SHORT TITLE

EFFECTS OF CALCIUM AND BORON ON FLAX

By

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EFFECTS OF VARIOUS LEVELS OF CALCIUM AND BORON NUTRITION ON FLAX.

by

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A

Thesis

submitted to

THE FACULTY OF GRADUATE STUDIES AND RESEARCH

in partial fulfilment of

the requirement for

THE DEGREE OF MASTER OF SCIENCE

in

PLANT PATHOLOGY

McGill University,
Montreal, Quebec.

August 1966.

" Tout déséquilibre des éléments minéraux assimilables, existant ou apparaissant dans le sol, soit du fait de son origine, soit comme conséquence des exportations par les récoltes, soit par suite de nos apports d'engrais ainsi que tout autre cause, doit être corrigé par les apports voulus d'éléments fertilisants, de manière à rétablir l'équilibre optimum des éléments du sol, qui permet d'obtenir une haute qualité biologique de la plante, tout en obtenant le plus fort rendement compatible avec cette haute qualité biologique." A. Voisin,1964.

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ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to Dr. W.E. Sackston, Professor of Plant Pathology, Macdonald College, for suggesting the topic, and also for his enthusiastic guidance and ungruding help throughout the course of this investigation and in the preparation of the manuscript.

My sincere thanks are due also to Dr. E.O. Callen, who assisted me with his most helpful advise and criticism during the writing of this thesis; to Dr. R.L. Pelletier for his suggestions in carrying out some of the techniques; and to Mr. T. Boerboom, Technician in the Department of Plant Pathology who helped in many ways when his help was most needed. To J. Harwig and to C.A. St Pierre, fellow students, for their help in gas chromatography techniques and in carrying computation at the computer Center at Macdonald College.

I am also extremely grateful to the Quebec Agricultural Research Council for financial assistance in the form of a scholarship during the two years of my studies, and for the grant supporting this project.

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I. INTRODUCTION

About 25,000 acres of flax for fiber was grown during the second world war in the Province of Québec when the traditional sources of supply in Europe were cut off (Anonymous). With the armistice, the prices dropped and farmers abandoned this crop because of competition from European countries. In the 1950's a few farmers became interested in growing flax for linseed oil, chiefly in the Montreal area. The acreage has increased steadily, so that by 1964 flax was being grown on about 35,000 acres in this Province (Shiller 1964).

Fusarium wilt (Fusarium oxysporum var. lini. Bolley) has been reported repeatedly on flax in Quebec (Lachance 1951, Lachance and Cubana 1954, Lachance and Leblond 1954.) Rust (Melampsora lini (Ehrenb.) Lev.), a major disease elsewhere in North America, has not been reported from Quebec.

An apical blight or dieback was observed in a 14 acre field of the flax variety Marine, near Magog in 1962 (Laganière and Sackston 1965).

The symptoms most closely resembled those of boron and/or calcium deficiency as described by Millikan (1951) and by Wallace (1961), although they were not identical. Numerous soils of the Province of Quebec are known to be deficient either in calcium, or in boron, if not in both (Ouellette 1964a,b 1966). Therefore, it was concluded that the most probable cause of this apical blight or dieback was either a boron deficient condition, poor calcium nutrition, or a combination of both. This thesis reports on experiments planned to show the effects of different levels of calcium and boron in combination, on the growth of Marine flax in nutrient solution.

The investigations also include a study of the recovery of plants suffering from deficiency, the phenomenon of fasciation that occured at recovery from boron deficiency, and anatomical and histological studies on the effects of the different levels of the nutrients. This thesis also includes studies on the effect of boron and calcium levels on water utilization by flax plants, and on seed production.

II. REVIEW OF LITERATURE

Mineral elements have been known for a number of years to be essential nutrients and components of plants. Justus von Liebig, in formulating his mineral theory, was the first to give an analysis of plant constituants (Russell 1961). During the second half of the 19th century, Sach, Knop, Nobbe, and others determined the elements essential to plant life (Hewitt 1966, Russell 1966, Hewitt 1966, Jones 1926), but according to True (1922), Salm-Horstman must be credited with proving in 1856 that calcium is necessary for the growth of higher plants. Reed (1907) mentions that Wolf in 1868 found that calcium had a stimulating effect on root growth, and that Stohman in 1862 showed that the growth of calcium deficient of corn plants was stunted, and the growing point died. However, Stohman found that these plants recovered when returned to a solution containing calcium.

It is only many years later that the function of microelements was discovered. These elements are required in such small amounts, that until salt purification techniques were perfected, the microelements could not be separated from the salts of major elements. In 1916 Agulhon (Hewitt 1966) was the first to show the beneficial effect of boron on plant growth. Mazé (1919) showed that boron was required for plant growth, but it is Warrington (1923) who showed that it was essential for all normal plant growth.

The exact requirements in nutrient elements for most of our important agricultural plants, are still not completely known today.

In the case of flax most of the work has been done in Europe, especially Russia, and Australia.

Flax was one of the first plants used in boron research. Somme and Lipman (1926) was the first to show that boron was indispensable to the growth of flax. Shkol'nik (1934) found that 0.8 to 1.6 mg of boron per kg of soil increased the yield by 21-22% (dry weight basis), and stated that boron is not only a stimulant, but also an essential element for flax growth.

Peive and Andrianova (1938) and Komarov (1939) were the first to state that boron deficiency in soil caused bacteriosis in flax and that boron fertilization increased yield.

Conducting nutrient experiments with minor elements, Bottini (1950) found that boron fertilization increased the yield of flax and induced increase in size of leaves or fruits, with a tendency to promote the absorption of mineral substances from the soil.

By her experiments in greenhouse and field, Mikhailova (1953) found that boron increased the yield and fibre content of the straw. The greater effects were in fields with soil near neutral in reaction and with a high calcium-humate content, and the least effects in sod-podzolic and peat-gley soils. She also stated that in dry soils, flax has a particularly high boron requirement, and that unless boron is applied, the plants become diseased. Fertilization with microelements, including boron, can be done by different methods.

Shcherbakov (1956) found that flax seeds treated with a mist of microelement solution consisting of : 0.02% $CuSO_4$, 0.65% H_3BO_3 ,

$MnSO_4$, and 0.05% $(NH_4)_6Mo_7O_{24}$ and planted in podzolic clay soil (pH 6.0) produced plants with higher grain and fibre yields; copper and boron increased the stem weight by 11-26% and seed yield by 9-20% ; boron, copper and zinc increased the proportion of long fibre by 52-58%.

Gubar (1956) reported that in carbonate-containing and podzolic clay soils, and in low-and high-humus peat soils, the total weight of flax was increased by 11-48% and fibre yield by 0.4-1.8% as a result of fertilization with P+K or N+P+K with 0.5 kilogram of boron per hectare. Boron alone increased yield only in neutral soils of high calcium-humate content.

Seed and soil treatment with manganese, boron, and molybdenum are reported by Smalik (1959) as increasing the oil concentration in flax grown in mountainous regions. Steklova (1959) found that boron fertilization increased the yield of seed in flax on high-lime soils, and stated that symptoms of boron deficiency observed in plants in high-lime soils are caused by the higher requirement of boron by the plants concerned.

Paribok (1958) in a complex experiment studied the supply and distribution of boron, molybdenum and manganese in flax and wheat during the vegetative growth as well as during flowering and maturation. He found that boron is most intensively absorbed before flowering time. Flax leaves were marked by the highest content of all microelements and flax seeds by the lowest. When boron and molybdenum were in increased doses in fertilizers, more of those

elements were accumulated in leaves, while they were stable in the seeds. In flax, 47% of the total content in boron was found in capsules and seeds.

Komarov (1938) was the first to describe the effects of boron deficiency in soil on flax. He mentioned that flax plants were shorter than normal, that the root system was reduced, and that there was decreased seed production. The absence of boron was reported by Shkol'nik (1933) to induce a poorly developed root system followed by the death of the plant. A complete description of the symptoms of boron deficiency on flax was given by Millikan (1951). Flax plants grown without boron develop very early chlorosis of the leaf margin which gradually spreads inwards to the midrib, while the lamina acquires a peculiar billowy curvature. The tops of the plants show a whitish-brown discoloration and die off. Shoots of boron deficient plants may ultimately die back almost to the seedling leaves. On the roots boron deficiency causes a swelling of the root tip, the growth is checked, and the root dies. Neales (1960) reported that in culture solution, the absence of boron reduced the total linear growth of flax radicles.

Millikan (1948) described the symptoms of boron toxicity on flax. The leaves in the middle portion of the stem are first affected, while youngest leaves are last to show the symptoms. These symptoms consist of greyish green transparent discolorations which commence at the tip of leaves, and gradually extend downwards until the whole leaf is dead. He also noted that no yellowing of the top Leaves occured.

By studying qualitative and quantitative analyses of chromatograms of ethanol extracts of stems, leaves, and roots of flax grown in nutrient solution with increasing quantities, (0-1.0 ppm) of boron, Neales (1959[b]) showed that boron deficiency depressed the sucrose, but not the glucose or fructose of stem tips. The stems and leaves of boron deficient plants contained more of the three sugars than the normal plants, and the roots of boron deficient plants more glucose and fructose.

According to Shkol'nik and Solov'eva-Troitskaya (1962) the addition of nucleic acid can partially eliminate the boron deficiency symptoms at low temperature, but not at high temperature. The author suggested that the increase of boron requirement of plants at high temperature is associated with the beneficial effects of boron on energy metabolism.

Jensen (1951) in his studies on the effects of boron on germination of flax seeds, and on respiration of seedlings, concluded that a concentration above 10^{-6}M and 10^{-8}M of boron lowered the rate of germination of flax seeds, and noted a constant depression of respiration of the seedlings at 10^{-8}M , 10^{-6}M , 10^{-4}M and 10^{-2}M , in comparison with the control.

In 1959[a] Neales found that the requirement of excised flax roots, grown in sterile culture, is satisfied by 0.05 ppm boron. The boron requirement of excised roots is similar to that of whole three week old flax seedlings.

Boron was found by Talybly (1935) to be beneficial to flax (greenhouse experiments) in counteracting overliming, and Shcherbakov

(1956) found that increased assimilation of calcium, magdnesium and phosphoric acid resulted from boron fertilization. Studies on liming of flax by Kataljmov (1935) showed that harmful effects of lime on flax can be corrected by boron. Truninger (1938) arrived at the same conclusion. Kostiuchenko (1938) found that liming had a negative effect on flax on podzolized soils when the soil moisture was insufficient. In the presence of sufficient moisture even large amounts of lime did not have a bad effect on flax. Boron prevents the harmful effect of liming by retarding the entrace of calcium into the flax plant. Yarusov (1940) discussed the harmful effect of lime on the yields of flax seed and fiber, and the power of boron to counteract this effect.

Jouis, Decacheux and Gauchy (1960) reported that boron deficiency in Caux region occured in patches in fields. In these patches, flax was stunted and turned yellowish-brown in color. The leaves turned yellowish or cholorotic and brownish at the growing point, and the growing point died. The plants recovered when a rainy period followed a drought period. Recovery was due to growth of axillary buds. The boron deficient patches were mainly on soil of ph 7.0 or higher, and were correlated with application of marl.

The first report concerning "withertop" (calcium deficiency) was made by Millikan (1944). He mentioned that when flax plants are under severe calcium deficiency condition, the plants did not survive the seedling stage. By using nutrient experiments, he found that

flax suffering calcium deficiency or showing "withertop" symptoms can recover when transferred to solution containing normal calcium level. He also described the symptoms of calcium deficiency on the flax as a sharp bending, approximately 2-4 inches below the tip of the plant. This part of the plant hangs vertically, soon loses its rigidity, turns brownish and dies. Among causes of calcium deficiency, water logged condition of the soil are reported by Millikan (1944); and overdosage of sodium, especially in combination with high potassium, induced calcium deficiency (wilting apex) and resulting lower grain yield in flax, according to Lehr and Wybenga (1955).

III. MATERIAL AND METHODS

A. SEEDS

The commercial flax seeds used in these studies, were of the variety "Marine", and were supplied by the Canada Linseed Oil Company of Montreal. This material showed a very high percentage of germination.

B. NUTRIENT SOLUTION

For the purpose of this work, it was found that modifications of the Hoagland and Arnon (1950) and McIlrath and De Bruyn (1956) nutrient solutions were most suitable. However, it should be noted that the concentration of minor elements is quite uniform in the solutions used by these workers. The differences are in the concentrations of the major nutrients and in the use of iron-EDTA as an iron source instead of iron citrate or some other iron salt.

