) found that boron added to water cultures, brought about normal growth of tobacco plants, while stunted growth was found when boron was not added.

The investigation to be discussed, was made at the Tobacco Experiment Station, Windsor, Conn. and is a continuation of previous work. Furthermore, it includes laboratory and field observations on the effect of manganese on tobacco.

REVIEW OF LITERATURE

Since Brenchley (1) in the new edition of "Inorganic Plant Poisons and Stimulants" has traced the history of boron and manganese investigations from the beginning up to the present time, it will seem superfluous to make an extensive review of literature. It may thus be sufficient to briefly summarize the conclusions of this author.

In summarizing the experimental work on the effect of boron and manganese compounds on plant growth, Brenchley states that very dilute solutions of the compounds mentioned decidedly increase growth; but while manganese is the more effective in stimulating barley, boric acid is far more potent for peas. While weak solutions of manganese sulfate have stimulated the vegetative growth of barley, ripening of the grain has been retarded with the same strengths of the compound used. Thus, certain physiological functions were expedited, while others were hindered by the action of manganese.

This author thinks that with respect to stimulative agents, two main theories hold the field at the present time: (1) that they act as catalytic agents; (2) that the stimulants themselves are of integral value for nutrition. In the former case the stimulant would act as a "carrier" of useful

food substances.

With respect to the relative importance of boron and manganese, Brenchley states that while boron seems to be associated with certain vital functions, such as formation of vascular tissues, it has not been clearly proved that manganese can be regarded as essential to the life of the plant.

SCOPE

This investigation was made in an attempt to answer the following questions:

1. Are boron and manganese essential for the growth of tobacco plants?
2. If essential, what concentration gives optimum growth?
3. At what concentration do these elements become toxic?
4. If not essential, are they in any way beneficial?
5. If beneficial, what is the optimum concentration?

METHODS

The first part of the experiments was carried out in the field at the Tobacco Experiment Station in Windsor, Conn. during the summer of 1927. Plots were laid out, measuring nine by three feet, that is, an area including six plants in a row of tobacco in ordinary farm practice. Boric acid, borax; sulfate, sesquioxide, and carbonate of manganese; sulfates of strontium and zinc, and potassium iodide were included in the test. All the chemicals were applied in different concentrations to the soil, which previously had received a dressing of commercial fertilizer. Tobacco plants were set on the plots on the 8th of July and harvested on the 17th of September and thereafter cured and stripped in the usual way. Weights of leaves were taken but no consideration

was given to quality.

The second part of the experiments was carried out in the Station greenhouse and included (1), pot cultures in common sand and pure silica sand, using earthen ware glazed crocks as containers; (2) water cultures, in which case common one quart mason jars were used. Proper care was taken to exclude the light from the solutions.

RESULTS

I. Preliminary experiments.

Field tests. For the purpose of comparing the effect of boron and manganese with other chemicals that could be thought of as possible plant stimulants, the sulfates of strontium and zinc, and potassium iodide were included in the experiment. Of these chemicals, the zinc sulfate only appeared to be beneficial to the growth of tobacco plants. Havana seed tobacco was used in this test.

The yield data are recorded in Table I A and I B.

Table I A. Yield of tobacco leaves from field test with Zn, Sr, and I - treatments. Ounces.

Treat-ments	A-p-p-l-i-c-a-t-i-o-n.					
	Check no treatm	1/2 lb per A = 1/4 ppm	1 lb per A 1/2 ppm	2 lbs per A 1 ppm	4 lbs per A 2 ppm	8 lbs per A 4 ppm
$ZnSO_4$	4.85	....	7.25	5.00	4.85	3.00
$SrSO_4$	4.60	....	4.85	3.65	4.60	1.75
KI	4.50	1.50	2.75	1.00	0.0	....

Table I B. Relative yield of tobacco leaves from field test with Zn, Sr, and I - treatments.

Treat-ments	1/4 ppm	1/2 ppm	1 ppm	2 ppm	4 ppm
$ZnSO_4$	....	1.56	1.09	1.00	0.64
$SrSO_4$	....	1.04	0.80	1.00	0.37
KI	0.32	0.58	0.22	0.0	....

"Blank spaces" in the table indicate that the treatment is not extended to respective concentrations. Where ciphers occur, the plants were killed by the treatment.

The figures in the tables above represent the average of two plots. Because of the fact that the "checks" showed very little variation, the average of the three checks (4.65 ounces) was taken as 1 in the computation of the relative yields. From the table it is seen that an application at a rate of one pound per acre of zinc sulfate increased the yield by 56 percent, while the effect of strontium sulfate was practically none. A depressing effect was shown by the iodine treatment at the very low application of one half pound of potassium iodide per acre.

Zinc may thus have a beneficial effect on the growth, which is in apparent agreement with Brenchley's (1) and Sommer and Lipman's (2) work on zinc compounds in plant nutrition.

Laboratory tests. For the water culture work to be carried on in the greenhouse, tests were made in order to obtain a suitable nutrient solution. After several trials the following was selected for the purpose:

Ammonium nitrate	0.200	gms.
Calcium "	1.000	"
Mono-potass. phosphate	2.400	"
Magnesium sulfate	1.800	"
Ferric citrate	0.005	"
Dist. water	1,000	"

A preliminary test was made to determine the effect of different manganese carriers on tobacco plants in the nutrient solution, recorded above. For this purpose $MnSO_4$, $MnCO_3$, Mn_2O_3 were added at concentrations of 0, 10, 50, and 200 parts of Mn per million. Treatments were in quadruplicate.

After the lapse of ten days, plants that were grown in these cultures all showed chlorosis, beginning with the top leaves and gradually spreading to the older ones. The roots were brownish and slimy.

It was obvious that no one of the manganese carriers was preferable to the others. As the lowest concentration, ten parts of Mn per million, had an injurious effect, weaker concentrations should be tried. Of the three Mn - compounds, manganous sulfate was selected as being the easiest to handle, because of its solubility and also its most frequent use in manganese studies, (Brenchley (1)). An experiment was started

with manganous sulfate, using concentrations of 0, 1, 2, 4, 8, and 10 parts of Mn per million; the experiment was carried on for about a month. With exception of the control plants no growth was made in this time. The injury as described above occurred very distinctly at the highest concentration - ten parts per million. Although the chlorosis was not so pronounced at lower concentrations the roots in all concentrations had turned brown to blackish.

From these preliminary experiments it was concluded that still weaker concentrations must be used to obtain a possible beneficial effect from manganese; possibly also the use of a different nutrient solution.

II. Boron Experiments.

Field tests. Applications of boric acid and borax were 0, 1, 2, 4, and 8 pounds per acre and were made in duplicate. After the tobacco (Havana seed) had been stripped it was packed up in a bundle to take on an even moisture content. After ten days the portions from the different plots and treatments were weighed and the yield data are recorded in Table II.

