THE EFFECT OF THE INTERRELATIONSHIP OF BORON AND MANGANESE ON THE GROWTH AND CALCIUM UPTAKE OF BLUE LUPINE (*LUPINUS ANGUSTIFOLIUS* L.) IN SOLUTION CULTURE

JAMES H. M. HENDERSON[1] AND MARY P. VEAL

(WITH SEVEN FIGURES)

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Introduction

A few years ago widespread interest was shown in the effect of fertilizers containing boron and manganese upon plant growth. These trace elements were used because (1) they exert a marked effect upon plant growth; and (2) they influence very materially the crop yield. The earlier work on the role of boron and manganese has been discussed by BRENCHLEY (**2**), later reviews are those of MCHARGUE and CALFEE (**7**), MCMURTREY (**8**), MINARIK and SHIVE (**10**), SCHUSTER and STEPHENSON (**11**), MEYER (**9**), and THATCHER (**13**). Blue lupine was of interest because it is a relatively new green manure crop in the southern sections of the United States (**6**), although as a plant, blue lupine has been known for hundreds of years. BEAUMONT (**1**) states that the ancient Romans and Germans used lupines in the role of leguminous green manure crops for the purpose of improving soils. It is with this latter purpose in mind that this study was undertaken.

A definite boron deficiency in many soils has been recognized throughout the country in a number of species of crops. As a result, several physiological diseases of plants have in recent years been attributed to this inadequacy of boron compounds in the soils. On the other hand boron may be present in the soils in sufficient amounts, but is rendered unavailable to crop plants, due to some chemical combination, soil reaction, etc.

Manganese is in many instances similar to boron in its effect on plants. THATCHER (**13**) states that manganese has been found by several investigators to be a specific remedy for chlorosis of plants due to excessive lime in the soil. However, its action is not uniform, in that on the same soil, or in the same culture medium, its beneficial effects will be shown by some crops and not by others.

Some investigators have related calcium uptake to the presence of certain minor elements in the soil. MINARIK and SHIVE (**10**) noted that the concentration of boron in the nutrient solution was an important factor for the production of tissues and for the accumulation of calcium in the leaves of the soybean plant. BRENCHLEY and WARINGTON (**2**) have shown that insufficient levels of calcium in solution cultures resulted in definite anatomical abnormalities in the broad bean plant.

[1] Present address: Kerckhoff Biological Laboratories, California Institute of Technology, Pasadena 4, California.

Materials and methods

The culture solution used was that of HOAGLAND and ARNON (**4**), and contained the following principal salts: 0.001 M KH_2PO_4, 0.005 M KNO_3, 0.005 M $(Ca(NO_3)_2 \cdot 4H_2O$ and 0.002 M $MgSO_4 \cdot 7H_2O$. One ml. of a 0.5% solution of ferric tartrate was added to a liter of solution, making a final concentration of 5 p.p.m. One ml. of a minor element solution containing 0.08 p.p.m. $CuSO_4$, 0.22 p.p.m. $ZnSO_4 \cdot 7H_2O$ was added to a liter of final solution. Boron and manganese were added where designated. Boron was added to H_3BO_3 and manganese as $MnCl_2$. Both boron and manganese are expressed in parts per million as the single ions, respectively, i.e., boron as B and not BO_3. The experiments reported in this paper contained boron and manganese in concentrations varying from 0.5 p.p.m. to 50 p.p.m.

Seeds which had been sterilized externally in calcium hypochlorite solution (10%) were germinated in sterilized petri dishes containing moistened filter paper. When the seedlings had reached a stage of 2–3 centimeters in length, which took place in from 3–5 days, they were transferred to quart-size Mason fruit jars and placed in holes in paraffined tops held on by the screw-top rings. In all cases the seedlings were grown over a period of fifteen days. There was no renewal of solutions during that period.