The three different levels of boron were used at each of three levels of calcium. The nine combinations were as follows (Table 1).

Table 1. Boron and calcium levels of nutrient solutions

Code	Combination	Parts per million Ca	B
CaL BL	Calcium low, Boron low	40	0.0
CaL BN	Calcium low, Boron normal	40	0.5
CaL BH	Calcium low, Boron high	40	2.5
CaN BL	Calcium normal, Boron low	200	0.0
CaN BN	Calcium normal, Boron normal	200	0.5
CaN BH	Calcium Normal, Boron high	200	2.5
CaH BL	Calcium high, Boron low	480	0.0
CaH BN	Calcium high, Boron normal	480	0.5
CaH BH	Calcium high, Boron high	480	2.5

The major element composition of the solutions for each of the three levels of calcium was as follows:

Calcium levels	Millimolar concentration					
	KNO_3	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$CaCl_2$	$NaNO_3$
40 ppm	6.0	1.0	2.0	1.0	--	6.0
200 ppm	6.0	5.0	2.0	1.0	--	---
480 ppm	6.0	4.0	2.0	1.0	8.0	---

Micronutrients were supplied as follows:

$MnCl_2$ . . . . . 0.5 ppm Mn.

$CuSO_4$ . . . . . 0.02 ppm Cu.

$ZnSO_4$ . . . . . 0.05 ppm Zn.

$Na_2MoO_4.2H_2O$ . . . . . 0.01 ppm Mo.

Stock solutions of the major nutrients salts were at molar concentration, and micronutrients at 1000 times the concentration used in the final nutrient solution. The stock solutions were stored in natural polyethylene bottles in the dark.

All solutions were made of salts grade of "Baker Analysed" grade by Canadian Laboratory Supplies Limited, which is the highest grade of purity. Distilled water used in stock solutions, as well as all distilled water used during the experiments, was produced in a stainless steel still, and kept in a natural polyethylene bottle. According to Neales (1959)[b] this kind of still gives the purest distilled water, and must be used in such experiments in plant nutrition as have been carried out here.

The containers used were filled with 850 ml of nutrient solution that was renewed twice a week. The level of the solution was 2 cm from the top of the container, to permit aeration of the solution by bubbling. When it was necessary, measured quantities of distilled water were added daily to maintain the volume of the solution.

A vacuum/pressure pump "little giant model 13152" made by Gelman Instrument Company, was used to blow air into the solution. It was arranged that the air flowed from the pump through a rubber tubing line to a 3-way valve, from which air was conducted to one container and on to the next valve by further rubber tubing (Fig. 3). Fig. 4 is a view of the arrangement for an individual container. The air coming from the main line was conducted from the 3-way valve, by a short length of rubber tubing to a plastic straw in the container. Plastic straw was used instead of glass piping to avoid boron contamination from glass. Plants were aerated for 3 hours daily, from 9 to 12 am.

C. CONTAINERS

The use of glass, enamel and metal containers presented some difficulties because of possible contamination. However, for a number of years polyethylene containers have been used by such workers as Odhoff (1957), and Shkol'nick, Krupnikova and Dmitrieva (1964). Since

polyethylene containers are easy to clean, unbreakable, relatively cheap, and contain only rare traces of metal, they were ideal for microelement work, and were therefore used throughout the experiments reported here.

Millikan (1948), and Millikan (1947) used 10 or 12 plants per 2 litre container in nutrient experiments with flax, therefore, 5 plants per 1 litre container were used in a preliminary experiment. As this gave good results, the same number of plants was used in all the other experiments.

To prepare the containers for the experiments, they were twice washed with hot water and detergent, rinsed 3 times with tap water, and then 3 times with distilled water (Pelletier 1965). As the containers are translucent, they were wrapped in aluminum foil, to avoid the growth of algae in the nutrient solution, and in order to keep off heat from the sun's rays.

For each container a 13 x 13 cm piece of "Masonite" hard-board, impregnated with melted paraffin wax was used to support the plants. Each piece was cut and trimmed, and 7 holes drilled in it, 5 for plants, 1 for stake, and 1 for the aerating system. Fig. 5 shows a Masonite support with plants in position.

D. PLANT SEEDLINGS

Since it had been decided to use seedlings as soon as possible after germination (Millikan 1948), the flax seeds

were germinated on moist filter paper in plastic petridishes. However, their tap root was too short to reach down into the containers. In a second attempt, the seeds were germinated on pure quartz sand, but the results were still unsatisfactory, as the roots were only 2 cm long, even after 4 days growth. Since the level of the nutrient solution was 2 cm from the top of the container in order to permit aeration by bubbling, some other way of germinating the seeds had to be devised.

To solve this problem, modifications of the Hoagland and Broyer (1936) method were tried. Instead of a metallic screen being used, cheese cloth was tied firmly over an enamel dish, with its edges floating in distilled water. This was to avoid contamination from the metallic screen (Millikan 1947, Hick and Grossa-Brauckmann 1964). This method was very satisfactory, as the four-day old seedlings developed roots 5 to 8 cm long. Fig. 1 is a diagramatic representation of the apparatus used, and fig. 2 shows the difference in root length of seedlings germinated on moist filter paper and on the apparatus developed.(Fig. 3).

The plastic flat and the enamel dish were washed twice with detergent, rinsed 3 times with tap water, finally 3 times with distilled water. The cheese cloth was treated in the same manner, and was then tied tightly around the edge of the enamel dish. The dish was then placed in the plastic flat, and both were filled with distilled water. However, in the dish, the surface of the water was not directly in contact with the cheese

cloth. The seeds on the cheese cloth received enough moisture from the edges of the cloth dipping into the water of the flat. To check evaporation, the whole apparatus was covered with a polyethylene sheet but with enough ventilation from the sides to aerate.

E STAINING TECHNIQUES

Material from plants growing under the different treatments was collected during the course of the experiments. These tissues were fixed and stored in FAA (formalin, Acetic Acid, Alcohol) (Sass 1964). The tissues were dehydrated by the n-Butyl alcohol method (Sass 1964). Paraffin wax (mp 53°C) was used for embedding, and sections cut on a rotary microtome at 9 µ. The sections, as a ribbon, were floated in a water bath thermostatically controlled at 40°C. Glass slides were immersed in the bath, and carried up under the sections which floated onto the glass slides. The sections were fixed to the glass slides by allowing them to dry on a warm plate (40°C) over night. The staining was done according to Gurr (1965) with Safranine O alcoholic and Fast Green F. C. C.. The stained sections were mounted in D.P.X. (a British Drug House product).

F EXPERIMENTAL CONDITIONS AND DESIGN

Three complete experiments were performed. All experiments were conducted in a greenhouse kept at 21°C, but the temperature went appreciably higher on sunny days, sometimes reaching

30°C for short intervals. Daylength was maintained at 15 hours (average summer daylength). Artificial light was provided by cool white fluorescents to extend daylength in the winter months, and to maintain light intensity at 700 ft-c on dull days. Light intensity on sunny days went up to 5000 ft-c.

All experiments were conducted in duplicate, with five plants in each replicate container at each nutrient level. The various treatments were randomized.

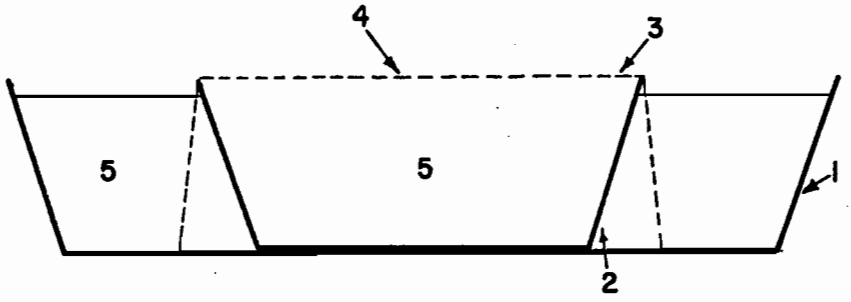
1. The first experiment was conducted as follows: 4-day old seedlings were subjected to the nine treatments described in table 1. After 33 days, the plants subjected to boron deficient treatments were changed to nutrient solution with normal levels of calcium and boron. Plants under CaL BN and CaL BH treatments were transferred to normal solutions after 51 days. The other treatments were kept as they were till the end of the experiment, i.e. for 112 days.

2. In the second experiment, seedlings were grown for one month in a complete nutrient solution, before being subjected to the different treatments for a further month. There after, plants in low calcium and low boron treatments were returned to normal solutions. Treatments combining both these elements at normal and excess levels were unchanged until the experiment terminated at the end of 118 days.

3. In the third experiment all seedlings were allowed to grow in a CaL BL treatment for 40 days, and were then

transferred to treatments combining low, normal and high levels of calcium with normal and high levels of boron. Sixty five days later, CaL BN and CaL BH treatments were changed to normal. The other treatments remained the same till the experiment was terminated at the end of 137 days.

Fig. 1. Diagramatic representation of apparatus developed



1. Plastic flat: 22 x 10 1/2 x 2 inches.
2. Enamel dish: 15 x 10 x 2 inches.
3. Two layers of cheese cloth.
4. Place for seeds.
5. Distilled water.

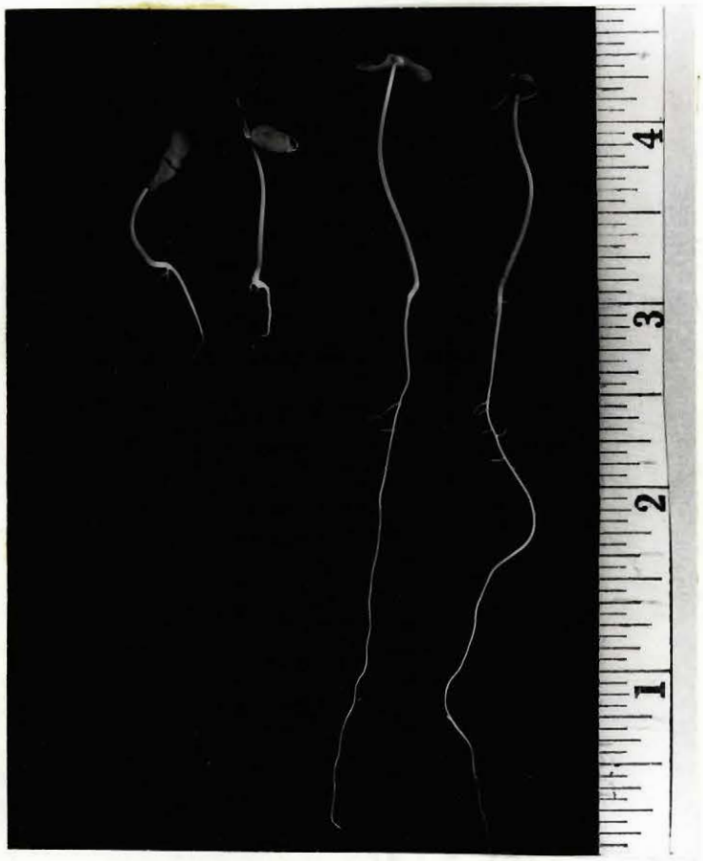


Fig. 2. Seedlings root length after 4 days: (A) in petri dish with moist filter paper, (B) On cheese cloth stretched over water.