Table II. Yield of tobacco leaves from field tests with boric acid and borax treatments. Ounces

Treatments	Application: Check no treatm	1 lb per A: ½ ppm	2 lbs per A: 1 ppm	4 lbs per A: 2 ppm	8 lbs per A: 4 ppm	Relative Yields: ½ppm	1ppm	2ppm	4ppm
H_3BO_3	3.75	7.40	3.00	1.75	0.40	1.90	0.90	0.50	0.10
$Na_2B_4O_7$	4.65	3.40	3.75	1.60	0.40	0.70	0.80	0.30	0.09

The figures in the table above represent the average of the duplicate treatments. Relative yields are calculated on the basis of the "checks", taken as 1. Of the two boron compounds used, it is seen that boric acid only gave a positive result at a rate of one pound to the acre; the increase in yield being as high as ninety percent. Doubling the amount had a depressing effect, which increased in proportion with further additions of the acid. Borax had a depressing effect at all concentrations. Although the actual amount of boron in two parts of borax per million is equivalent to less than one half part of boron per million, the deleterious effect is rather pronounced. The action of borax is not so easily explained, unless the depression may be attributed to the sodium radical. The question may be raised, whether borax would have rendered a stimulating action at an application of one half pound to the acre. Against this speaks the fact that the second concentration of borax (two pounds per acre) gave slightly higher yield than the first one, apparent-

ly giving a slight response to the increased quantity of boron.

Pot culture tests. In the first pot experiment a comparison was made between boric acid and anhydrous borax when applied in solution to the growing media. Common sand was transferred into four gallon crocks to a quantity of thirteen kilograms. To each crock nutrients were added in solution form in the following quantities:

Ammonium nitrate	0.200 gm.
Calcium "	0.200 "
Mono-potass. phosphate	0.500 "
Magnesium sulfate	0.440 "
Ferric citrate	traces

The same quantities of nutrients were added after three and six weeks of growth.

One tenth per cent solution of boric acid and borax were added to the crocks in amounts of 3, 9, and 27 cc. This corresponds to 0.23, 0.69, and 2.07 parts per million of boric acid and borax. The treatments were carried on in triplicate; three crocks received no boron and served as check for the series as a whole. Havana seed tobacco was used here.

This experiment was planned to furnish further information on the effect of the two boron compounds under better controlled conditions than in the field. The following table (III) records the dry weights of the plants, which were harvested after a growth of 75 days.

Table III. Dry weights of tobacco plants in common sand cultures with boric acid and borax treatments.

Grams.

No.	Treatment.	Boron Equiv	Tops	Roots	Total	Ave. of 3 plants	Relative weights
x1	0	....	3.50	0.50	4.00		
x2	0	....	3.00	0.40	3.40	4.80	1.0
x3	0	....	5.50	1.50	7.00		
1	0.23 pm H_3BO_3	0.04	7.50	1.50	9.00		
2	"	"	5.50	1.50	7.00	7.83	1.63
3	"	"	5.00	2.50	7.50		
4	0.69 "	0.12	6.50	1.50	8.00		
5	"	"	8.50	1.00	9.50	8.83	1.84
6	"	"	7.50	1.50	9.00		
7	2.07 "	0.36	7.50	1.40	9.00		
8	"	"	7.50	1.50	8.90	9.30	1.94
9	"	"	8.00	2.00	10.00		
10	0.23 $Na_2B_4O_7$	0.05	7.00	1.50	8.50		
11	"	"	6.00	1.00	7.00	8.30	1.72
12	"	"	7.50	2.00	9.50		
13	0.69 "	0.15	6.50	1.70	8.20		
14	"	"	9.00	1.50	10.50	10.60	2.20
15	"	"	10.00	3.00	13.00		
16	2.07 "	0.45	4.70	.70	5.40		
17	"	"	5.00	1.00	6.00	6.10	1.27
18	"	"	5.50	1.50	7.00		

Remark:

An extra set of plants with .23 ppm treatment of H_3BO_3 had a total wt. of 8.0, 6.10, and 9.0 gms respectively. Av. Wt. 7.7 gms. Relative yield, 1.60.

In this experiment a maximum concentration of about two parts per million of the boron compounds was chosen, as it was learned from the field tests that applications above this limit had no beneficial effect.

From table III it is evident that there was a steady increase in yield, corresponding to the added increments of boric acid up to 2.07 parts per million. A very distinct response to the application of boron in both forms is noticed at the lowest concentrations in that 23 parts of H_3BO_3 and $Na_2B_4O_7$ in one hundred million gave 63 and 72 per cent increase, respectively. Data on the lowest concentration of boric acid is further manifested by an extra replication, shown under "remarks" of table III, in which case the increase was 60 percent.

Somewhat surprising is the result obtained by the second concentration of borax, which gave greater yield than the highest concentration of boric acid, or 120 percent increase. This seems to indicate that borax might have had a stimulating effect, if it had been applied to the soil in quantities, considerably less than two parts per million. The highest concentration of borax had a ~~similar~~ depressing effect as in the field.

It is doubtful, if the actual amount of boron present, less than one half part per million, could have caused the retardation of growth. It was shown in previous work (3) that plants continually, though slightly, increased in growth, following the application of a concentration of one half part per million of boron. Other investigators (1, 2) have obtained similar results on

plants other than tobacco.

The sodium radical may therefore have played an important part in the depressing effect on growth. There may be several explanations as to the action of the soluble sodium in soil or sand. If taken up by the plant, it is questionable, if this small amount could have retarded growth to this extent. Sodium, added to the soil in amounts comparable to quantities applied in ordinary commercial fertilizers, usually is not followed by any noticeable detrimental effects, although the amount of sodium added is much greater.

There may be a possibility of boron being fixed with silica compounds in the soil and the sodium radical combined with OH - ions to form NaOH, which would explain the immediate deleterious effect of borax when added in the same quantity as boric acid. The reason why the NaOH formed would be deleterious in soils but not in water cultures (1) is plainly this, that the free soil water does not offer the same degree of dilution of NaOH as does a water culture.

On the other hand, boron itself is deleterious to growth, as was found in the field experiment when one part per million of H_3BO_3 was applied. The deleterious effect of a half part per million of borax in the field is thus likely to be due to the sodium present and this is more probable as

the sand culture test showed a slight stimulation at this concentration of borax.

