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The effect of the calcium ion on the development of soy bean seedling and the antagonism of this ion to arsenic, boron, and selenium ions.

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THE EFFECT OF THE CALCIUM ION ON THE DEVELOPMENT OF SOY BEAN SEEDLING AND THE ANTAGONISM OF THIS ION TO ARSENIC, BARON, AND SELENIUM IONS

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The Effect of the Calcium Ion on the Development of Soy bean seedling and the Antagonism of this Ion to Arsenic, Baron, and Selenium Ions

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TABLE OF CONTENTS

INTRODUCTION

The recognition of the practical importance of arsenic, boron and selenium as related to Agriculture is one of the recent interesting contributions to Science. The failure to realize the full importance of these minor elements earlier may be attributed to many factors. One of these factors is the lack of a method of analysis sensitive enough to determine the small amount usually present in soils and plants. Furthermore, arsenic, boron and selenium as required by plants are confined to such a narrow range of concentration that it is difficult to avoid toxicity on one hand and deficiency on the other.

Arsenic has been known in Agriculture for many years as to its effect on insects in spray mixtures. Little is known of its nutritive value in plant culture. Steward, (58) however, in 1922 stated that sodium arsenate showed a beneficial influence in low concentrations upon beans, peas, wheat and radishes. A thorough knowledge of arsenic and its effect upon plant growth is essentially important in our present day Agriculture.

In the past few years more attention has been given to the investigation of boron than to that of arsenic and selenium. The first period, 1837-1900, in which only 18 papers reported, was marked chiefly by the detection of boron in plants; second, as shown, 1900-1915, is most important since it included the discovery of the essential nature of boron by Maze (46) in 1914.

From 1915-1925 much interest in boron injury was shown in this Country. This was found in the potassium fertilizers and was soon eliminated by the chemists by taking the borax out of the potassium salt, From 1925-1930 further proof of the necessity of boron in normal plant growth was obtained, but interest was not as great as during the previous period. Interest suddenly arose in the period of 1930-1936 and there were approximately 100 papers reported on boron, most of which were stimulated by the work of Bradenburg (16) in 1931, who reported that heart rot in sugar beets was caused by boron deficiency rather than by an organism. The period beginning in 1936 may be characterized by the recognition of the fertilizing value of boron, and in this recent time approximately 350 investigations have been reported on.

Calcium has been recognized to be essential to the growth and development of all green plants with the exception of some of the lower fungi. According to

LITERATURE REVIEW

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ARSENIC AND ITS BEHAVIOR

Albert (3) discovered that the use of heavy applications of calcium arsenate for boll-weevil control on cotton increased the arsenic content of some sandy soils sufficiently to cause arsenic toxicity to crops. The concentration of soluble arsenic as measured by collodion bag dialyzates was found by Albert and Arndt (4) to be a more reliable index of arsenic toxicity than is the total arsenic present in the soil. Liming and the use of fertilizer alone with the ions and clay compounds of the soil played an important role in rendering arsenic harmless to sensitive crops. It was also observed that an addition of one part per million of arsenic definitely retarded root and top growth of cow peas growing in the greenhouse. It is also pointed out that concentration of one part per million of soluble arsenic as measured by the collodion bag test is not unusual in heavily arsenated fields.

A concentration of M/40,000 potassium arsenate in a basal nutrient solution was sufficient to reduce greatly the growth of cotton plants growing in water cultures. Potassium arsenite was approximately four

Bamford (9) the necessity of calcium for plant growth was first demonstrated by Salm-Hortsmar (57) 1856. Since that time numerous functions have been ascribed to calcium, though many of these are little understood. Therefore a better understanding of calcium and its function in the soil and plants is of vital importance in our present system of Agriculture.

Selenium, which occurs in the soil in varying amounts over various areas of Nebraska, South Dakota, Wyoming and other sections of the Western States was discovered in 1817, and in 1856 was brought to the attention of the scientists by causing a disease in animals. This disease today is known as alkali disease. Although during the last decade much interest has been stimulated as to the behavior of the element, our knowledge is still very limited as to the characteristic behavior of it in the soil and plants.

Therefore, the major and minor elements, as they simply occur in nature, complex to understand and essential in the normal development of plant growth, lend themselves to further scientific investigation as to the roles they play in the soil and plants in relationship to the physiological balance.

times as toxic as potassium arsenate.

Albert and Paden (5) believed that the addition of calcium arsenate to light sandy loam soils may be expected eventually to interfere seriously with growing such arsenic-sensitive crops as cow peas, cotton and various grasses.

Bouilhac (15) states that a number of fresh water algae are said to absorb arsenic acid from arsenates without apparent injury.

Brenchley (19) has found that the toxic effect of arsenic upon higher plants was much more marked with arsenious acid and its compounds than with arsenic acid and its derivatives.

Hurd-Karrer (41) found that the presence of phosphorus greatly inhibited arsenic injury of plants.

Morris and Swingle (48) conclude that incorporation of arsenical compounds in the soil is a dangerous practice and may cause considerable injury as the concentration of arsenic increases.

Plants differ in their ability to withstand arsenic. Some crops remain approximately normal when arsenic in some form is present while other crops in the same environment are killed. Beans and cucumbers were found to be very susceptible to arsenic, but cereals and grasses were more resistant.

Stewart and Smith, (58) found that arsenic in the form of disodium arsenate, showed a beneficial influence in low concentration upon beans, peas, wheat and radishes. Beans, peas and wheat were stimulated by concentration of disodium arsenate up to 75 p p m. Radishes were stimulated by concentration up to and including 240 p p m. The beans were the most sensitive to this element.

Stoklasa (59) in his experiment with oats concludes that while arsenic acid cannot replace phosphoric acid in living cells, it is able to increase the development of the organ of assimilation in the plant.

Voelcker (64) states that the injurious action of arsenic depends upon the solubility of the compound to which it is added. When in soluble form, like arsenious acid, the higher concentrations were used without injury to germination of crops. The more soluble compounds, like arsenic acid, sodium, arsenite and arsenate, decreased the crop when the element arsenic was added in amounts up to 2 per cent and caused the crop to fail when 5 per cent of arsenic was added. Soluble arsenic added up to the amount of 5 per cent retarded germination and 1 per cent entirely inhibited it. The limit of safe use appeared to be . 1 per cent of arsenic, representing an application of about 825 pounds of arsenic acid per acre.

Waber (65) in his investigation of the toxicity of arsenic acid on various plants, such as oats, wheat, maze, rye and legumes, found that the legumes were more susceptible to poison than other plants.

Bailey and Robinson (8) state that when calcium arsenate was used as a dust to control boll weevils, the average increase in cotton yields per acre was 40 pounds yearly on plots that were not fertilized and 203 pounds on fertilized plots.