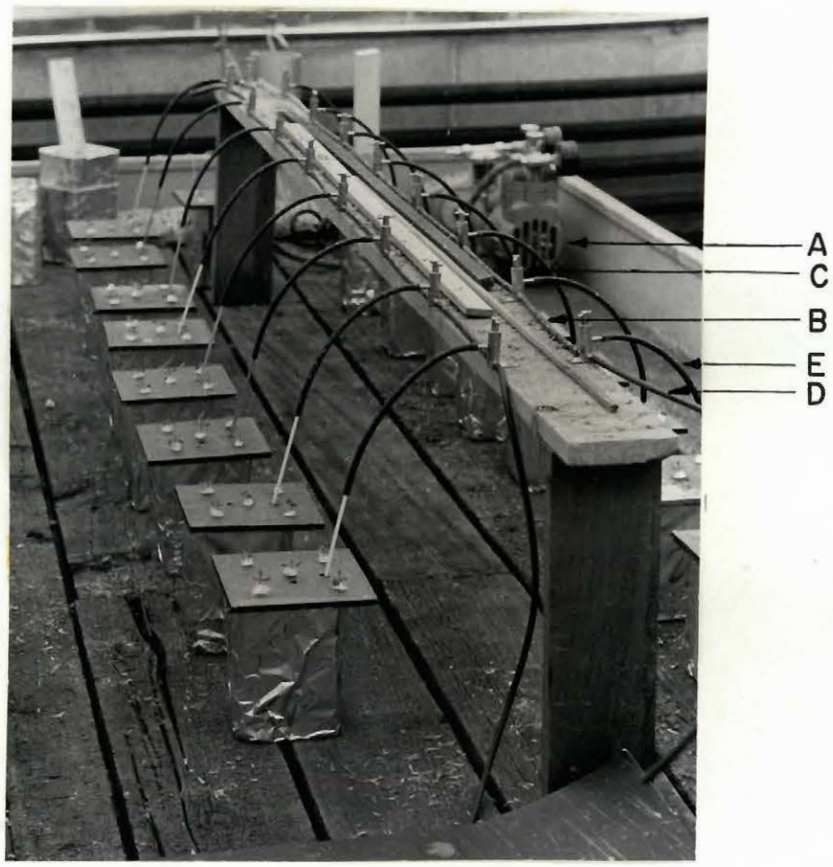


Fig. 3. General view of apparatus: (A) air compressor, (B) Main line for air distribution, (C) 3-way-valve, (D) main line for supplying next set-up, (E) secondary line delivering air to individual container.

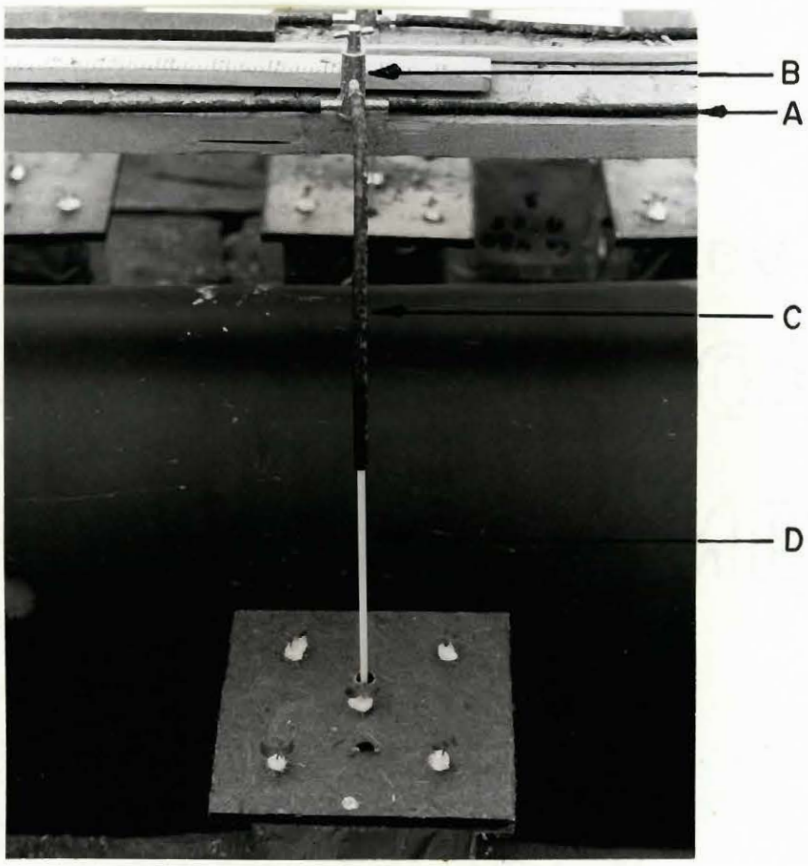


Fig. 4. Arrangement for aeration of each container: (a) main air line, (b) 3-way valve, (c) connective rubber tubing, (d) plastic straw entering container.

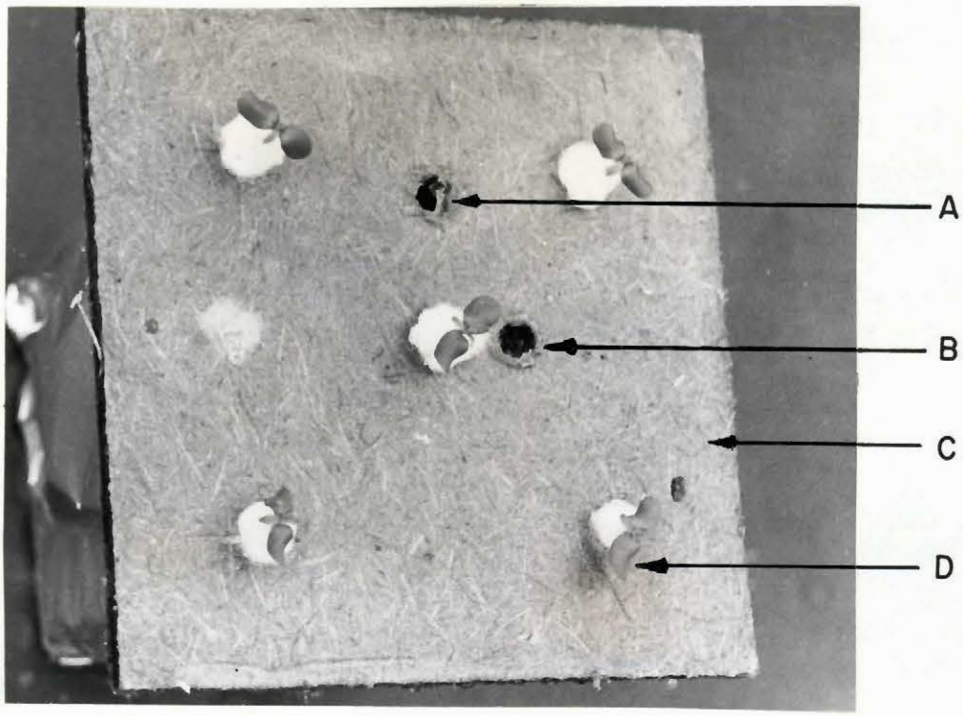


Fig. 5. Container with its 5 seedling flax plants: (a) hole for stake, (b) hole to receive plastic straw for aeration, (c) 5" x 5" piece of "Masonite" hardboard impregnated with paraffin was, (d) flax seedlings with first true leaves.

IV. RESULTS

A. BORON DEFICIENCY

1. General symptoms

In the first as well as the third experiment, 4-day old seedlings were transferred to boron deficient condition (0.0 ppm boron) from the very beginning. The first symptoms occurred 21 days later. The level of calcium in the solution did not make any difference.

a. On stem

The first sign of boron deficiency in flax is the unfolding of the leaves of the tip just as if they were petals of a flower (Fig. 6). Later these leaves became paler green and gradually turned yellow, the growing point died, and the stem tip collapsed (Fig. 7). The mid and marginal veins turned darker taking on a brownish-red color. In a few days, the leaves had dried out, and had turned a pale brown, but the veins still remained reddish (Fig. 8). The chlorotic and dried leaves had a tendency to curve (Fig. 9). The yellowing of the leaves spread gradually down onto the stem (Fig. 10). When the first symptoms developed at the tip of the main stem, the buds in the leaf axils developed into short shoots at almost every node (Fig. 11). This phenomenon started first at the cotyledon level (Fig. 12). On the middle part of the stem, these shoots seem to grow more slowly (Fig. 11), but none of those shoots grew for long since the tip dried up just as with the main stem (Fig. 13).

The only effects observed between different calcium levels with boron deficiency, was that on some plants grown with a high level of calcium, the side shoots grew quite long (4 -5 inches) before the growing tip died (Fig. 14), while under normal and low levels of calcium they did not grow longer than 1 - 2 inches.

b. On roots

Flax plants grown in boron deficient conditions, whatever the calcium level was, showed a poorly developed root system as compared with the controls (Fig. 15). These roots failed to develop in length; instead they swelled up and took on a brownish color. The tips of those roots enlarged, turned dark, became necrotic and died (Fig. 16). Further back along the root, secondary roots started to develop, but they just died as did the primary root. On the secondary root, tertiary roots started to grow as shown in Fig. 17.

2 EFFECTS OF CALCIUM LEVELS

In the second experiment, flax plants were grown for one month in a normal or complete nutrient solution, before the boron deficiency treatments were started with the different levels of calcium.

a) In CaL BL treatment

The first symptoms were observed 18 days after the beginning of the treatment. At first, about 1 inch (2-3 cm) of the tip of the stem bent over and hung vertically. On the bent portion the leaves looked healthy when the bending occurred, but later, turned dark green and then greyish green in color. Meanwhile the leaves below the bending point gradually turned yellow progressively on the stem. Later the leaves on the bent portion of the stem turned brown, but the leaves just at the growing point remained greyish green. Leaves on the first two inches of the stem below the bent tip turned brown at the base at the point of attachment to the stem (Fig. 18).

Excessive growth or shooting of the axillary buds was observed almost exclusively at the cotyledon level. They grew for about 15 days after the first symptoms occurred. Shooting of the buds on the middle and upper part of the stem stopped when the shoots were only a few mm long.

b. CaN BL treatment

The symptoms developed 4 days later than with CaL BL, i.e. 22 days after starting the treatment. These symptoms are about the same as those observed in the first experiment (Fig. 6).

c. In CaH BL treatments

The first symptoms developed 2 days later than with the CaL BL treatment, i.e. 20 days after, and were the same as those of the first experiment. It was noted, however, that shooting of the axillary buds occurred at the same time as the first symptoms on the main stem. The tillers showed the same symptoms as the main stem, and at the same time.

It should be noted that in the above experiments, the death of the growing point occurred just as the first flower buds were being formed. The dying back of the tip was actually very slow, about two inches in one month. There was a clear cut demarkation between the yellow and the green leaves with the high calcium level, but under low and normal calcium levels there was a certain progression in the yellowing.

The side shoots that developed from the axillary buds grew well, some being up to 4-5 inches long (Fig. 14).

Fig. 6. Early symptoms of boron deficiency on flax stem: note tip leaves opened, lighter green upper most leaf tips dried up.

Fig. 7. Dead tip of boron deficient flax plant: note tip necrotic, leaves yellow to light brown.

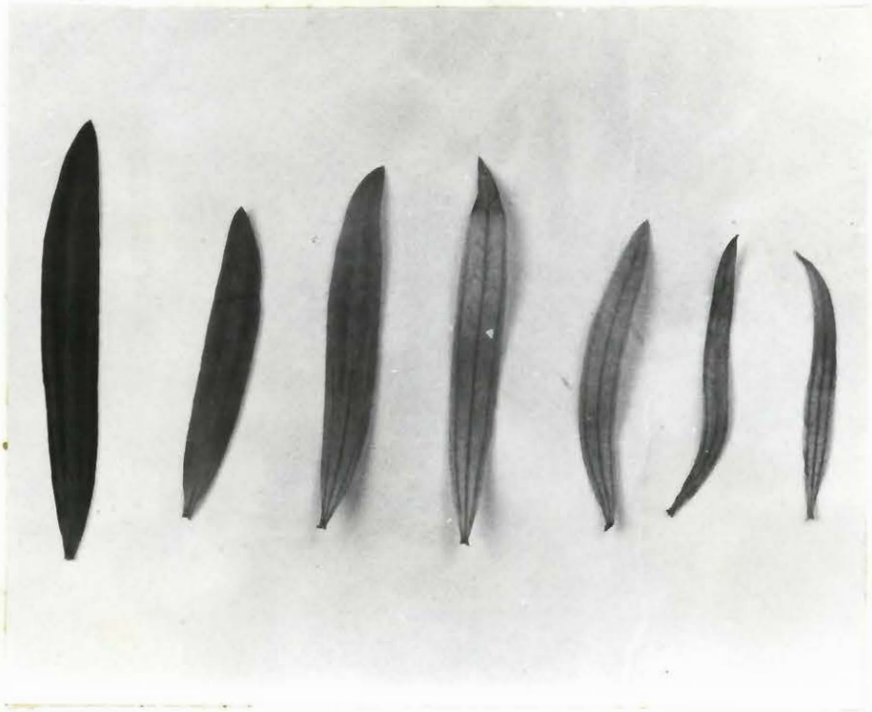


Fig. 8. Leaves from base to tip (left to right) of boron deficient plant: note gradual yellowing, then browning with reddening of veins.