In an attempt to gain further knowledge on the action of boron as a nutritive element, an experiment was arranged in the following way:

Twenty 2 - gallon crocks were filled with pure silica sand. To each pair of crocks nutrient solution was added as follows:

1.	Complete solution (see page 8)		no boron.	
2.	Excluding potassium		"	
3.	"	phosphorus	"	
4.	"	magnesium	"	
5.	"	calcium	"	
6.	Complete solution as above with boron.			
7.	Excluding potassium		"	"
8.	"	phosphorus	"	"
9.	"	magnesium	"	"
10.	"	calcium	"	"

For 2 and 7, $KH_2(PO_4)_2$ was replaced by $Ca_3(PO_4)_2$; 3, 5, 8, and 10 received KNO_3 to exclude phosphorus and calcium respectively; 4 and 9 received the sulfate as $CaSO_4$. Boron was added to 6 - 10 at the rate of three parts of H_3BO_3 per million.

In this series, with half of it treated with boron and

the other half without, it was the object to observe, if boron could take the place of any of the elements excluded. Tobacco plants (Havana seed) that were set in the pots were photographed after a growth of about two months. The results in growth in this time are recorded below:

1.	Complete	- B	developed	8	new	leaves;	height	5 in.
6.	"	+ B	"	11	"	"	"	8 "
2.	No potassium	- B	"	6	"	"	"	2 "
7.	" "	+ B	"	"	"	"	"	" "
3.	" phosphorus	- B	"	3	"	"	"	½ "
8.	" "	+ B	"	"	"	"	"	" "
4.	" magnesium	- B	"	5	"	"	"	1 "
9.	" "	+ B	"	"	"	"	"	" "
5.	" calcium	- B	"	0	"	"	"	
10.	" "	+ B						

In the "complete" series, where boron was excluded, the plants showed an apparent healthy growth, although the leaves were of a lighter green color and of smaller size than were the plants receiving boron. This may be better vizualized in figures 1 and 2. With the exception of the complete treatment practically no differences were found in the other treatments between the part that received boron and the one without. Very typical injuries, however, occurred on the leaves, where respective elements had been left out.

Fig 1.

Fig 2.

Fig. 1 and 2 show the growth with and without boron, respectively. The numbers indicate: 1, complete nutrient solution; 2, lack of potassium; 3, lack of phosphorus; 4, lack of magnesium; 5, lack of calcium.

Lack of potassium resulted in the crinkling and curving of the lower leaves, progressing upwards; brown spots developed later at the tips of the leaves progressing toward the midrib.

Where phosphorus was omitted the plants were much stunted in growth; the leaves took on the shape of a spatula, but otherwise the leaves were smooth and of dark green color.

Excluding magnesium resulted in a stunted growth and yellowing of the lower leaves, gradually progressing to the upper ones.

Lack of calcium prohibited entirely the development of new leaves. Rudimentary top leaves turned grayish at the tips. While plants in the two cultures, receiving no boron, died after four weeks, the boron treated ones were still alive after two months. This condition suggests that calcium has a closer relationship to boron than any of the elements mentioned. This corroborates the observations made by Brenchley (1), who moreover states that none of more than fifty elements tested can function in the same way as boron.

Nitrogen, the very important nutrient element, was not included in the test as in previous work (3) it was shown that boron necessarily should be present in order to make it possible for the plants to assimilate nitrogen.

From this experiment it may be learned that boron has a definite function in plant nutrition, being confined to a direct promotion of new growth, which, however, is impossible in the absence of calcium.

In an attempt to secure still further evidence on boron as an essential element in plant nutrition, an experiment was carried on with cultures in pure silica sand.

Fifteen crocks were used for this test. All received a nutrient solution, that had been successfully used in nutrition work by Dr. A. B. Beaumont, Massachusetts Agricultural College. It was a modification of Crone's solution and had the following partial - volume molecular concentration:

Tricalcium phosphate	0.0022
Ferric "	0,0019
Magnesium sulfate	0.0114
Calcium "	0.0052
Potassium chloride	0.0075
Sodium nitrate	0.0278

Sodium nitrate was replaced by ammonium nitrate in the above formula and used in a partial - volume molecular concentration of 0.0250. To each crock, which contained 20 lbs of pure silica sand nutrients were added, equivalent to two liter of the above solution. Sufficient water was supplied to secure a proper moisture content.

Boric acid was applied in the following concentrations, to duplicate pots: 1, 2, 4, and 8 parts per million of dry

sand. One crock received 50 parts of boric acid per million, another 50 parts of anhydrous borax.

Five cultures, receiving no boron served as "checks". Three tobacco plants (Turkish tobacco) were set in each crock but were later on thinned out to one. During the whole growing period of 65 days none of the plants showed any vigorous growth as compared with the plants in water cultures. In table IVA and IVB yield data may be found.

Table IV A. Green weights of tobacco plants grown in pure sand cultures with boron treatment. Ounces.

Treatment & number		Tops	Roots	Total	Relative average weights
Check 1		1.00	0.25	1.25	
" 2		1.00	0.25	1.25	
" 3		1.25	0.20	1.45	1.00
" 4		1.25	0.25	1.50	
" 5		1.70	0.25	2.00	
1 ppm H_3BO_3	1	1.50	0.25	1.75	0.98
	2	1.00	0.20	1.20	
2 ppm H_3BO_3	3	1.00	0;20	1.20	0.82
	4	1.00	0.25	1.25	
4 ppm H_3BO_3	5	1.00	0.25	1.25	0.67
	6	0.60	0.10	0.70	
8 ppm H_3BO_3	7	0.50	0.08	0.58	0.35
	8	0.40	0.05	0.45	
50ppm B_3BO_3	9	....	....	0.25	0.16
50ppm NaB_4O_7	10	....	....	0.0	0.0

Table LV B. Dry weights of tobacco plants grown in pure sand cultures with boron treatments. Grams.

Treatment and number		Total	Relative average weights
Check	1	5.00	
"	2	5.00	
	3	5.00	1.00
	4	5.50	
	5	8.00	
1 ppm of H_3BO_3	1	5.50	0.92
	2	5.00	
2 ppm of H_3BO_3	3	4.00	0.70
	4	4.00	
4 ppm of H_3BO_3	5	3.50	0.43
	6	1.50	
8 ppm of H_3BO_3	7	2.00	0.26
	8	1.00	
50 ppm H_3BO_3	9	1.00	0.18
50ppm $Na_2B_4O_7$	10	0.0	0.0

In this experiment, where the modified Crone's solution was used, it was found that the solution alone gave better results than any of the boron treatments. One explanation of this fact may be that traces of boron were present and actually detected in some of the salts used ($Ca_3(PO_4)_2$ $CaSO_4$, $Fe\ PO_4$). Boric acid may have been liberated to the extent needed (two to three parts per million) for providing normal growth. A surplus of one part per million of H_3BO_3, approaching four parts per million, would retard growth as shown in the field test and in a previous experiment (3). The retardation in growth is plainly illustrated in figure

Fig 3. Growth influenced by varying amounts of boric acid as indicated in the photograph , plant grown in pure silica sand.