Cobet (25) has found that arsenious acid at a concentration of 1:200,000 is strongly poisonous to plant roots, dwarfing and killing them. Weaker concentrations were not found to favor growth.

Cooper, Paden, Hall, Albert, Rogers and Riley (28) discovered that frequent applications of calcium arsenate designed to control the boll weevil markedly depressed the yield of some crops. The crops on soils of low iron content were the ones most depressed.

Greaves (34) states that the results obtained from an investigation, conducted to determine the effects of different amounts of sodium arsenate, zinc arsenite, lead arsenate, arsenic trisulphid and Paris Green on the ammonification and nitrification of dried blood in a sandy soil rich in calcium and iron and abundantly supplied with plant food, with the exception of nitrogen, varied with the different compounds. However, all exerted a stimulating effect at the lower concentrations and a toxic action in the higher.

Lead arsenate is the least toxic. Next come zinc arsenite and arsenic trisulphid. Paris Green exerted the greatest degree of toxicity, whereas lead arsenate exerted the greatest degree of stimulation.

Paden and Albert (51) conclude, from tests made on soils in which crops had been treated with large quantities of calcium arsenate, that there is a relationship between the unproductivity of certain soil types and the accumulation of soluble arsenic in the soil.

They observed that an application of lime improved the growth of cotton in the poisoned soils. Soil relatively low in iron and other materials that would be expected to render arsenic insoluble were found to be the most seriously injured by the arsenate.

BORON AND ITS BEHAVIOR

Agulhon (1) states that in experiments made with wheat, oats, peas, radishes, etc., grown in sterile liquid media in sterilized soil and in open fields, the different species reacted differently toward boron; but for each there was an optimum which fávored the growth and yield of plants. Greater quantities than the optimum were found to reduce the chlorophyll content of plants and to check their root development.

Agulhon (2) states that boron in small amounts has marked effect on various crops, but especially on Gramineae. Applications of less than 2 kg. per hectare have proven especially beneficial to corn.

Bobko (10) states in a report of a meeting at Tashkent that cotton plants were found to develop unsatisfactorily if boron was left out of the solution. Wilt was decreased and yields increased when boron was added.

Bobko and Zerling (11) found that plants developed better with H_3BO_3 present than with sodium borate, the boron content being the same in both cases.

Bobko, Syvorotkin and Filippov (12) state that one-tenth per cent boron added to soil in pots with

$CaCO_3$ up to twice the hydrolytic CaO requirement caused increase nearly proportional to $CaCO_3$ applied, but the latter without boron caused corresponding decreases in yield of turnips.

Bobko and Syvorotkin (13) found that small additions of boron neutralized the harmful effects of over liming and increased yields, but reduced the nitrogen and ash contents of the plants.

Bobko, Matveeva, Doubachova, Phillipav (14) and Naftel (49) suggest that over liming stimulates microbiological activities which in turn depletes the soil solution of available boron.

Brenchley (17) states that among the boron compounds boric acid is said to be less harmful than are the compounds of copper, zinc and arsenic. There seems to be evidence that below a certain limit of concentration boron exercises a favorable influence upon plant growth, encouraging the formation of strong roots and shoots.

Brenchley (18) points out that certain plants, especially many of the leguminosae, require traces of boron, one part in 2,500,000 being ample. In the absence of boron, the plants die from the apices, anatomical changes, disintegration, and blacking occurring in the tissues.

Collings (27) found that in solution and sand cultures small concentrations of boron compounds were toxic to soy beans. There was no distinct evidence of a stimulating effect.

The more recent work of Davis, (29) 1937, Dennis, (30) 1937, Hill, (38) 1938, Millar and Tucker, (47) 1938, and Naftel, (49) 1937, infer that boron deficiency occurs more frequently in alkaline than in acid soils.

Kataluimov, (43) observed that the presence of boron improved the harvest of flax in acid soils and of wheat in carbonate saline soil treated with $CaSO_4$. The presence of boron not only neutralized the injurious effect of lime on flax and mustard, but actually improved the harvests.

Naftel (49) found that excessive liming of a Norfolk loamy sand resulted in over liming injury to vetch, turnips, oats, cabbage, tomatoes and soy beans. The addition of large amounts of phosphorus and manganese, did not overcome these injuries, but when boron was added it completely prevented the injurious effect of over liming. From these facts the author concludes that the over liming was due to boron deficiency.

Nowotnowna (50) states that in the experiments performed with the addition of 1.5-60 mg. H_3BO_3 per pot (9.5 Kg sand) with soy beans and 25-1600 mg. $Na_2B_2O_7$ per pot (36 Kg sand) with sugar beets, the results indicate that boron is absolutely necessary for the normal development of soy beans. The optimum concentration of H_3BO_3 per pot was 3-12 mg. An excess of boron produced yellowing and deformation of the leaves.

Askew (7) states that boron, calcium, magnesium and zinc are all essential to the healthy growth of cultivated plants. Various disorders found to be associated with deficiencies of these four elements mentioned.

Observations have been made by Headden (36) 1921, Parker and Troug, (52) 1920, Richardson (53) 1920, and Trelease and Martin, (61) 1936, and others in regard to the different proportions of the elements contained in plants of different species growing under the same conditions.

Robinson, Steinkoenig and Millar, (55) 1917, made extensive analysis of a large number of plants from different regions for rare elements.

Arnon (6) has found that asparagus and lettuce in culture solution containing no micro element made poor growth and showed nutrient deficiency symptoms.

Eaton (31) states that when Acala cotton plants were grown in sand beds with boric acid and in concentrations of 0, 1, 5, 10, 15 and 25 p p m of boron, plants in the "0 bed" were much shorter than those in the 1-5 p p m beds.

From these observations Eaton is of the opinion that gratifying results might follow the use of boron as a fertilizer in the Cotton Belt of the South.

French (33) has made these observations: Tests with culture solutions with tomatoes showed the indispensability of boron to normal plant growth. Concentrations of 2.1-7.0 p p m of boron in the nutrient solution produced plants free from leaf curl and die-back.

Holley and Dulin (39) found that the addition of 10 p p m of boric acid resulted in a decided improvement in growth of cotton plants in nitrate nutrient solutions in spring cultures not constantly renewed.

CALCIUM AND ITS BEHAVIOR

Brioux and Jouis (20) believe that when calcium is added to the soil an insoluble calcium borate is formed.

Colby, (26) 1933, noticed in young French pruned trees that a lack or scarcity of either, potassium, magnesium or phosphorous seriously depressed nitrate absorption and even caused a loss of this ion from the roots late in the season. The lack of calcium prevented root growth and the trees absorbed very little nitrate.

Loew (45) believes that the contraction of the nuclei in cells subjected to potassium oxalate solutions of 0.5 to 2 per cent strength is due probably to the extraction of the water of imbibition or the withdrawal of the calcium from the nucleus.