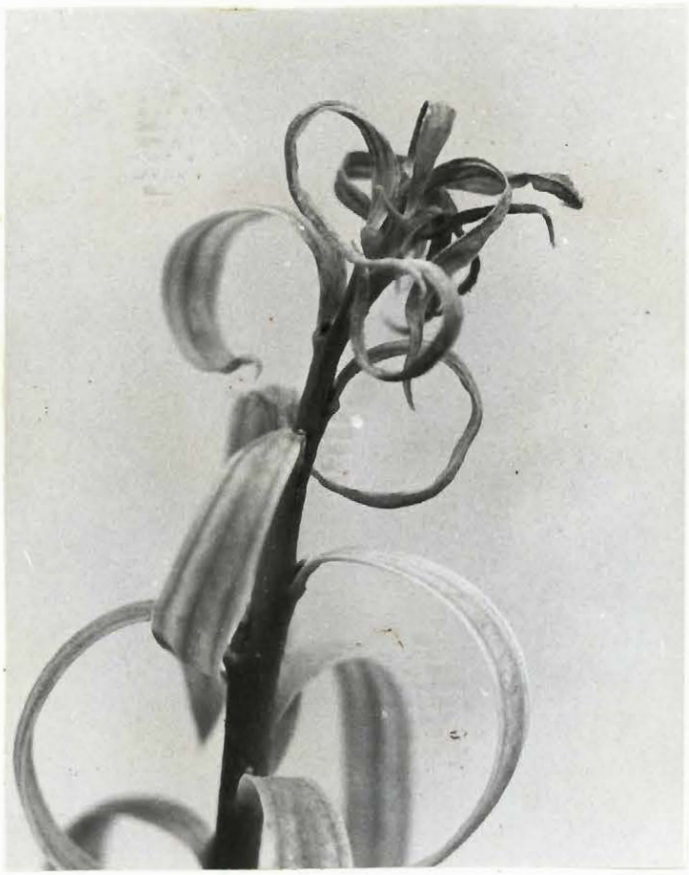


Fig. 9. Tip of flax plant in boron deficient conditions: note rolling of chlorotic and dried leaves.

Fig. 10. Shoots forming in leaf axils of boron deficient plant.

Fig. 11. Axillary shoots on boron deficient plants: note better developed at cotyledon level, Basal shoots called tillers.

Fig. 12. Numerous shoots at cotyledon level of boron deficient plant.

Fig. 13. Tillers of boron deficient flax plant: note dead growing points.

Fig. 14. Boron deficient flax plant grown with high calcium level: note 4-5 inch long shoots.

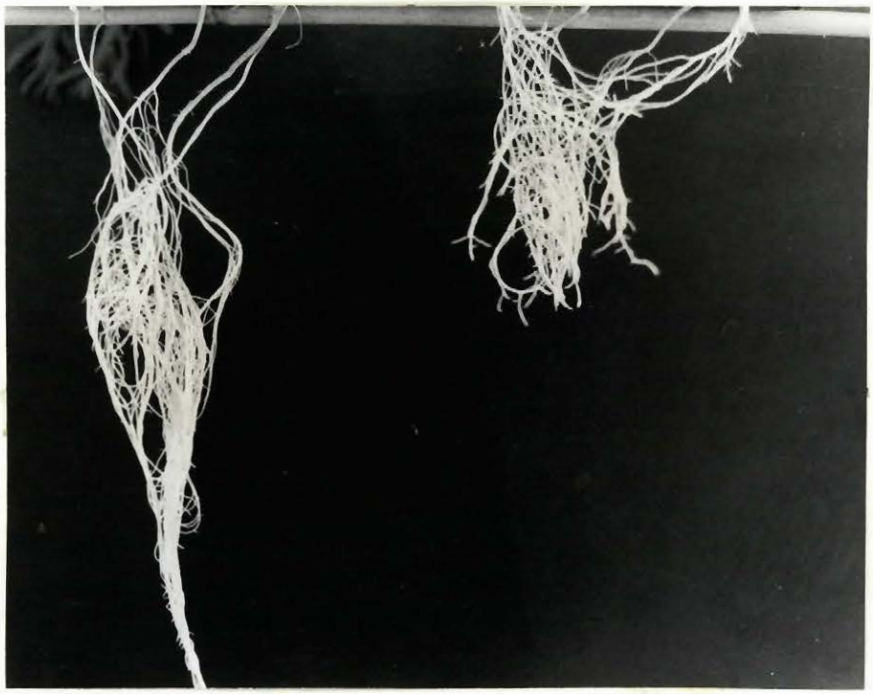


Fig. 15. Flax root system: left normal, right boron deficient plant with shorter swollen roots.

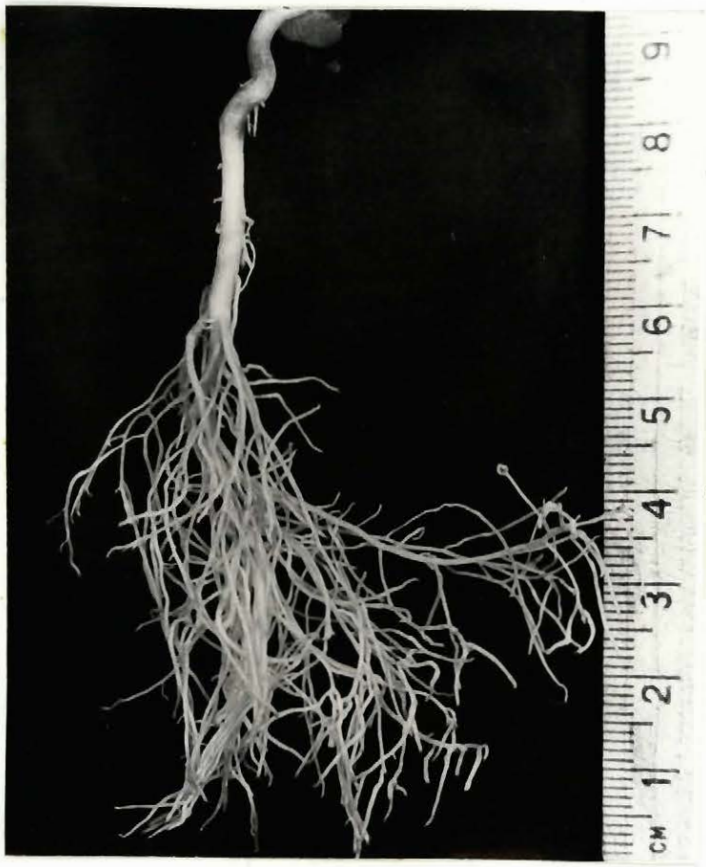


Fig. 16. Roots of boron deficient flax plant: note profuse branching.

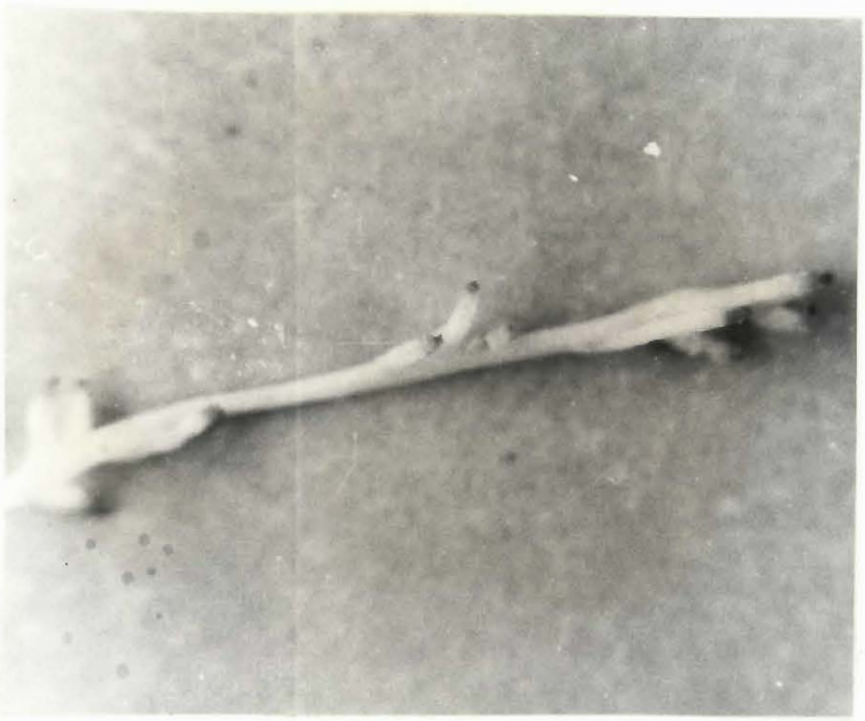


Fig. 17. Flax plant root, showing dead necrotic tips resulting from growth under boron deficient conditions. Note secondary growth.

Fig. 18. Boron deficiency symptoms on flax plant grown first in complete solution for one month before CaL BL treatment. Note bent part and yellow leaves.

3 HISTOLOGICAL EFFECT

a) Stem

In addition to describing the external symptoms, sections of boron deficient plants were examined. Longitudinal sections of the dead tip of flax plant, showed that the cells of the meristems were disorganized, the last leaf primordia were necrotic, and that the cell walls of many cells were disintegrating (Fig. 19). A section of normal stem tip showed (Fig. 20) that leaf primordia were formed of well organized cells and that procambium was present. In boron deficient plants the procambium was not visible in the meristematic region.

b) Root

A longitudinal section through the root tip of a boron deficient flax plant showed numerous secondary root primordia (Fig. 21). The primary root tip was necrotic, with thick epidermal cell wall, and disorganized cells in the meristematic region. Some of the root primordia aborted (Fig. 21). In some cases, these secondary root primordia were developing very close together (Fig. 22). Two days after the transfer of boron deficient plants to complete nutrient solution, normal root primordia were developing on the boron deficient roots (Fig. 23).

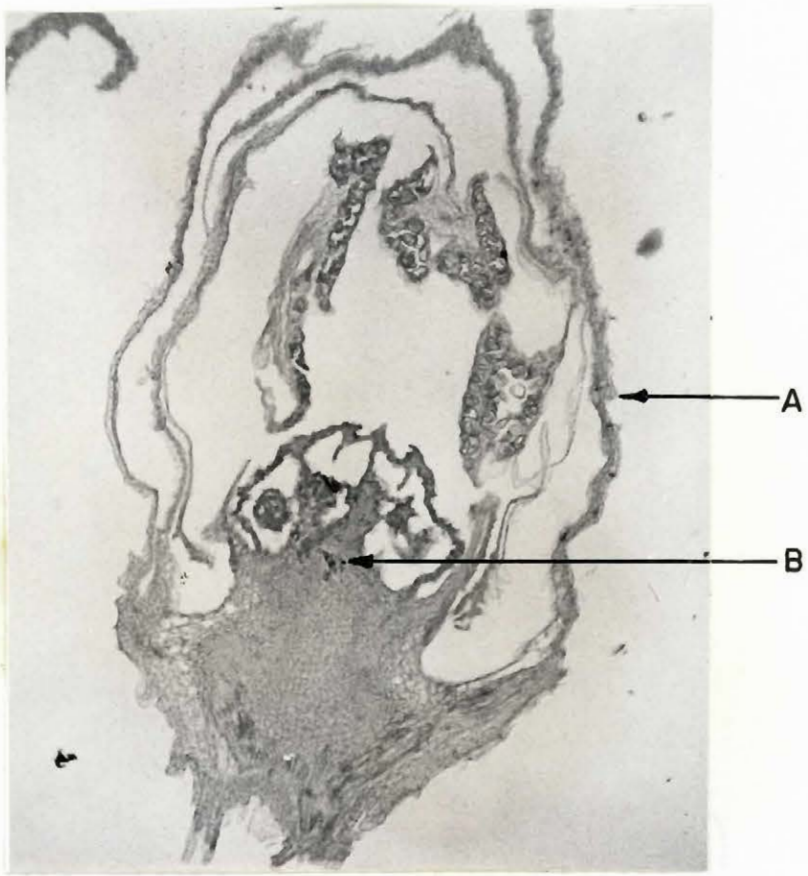


Fig. 19. Stem tip of flax plant grown in boron deficient conditions: note (a) necrosis of leaves, (b) the complete disorganization of cells of the apical meristem. (180 X.)