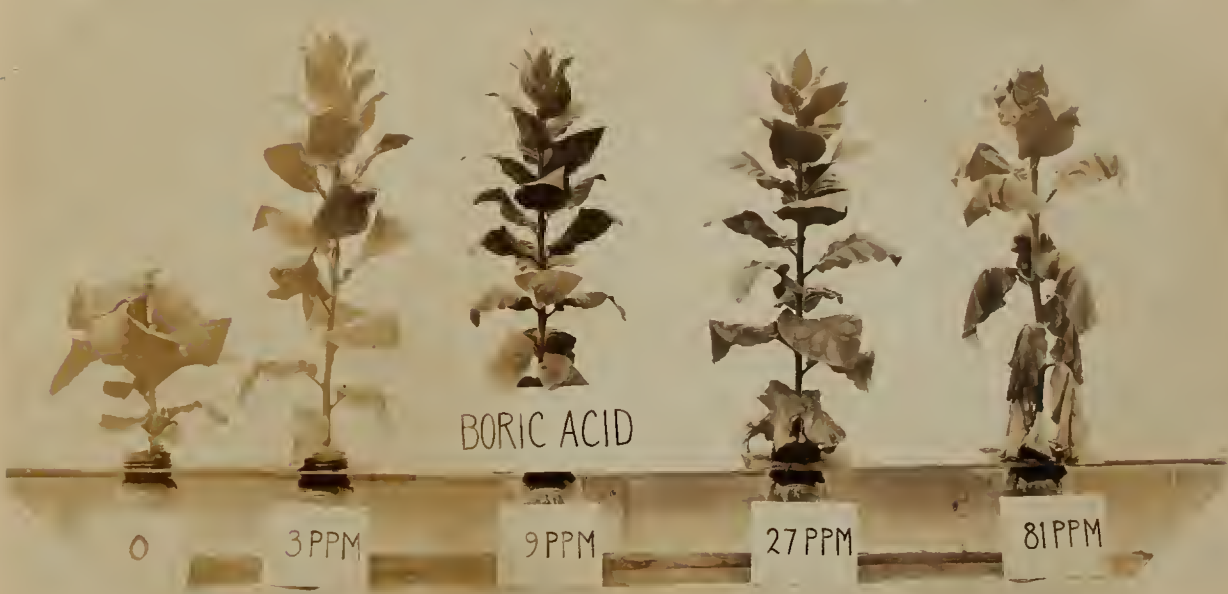

Fig 4. Growth influenced by boric acid in water cultures. Concentrations indicated in the photograph.

three and is confirmed by the figures of relative weights in tables IV A and IV B. The experiment goes to prove that pure salts should be used in nutrition experiments.

Water cultures. In previous experiments (3) it was found that two parts of boric acid per million gave optimum growth of tobacco plants in water cultures. A curve of growth would have shown a distinct point of inflection at the above concentration and the continuation of the curve a rather smooth running plateau.

It was with the aim of making further observations on the general trend of the "plateau" that the following experiment was undertaken.

Crone's modified solution, with ammonium nitrate as source of nitrogen, was transferred into twenty jars to make quadruplicate cultures for the following treatments with boric acid in concentrations of 0, 3, 9, 27, and 81 parts per million. One tobacco plant of Havana seed was set in each jar. During the entire period of growth the solutions were not changed but distilled water was supplied to the extent needed. After about six weeks of growth the control plants showed stunted growth, the buds turned brown, beginning first below the young leaves and spreading upwards. This phenomenon has

been observed by Brenchley (1) and others on plants lacking boron in the nutrient media, and was also observed on tobacco plants in previous work (3). While it was observed that the boron treated plants in all cases developed normally and produced flower buds, the three higher concentrations caused a yellowing of the bottom leaves, the injury increasing with the concentrations. This corroborates the findings of other investigators on various plants and the writer's (3) observations on tobacco plants.

The known fact that boron injury first occurs on the bottom leaves of plants investigated (1,2) as well as on tobacco plants (3) may lead to the assumption that an accumulation of boron compounds takes place in the lower leaves, which may become a storage place for the boron compounds not needed for promotion of top growth.

To some extent this may be supported by analytical data on tobacco leaves, secured by Dr. E. M. Bailey and Mr. H. Fisher at the Agricultural Experiment Station, New Haven, Conn.

Seven groups of samples of Havana seed tobacco, each containing top leaves and lower leaves, were analyzed with respect to boron.

The results are summarized as follows:

	Top leaves % B_2O_3	Lower leaves %
Group 1	0.005	0.005
2	0.010	0.013
3	0.020	0.025
4	0.029	0.030
5	0.029	0.035
6	0.026	0.026
7	0.032	0.031

Percentages are computed on the basis of dry matter.

The tobacco was obtained from Havana seed plants grown in the field. It is seen that there is a slight tendency of an increase of boron in the lower leaves. Moreover, it is surprising how relatively large amounts of boron actually are taken up by the plants, considering the small amounts needed and tolerable in the growing media.

Further analyses made on tobacco leaves from manganese - treated plots (table V) show that the intake of boron is somewhat related to the form of manganese present in the soil. They also show the distribution of boron compounds within the leaves.

Table V. Determinations of boron in tobacco leaves, calculated as per cent of B_2O_3.

Plot	Whole leaf	Midrib	Remainder of leaf
Non-treatment	0.020	0.020	0.023
Mn CO_3	0.027	0.017	0.027
Mn_2O_3	0.030	0.020	0.030
Mn SO_4	0.033	0.023	0.030

Boron is noted to be present in greater amount in the leaf than in the midrib. This suggests some studies on the relation of boron to the effect on burn of tobacco.

It is also noted that the different manganese compounds had promoted the intake of boron and in proportion to their solubilities. The sulfate was thus the most effective and the carbonate the least effective compound. No boron had been added to the manganese plots, hence it is obvious that the soil contained boron, which became more available thru the manganese treatments.

After 65 days of growth the plants in the water cultures were harvested and the yield data are given in Tables VI A and VI B.

Table VI A. Green weights of tobacco plants grown in water cultures with boric acid treatment. Ounces.

Treatment & number		Tops	Roots	Total	Relative average weights
Check	1	2.20	0.50	2.75	
"	2	1.50	0.20	1.70	1.00
"	3	1.75	0.25	2.00	
"	4	1.75	0.25	2.00	
"	5	2.50	0.50	3.00	
3ppm of H_3BO_3	6	2.75	0.60	3.35	1.62
	7	2.90	0.65	3.55	
	8	3.00	0,75	3.75	
	9	2.00	0.50	2.50	
9 " " "	10	2.75	0.50	3.25	1.35
	11	2.70	0.50	3.20	
	12	2.00	0.40	2.40	
	13	2.90	0.75	3.65	
27 " " "	14	3.00	0.80	3.80	1.70
	15	2.40	0.50	2.90	
	16	3.00	0.75	3.75	
	17	2.50	0.50	3.00	
81 " " "	18	2.75	0.60	3.35	1.50
	19	2.75	0.50	3.25	
	20	2.60	0.50	3.10	

Table VI B. Dry weights of tobacco plants grown in water cultures with boric acid treatments. Grams.