Robert (54) states that pea seedlings, when transferred to a medium containing 25 per cent of magnesium sulphate, 5 per cent of hydrogen potassium phosphate and 5 per cent of ammonium nitrate, showed arrest of development. This however, was overcome by the addition of 5 per cent of calcium carbonate or calcium sulphate.

SALTS AND THEIR BEHAVIOR

Eisenmenger, (32) 1928, and Trelease and Trelease, (60) 1926, demonstrated that the roots of young wheat seedlings can be used satisfactorily for demonstrating the growth-retarding effect of single salt solution and the antagonistic action of mixed solutions.

Hansteen, (35) is of the opinion that lime exerts its antagonistic effect only when in conjunction with other salts, acting as an external protection to the roots.

Hibbard, (37) 1927, calls the attention to the fact that under field conditions the proportions of various salts available to plants must vary from time to time because of the fluctuation of the salt content of the ordinary soils. Similar conclusions were drawn by Eisenmenger (32) 1928, in studying the rate of elongation of wheat roots supplied with simple solutions of mono potassium phosphate, calcium nitrate and magnesium sulphate, and with solutions containing pairs of the salts. He considered that the wide range of salt proportions that allow nearly optimum growth is one of the conspicuous features of his results.

SELENIUM AND ITS BEHAVIOR

It is the opinion of Byers, (21, 23, 24) 1935-1936, Byers and Knight, (22) 1935, Hurd-Harrer, (42) 1935, and Robinson, 1936, that the quantity of selenium absorbed by plants and their subsequent toxicity depends upon numerous factors, Chiefly among these are the composition of the soil, the moisture relations of the soil, the concentration of this element in the soil, the species of plants, the stage of its development, the available sulphur and the crop previously grown.

Hurd-Karrer, (40) 1933 and (41) 1934, found that wheat plants grown in soil to which sodium selenate had been added at the rate of 15 p p m or less became characteristically chloratic. The young leaves frequently became almost snow white with green tips and green mid veins. When the selenate was added to the pots containing older plants, white chlorasis appeared only on the leaves emerging subsequent to the addition of selenium.

Hurd-Karrer (41, 42) also noted that the toxicity of sodium selenate is determined by the amount of sulphur available to the plants. Thus, in cultural solutions concentrations of selenium as low as 0.1 p p m produced distinct injury after a few weeks if no sulphate was present, whereas a concentration of 18 p p m was required for this degree of injury if the solution contained 192 p p m of sulphur.

According to Levine, (44) 1925, selenium compounds in concentrations of 1 per cent and above exerted a detrimental influence on the growth of white lupine and timothy seedlings. On further investigation he states that he obtained increased growth of these plants in solutions of selenium dioxide and selenic acid with concentrations of 0.01 and 0.1 per cent respectively.

Trelease and Martin, (61) 1936, state that western wheat grass growing on various soils accumulated 1 to 60 p p m of selenium, while Astragalus bisulcatus growing on the same soil accumulated 200 to 4,300 p p m.

Trelease and Trelease (62 and 13) state that many plants that seem to be indicators for selenium in the soil absorb considerable quantities of it. Growth of Astragalus racemosus was stimulated by selenite in concentrations of 1-27 p p m in solution and sand culture. They also state that sulphur plays an important role in the amount of selenium that will be absorbed by various plants, although range plants accumulate enough selenium to make them potentially lethal to animals.

OBJECT OF THE INVESTIGATION

In the field of Science it is internationally known that a physiological balance solution is mutually essential in the normal development of plant growth.

Arsenic, boron and selenium, being minor elements in nature, yet complex in their behavior toward plants, have long been neglected by scientific investigation in the field of Agricultural Science.

The reversion of scientific investigation has brought to the attention of the scientists the importance of a thorough basic knowledge as to the behavior of these elements in soil and plant development. Therefore, the object of the investigation was to ascertain the katabolic and anabolic effects arsenic, boron, calcium and selenium have upon plant growth, singly and in combination with calcium.

It was thought that any knowledge obtained that would reveal a better understanding of the constructive and destructive effects of these elements upon plants would help to reduce some of the risk bearing situations in some of the practical phases of Agriculture and would serve as a guide in further investigations of these elements.

METHODS, TECHNIQUE AND PROCEDURE

The chemicals used calcium nitrate, boric, arsenic and selenous acid. The seeds used in germination for the experiment were Dunfield soy beans. These were washed thoroughly in water, and carefully graded or assorted. On completion of the washing and assorting they were treated with a 1 per cent of a 40 per cent solution of formaldehyde. The formaldehyde inhibited the development of molds which readily form on plants grown under a dark warn moist condition.

After treating the seeds with formaldehyde they were rinsed thoroughly with distilled water and transferred to clean, carefully prepared germinating dishes. The dishes were then placed in a dark warm place, temperature ranging from 21° - 24°C. When the seed sprouts had reached 20-30 mm they were transferred to the solution with sterilized forceps, the roots being dipped into the solution.

As to the growth of the seedling, root elongation with respect to its behavior in various solutions was the major factor considered. In the early stage of growth the plant is naturally self-sustaining and there was no sign of starvation of plants grown in distilled water.

The investigation involved the toxicity of calcium nitrate, boric, arsenic and selenous acids. The concentrations of each compound used singly consisted of 5, 25, 50, 75, 95 and 100 per cent of .002M arsenic acid, .0005M selenous acid and .05M calcium nitrate.

In combination or mixed solutions seven sets of percentages molecular proportions were used. The sets of percentage used were:

0	5	25	50	75	95	100
100	95	75	50	25	5	0

Calcium Nitrate reads from 0 to 100 and Arsenic, boric and selenous acid reads from 100 to 0.

The methods treating upon roots were the same as those described by Eisenmenger (32) and Trelease and Trelease, (60) 1926. For each culture two lipless pyrex beakers were used. The smaller one was of 300 cc capacity and the larger one was of 600 cc capacity. The top of each small beaker was covered with a piece of paraffined mosquito netting which was secured below the rim by a ligature of paraffined thread. The small beaker was placed inside the large one and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at the top.

When the seedlings from the germination dish were of the desired length they were placed on the mosquito netting so that the roots dipped into the culture solution.

For each experimental solution duplicate cultures of 25 plants were used, making a total of 50 plants for each concentration. The cultures were placed in a dark room and the seedlings were allowed to develop until the primary roots of the control had elongated to 100 - 120 m m.

The length of the primary root of each individual plant was measured, recorded, and the average computed. From the average of root length was deducted the average length of the roots at the time the seedlings were taken from the germination dish. The difference constituted the average root elongation value for each culture concentration. Each relative growth value as expressed in percentage was obtained by dividing the elongation of roots of a given culture by the average elongation of roots of the control culture and the quotient multiplied by 100.