Fig. 20. Stem tip of flax plant in complete nutrient solution: no bud primordia are present. (400 X.)

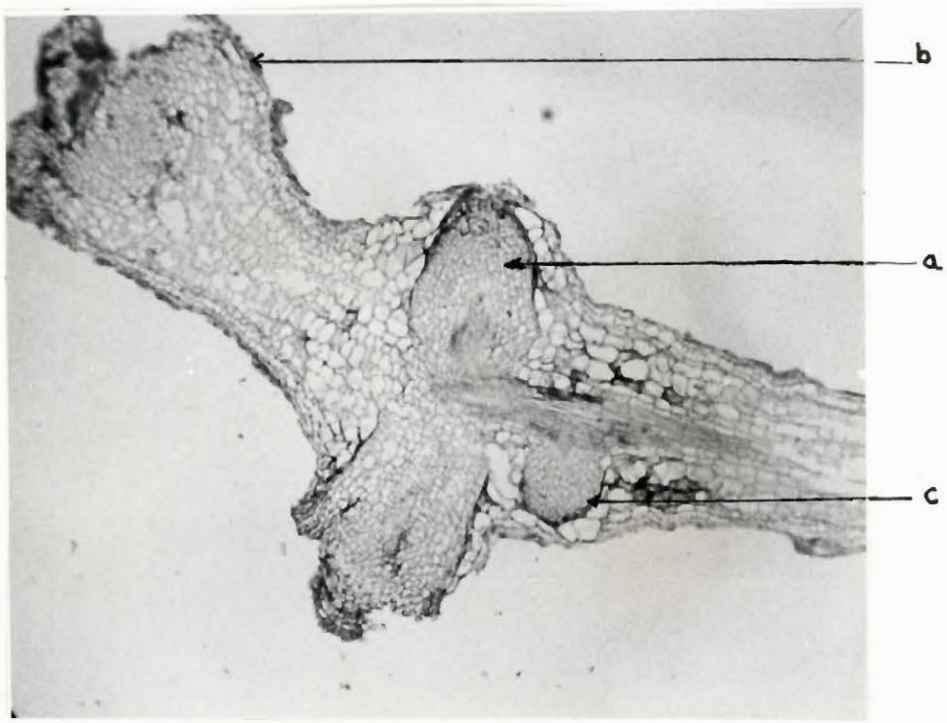


Fig. 21. Longitudinal section of root tip of flax plant in boron deficiency solution. Note (a) the abundance of secondary root primordia, (b) the necrotic root tip, (c) abortive root primordia. (185 X.)

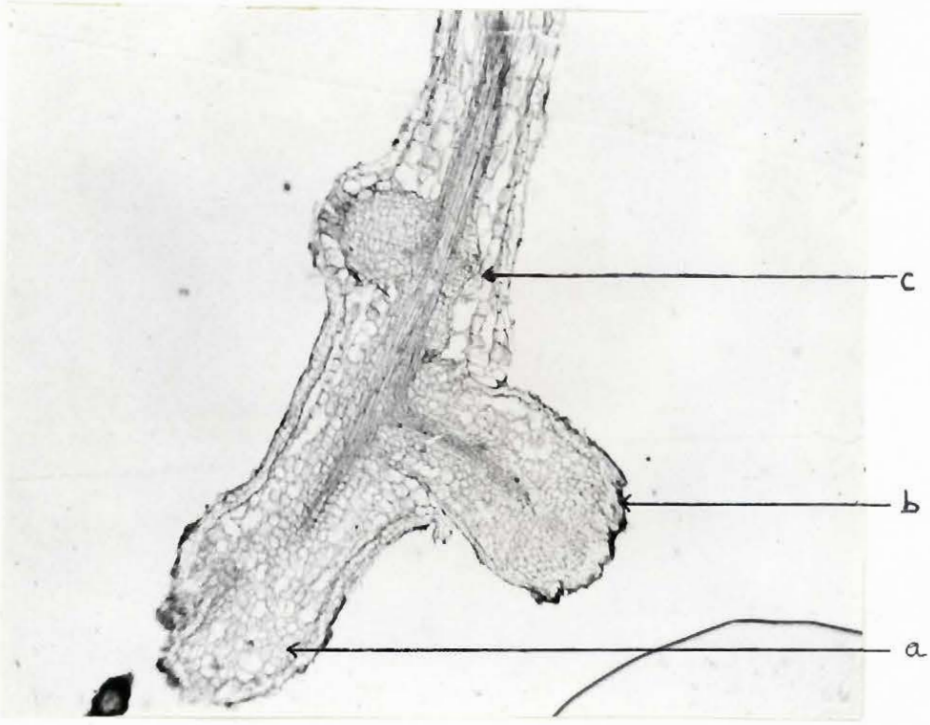


Fig. 22. Longitudinal section of root tip of flax grown in boron deficient solution. Note (a) the necrosis of the original root tip, (b) necrosis on tip of secondary root, (c) two root primordia developing close together. (180 X.)

4. RECOVERY FROM BORON DEFICIENCY

Boron deficiency symptoms could be induced on flax plants without any great difficulty. After one month they were stunted, and if the treatment was continued, the plant died eventually. In an attempt to find out if plants suffering from boron deficient conditions could recover from this condition, they were transferred back to a complete nutrient solution.

a. Results

(a) On the stem

Figure 24 shows a flax plant before being transferred to the complete nutrient solution. One week later, new healthy shoots were growing out along the stem, but mostly at the cotyledon level, the level at which the tillers grow (Fig. 25). These new shoots developed normally, had flowers and set seed.

However, in the first experiment, 2 weeks after the transfer to normal conditions, it was observed that some of those shoots flattened and produced a bunch of leaves at their tip (Fig. 26). This is the phenomenon of fasciation.

(b) On the roots

Roots of boron deficient flax plants were light brown in color, with a necrotic tip (Fig. 17). One week after the transfer to a complete nutrient solution, new roots developed. These roots were white, long and narrow, i.e. quite normal, (Fig. 27). These new roots were developing from primordia on the

necrotic roots (Fig. 28).

4. FASCIATION

Since the appearance of fasciation in plants recovered from boron deficiency was quite unexpected, a search of the literature was conducted to find out which conditions are believed to lead to fasciation.

a. Review of fasciation literature

According to the review on this subject by White (1948), this phenomenon of fasciation or monstrosity on plants has been known for many years, apparently from the 17th century. According to the same author, fasciation is the phenomenon by which plant stems or shoots, instead of having normal round or quadrangular stems develop flat, ribbon-like ones. These plants can grow to maturity. The tip of the affected plant may turn over as a shepherd's crook or roll on in a snail-coil like helix. The main over all effect is an increase in volume and weight of the affected plant.

Fasciation seems to be related to certain growth conditions. The following causes have been suggested: environmental factors such as drought that keeps the plants just at the point of wilting for a period, and then returned to normal conditions (White 1948); unfavorable weather with high precipitation, and low temperature followed by normal conditions

(Danilova and Luzina 1962); bacterial invasion (Samuels 1961); abrupt increase of available nutrients (White 1948).

However, even though it is known, that under certain conditions fasciation can be manifested, the fundamental cause of this monstrosity is still not exactly known. White (1948) reported that Moquin-Tandon had a theory that fasciation results from flattening or enlargement of a single growing point. He also mentioned the theory of Linnaeus, which stated that fasciation results from an increase in the number of growth points on buds, and that owing to crowded conditions, these subsequently fuse. A third theory mentioned by White (1948) is that of De Vries. According to that theory, fasciation is the phenotypic expression of an unknown gene, that is expressed only under certain environmental conditions. This theory seems to fit with what has been observed on flax in Taiwan, according to Shibuya (1939) in White (1948), who reported that when seeds were sown thickly, fasciations were infrequent or absent. The greater the spacing, the more frequent the fasciation. When seeds were sown monthly in Taiwan, plants from the August, September and October sowings have the largest and most frequently fasciated individuals, this being the season of vigorous growth. Decline in fasciation was gradual in November and December sowings, until there were none at all in January to May, the most unfavorable growing period.

More recently Tyutyunik (1962) believed that the mechanism of fasciation in grape shoots involves the unequal division of cells

in the apical meristem resulting from certain factors. This causes the division of the apical cone into several sections, which develop more or less independently.

b. Observed fasciation

(i) Method

Since fasciation had been observed in the experiment in which boron deficient flax plants were returned to a complete nutrient solution, two further experiments were conducted to find out if fasciation could be induced under slightly different treatments. In the second experiment, plants were grown for one month in a normal solution, before being subjected to one months boron deficiency treatment, and then returned to the complete nutrient solution. The third experiment was to study the effects of the following treatments: normal and high levels of boron, combined with low, normal, and high calcium levels: However, the 4-day old seedlings were grown under boron deficient calcium levels, before being transferred to these treatments for experiment three.

(ii) Results

Fasciated shoots were observed in both the 2nd and 3rd experiments. In the third experiment the fasciated shoots were almost identical no matter what the treatment.

c. Description of fasciated shoots

The stems of normal fasciated shoots have a ribbon like

shape. This flat stem seems to be formed of 5-6 stems stuck together, because small bumps, the size of ordinary shoots or branches, could easily be distinguished. The dimensions of the stem were: $\pm$ 1cm X $\pm$ 2mm., or the equivalent of 5-6 normal stems (Fig. 29).

Leaves of the fasciated stems were growing at irregular intervals, and in some cases more than one leaf occurred at the same node. At the tip of the shoot, leaves were growing in a bunch. Some of those leaves looked normal, but about half of them were distorted or twisted (Fig. 26).

Fasciated shoots set flowers, but always only one abnormal flower (Fig. 30). This fasciated flower was not round or circular as is normal, but rectangular in shape, about 2 cm long by 1 1/2 cm wide. In the center of that flower, the pistils were in one row with a row of stamens on either side equivalent to about 4-5 normal flax flowers. Under greenhouse conditions, petals of ordinary flowers remain attached for one or possibly two days, but with the fasciated flower, the petals remained for at least 10 days under greenhouse conditions. No seed was set from the fasciated flowers.

At this stage of growth, flowering time, normal branches arose from the fasciated shoots, but mainly from last 2-3 inches at the top of the stem. These new branches grew normally and later set flowers and seed (Fig. 31).

Some variations in the fasciation have been observed. (i) Some plants developed a flat stem as described before. However, a short time before flowering, the tip of the stem started to curve and soon developed into a coiled structure (Fig. 32 and 33). A flat terminal bud appeared, but did not open fully. Later, 2 to 4 normal branches grew from just under the tip of this coiled mass of leaf and stem (Fig. 34). (ii) In another type of variation, the plant grew a more or less flat fasciated stem to begin with, but then 5 shoots developed at the top and grew on as normal shoots (Fig. 35). (iii) On a few stems, fasciation occurred only on the pedicel. Two pedicels would stick together (Fig. 36) and set a double flower (Fig. 37), that set twin bolls (Fig. 38). (iv) Some fasciated shoots developed a whirl of leaves at very young stage (Fig. 39), or had 2, 3, and even 4 leaves growing from the same node. (v) In one peculiar plant (Fig. 40), the fasciated shoot divided into two. One shoot remained fasciated, and produced a coil-shaped tip, while the second shoot grew normally, i.e. was not fasciated.

Another abnormality that was observed, was the repeated branching of shoots. This may not be fasciation. As shown in figure 41, one shoot would grow apparently normally to a point where it split into two to form two further shoots, a type of dichotomous branching. Eventually those shoots branched normally and set fruit. This branching of shoots mostly occurred close to the main stem.

In general, one plant in five showed fasciation or other abnormality on one or more of its shoots, but never on the tillers.