Treatment & number		Total	Rel. Average weights
Check	1	8.50	
"	2	6.00	1.00
"	3	8.00	
"	4	8.00	
"	5	11.00	
3 ppm of H_3BO_3	6	13.00	1.63
	7	14.00	
	8	12.00	
	9	10.00	
9 " " "	10	11.50	1.36
	11	11.00	
	12	9.00	
	13	11.50	
27 " " "	14	15.00	1.70
	15	11.00	
	16	14.50	
	17	10.00	
81 " " "	18	13.00	1.56
	19	10.50	
	20	14.00	

The data in tables VI A and VI B reveal the fact that three parts of boric acid per million gave better growth than nine parts per million, but again, twenty-seven parts per million gave slightly better results in weight than the first concentration. At eighty-one parts per million again a decrease is noticed. The sudden drop in yield when the concentrations are doubled was observed in previous work (3) and by increasing the intervals it is now shown that the effect is more pronounced. The amount of boron that can safely be supplied to water cultures is thus about one half

part per million, as at all higher concentrations boron injury occurred on the lower leaves. At the time of harvesting, plants in the highest concentration were wilting as indicated in figure 4, which also points out the slight differences in growth due to the varying amounts of boric acid supplied.

Aside from the injurious effect of boric acid at concentrations above three parts per million, it may be of interest to observe that the curve of growth is bimodal rather than a "plateau". This is illustrated in figure 5. Further investigations are needed in order to give an explanation to this phenomenon, which probably will be of little practical importance because of the injurious effects at higher concentrations. It may also be desirable to obtain further data in order to trace the actual trend of the curve.

III. Manganese Experiments.

Field tests. Field tests on manganese compounds were carried out in a similar way as were those on boron. The sulfate, and carbonate of manganese were used in applications at a rate of 0, 1, 2, and 4 pounds per acre, and the sesquioxide at 0, 1/2, 1, and 2 pounds acre. The treatments were in duplicate. In other respects the procedure was the same as for the boron field test; Havana seed tobacco was used also in this field test.

The yield figures from the plot test may be found in Table VII.

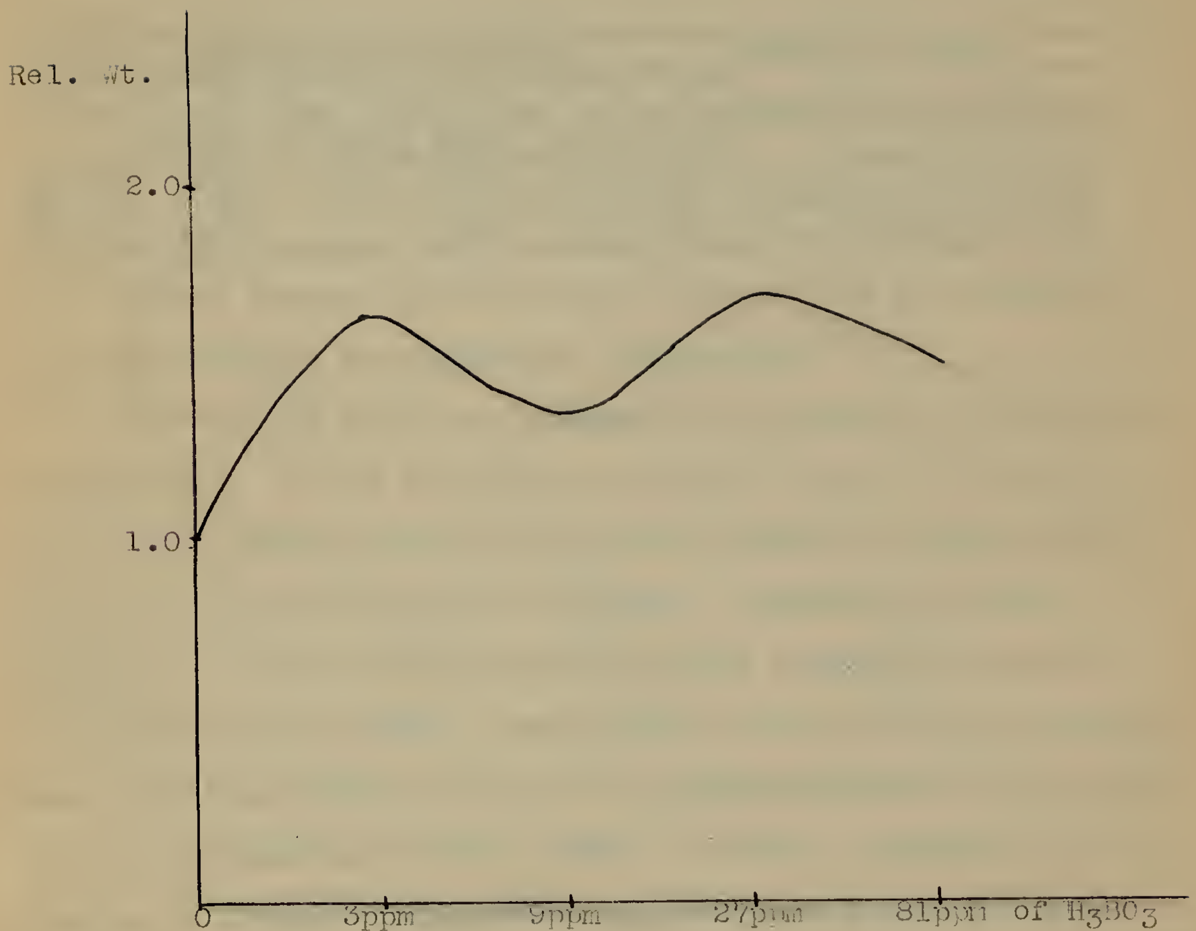

Fig. 5 Growth influenced by varying amounts of boric acid.

Table VII. Yield of tobacco leaves from field tests with sulfate, sesquioxide, and carbonate of manganese.

Ounces.

Treatment	Check no Treat.	½ lb. per A ¼ ppm	1 lb. per A ½ ppm	2 lbs. per A 1 ppm	4 lbs per A 2 ppm	¼ppm	½ppm	1ppm	2ppm
	A-p-p-l-i-c-a-t-i-o-n					Relative Yields			
$Mn\ SO_4$	4.00	....	7.50	7.40	6.00	....	1.87	1.85	1.50
$Mn_2\ O_3$	4.65	4.10	6.40	8.15	4.25	0.90	1.40	1.75	0.90
$Mn\ CO_3$	4.00	....	6.50	7.50	7.00	....	1.62	1.87	1.72

"Blank spaces" in the table indicate that the treatment was not extended to respective application.