In order to determine the effectiveness of boron and manganese on calcium uptake, and since the present micro-methods for determining manganese and boron are less accessible, a method for determining calcium was used. To make analysis for the absorption of calcium, duplicate samples of 500 ml. each were measured from the jars, evaporated to 200 ml. and determined according to the method described by KOLTHOFF and SANDELL (**5**) in the section on "Determination of calcium after precipitation as calcium oxalate."

Experimental results

Data on the dry weight yields and length of stems and roots for experiments are summarized in tables I–III. The data reported for dry weights, length, and milligrams of calcium are averages of the results for each particular group.

The results for the first set of experiments, containing 0.5 p.p.m. of B and/or Mn, show a distinct difference between plants grown in boron and those grown in manganese, especially in the case of the roots (table I). The results for manganese are invariably the same as, or lower than, those for solutions containing neither manganese nor boron. From a similar comparison between plants growing in solutions containing neither boron nor manganese, it is obvious that boron is significantly effective in its influence on growth. Thus, it is logically assumed that it is the presence of boron and not the absence of manganese which causes the difference between groups I–II and III–IV. Since the results in group IV are not ap-

TABLE I

GROWTH OF BLUE LUPINE SEEDLINGS IN SOLUTION CULTURE CONTAINING 0.5 P.P.M. OF B AND MN, AND CA UP-TAKE

PLANT GROUP	SOLUTION		AVERAGE LENGTH IN CM.			AVERAGE WEIGHT IN MG.			AVERAGE MG. OF CA ABSORBED	
			STEMS	ROOTS	TOTAL	STEMS	ROOTS	TOTAL	PER GROUP	PER GRAM DRY WT.
I	Control*	(41)	14.2	12.0	26.2	96.0	15.6	111.6	7.8	70
II	+ H_3BO_3	(41)	14.0	11.0	25.0	101.8	14.3	116.1	7.5	65
III	+ $MnCl_2$	(41)	13.3	4.7	18.0	91.5	10.4	101.9	7.1	70
IV	− Both	(39)	13.1	5.0	18.1	89.5	10.7	100.2	6.6	66

* Control contained both boron and manganese.
Parenthesis ( ) indicates total number of plants per group accounted for in average.

preciably better than those in group III, one can dispense with the idea of manganese toxicity as being the direct cause for the lower result shown in group III in comparison with groups I and II.

The greatest amount of calcium was absorbed when both boron and manganese were present and the lowest amount of absorption took place in plants grown in solution containing neither manganese nor boron (fig. 1 and table I). The absorption of calcium may be due indirectly to the growth of the plant.