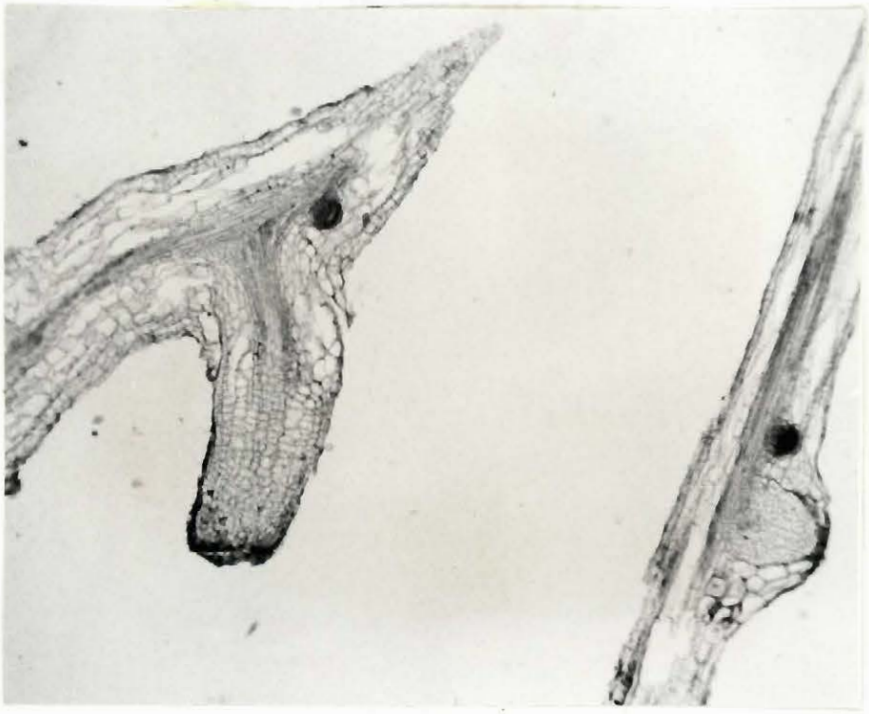


Fig. 23. Longitudinal section of root tip of flax plant grown in boron deficient solution and transferred to normal conditions for 3 days. Note root primordium that looks healthy. (180 X.)

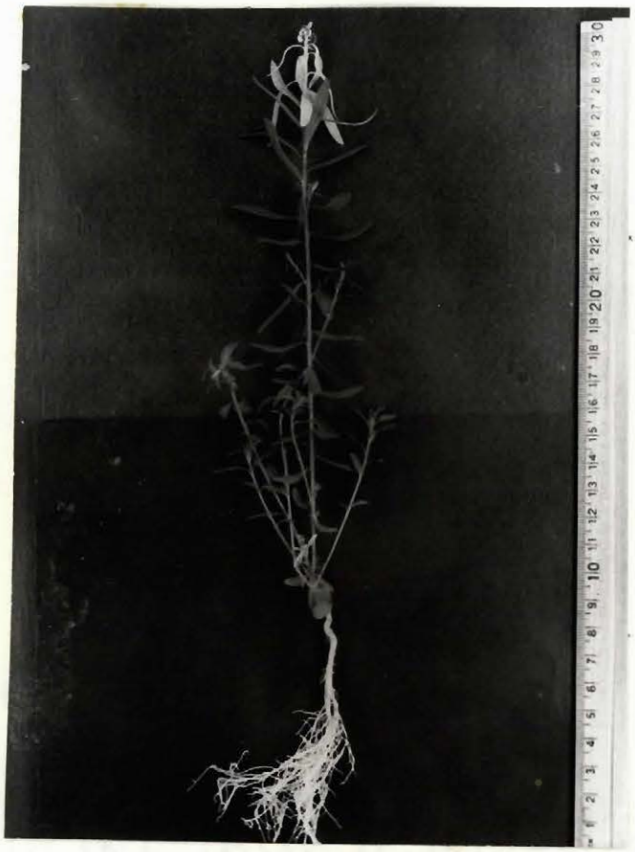


Fig. 24. Plant grown in boron deficient solution for 30 days: note dead growing point, the yellow tip, the poorly developed root system and irregular development of side shoots.

Fig. 25. Boron deficient flax plant transferred to complete nutrient solution for 7 days, note healthy shoots growing mainly at the cotyledon level.

Fig. 26. Cluster of leaves at the tip of fasciated flax shoot.

Fig. 27. Root system of boron deficient flax plant transferred for 7 days in complete nutrient solution. Note thin healthy roots as constrated with thick boron deficient roots.

Fig. 28. Roots of boron deficient flax plant showing new healthy root developed after transfer to normal nutrient solution.

Fig. 29. Fasciated flax stem with flower.
Note bumps or ridges on the stem.

Fig. 30. Abnormal flower of fasciated flax plant stem.

Fig. 31. Fasciated flax plant produced apparently normal side shoots or branches.

Fig. 32. Fasciated flax plant starting to curl at the tip.

Fig. 33. Fasciated flax plant with fully coiled tip.

Fig. 34. Fasciated flax plant with coiled tip producing normal shoots or branches.

Fig. 35. Fasciated flax plant that has branched at the tip to give 5 normal (non-fasciated) shoots.

Fig. 36. Normal flax plant with fasciated pedicel.

Fig. 37. Flax plant showing two flowers arising from a fasciated pedicel.

Fig. 38. Twin bolls from fasciated pedicel of flax plant.

Fig. 39. Whorled leaves on fasciated flax plant.

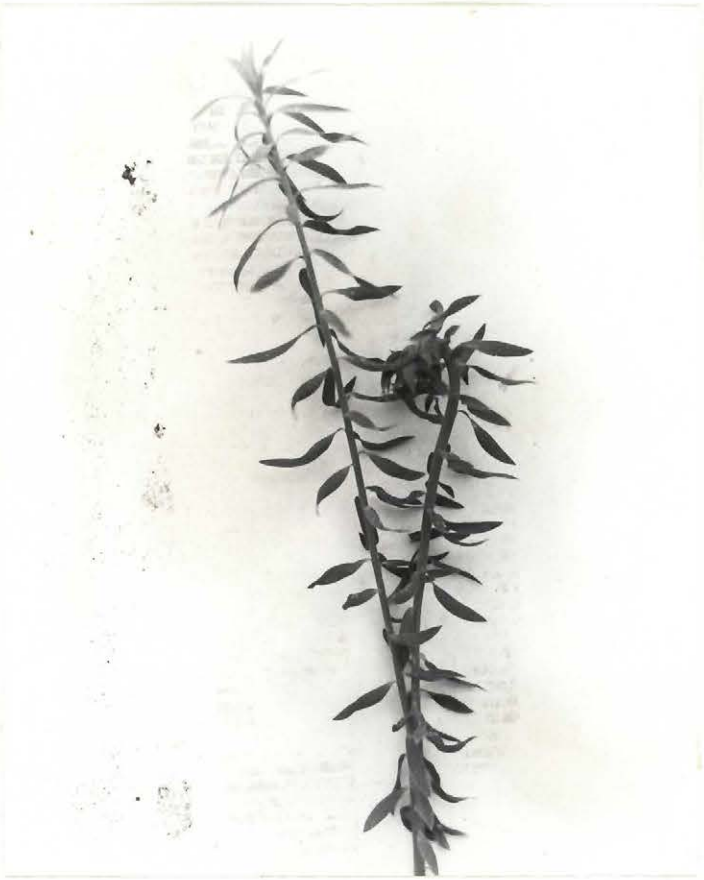


Fig. 40. Fasciated flax stem that split in to two, one part now normal.

Fig. 41. Dichotomous branching of flax plant which may not be due to fasciation.

d) Histological effects

According to White (1948), one theory of fasciation states that the phenomenon results from a flattening or enlargement of the growing point. To find out if this theory could be applied to fasciated flax plants, longitudinal sections of shoot tip were made (Fig. 42). No such flattening or enlargement of the growing point could be seen. The meristem looks normal and round, as the meristem of flax stem growing in complete nutrient solution (Fig. 20). However, it was noted that there were more leaves at the tip, than is normal. A cross section of a young flat fasciated stem, showed that there were bunches of vascular bundles all around the flat stem (Fig. 43). Because of the immaturity of that shoot, the fibres could not be distinguished.

Fig. 42. Stem tip of a fasciated shoot of flax in nutrient solution. Note that there are no bud primordia, but leaves are numerous. (400 X.)

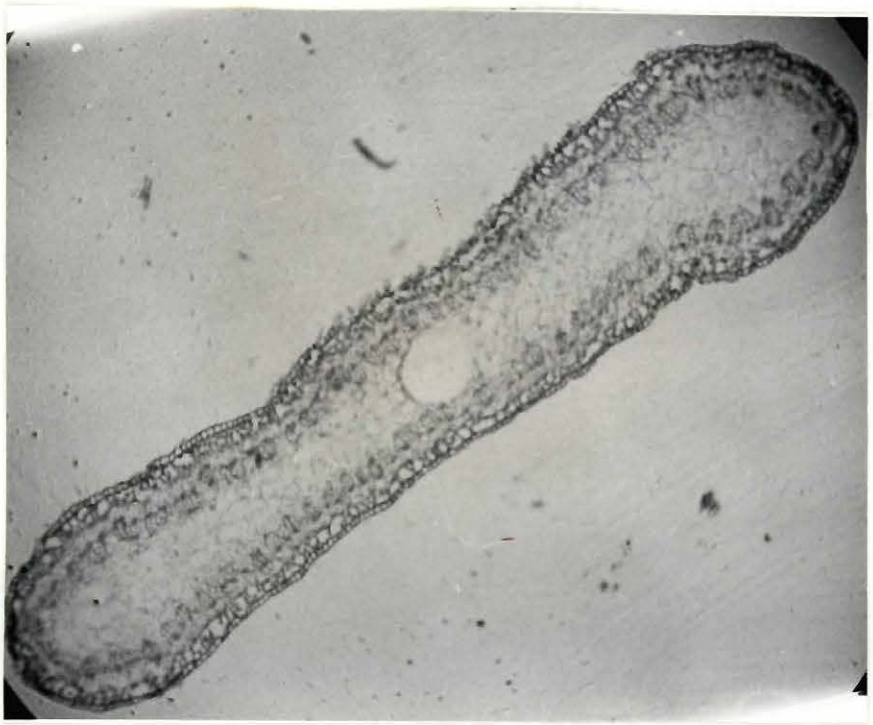


Fig. 43. Cross section of a young fasciated flax shoot. Note the ring of vascular bumdles. (150 X.)

B. BORON EXCESS

Boron toxicity symptoms developed on flax plants grown in 2.5 ppm boron after 15 days whether the plants were started in that solution, or grown in a normal nutrient solution for one month before the treatment.

When cotyledons were present, they were the first to be affected, otherwise it was the first true leaves. The first symptom was a yellowing of the tip of the leaf, then the yellow changed to light brown and the tip started to curl (Figs. 44 and 45). The yellowing spread down the midrib and gradually the whole leaf turned light brown and became brittle. Leaves thus affected by the boron toxicity then fell off, though sometimes they fell before being completely brown and dry. These symptoms occurred on the main stem and on the tillers. Different levels of calcium caused very slight differences in time and in development of the boron toxicity symptoms.

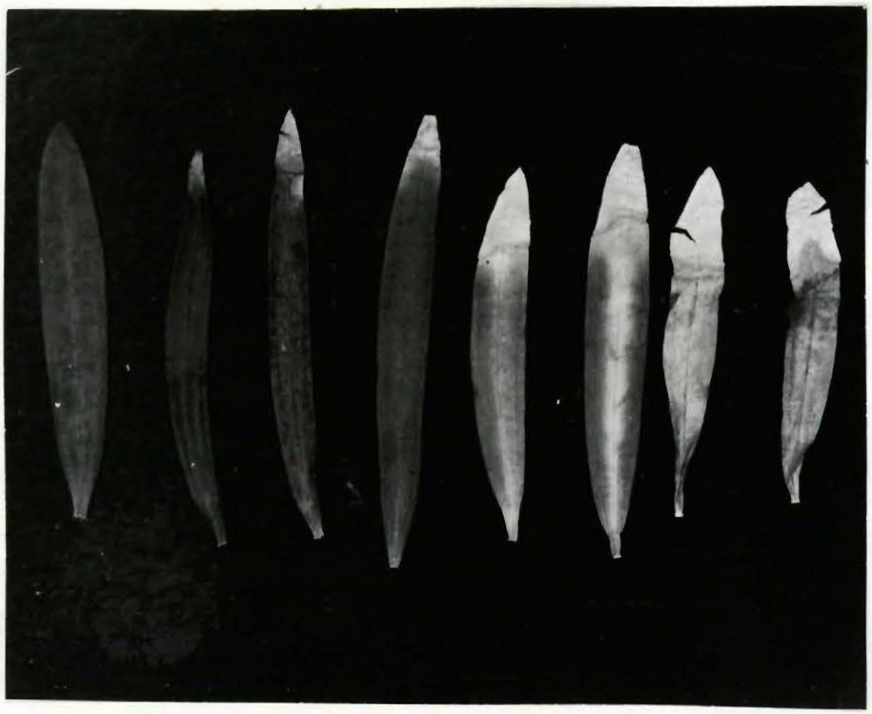


Fig. 44. Boron toxicity symptoms on flax leaves: healthy (left) to dead (right).