Each group was accompanied by a control which consisted of two beakers, having 25 individual plants.

These 50 plants were grown in a complete nutrient medium known as Knop's Solution, which consisted of the following compounds: $Ca(NO_3)_2$ - KNO_3 _ K_2HPO_4 _$MgSO_4$- $FePO_4$ - and H_2O. A culture of distilled water of two beakers each containing 25 plants was also used with each group of plants. During the growth period of the seedlings the temperature ranged from 21° - $24^{\circ}C$.

The period of germination of the seeds varied from two to three days, but the growth period of the seedlings in the various cultures or concentrations (five days or one hundred twenty growing hours) was constant.

The Beckman Method was used to determine the pH of all the (freshly prepared) solution.

The distilled water was obtained from an improvised Barnstead still.

The experiment was conducted in the Soil Laboratory.

DISCUSSION

Previous investigators have stated that there is a great degree of variation in plants, relative to their resistance to plant poisons. Plants of the grass family are the most resistant, whereas those of the leguminous family are the most sensitive. It is also the opinion that toxic elements at low concentrations have a stimulating effect on plant growth in water and sand cultures.

In the recent experiment it was observed that a .5 per cent of .002M arsenic acid exerted a stimulating effect upon the soy bean roots in a water culture solution. This amount is approximately equal to 0.1 p p m of arsenic (Table 1, Figure 1). Amounts higher than this exerted a decidedly retarding effect on roots as compared with that at .5 per cent of 0.002M. The plants that were principally affected showed lesions of the primary roots. On later development the roots turned light brown and the ends were dwarfed. Eventually the roots assumed the nature of an opened wound where modification had gone to completion. This was an indication that the cells had lost their turgor and had become plasmolized. The lateral roots were marginal and showed lesions similar to those of the primary roots. Those that were near the surface were more developed than those in the lower part of the solution. Further inspection disclosed that the tops were the same color as the roots, but the degrees of injury were more pronounced in the primary roots.

MIXTURES OF ARSENIC ACID AND CALCIUM NITRATE

The relative effects on plants grown in mixtures of acid and salt solutions, called "nutrient solution", are more easily shown, for the growth effects are, for the most part, those produced by the cation.

In this investigation growth data were compared when soy bean seedlings were grown in arsenic acid alone, in calcium nitrate and mixtures of the two compounds; the molecular concentration of arsenic acid being .002M and calcium nitrate .05M.

In order to express more adequately the effect of these compounds when in mixture, it is essential to know the effect of calcium nitrate when used singularly (Plate 4, Table 4); the molarity being .05M calcium nitrate. The curve (Figure 1) indicates that low concentrations of calcium nitrate are stimulating to growth, but at higher concentrations the growth curve readily decreases. The decline in the curve indicates that calcium nitrate at a high concentration is toxic to plant growth.

It is the opinion that the stimulating effect is due to the low calcium ion in solution and the retarding to the higher concentration of the calcium ion in solution.

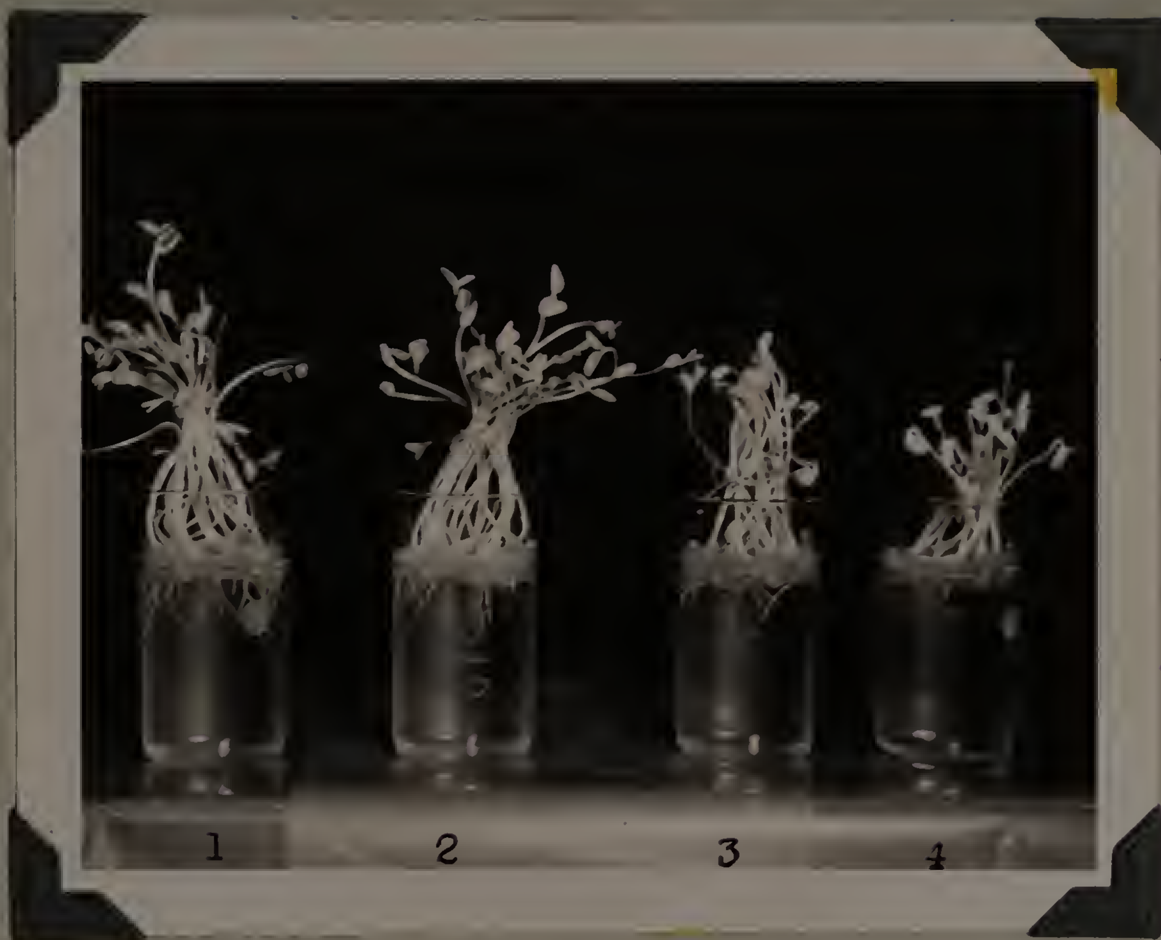
One of the most outstanding characteristics of growth relationship was observed when the seedlings were grown in a mixture of arsenic acid and calcium nitrate (Table 1a). This was due, in part, to the neutralization of the arsenic acid. At lower concentrations of calcium nitrate with higher concentrations of arsenic acid, the arsenic acid reduced the stimulating effect of calcium nitrate, but as calcium nitrate increased and arsenic acid decreased the degree of root and top growth increased likewise. The greatest degree of growth was obtained when calcium nitrate and arsenic acid were in the ratio of 95: 5 per cent of their respective molar concentrations (Figure 1).