In order to test for toxicity and the effect on growth of these elements on

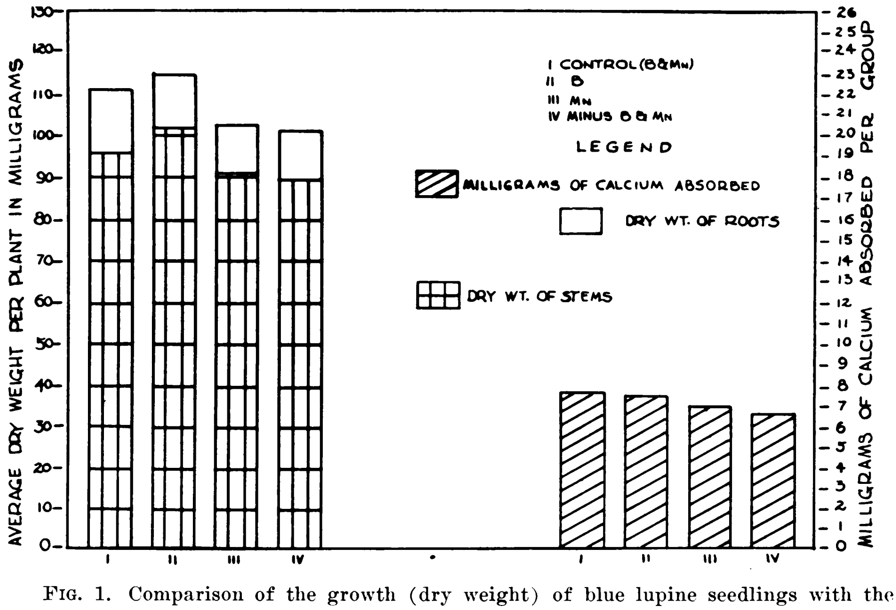

FIG. 1. Comparison of the growth (dry weight) of blue lupine seedlings with the uptake of calcium in solution cultures, containing 0.5 p.p.m. of boron and manganese.

TABLE II

GROWTH OF BLUE LUPINE SEEDLINGS IN SOLUTION CULTURE AT VARIOUS CONCENTRATIONS OF BORON AND MANGANESE (3.75–7.5 P.P.M.) AND CALCIUM UP-TAKE

Plant group	Concentration		Average length in cm.			Average weight in mg.			Average mg. of Ca absorbed	
	Mn	B	Stems	Roots	Total	Stems	Roots	Total	Per group	Per gram dry wt.
I (28) (Control*)	3.75	3.75	15.3	14.4	29.7	94.3	16.6	110.9	12.9	116
II (30)	3.75	None	12.1	11.6	23.7	82.2	13.1	95.3	9.7	102
III (28)	None	5.0	14.9	13.6	28.5	94.3	16.1	110.4	13.3	120
IV (30)	5.0	None	11.5	10.3	21.8	81.1	12.6	93.7	8.8	94
V (28)	None	7.5	14.2	13.9	28.1	92.8	11.7	104.5	14.0	135

* Control contained both boron and manganese.
Parenthesis ( ) indicates total number of plants per group accounted for in average.

blue lupine, the concentrations of these elements in a second series of experiments was increased. In this series of experiments (table II), containing boron and manganese at concentrations 3.75, 5.0, and 7.5 p.p.m., plants receiving 5.0 p.p.m. of boron gave results almost equal to those of plants receiving 3.75 p.p.m. of boron and manganese. When compared with group I, it might be interesting to note that plants receiving 5.0 p.p.m. of boron (group III) showed similar results in dry weight of stems and roots. Plants receiving 7.5 p.p.m. of boron (group V) gave results that were similar to those obtained in groups I and III. There is the possi-