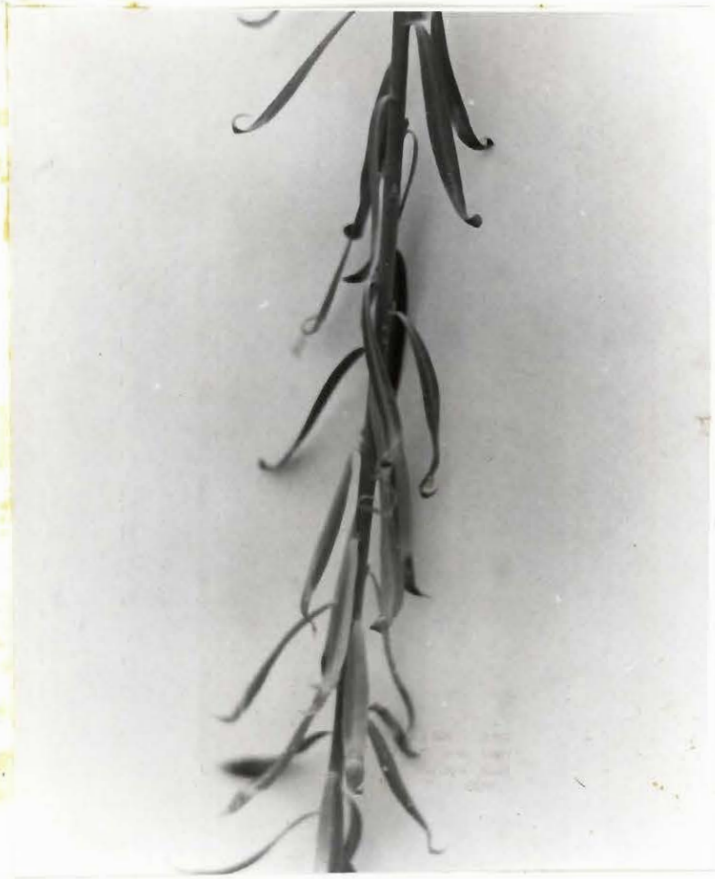


Fig. 45. Boron toxicity symptoms on flax plant, note curling of leaf tips.

C. CALCIUM DEFICIENCY

When flax plants were grown under calcium deficient conditions right from the beginning, it was impossible to observe calcium deficiency symptoms when the nutrient solution was at a low level of boron. The reason for this was that boron deficiency symptoms occurred before the calcium deficiency symptoms could be manifested. On the other hand, a low level of calcium (40 ppm) combined with either a normal or a high level of boron permitted the plant to grow up to the budding stage, or for 42 days.

I. SYMPTOMS

The first symptoms of calcium deficiency was that the buds, instead of flowering as in the control, collapsed, and failed to flower. The pedicels bent over either at their point of attachment or half way up. When the pedicels were not completely formed, it was the terminal 2 to 8 cm of the apex of the stem that bent over and withered (Fig. 46). This phenomenon is the "withertop" symptoms as mentioned by Millikan (1944).

At the bending point, the stem or the pedicel lost its rigidity, shrank and became necrotic. Two or three days later, the leaves of the bent part of the stem turned dark green or greyish-green in color, and then changed to light brown and died (Fig. 47).

When the stem tip bent over, shoots started to develop

in the leaf axils below the bending point (Fig. 47). Those shoots first seemed to grow normally, but within a week, the bending occurred on these new shoots, and they in turn started branching. This branching on branches gave a bushy appearance to the calcium deficient plants (Fig. 48).

In the case of plants grown for the first month in complete nutrient solution and transferred to a calcium deficient solution thereafter, the first symptoms of withering occurred 31 days later. This was possible only when boron was at normal or at high concentration in the nutrient solution. However, different kind of symptoms were observed on the tillers and on the stems that were not producing flower buds, i.e. they were in a vegetative stage. The first symptom observed was that the leaves of the tip of the stem were closely pressed together upwards along the stem (Fig. 49), and the necrosis took place at the tip of those leaves, and that necrosis took place at the tip of those leaves and progressively invaded the whole leaf. Simultaneously shoots appeared mostly in the upper leaf axils (Fig. 49). Necrosis (Fig. 50) was also noted at the tip and middle of the leaves below the growing point. Later the apex leaves separated from each other (Fig. 51), and necrosis invaded the shoot at the leaf axils (Fig. 52). In the last stage, the necrotic point bent over (Fig. 53).

2. RECOVERY FROM CALCIUM DEFICIENCY

Whether the flax plants had been grown in complete nutrient solution or not before being subjected to calcium deficiency, they react in the same way when transferred to a normal solution. In a week, new apparently healthy shoots were developing from the leaf axils below the withering point (Fig. 54). However, although most of those new shoots grew normally, setting flowers and seeds, a good number produced buds that collapsed and the pedicels bent over.

Again these branches produced further branches, as in calcium deficient conditions (Fig. 55), giving a bushy appearance to the plant.

Fig. 46. Calcium deficient plants: note bending over of tips.

Fig. 47. Calcium deficient plant: note (a) the necrosis of the bent part, (b) the shooting at leaf axils.

Fig. 48. Calcium deficient plant: note the numerous shooting from leaf axils and further shooting from shoots.

Fig. 49. Calcium deficient flax plant: note leaves pressed together at the tip.

Fig. 50. Calcium deficient flax plant: note necrosis at the leaf tip, and shooting at leaf axils.

Fig. 51. Necrotic leaves separated from each other on calcium deficient flax plant.

Fig. 52. Calcium deficient flax plant: note the necrosis on shoots at leaf axils.

Fig. 53. Bending over of the necrotic tip of calcium deficient flax plant.

Fig. 54. Apparently healthy shoots on calcium deficient plant transferred to normal solution. Note dead growing tip.

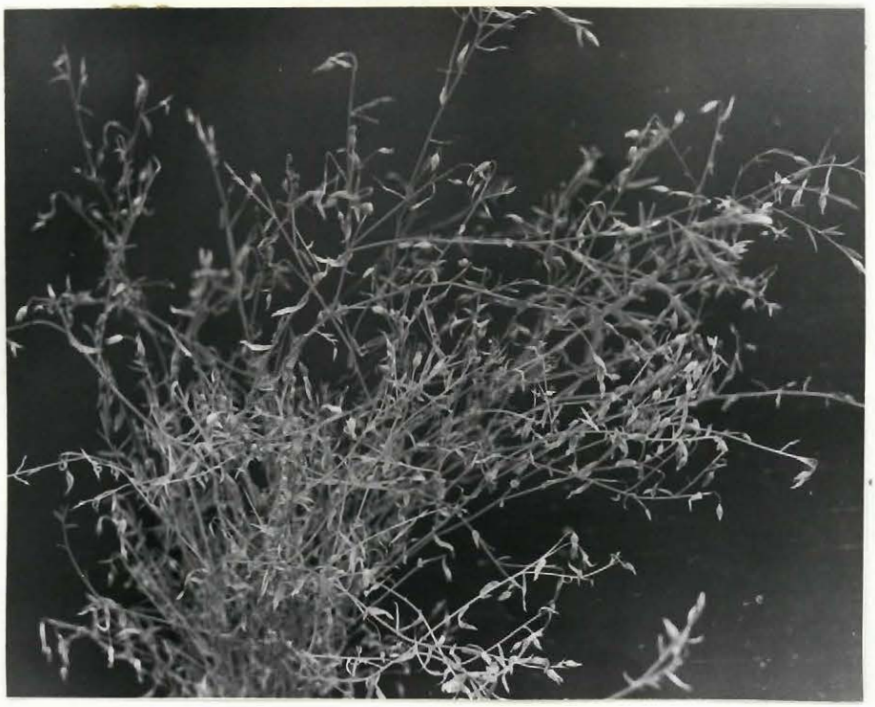


Fig. 55. Bending over of pedicel on shoots after transfer of calcium deficient flax plant to normal complete nutrient solution.

Histological effects

It was observed that buds in leaf axils developed into shoots, when flax plants were growing in calcium deficient conditions (Fig. 49). A longitudinal section of the stem tip (Fig. 56) showed that buds in the leaf axils developed very early in the meristematic region, even in the axils of the first leaves. Not only one bud developed, but secondary bud developed also in the leaf primordia of the first bud. On normal stem no buds were observed, and longitudinal sections showed that there were no buds either (Fig. 20). The internode length of normal stem is shorter than with stem from low calcium treatments.

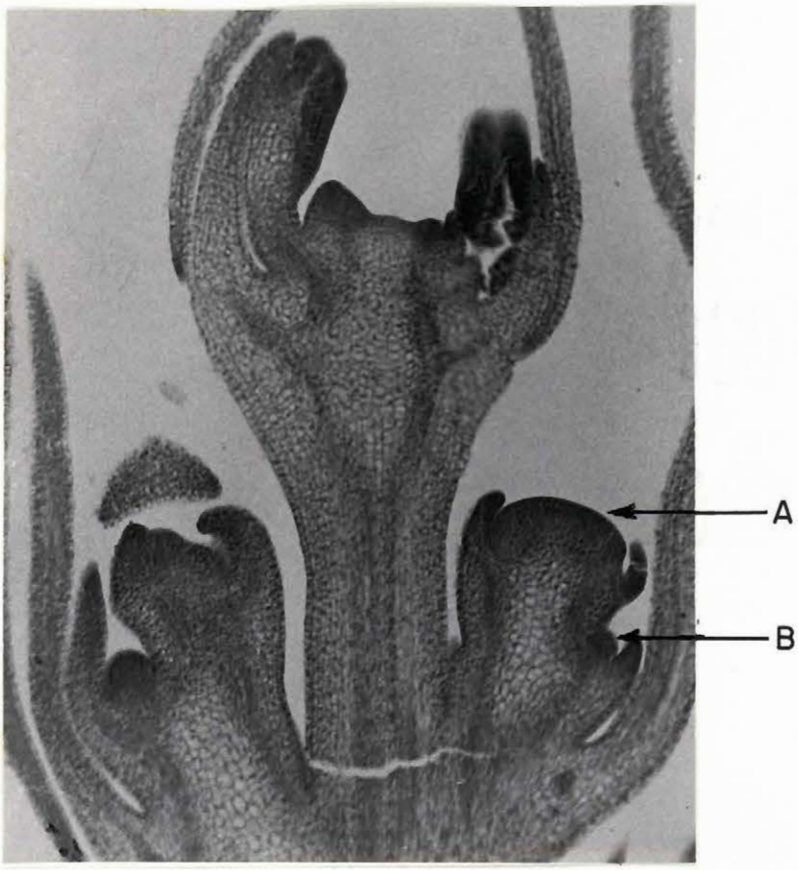


Fig. 56. Stem tip of flax plant growing in calcium deficient solution: note (a) first axillary bud developing at the leaf axil, and (b) second axillary bud developing on the first. (400 X.)

D. CALCIUM EXCESS

Calcium excess (480 ppm) in the nutrient solutions did not induce very marked symptoms. It was observed that the bottom leaves yellowed slightly, very slowly after 40 days of treatment. It took from 4 to 5 days for leaves to yellow and dry out, (Fig. 57). About half the leaves were dead by the time the plants were mature. Flowering and seed production did not seem to be affected. The identical symptoms appeared, whether, the flax plants were grown in excess calcium from the beginning, or first grown for a month in complete normal nutrient solution before being transferred to excess calcium conditions.

The symptoms reported above are visible only when high calcium is combined with normal boron in the nutrient solution, since boron excess and deficiency symptoms manifest themselves more rapidly than those of excess calcium.

Fig. 57. Calcium toxicity symptoms on lower leaves of flax plant: note yellow and dried leaves.