Plate I, Numbers 1 and 2, as compared with Numbers 3 and 4, show the relative effect calcium nitrate has upon plant growth in combination with arsenic acid; arsenic acid being 50 per cent of .002M in all of the four numbers, with the addition of 50 per cent calcium nitrare of .05M to Numbers 1 and 2, making a mixture of 100 per cent arsenic acid and calcium nitrate in Numbers 1 and 2, and only 50 per cent arsenic acid in

3 and 4. The primary and lateral roots in 1 and 2 showed no degree of lesion and assumed a silver color. The tops were likewise where the growth was most pronounced. Numbers 3 and 4 showed a high degree of lesion, which has been alluded to before. The primary roots turned brown and the ends were dwarfed. They later assumed the nature of an opened wound where modification had gone to completion.

From this investigation it can be definitely stated that calcium nitrate at high enough concentration will reduce the toxicity of arsenic as related to plant growth.

PLATE I.



No. 1 & 2 Growth of soy bean seedling in a mixed solution fifty per cent of .002M Arsenic Acid and fifty per cent of .05M Calcium Nitrate.

No. 3 & 4 Growth of soy bean seedling in a single solution, fifty per cent of .002M Arsenic Acid.

TABLE I.

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTION OF ARSENIC ACID

No.	Percentage of .002M concentration	p H	p p m	Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1	0	-	-	22.2	30
2	5	5.1	0.1	33.4	53
3	25	4.2	0.5	16.7	26
4	50	4.0	1.0	10.2	16
5	75	3.9	1.5	9.10	15
6	95	3.5	1.9	5.4	8
7	100	3.5	2.0	0.0	0

TABLE Ia.

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ARSENIC ACID AND CALCIUM NITRATE

No.	Percentage of As_2O_5 .002M + $Ca(NO_3)_2$ .05M concentrations	p H	p p m	Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1	0	-	-	22.2	30
2	0 + 100	3.3	0 + 2.00	0.0	0
3	5 95	3.7	590 1.90	7.2	12
4	25 75	3.8	2,950 1.50	17.3	28
5	50 50	4.0	5,900 1.0	25.8	41
6	75 25	4.3	8,850 0.5	54.6	80
7	95 5	4.7	11,210 0.1	55.8	82
8	100 0	5.8	11,800 0.0	26.8	43

Under concentrations "O" represents distilled water.

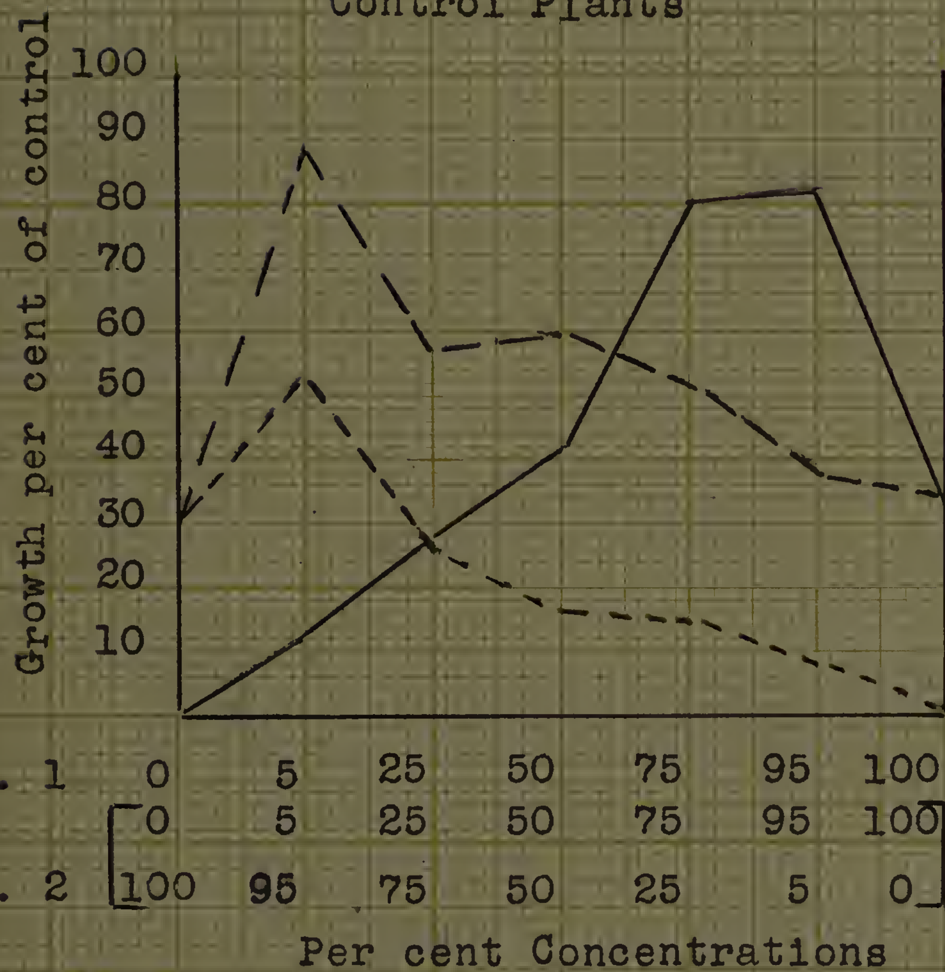
Under concentration and p p m all numbers reading left represents Calcium Nitrate.

FIGURE I.

Soy Beans

Root Elongation

Control Plants



(a) ------- As_2O_5

(b) - - - - $Ca(NO_3)_2$

(c) ——— $As_2O_5 + Ca(NO_3)_2$

No. 1 represents the per cent concentration of single solutions of .002M Arsenic Acid or .05M Calcium Nitrate.

No. 2 represents the per cent concentration of mixed solutions of .002M Arsenic Acid plus .05M Calcium Nitrate.

In No. 2 Calcium Nitrate reads from 0 to 100 and Arsenic Acid reads from 100 to 0.

In the past few years more work has been done toward the investigation of boron than any other of the minor elements.

In the recent investigation of boron it was found that soy bean seedlings grown in a single water culture solution of 5 per cent of .0005M boric acid had approximately the same effect as that of 75 per cent of .0005M; the former being approximately .07725 p p m and the latter 1.158 p p m.