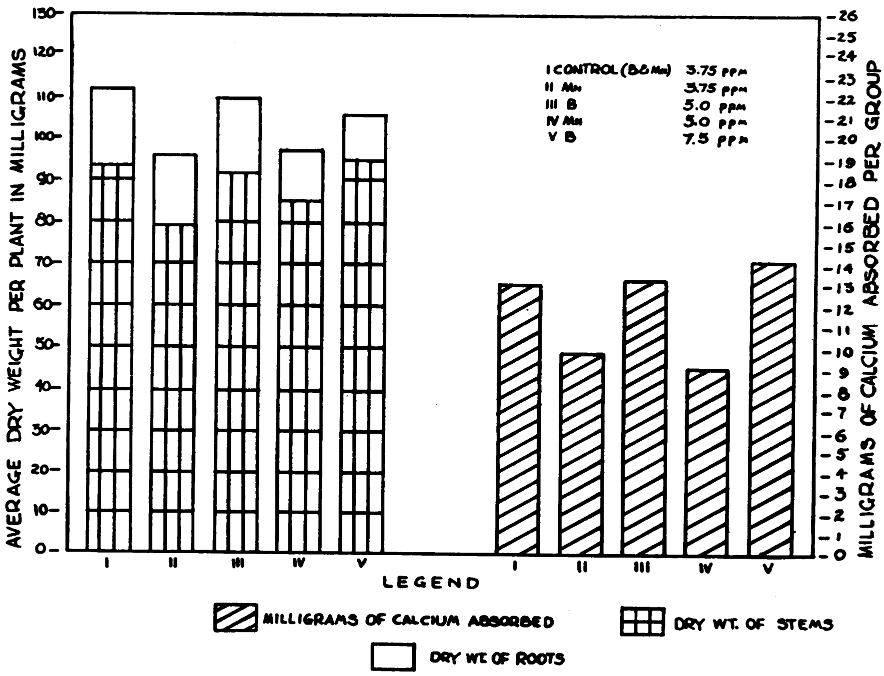

FIG. 2. Comparison of the growth (dry weight) of blue lupine seedlings with the uptake of calcium in solution culture, containing boron and manganese at varying concentrations (3.75–7.5 p.p.m.)

bility that here boron may have reached a concentration which is slightly toxic to the roots. Plants receiving 3.75 p.p.m. of manganese (group II) gave low results when compared with plants receiving similar amounts of boron. Plants receiving 5.0 p.p.m. of manganese gave results that were lower than plants receiving 3.75 p.p.m. of manganese. Although there are a number of plants known to have requirements above 1 p.p.m., there are few known which will tolerate concentrations of boron as high as those applied above to blue lupine. MEYER (9), in an investigation on the effect of various concentrations of boron on the development of kok-saghyz, found that the maximum root yields were obtained at 10–15 p.p.m. of boron, although moderately good development of the plants occurred over a consid-

erably wider range of boron concentrations (5–25 p.p.m.). THATCHER (**13**) states that boron in the form of borax is very toxic to certain crops, if in concentration of more than 3 p.p.m.

The plants in group V containing 7.5 p.p.m. of boron tend to show greater absorption of calcium than those in groups I and III when calculated on a basis of milligrams of calcium per gram of dry weight (table II and fig. 2). Plants receiving 3.75 p.p.m. and 5.0 p.p.m. of manganese show a decrease in the number of milligrams of calcium absorbed as compared with plants receiving similar amounts of boron.

The third series of experiments (table III) for plants grown in solution cultures containing 5.0, 7.5, and 10.0 p.p.m. of boron, respectively, show

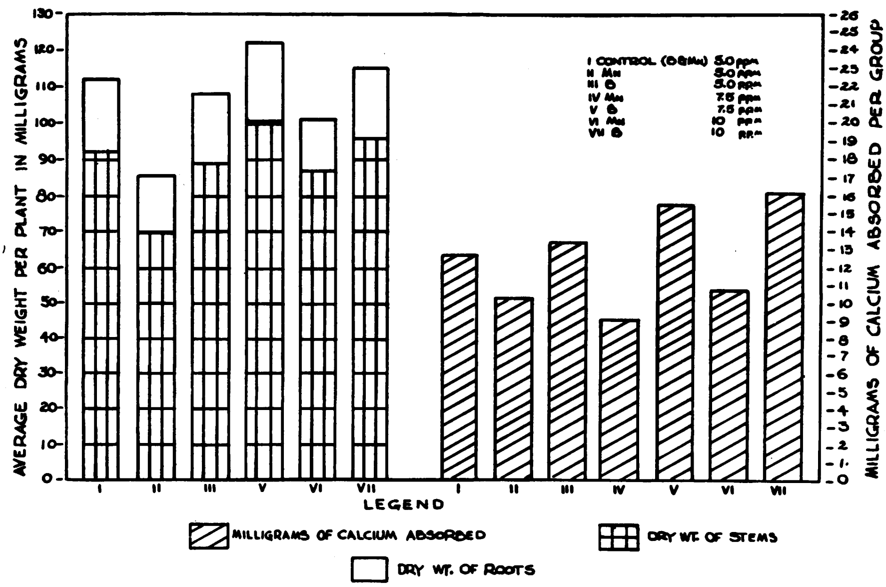

FIG. 3. Comparison of the growth (dry weight) of blue lupine seedlings with the uptake of calcium in solution cultures containing boron and manganese at varying concentrations (5.0–10.0 p.p.m.)

that the lowest results were obtained from plants receiving 5.0, 7.5, and 10.0 p.p.m. of manganese (group II, IV, and VI). It is significant that although the optimum concentration for blue lupine appears to be about 7.5 p.p.m. of B, excellent growth was obtained at higher and lower concentrations. In the first series (table I), in which small traces of boron were added, moderately good results were obtained. Thus it is evident that excellent growth of blue lupine has been obtained in solution cultures over a wide range of boron concentrations.