The data in Table VII represent the average of duplicate treatments. It may be noticed that the "checks" for $Mn\ SO_4$ and $Mn\ CO_3$ show an equal weight which makes the results from these two compounds fully comparable. Manganous sulfate applied at a rate of one pound per acre caused an increase in yield by 87 percent. Two pounds per acre did not increase the yield any further and a slight depressive effect was found at the application of four pounds per acre. Manganous carbonate as a less soluble compound, gave the highest increase at the application of two pounds per acre. This suggests that from the carbonate, applied at this rate, about an equal amount of soluble manganese may have been liberated that was most favorable to growth in the case of the $Mn\ SO_4$, viz., one pound per acre. This holds true for the sesqui-

oxide also, though the depression is more pronounced at the application of four pounds per acre, which in turn may be due to the larger quantities of Mn present in an aliquot amount of sesquioxide as compared with the sulfate and carbonate.

Determinations of Mn, made by Dr. Bailey and Mr. Fisher (see above) on tobacco leaves from manganese-treated plots are recorded in Table VI. Analyses were calculated on a water free basis.

Table VIII. Determinations of manganese in tobacco leaves of Havana seed tobacco calculated as Mn_3O_4. Percent

Plot	Whole leaf	Midrib	Remainder of leaf
Non-treatment	0.010	0.005	0.010
$Mn\ CO_3$	0.055*	0.002	0.007
$Mn_2\ O_3$	0.010	0.004	0.010
$Mn\ SO_4$	0.050	0.020	0.080

*This analysis is not satisfactory, as the amount of manganese in separate parts does not check up with the total; in fact, the carbonate did not become fully effective until twice the amount had been applied as compared with the sulfate.

From the data in table VIII it is noticed that manganese to a considerable extent is stored in the leaf proper rather than in the woody parts. Considering the data tabulated under "remainder of leaf", it is seen that the intake of man-

ganese is directly proportional to the solubility of the three compounds, which suggests that less soluble manganese compounds should be used, if it is not desirable to have an abundance of manganese in the crop.

The field test with manganese treatment has shown that manganous sulfate, the most readily available compound of the three, gave the best results when applied at a rate of one pound to the acre, and that the less soluble, sesquioxide and carbonate of manganese may be applied at a rate of two pounds per acre in order to obtain similar results.

Pot cultures. An experiment with sand cultures on the effect of manganous sulfate was arranged in the following way:

The same cultures as mentioned previously for the boron test were used. After the plants in previous experiment had been harvested, nutrients were added to the crocks as before to the boron series. (see p. 11) and additional amounts after three and six weeks. Manganous sulfate was added to the cultures in the following concentrations: 3, 9, 27, 81, 243, 729 parts per million, each concentration being in triplicate. Plants (of Turkish tobacco) were set, one in each crock, as soon as nutrients and treatments were applied and proper moisture obtained.

After about two weeks of growth a distinctly injurious

effect, due to the manganese treatments, occurred on the plants, except for the three lowest concentrations. The injury thus first occurred on the treatment of 81 parts per million and increased with the increased concentrations. The first symptom of the injury was a yellowing of the top leaves. Plants in the 81 ppm-concentration overcame rather soon the injury and grew normally, while those in the two highest concentrations, having retarded growth, continually developed leaves showing the injury. This injury may be described as follows: The top leaf to begin with has a yellow-green color. When the leaf is fully developed the yellowish color is minutely distributed in the interspaces of the finest ramifications of the leaf veins, the color being more pronounced toward the tip. In later stages the whole leaf takes on a more yellow green color, but the "pattern" remains the same. In still later stages the leaf may crinkle and brown irregular spots develop on the tips,

The plants in this series were harvested after a growth of 65 days. Yield data are recorded in tables IX A and IX B.

Table IX A. Green weight of tobacco plants grown in common sand, treated with Mn SO_4. Ounces.

Treatment & number		Tops	Roots	Total	Rel. Average weights
Check	1	3.50	0.30	3.80	
"	2	2.00	0.25	2.25	1.00
"	3	3.25	0.30	3.55	
	1	2.75	0.25	2.95	
3 ppm of Mn SO_4	2	2.50	0.25	2.75	0.85
	3	2.25	0.20	2.45	
	4	2.70	0.30	3.00	
9 " " "	5	3.00	0.25	3.25	1.00
	6	2.75	0.30	3.05	
27 " " "	7	3.00	0.30	3.30	
	8	3.10	0.30	3.40	1.04
	9	3.00	0.30	3.30	
81 " " "	10	2.70	0.25	2.95	
	11	2.75	0.25	3.00	0.91
	12	3.10	0.25	3.35	
	13	1.25	0.10	1.35	
243" " "	14	2.25	0.20	2.45	0.65
	15	2.20	0.25	2.45	
	16	2.00	0.20	2.20	
729" " "	17	1.90	0.20	2.10	0.68
	18	2.00	0.20	2.20	

Table IX B. Dry weights of tobacco plants grown in common sand, treated with $MnSO_4$. Grams.

Treatment & number		Total	Relative Average weights
Check	1	14.00	
"	2	7.00	1.00
"	3	14.00	
	1	9.50	
3ppm of $MnSO_4$	2	8.50	0.77
	3	9.00	
	4	9.00	
9 " " "	5	9.50	0.78
	6	9.00	
	7	11.50	
27 " " "	8	12.00	1.00
	9	11.50	
	10	8.00	
81 " " "	11	10.00	0.85
	12	12.00	
	13	4.00	
243 " " "	14	8.00	0.60
	15	8.00	
	16	6.00	
729 " " "	17	6.00	0.51
	18	6.00	

The cultures used in this experiment had previously carried a crop of tobacco treated with boron; the numbering of the crocks corresponding exactly to those in table III. The check cultures received an application of three parts of boric acid per million in order to make them similar to the rest of the cultures.

Taking the average of the three "checks" as 1, from Tables IX A and IX B it is seen that the check is better than

the treatment of three parts of Mn SO_4 per million. Twenty-seven parts of Mn SO_4 per million gave an increase of four per cent above the check in green weight, but was equal to the check with respect to dry weight. At higher concentrations where the manganese salt caused injury to the plants it is seen that the growth was considerably retarded. The fact that the injury was most severe at the highest concentration did not seem to retard growth correspondingly as the yield figures show a slight increase above the next highest concentration.

From this experiment it may be concluded that in cases where boron is present in sufficient amount in the growing media an addition of manganese has no beneficial effect. The apparent stimulating effect of manganese in the field may thus be attributed to the amount of boron liberated in the soil thru the use of manganese compounds, as the analyses mentioned above, seemed to indicate, rather than the manganese itself.