To ascertain this, three series were run and each time the results were apparently the same. Table 2 and (Figure 2) indicate that 5 per cent and 75 per cent of .0005M boric acid stimulated growth. The greater degree of stimulation occurred at 75 per cent of .0005M. Beyond that concentration, the growth curve continued to decline. This was an indication that boric acid had become toxic to the seedlings. The most peculiar occurence observed in this experiment was in the concentrations ranging from 5 per cent to 75 per cent of .0005M.

In each of the three series run, it was observed that a black lesion occurred throughout the stem of some of the seedlings and cotyledons. The exact cause of this is not definitely known, but Brenchley (19) states in one of her investigations that such blacking occuring in tissues of the plant is due to boron deficiency.

It was also observed that seedlings grown in distilled water, "nutrient solution" (Knop's Solution) and concentrations exceeding 75 per cent of .0005M showed no sign of blacking of plant tissues. There was a marked degree of lesion on the primary and lateral roots, and on the ends of the primary roots a small amount of flexible matter accumulated. The lateral roots were most numerous at the surface of the solution. The tops showed slight indication of a brown tint. At higher concentration the primary roots became dwarfed and blunt on the ends.

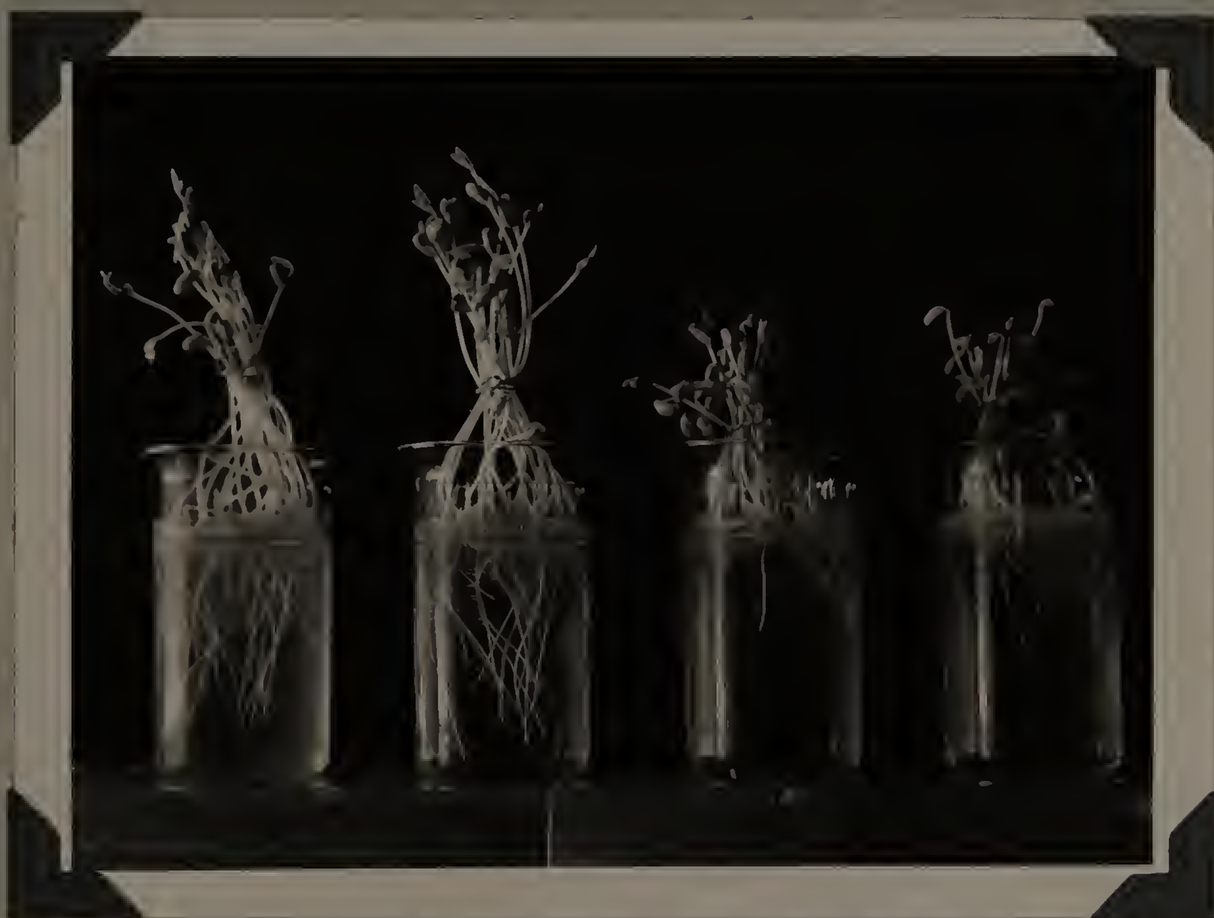
III

MIXTURES OF BORIC ACID AND CALCIUM NITRATE

When soy bean seedlings were grown in a mixture of boric acid and calcium nitrate solution, the 95 per cent of .0005M boric acid retarded the stimulating effect of .5 per cent of .05M calcium nitrate, as compared with the growth of 5 per cent of .05M calcium nitrate singular. However, Figure 2 shows that at all concentrations calcium nitrate ameliorates the toxic effect of boric acid. The primary and lateral roots were the same color as those of the control (Knop's Solution). They were bright and assumed a silver color. There were no signs of lesion in any of the concentrations.

Plate 2, Table 2a and Figure 2 shows the effect calcium nitrate has upon plant growth singly and in combination with boric acid. Plate 2, Numbers 1 and 2, have a mixture, each 50 per cent of .0005M boric acid and 50 per cent of .05M calcium nitrate, whereas Numbers 3 and 4 have only 50 per cent of .0005M boric acid.

PLATE II.



No. 1 & 2 Growth of soy bean seedlings in mixed solution, fifty per cent of .0005M Boric Acid and fifty per cent of .05M Calcium Nitrate.

No. 3 & 4 Growth of soy bean seedlings in a single solution, fifty per cent of .0005M Boric Acid.

TABLE II.

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTION OF BORIC ACID

No.	Percentage of .0005M concentration	p H	p p m	Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1	0	-	-	22.2	30
2	5	6.6	.077	26.4	39
3	25	6.4	.386	19.6	29
4	50	6.3	.772	22.3	33
5	75	6.2	1.115	29.1	43
6	95	6.1	1.457	27.0	40
7	100	6.0	1.545	22.2	30

TABLE IIa

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTION OF BORIC ACID AND CALCIUM NITRATE

No.	Percentages of H_3BO_3 .005M + $Ca(NO_3)_2$ .05M concentrations			p H	p p m			Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1		0		-		-		22.2	30
2	0	+	100	5.2	0	+	1.545	22.2	30
3	5		95	5.9	950		1.457	50.0	50
4	25		75	6.0	2,950		1.115	65.7	68
5	50		50	6.2	5,900		0.772	52.6	54
6	75		25	6.3	8,850		0.386	54.9	56
7	95		5	6.4	11,210		0.077	47.5	48
8	100		0	5.8	11,800		0.0	41.4	39

Under concentration "0" represents distilled water.