The data in figure 3 and table III indicate that the highest rate of absorption of calcium was obtained where manganese was lacking and boron was present at a concentration of 10 p.p.m. (group VII). Plants receiving 7.5 p.p.m. (group V) gave results that were almost equal to those of group

TABLE III

GROWTH OF BLUE LUPINE SEEDLINGS IN SOLUTION CULTURE AT HIGHER CONCENTRATIONS OF BORON AND MANGANESE (5–10 P.P.M.), AND CALCIUM UP-TAKE

Plant group	Concentration		Average length in cm.			Average weight in mg.			Average mg. of Ca absorbed	
	Mn	B	Stems	Roots	Total	Stems	Roots	Total	Per group	Per gram dry wt.
I (12) (Control*)	5.0	5.0	20.8	18.8	39.6	92.8	20.1	112.9	12.5	110
II (10)	5.0	None	10.7	8.3	19.0	68.9	16.5	85.4	10.0	117
III (12)	None	5.0	19.9	18.1	38.0	88.8	19.4	108.2	13.4	124
IV (7)	7.5	None	10.3	5.4	15.7	......	......	......	9.1	......
V (10)	None	7.5	21.5	19.1	40.6	101.3	22.2	123.5	15.8	128
VI (8)	10.0	None	10.2	8.5	18.7	86.6	15.4	102.0	11.0	108
VII (12)	None	10.0	20.4	19.7	40.1	96.0	21.8	117.8	16.3	139

* Control contained both boron and manganese.
Parenthesis ( ) indicates total number of plants per group accounted for in average.

VII. There was a slight decrease in the amount of calcium absorbed by groups I and III compared to V and VII. The lowest amount of absorption of calcium obtained was by plants grown in solution culture receiving manganese. The milligrams of calcium tend to increase proportionately with the increase in the concentration of boron in the solution culture. This seems to indicate that either directly or indirectly boron enhances the uptake and possible utilization of calcium.

A final experiment was set up in order to establish a general toxicity range on blue lupine. The trend of results tends to show that plants grown in solution cultures containing boron would tolerate up to 15 p.p.m. of boron. Plants grown in solutions having a higher concentration of boron tended to show definite toxic symptoms. It might be of some interest to

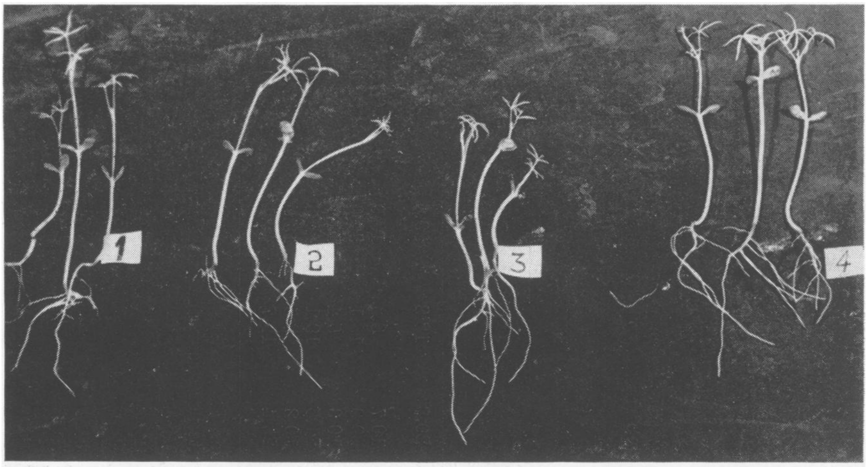

FIG. 4. Blue lupine seedlings at the end of 15 days taken from solutions containing 5 p.p.m. of boron and manganese (control).