Figure six brings out the development of growth due to the varying amounts of manganous sulfate added. It is seen that the "check" plant is somewhat larger in size and the rest of the plants correspond to the data presented above.

Another experiment on the effect of manganous sulfate wàs conducted in pure-sand cultures in a similar way as mentioned above in the boron test, using pure silica sand; in fact, the control plants in that test were used as check also against the manganese treated plants (Turkish tobacco).

The treatments, all in duplicate, were the following concentrations of manganous sulfate:

1, 2, 4, 8, and 50 parts per million. During the early growth it was noticed that the highest concentration caused injury to the plants, which, however, overcame this effect in later stages of development. As before, three plants were set in each crock and later thinned out to one. The plants were harvested after 65 days of growth and yield records taken which are tabulated below. (Tables X A and X B)

Table X A. Green weights of tobacco plants, grown in pure sand cultures, treated with Mn SO_4. Ounces.

Treatment & number		Tops	Roots	Total	Relative Average weights
Check	1	1.00	0.25	1.25	
"	2	1.00	0.25	1.25	
"	3	1.25	0.20	1.45	1.00
"	4	1.25	0.25	1.50	
"	5	1.70	0.25	1.95	
1 ppm of Mn SO_4	6	1.25	0.25	1.50	0.78
	7	0.75	0.15	0.90	
2 " " "	8	1.00	0.25	1.25	0.93
	9	1.25	0,30	1.55	
4 " " "	10	1.00	0.25	1.25	0.66
	11	0.50	0.20	0.70	
8 " " "	12	0.70	0.20	0.90	0.72
	13	1.00	0.25	1.25	
50 " " "	14	1.10	0.25	1.35	0.96
	15	1.25	0.25	1.50	

Fig 6. Growth influenced by varying amounts of manganous sulfate applied to common sand. Concentrations indicated in the photograph.

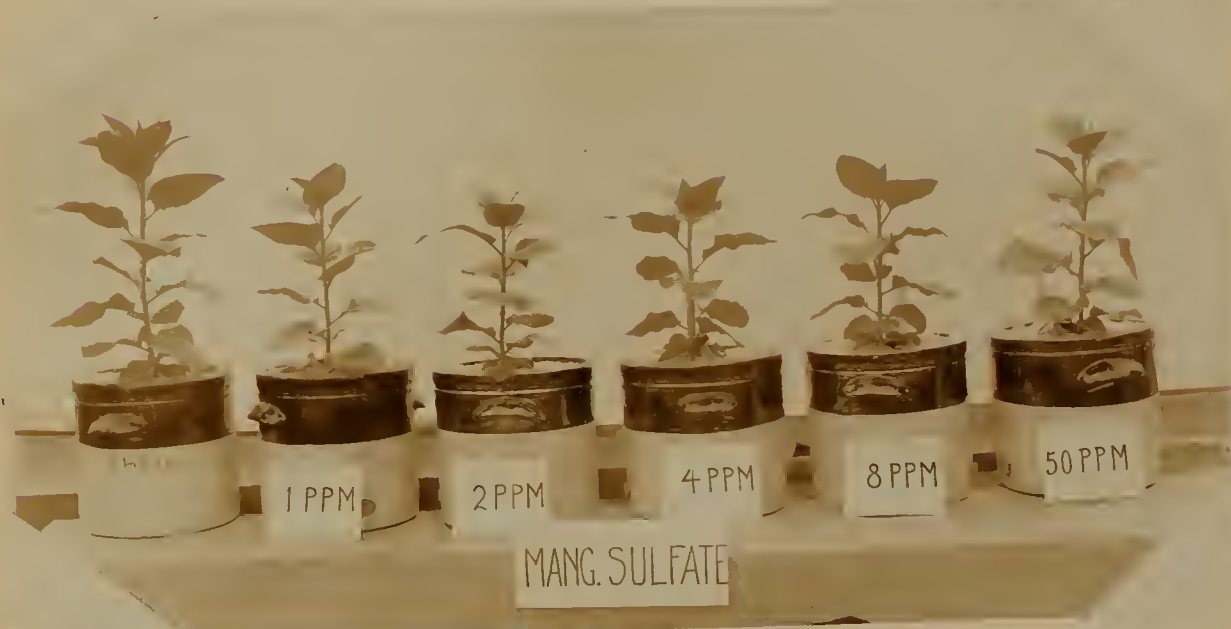

Fig. 7. Growth influenced by varying amounts of manganous sulfate applied to pure silica sand. Concentrations indicated in the photograph.

Table X B. Dry weights of tobacco plants, grown in pure sand cultures, treated with Mn SO_4. Grams.

Treatment & number		Total	Relative Average weights
Check	1	5.00	
"	2	5.00	
"	3	5.00	1.00
"	4	5.50	
"	5	8.00	
1 ppm of Mn SO_4	6	5.00	0.66
	7	2.50	
2 " " "	8	5.50	1.00
	9	6.00	
4 " " "	10	5.00	0.70
	11	3.00	
8 " " "	12	4.00	0.79
	13	5.00	
50 " " "	14	5.00	0.96
	15	6.00	

From tables X A and X B it is seen that the results obtained further substantiate the findings in previous experiments. No beneficial effect was obtained by the use of manganous sulfate. Crone's modified solution, apparently providing sufficient boron (see under boron experiments) determined the result. If at all beneficial, manganous sulfate should be used in lower concentrations than one part per million. The relative weights seem to indicate that the curve of growth would have a bimodal trend, as the treatment of fifty parts per million is nearly as good as that of two parts. This is plainly emphasized in figure 7.

Water cultures. An experiment was undertaken in an attempt to answer the question whether manganese can be substituted for iron in plant nutrition, viz., in the production of chlorophyll.

Plants were grown in a nutrient solution, which was entirely lacking iron. When after three weeks all the leaves were strongly etiolated, taking on a cream yellow color, the series was divided in halves, one receiving an adequate amount)8ppm) of ferric citrate, the other a small amount (5ppm) of manganous sulfate. The iron added resulted in return of the green color to all of the plants so treated. Manganous sulfate, on the other hand, had no such effect. Though the experiment may not definitely prove that manganese may not serve the same purpose as iron, it seems doubtful whether manganese has to do directly with the formation of chlorophyll.

From preliminary tests with manganous sulfate in water cultures it was learned that none of the concentrations used gave positive results. It was thought well to try a different nutrient solution. Crone's modified solution (see page18) was selected for this purpose. In order to obtain a finer gradation the following concentrations of manganous sulfate were used:

1, 3, 6, 9, and 12 parts per million. The treatments

were in quadruplicate and the same checks were used as in the boron series mentioned on page 18, as the two series were started simultaneously. Plants grown in the manganese treated cultures showed the injurious effect after the first week of growth. Plants in the lowest concentration soon recovered and had the appearance of healthy growth. The roots were also healthy and white. In the higher concentrations plants were either killed or they recovered from the injury, but none of the plants showed normal growth at the end of the experiment (after 65 days), with exception of two plants in the lowest concentration. No yield data was taken, but the following summary may serve as indication of the response of the manganese treatments.