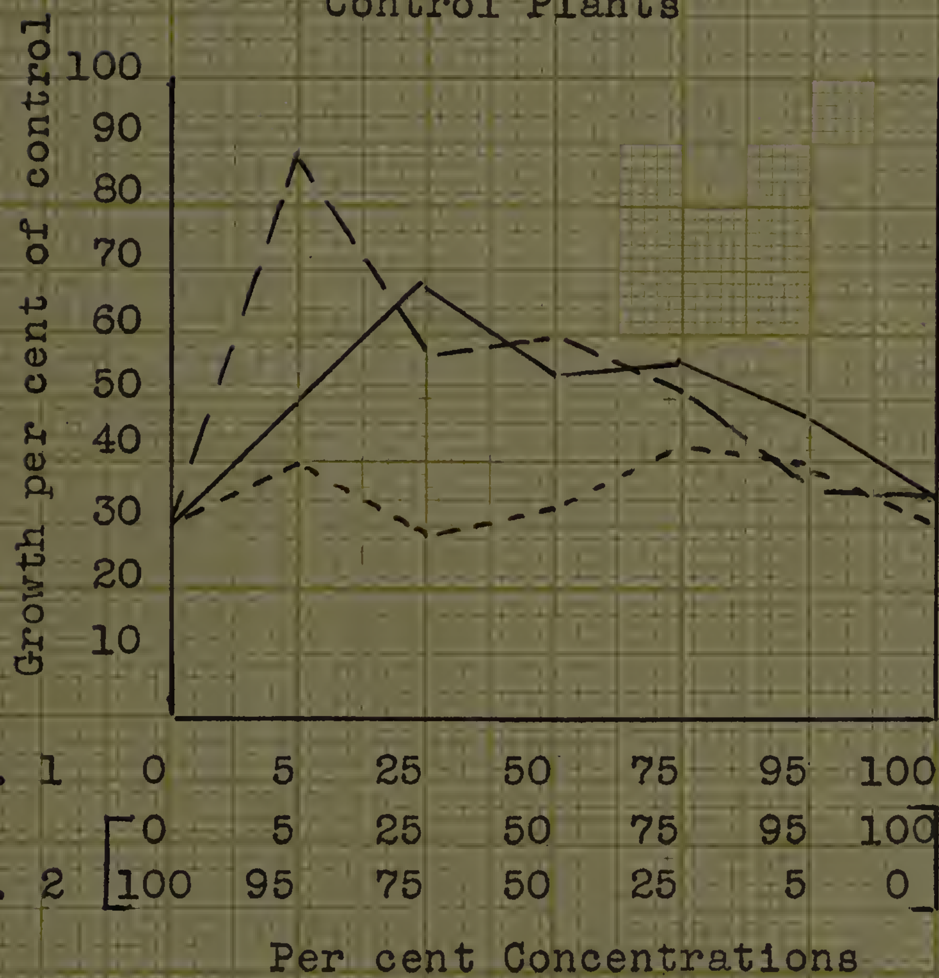
Under concentration and p p m all numbers reading left to right represents calcium nitrate

FIGURE II.

Soy Beans

Root Elongation

Control Plants



(a) ------- H_3BO_3

(b) - - - - $Ca(NO_3)_2$

(c) ——— $H_3BO_3 + Ca(NO_3)_2$

No. 1 represents the per cent concentration of single solutions of .0005M Boric Acid or .05M Calcium Nitrate.

No. 2 represents the per cent concentration of mixed solutions of .0005M Boric Acid plus .05M Calcium Nitrate.

In No. 2 Calcium Nitrate reads from 0 to 100 and Boric Acid reads from 100 to 0.

Soy bean seedlings grown in single solutions ranging from 5 to 95 per cent of .005M selenous acid showed similar characteristics as those grown in 5 to 95 per cent of .002M arsenic acid solution. The degree of arrest, however, was less than that of arsenic acid.

A 5 per cent of .005M selenous acid solution (Figure 3) indicates stimulation of growth, whereas at higher concentrations the curve continued to wane. Light brown dwarfed primary roots in higher concentrations with an accumulation of a jelly-like substance on the end indicates that selenous acid at high concentration is toxic to plant growth.

Table 3. The lateral roots had the same characteristics of the primary roots, but the degree of arrest was less pronounced near the surface of the solution. The stems also showed lesions similar to those appearing on the primary roots.

The greatest degree of arrest was found to be in the stock solution, .005M. This was approximately 5 p p m.

MIXTURES OF SELENOUS ACID AND CALCIUM NITRATE

Relative growth of soy bean seedlings is promoted by the medium concentration of calcium nitrate rather than by lower concentrations. The root system is more markedly stimulated by medium proportions, approximately 50 per cent of .05M calcium nitrate and 50 per cent of .005M selenous acid; calcium nitrate being 5,900 p p m and selenous acid 2.50 p p m.

From Figure 3 it is well indicated that high concentrations of calcium nitrate with low concentrations of selenous acid retards growth as compared with 50 per cent of .05M calcium nitrate and 50 per cent of .005M selenous acid in combination.

Mixed proportions, 50 per cent of .05M calcium nitrate and 50 per cent of .005M selenous acid, produced growth greater than that of the control (Knop's Solution equals 100 per cent).

Figure 3. The stems and primary roots with their well developed lateral roots and lustrous appearance, varying according to the different concentrations, showed no degree of physiological defects.

Plate 3, in Numbers 1 and 2, well exemplify the detoxifying effect calcium nitrate has in combination with selenous acid as compared with 3 and 4. Numbers 1 and 2 have 50 per cent of .05M calcium nitrate and 50 per cent of .005M selenous acid, whereas 3 and 4 have only 50 per cent of .005M selenous acid.

PLATE III

No. 1 & 2 Growth of soy bean seedlings in a mixed solution fifty per cent of .005M Selenous Acid and fifty per cent of .05M Calcium Nitrate

No. 3 & 4 Growth of soy bean seedlings in a single solution fifty per cent of .005M Selenous Acid.

TABLE III.