know that the roots of plants grown in solutions containing boron were superior to those grown in solution cultures lacking boron (figs. 4, 5, 6, 7). In most cases there were only a few secondary roots on plants which received small quantities of boron. There were definite gross anatomical abnormalities on plants grown in solution cultures containing no boron. In general when boron was not used there was retardation of growth, a browning at the tip of young leaves and a decrease in their weight.

From the results obtained on the growth of blue lupine seedlings in culture solutions containing manganese, it is evident that manganese when used singly gives results which show no improvement over the results obtained when this element is absent. These results seem to agree with those obtained by SKINNER and SULLIVAN (**12**), who showed that manganese is often ineffective; occasionally injurious. Its effect depends upon the character of the soil.

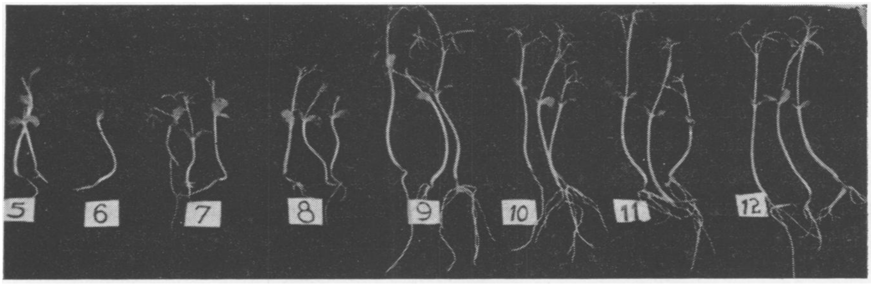

FIG. 5. Seedlings at end of 15 days. 6, 7, and 8 from solutions containing 5.0 p.p.m. of manganese; 9, 10, 11, and 12 from solutions containing 5.0 p.p.m. of boron.

FIG. 6. Seedlings at end of 15 days. 13, 14, 15, and 16 from solutions containing 7.5 p.p.m. of manganese; 17, 18, 19, and 20 from solutions containing 7.5 p.p.m. of boron.

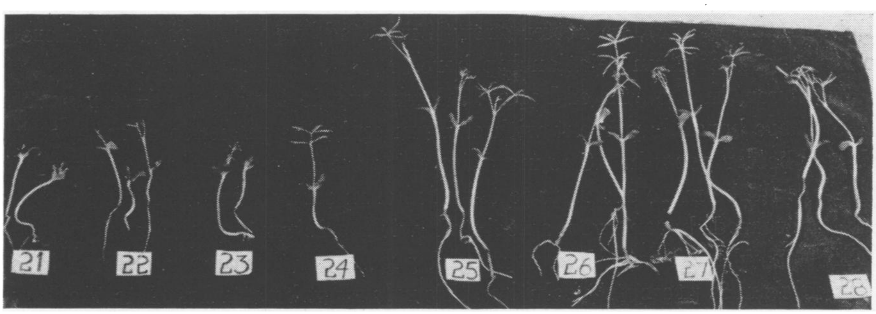

FIG. 7. Seedlings at end of 15 days. 21, 22, 23, and 24 from solutions containing 10 p.p.m. of manganese; 25, 26, 27, and 28 from solutions containing 10 p.p.m. of boron.