Observations on conditions of plants grown in water cultures with $Mn\ SO_4$, after 65 days:

No treatment recorded under boron treatment., see p. 19.

1 ppm of $Mn\ SO_4$	1	stunted	growth,	browntop	
	2	normal	"	upper leaves crinkled	
	3	"	"		
	4	"	"		
3 ppm of $Mn\ SO_4$	5	stunted	growth,	brown	top
	6	killed			
	7	stunted	"	"	"
	8	"	"	"	"
6 " " "	9	"	"	"	"
	10	killed			
	11	"			
	12	stunted	growth,	brown	top
9 " " "	13	killed			
	14	"			
	15	stunted	growth,	brown	top
	16	"	"	"	"

12 ppm of $Mn\ SO_4$ 17 killed
18 "
19 stunted growth, brown top
20 killed

From this summary it is seen that some plants were killed by the three higher concentrations; plants in the highest one being most affected. The solutions were not changed during the growing period, which may explain the recovery of some of the plants. As the manganese taken up by the plants possibly may have been stored in the injured leaves, the solutions gradually contained less manganese. Although attempts were made to select plants of equal size at the start, some of the plants were evidently stronger than the others.

This experiment has thus shown, that if manganese stimulates the growth of tobacco plants at all, a considerably lower concemtration than one part per million of $MnSO_4$ should be available at any one time.

DISCUSSION OF RESULTS

Throughout the experiments with boron it has been found that at certain low concentrations the element stimulated the growth of tobacco plants. Moreover, when growth was observed under best controllable conditions of essential nutrients, as in water cultures, it was found that boron necessarily must be present in the growing media in order to produce normal development of leaves and reproductive organs.

Traces of soluble boron were detected in the salts used for Crone's modified solution. This circumstance may serve as an explanation to the relatively good growth of the control plants, as for instance, is illustrated in figure four. As the solutions were not changed during the experiment, it seems plausible that at the time when the stunted growth occurred the boron present in the solution may have been exhausted.

Soluble boron was not detected in the glass sand used and where pure salts were used a striking difference occurred in growth between boron treated plants and those receiving no boron. This is emphasized in figures one and two, of plants photographed after 60 days of growth; and in figure eight, where the plants receiving full nutrient solution were

Fig 8. Tobacco plants (Havana seed) after 75 days of growth: 1 and 2 with boron; 3 and 4 without boron. Duplicate of plants in fig. 1 and 2 receiving full nutrient solution.

left to grow for two more weeks.

In view of the results in growth due to boron treatment and the fact that boron can not be substituted for any of the main elements: N, P, K, Mg, Ca, and for any other fifty elements investigated (Brenchley (1)), it seems reasonable to assume that boron is essential to the growth of tobacco plants.

In view of the demonstrated necessity of boron for the proper development of tobacco plants, is it likely that the tobacco crop in the field would be benefited by the inclusion of boron in fertilizer applied to the soil? This question cannot be answered at present for all soils. No symptoms resembling boron starvation have been observed in the fields. Limited chemical analyses have shown boron to be present in the leaves of the plant and also in the soil. It is quite probable that such organic fertilizer compounds as cottonseed meal contain boron. Under these circumstances and in view of the known harmful effect of excess of this element, it would not seem wise to add boron. Further work, however, may show that there are certain conditions under which it may be beneficial.

In previous work (3) it was found that two parts of

boric acid per million gave optimum growth of tobacco plants. Three parts per million were used in this work in order to cover the optimum range and still keep within the limit, found by Sommer and Lipman (2). This concentration gave optimum growth without injury to the plants. While this concentration may be the maximum that can safely be used, the lower concentration will provide sufficient boron for normal growth of tobacco.

Although injurious effects of boron were shown in pure sand when a concentration of fifty parts of borax per million was applied, the injury in the field test occurred at a concentration of only four parts of borax per million. In water cultures the harmful effect was shown at a concentration of nine parts per million.

In all experiments carried on with manganese, no evidence was found that this element is essential to the growth of tobacco plants. If essential, the concentration would necessarily fall below one part per million. No manganese was detected in the salts or the pure sand used in the experiments. By a qualitative test, however, very faint traces of manganese were found in the plants receiving no manganese, but on the other hand, definite traces of manganese were found in plants receiving one part of manganous sulfate per million.

Applying fifty parts per million to pure sand caused no visible symptom of injury to the plants, but when eighty-one parts were applied to common sand a definite injurious effect was noted. From this it may be concluded that when the root hairs are functioning normally, as in soil or sand, the intake of manganese by the plant proceeds at a much slower rate than in water cultures. This may support the idea that manganese in solid substrata may be useful in processes taking place in the root hair zone and is accidentally taken up by the plant. There is also the possibility that manganese may be fixed in the soil and thus made less injurious, but against this speaks the circumstance that manganese supplied to the soil as $Mn\ SO_4$ was taken up in eight times larger quantity than the carbonate without causing any harmful effect.

Finally, if manganese is a desirable constituent in tobacco, an application of one to two pounds per acre may produce beneficial.effects.

The present state of our knowledge of the effect of manganese does not warrant the inclusion of this element in fertilizer mixtures nor is there any occasion to fear that the tobacco crop may suffer by constant use of fertilizers which do not contain manganese.

SUMMARY

1. This paper gives the results of studies on the effects of boron and manganese on the growth of tobacco plants.

2. It was found that boron must be present in the nutrient media to produce normal growth.

3. Boric acid applied to soil at a rate of one pound per acre gave beneficial effects,while two pounds retarded growth. No beneficial effect was found by using borax at the same rates.

4. Boric acid applied to common sand in pots gave increase in growth relative to amounts used (up to about two parts per million). Borax under the same conditions showed beneficial effects in concentrations lower than two parts per million.

5. Boric acid applied to cultures of pure silica sand, receiving full nutrients in the form of Crone's solution showed no beneficial effect, indicating that this solution contains traces of boron.

6. Boric acid applied to pure silica sand, receiving full nutrients in the form of soluble salts, showed beneficial effect. (Photographic evidence)

7. Three parts of boric acid per million produced normal growth without injury in the presence of other essential nutrients in water cultures.

8. Although manganese in certain cases seemed to stimulate growth no evidence was found to support a belief that manganese is essential to the growth of tobacco plants.

9. In the field one pound of Mn SO_4 per acre or two pounds of either Mn CO_3 or Mn_2O_3 produced about an equal optimum stimulating effect; in water cultures, however, one part per million of Mn SO_4 was injurious to the plants.

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