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTION OF SELENOUS ACID

No.	Percentage of .005M concentration	p H	p p m	Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1	0	-	-	22.2	30
2	5	6.0	.25	34.2	54
3	25	5.7	1.25	29.8	48
4	50	5.3	2.50	27.3	44
5	75	5.1	3.75	21.2	34
6	95	4.9	4.75	21.6	35
7	100	4.7	5.00	17.0	27

TABLE IIIa

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF SELENOUS ACID AND CALCIUM NITRATE

No.	Percentage of H_2SeO_3 .005M + $Ca(NO_3)_2$ .05M concentrations		pH	ppm		Average elongation of seedling roots in mm	Average Elongation of seedling roots based on per cent of control
1	0		-	-		22.2	30
2	0 +	100	4.8	0 +	5.00	17.0	27
3	5	95	5.0	950	4.75	28.5	46
4	25	75	5.5	2,950	3.75	60.1	96
5	50	50	6.2	5,900	2.50	63.7	102
6	75	25	5.8	8,850	1.25	50.2	80
7	95	5	6.0	11,210	0.25	52.1	83
8	100	0	4.7	11,800	0.0	22.8	34

Under concentration "6" represents distilled water.

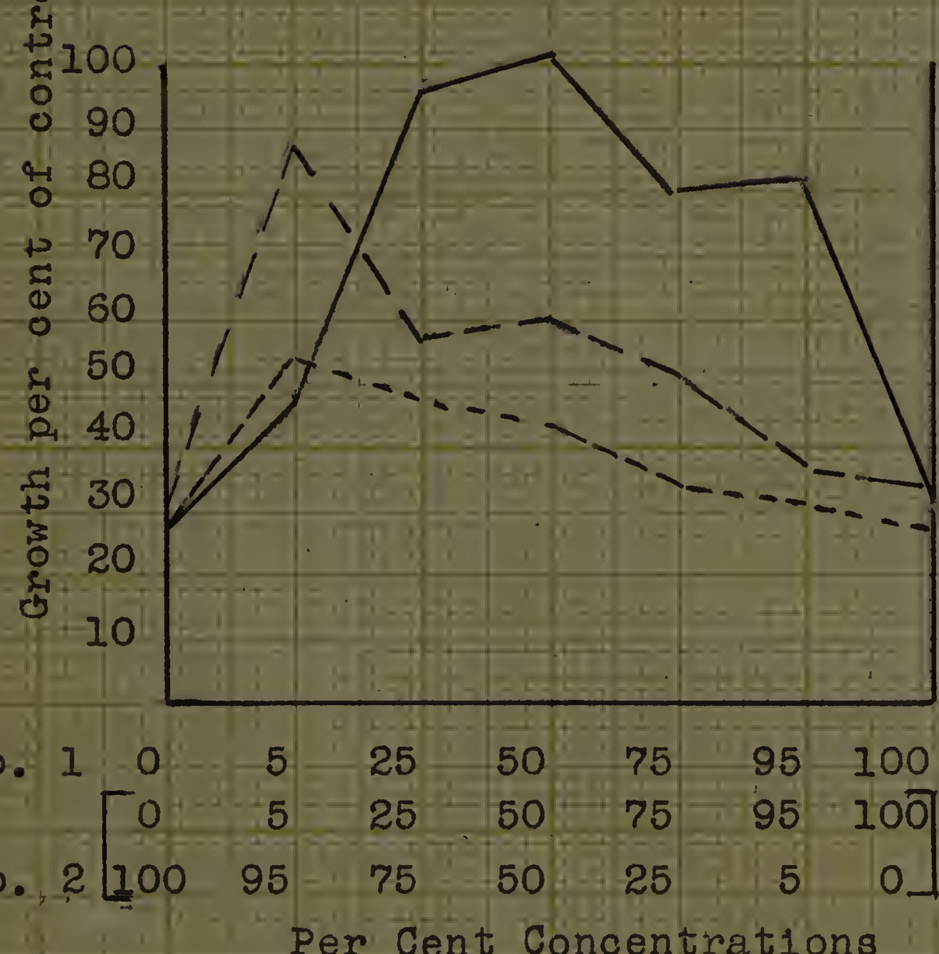
Under concentration and p p m. All numbers reading left represents Calcium Nitrate.

FIGURE III.

Soy Beans

Root Elongation

Control Plants



(a) ------- H_2SeO_3

(b) - - - - $Ca(NO_3)_2$

(c) ———— H_2SeO_3 + $Ca(NO_3)_2$

No. 1 represents the per cent concentration of single solutions of .005M Selenous Acid or .05M Calcium Nitrate.

No. 2 represents the per cent concentration of mixed solutions of .005M Selenous Acid plus .05M Calcium Nitrate.

In No. 2 Calcium Nitrates read from 0 to 100 and Selenous Acid reads from 100 to 0.

PLATE IV.

No. 1 Growth of soy bean seedlings in distilled water.

No. 2, 3, 4, 5, 6 and 7 Growth of soy bean seedlings in a single solution of 5, 25, 50, 75, 95, and 100 per cent of .05M. Calcium Nitrate.

No. 8 Growth of soy bean seedlings in Knop's solution which was used as a control.

TABLE IV.

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTION

OF CALCIUM NITRATE

No.	Percentage of .005M concentration	p H	p p m	Average elongation of seedling roots in m m	Average elongation of seedling roots based on per cent of control
1	0	-	-	22.2	30
2	5	6.0	590	64.2	89
3	25	6.10	2,950	41.1	57
4	50	6.12	5,900	43.3	60
5	75	6.15	8,850	37.6	52
6	95	6.17	11,210	26.8	37
7	100	5.8	11,800	22.8	34

SUMMARY

Arsenic, boric and selenous acid and calcium nitrate each at low concentrations stimulated plant growth as compared with that of distilled water, which indicated that distilled water is toxic to plant growth, and these elements at low concenttations overcame the toxicity of the distilled water. The stimulation is also attributed to the absorption and assimilation of the elements by the plants without any injurious effect to the plant tissues; but with increasing concentrations the elements are toxic to plant growth. The toxicity is due in part to absorption and assimulation, absorption and accumulation of the elements by the plants, thus causing incipient plasmolysis of the plant cells and tissues.

Calcium Nitrate solution mixed with arsenic boric and selenous acid solution in various molecular proportions,ameloriates the toxic effect of each element as compared with the single solutions. The cation of the calcium neutralized the toxic effect of arsenic boron and selenium to the extent that a degree of antagonism occurred, thus imparting bright color to the plants and increasing root elongation. Calcium Nitrate acted as

an antidote with arsenic, boric and selenous acid. Therefore it can be definitely stated that

(1) Arsenic, boric and selenous acids at low concentrations stimulate growth.

(2) Calcium nitrate at low concentration promotes growth.

(3) Arsenic, boric and selenous acids exert a distinct toxic effect on the growth of soy bean seedlings.

The toxicity increases with increased concentration.

(4) Calcium nitrate at high concentration is deleterious to soy bean seedlings.

(5) The toxicity of arsenic, boric and selenous acids could be very effectively overcome by calcium nitrate.

(6) More harm can be done by over-liming than by under-liming.

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