Discussion

In most cases plants grown in solution cultures containing boron had more secondary roots than plants grown in solutions lacking this element; there was, also, an increase in both the dry weight and length of stems and roots as compared with solutions containing manganese and lacking boron. Coincident with the increase in growth was an increased uptake of calcium in those plants grown in solutions containing boron. This supports the findings of GLASSTONE (**3**), who reported that manganese and zinc are not

apparently needed in detectable amounts for the essential growth of tomato roots, although they do play an important part in photosynthesis. It would seem, therefore, that it is the presence of boron and not the absence of manganese which might be responsible for the favorable growth results that were obtained in these experiments.

From another point of view, the use of these three elements (ions), boron, manganese, calcium, suggests a possible antagonism among them. Antagonism may be defined from the point of view of basic cellular reactions as the mutual inhibitory or retarding effect of one ion in solution on the absorption of another, depending on the chemical or physical properties of the ion. Then, when considering the combinations possible in this experiment in relation to elements under investigation, there are three: (1) manganese: boron; (2) manganese: calcium; and (3) boron: calcium. The first, manganese: boron, may be eliminated by the control which showed the best or equal growth to other groups. The second, manganese: calcium, may be eliminated on a comparison of groups containing manganese alone and where both manganese and boron are lacking (calcium is present in all groups throughout). In solutions containing calcium alone the growth was no better than where manganese and calcium were present, thus showing that manganese in no way influenced the effect of calcium on the growth.

Antagonism, in another sense, may be looked upon, especially in the case of toxic ions, as the influence of one ion on another, both of which may be toxic alone, in overcoming this toxicity and stimulating better growth. In this sense then, calcium may appear as somewhat "toxic" (group IV, table I, and fig. 1); but since in solutions containing both manganese and calcium (group III), no better growth resulted, we may again say there is no maganese: calcium relationship here.

However, in the case of boron: calcium we do have a positive response, since where boron is present with calcium or in the presence of manganese, there is better growth. One may cite this as a boron: calcium antagonism, but it would be more accurate to term it boron stimulation. Thus, in either case, increased boron was associated with calcium uptake and possible utilization which resulted in increased growth of the plants. Since in all cases calcium is present in solution, this role must be attributed to the boron in solution. It would seem then, that boron in contrast to manganese aids in the absorption of calcium, the ultimate result being increased growth of blue lupine seedlings. It is not necessarily implied that this result is a direct one. It is quite logical to assume that boron may be acting in one of several ways, primarily as a catalyst.

Summary

1. Blue lupine seedlings grown for 15 days in a Hoagland and Arnon nutrient solution were supplied with boron and manganese at different levels of concentration, ranging from 0.5 to 15 p.p.m.

2. At the low concentration of boron and manganese of 0.5 p.p.m., the

seedlings exhibited marked differences between solutions containing boron and those containing manganese, with the latter of little or no effect on the development of the seedlings. Where neither was present, the results were similar to those of solutions containing manganese alone.

3. At higher concentrations of boron and manganese (3.75–7.5 p.p.m.) similar results were obtained. Calcium uptake corresponded closely with the results, i.e., higher uptake where boron was present.

4. At yet higher concentrations of boron and manganese (5.0–10.0 p.p.m.) growth was similar, indicating a wide range of tolerance for boron. Calcium uptake was equally as good here as for the previous concentrations. Both growth development and calcium uptake were retarded by manganese.

5. For blue lupine optimal concentrations of boron appear to be about 7.5 p.p.m. with a range of tolerance from 0.5 to 15 p.p.m., which is generally higher than most plants will tolerate.

6. Definite anatomical symptoms are shown by plants grown in solution cultures lacking boron. The root system was reduced and top growth retarded generally, especially meristematic tissue. Browning of the tips of the leaves also resulted.

7. An interpretation from the point of view of antagonism between the three main ions concerned (B, Mn, and Ca) is attempted. The conclusion is reached that antagonism as such plays little role, but rather, that boron acts in a stimulatory manner in causing the better growth and development of blue lupine seedlings.

DEPARTMENT OF PLANT SCIENCE
TUSKEGEE INSTITUTE
TUSKEGEE INSTITUTE, ALABAMA

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