E. SEED PRODUCTION

Total seed production or yield, quantity and quality of oil contained in flax seeds are dependant on a number of factors. Flor (1944), Sackston (1950), Sackston and Carson (1951), and Frederiksen and Culbertson (1962) reported the effects of fungal diseases on the yield of flax. Henne and Friesen (1962) mentioned that herbicides affected the oil content and quality of flax seeds. Fertilization with major elements has been reported to affect the oil content of flax seeds both quantitatively and qualitatively by Yermanos, Holland and Burge (1964) Dybing, and Dean (1964), Sinha and Saxena (1965). Dybing and Zimmerman (1965) as well as Yermanos and Goodin (1965), Ford and Zimmerman (1964), and McGregor and Carson (1961) reported on the effects of temperature on fatty acid composition of linseed oil. According to Sosulski and Gore (1964) the photoperiod has effect on the quantity and the quality of oil of flax seeds, and compared longer photoperiod to cooler temperature.

In a first exploratory experiment, flax plants were grown under the following treatments: CaH BH, CaH BN, CaN BH and CaN BN. It was found that their yields had a tendency to differ from one treatment to the other. However, because of the low number of plants used and the nature of the experiment, no results were analysed.

1. Materials and methods.

In experiment II, plants were grown for 1 month under

CaN BN, or normal level of calcium and boron, before being transferred in the different treatments. Plants under normal and high boron levels combined with normal and high level of calcium grew well till the end of the experiment. Those four treatments were therefore maintained for 88 days.

At the end of the experiment, ripe bolls were harvested, counted, and threshed, while the green bolls were simply counted. Threshed seeds were divided into good or bad (seeds full or empty), and both were counted. Weight of thousand seeds (mixed) seeds) was taken.

2. Results

All the figures of the results, except weight of a thousand seeds and the number of seeds per boll are on the basis of one plant. Table 2 gave the mean per plant of 5 plants per treatment, each treatment being in 2 replicates. From these results, it can be noted that a high level of calcium combined with a normal level of boron give a significantly higher number and percentage of good seeds per plant, over 5% level, than CaN BH only, but a nonsignificant number of total seeds (good bad) per plant. This treatment also gave a higher weight for a thousand seeds, more ripe bolls per plant, more bolls per plant and more seeds per boll. However, these differences were nonsignificant for the four treatments. The treatment combining normal level of both calcium and boron gave results which were not significantly different from the others.

Even if the results showed that there was a significant difference for the number of good seeds per plants and the percentage of good seeds only, it should be noted that there was a regular tendency for the CaN BH treatment to give the lowest yield, and for CaH BN to give the highest; the control and CaH BH treatments were almost identical. Since this experiment was only carried once, with two replicates, the margin for error was small.

Most of the results did not show a significant difference between treatments, but because of the clear tendency shown by CaN BH results, it would be expected that the same experiment repeated with more replicates could show results with significant differences. Such an experiment was not feasible, however, because of the limitations of materials and greenhouse space.

To complete the analysis of seed production, an analysis of the oil was made by the gas chromatography techniques, in order to find out if there was a difference in the percentage of oil, and in the relative content of fatty acids.

F. FATTY ACID COMPOSITION OF OIL

1. Materials and methods

Ripened bolls from the different treatments were threshed and the seeds allowed to dry at room temperature. From each treatment, 80 seeds were sampled and weighed.

Each sample was homgenized in a "Virtis 23" homogenizer

with 15 ml redistilled petroleum ether (bp 30-60 C), for two minutes at 23,000 rev/min. The homogenate was transferred (using a funnel) to a 10 x 50 mm thimble and placed in a 50 ml soxhlet apparatus. Lipids were extracted using redistilled petroleum ether at the rate of 13 solvent changes per hour for one hour (Dybing 1963). The solvent containing the extracted lipids was evaporated in a water bath at 50 C under a jet of nitrogen. For drying, the evaporated samples were placed in a desiccator under vacuum over activated alimina before methylation.

Methyl esters were prepared according to Metcalfe, Schmitz, and Pelka's (1966) technique. Lipids from the sample (about 120 mg) were transferred to a 50 ml volumetric flask. Four millilitres of 0.5 N methanolic sodium hydroxide was added to the mixture which was heated for 5 minutes on a steam bath until the fat globules went into solution. Five ml of BF_3 - methanol (125 g BF_3 in 1000 ml methanol) was added to the flask and the mixture was boiled for 2 minutes. A saturated sodium chloride solution was added to float the methyl esters up into the narrow neck of the flask. Then the entire mixture was transferred to a separatory funnel. About 20 ml of redistilled petroleum ether was added to the separatory funnel. This funnel was shaken vigorously for 1 minute, and the layers allowed to separate. The aquaeous layer was drained off and discarded. The petroleum ether layer was then poured through filter paper into a 50 ml beaker. The solvent was evaporated in a 50 C water bath with the aid of a stream of

nitrogen. The samples were allowed to dry in a vacuum desiccator filled with activated alumina. The samples were then stored in another desiccator which was flushed with nitrogen and placed in a refrigerator at 7° C.

A Perkin - Elmer gas chromatograph, model 154 D, provided with a thermal conductivity cell, was used for the fatty acid determinations. The chromatograph was connected to a 1 mV Philips Recorder with a chart speed of 160 cm/hour. The column temperature was 210 C, the flow rate of helium as carrier was 60 ml/minute, column inlet pressure was 25 lbs, and the detector voltage was 8 V. A 1 µl sample of methyl esters was injected with a Hamilton syringe.

The column used was provided by Dr. B. M. Craig, and has the following specifications. It is a 10' x 3/16" O.D. copper tubing filled with O-phthalic glycol polyesters on a A.W. C-22 fire brick (60-80 mesh) in the ratio of 45 : 1 (W/W).

This column separated the fatty acids as follows: palmitic first, then stearic, oleic, linoleic, linolenic, and arachidic in that order.

The calculation of the relative fatty acid composition from the chromatogrom was done according to the procedure of Bartlett and Iverson (1966).

2. Results

Lipids extracted were weighed and reported as percentage of seeds weight (dried at room temperature). The results are

reported in table 3. Lipids extraction was carried out for one sample only from each treatment, and the methyl esters samples (1 µl) were injected twice for each treatment into the gas chromatograph, to have two analyses for each treatment.

The results are per force only indications, since there were no repititions of the lipids extractions. However, from table 3, it can be noted that a higher percentage of oil was extracted from seeds produced by plants growing in CaH BH, than in other treatments. Lipids extracted from seeds coming from CaN BN and CaH BN treatments contained a higher relative percentage of oleic acid, and a lower relative percentage of linoleic and linolenic acids, than the lipids from CaH BH and CaN BH.

Discussion

It is believed that the technique used did not permit the extraction of all the oil from the seeds, since results indicate that those seeds contained 27 - 29% of oil, and commercial seeds used in the experiment 30%. McGregor and Carson (1961) found 39-40% from Marine flax seeds produced under field conditions in Western Canada. Because of this difference in extraction, it is very possible that the relative composition might change. According to Rheinick (1965), the linseed oil components in fatty acids are: linoleic 17%, oleic 22%, linolenic 51% and saturated acids 10%. The present results indicate that the seeds of this experiment contained less linoleic (10-12%), more oleic acid (25-30%), more linolenic

(52-56%), and 6-7% of saturated acids. The total of unsaturated fatty acid is therefore, 93-94% in the experiment against 90% as reported by Rheinick.

The present analysis will have to be supplemented by further work. The future analysis should try to get a higher oil extraction by either longer homogenization or longer extraction by the Soxhlet apparatus (Dybing 1963). It might also be advantageous to inject a larger volume of methyl esters into the gas chromatograph (i.e. 2 or 3 µl) (Craig and Murty 1959) instead of 1 µl as used in the present analysis.

Table 2. Yield data of flax plants started in complete solutions for 30 days, then transferred to test solutions for 88 days.

Treatments	Bolls per plant	% ripe bolls	Seeds per plant	% good seeds	Weight of 1,000 seeds
CaN BN	54.3	65.4	263.6	1/ 88.8[a]	4.70
CaN BH	53.6	60.5	234.1	69.2[b]	4.45
CaH BN	56.9	70.3	299.0	97.2[a]	4.82
CaH BH	52.2	67.6	260.7	96.0[a]	4.75

1/ Note: Means followed by the same letter are not significantly different at the 5% level (L.S.D. - 9.85).

Table 3. Fatty acid composition of linseed oil from flax plants started in complete solutions for 30 days, then transferred to test solutions for 88 days

Treatments	Oil extracted	Relative percentage of fatty acids				
	% weight of seeds	Palmitic	Stearic	Oleic	Linoleic	Linolenic
CaN BN	27.89	4.59	1.29	30.24	10.42	53.28
CaN BH	27.65	4.97	1.35	26.24	12.31	54.99
CaH BN	27.89	4.38	1.44	30.97	10.50	52.68
CaH BH	29.52	4.54	1.29	25.27	11.94	56.86

G WATER UTILIZATION.

I INTRODUCTION

In all experiments with nutrient solutions, whether plants were growing in sand or in solution, water had to be added to the medium almost every day to keep the solution at the concentration desired and to replace the water used or absorbed by the plants (Hewitt 1966). During the first experiment, it was noted that the volume of nutrient solution used daily by the plants was different in each treatment. This seemed worth investigating.

a) Measurement of water utilization

In an attempt to check the importance of this observation, the volume of the nutrient solution absorbed by the plants in the second experiment was measured daily. Since all the plants grew for one month in normal solution, they were of the same size when transferred to the different treatments. The volume of water used was measured by finding the volume of distilled water that had to be added daily to bring the nutrient solution up to the level of the day before.

b) Computation.

The figures of water utilization for each treatment were obtained by taking the mean per plant for the two replicates, and transforming it into a percentage of the control. This was to avoid possible daily variations due to temperature, light and humidity in the greenhouse. All calculations were computed using

an I.B.M. Fortran program.

2. RESULTS

a) Effects of boron deficient treatments

There were three boron deficient treatments, one with low calcium, a second with normal calcium, and a third with a high level of calcium. All boron deficient treatments showed a highly significant negative correlation between time and water absorption for the 30 days of the treatments (Table 4). The different calcium levels did not affect the correlation factor very much, even though it was possible to note a certain tendency for calcium interaction.

Once the low boron level treatments were changed to complete nutrient solution, a decrease in water uptake was still observed for about 7 days (Table 4, Fig. 58). On the seventh day after the transfer, the water uptake was about 2% of the control. The two other boron deficient treatments did not come so low (Table 4, Fig. 59 and Fig. 60).

A projected line based on the regression line of data from the 30-day treatment, comes down to just about that value. Therefore, this might be used as a mean for predicting when the plant should stop water uptake. According to this, plants in CaL BL treatment would stop to absorb water after 38 days, after 45 days in the CaB BL treatment, and 52 days in the CaH BL treatment.

From those different projected lines, it would appear that a certain amount of calcium was used by the flax plants to replace the boron that was deficient in the solutions. In a comparison with CaL BL, CaN BL has 5 times more calcium in the solution, and the water uptake would stop 7 days later, while in CaH BL, there is 12 times the concentration of calcium of CaL BL, and the water uptake would cease 14 days later. Since nutrient solution with 200 ppm of calcium would add 7 days of water uptake, and 480 ppm calcium a further 7 days, it could be stated that 160 ppm calcium were necessary for those 7 days, and 280 ppm calcium for the next seven days. It should therefore be possible to reach a concentration of calcium that would not add any more time, and probably with a further calcium increase beyond that there would be a decrease in the number of days of water uptake.

After this seven day period, the water utilization increased regularly with the growth of shoots and new roots on the boron deficient plants. However, it was observed that recovery in water absorption was faster in CaN BL than in CaL BL or CaH BL, even though there was a highly significant positive correlation for the three treatments (Table 4).

b) Effects of calcium deficient treatments

It was very difficult to compare the different calcium deficient treatments, since boron deficiency symptoms developed first, when low boron level was used. However, if CaL BN and CaL BH are compared, the high level of boron seems to give partial

compensation for a lack of calcium (Table 4).

c) Effects of combination of normal and high levels of both elements.

In the first month of treatments, there was no difference between treatments (Table 4). In the second month, there were slight changes; the correlation for CaN BH, changed from 0.08 to -0.23, while the CaH BN and CaH BH kept positive, passing from a correlation coefficient of 0.09 to 0.04 to 0.34 respectively (Table 4).

However, if the complete period of treatments is considered, it is found that for CaN BH the correlation coefficient is highly significantly negative, significantly positive for CaH BN, and highly significantly positive for CaH BH (Table 4).

Table 4. Correlation coefficients for time in test solutions and water uptake of flax plants started in complete nutrient solutions for 30 days

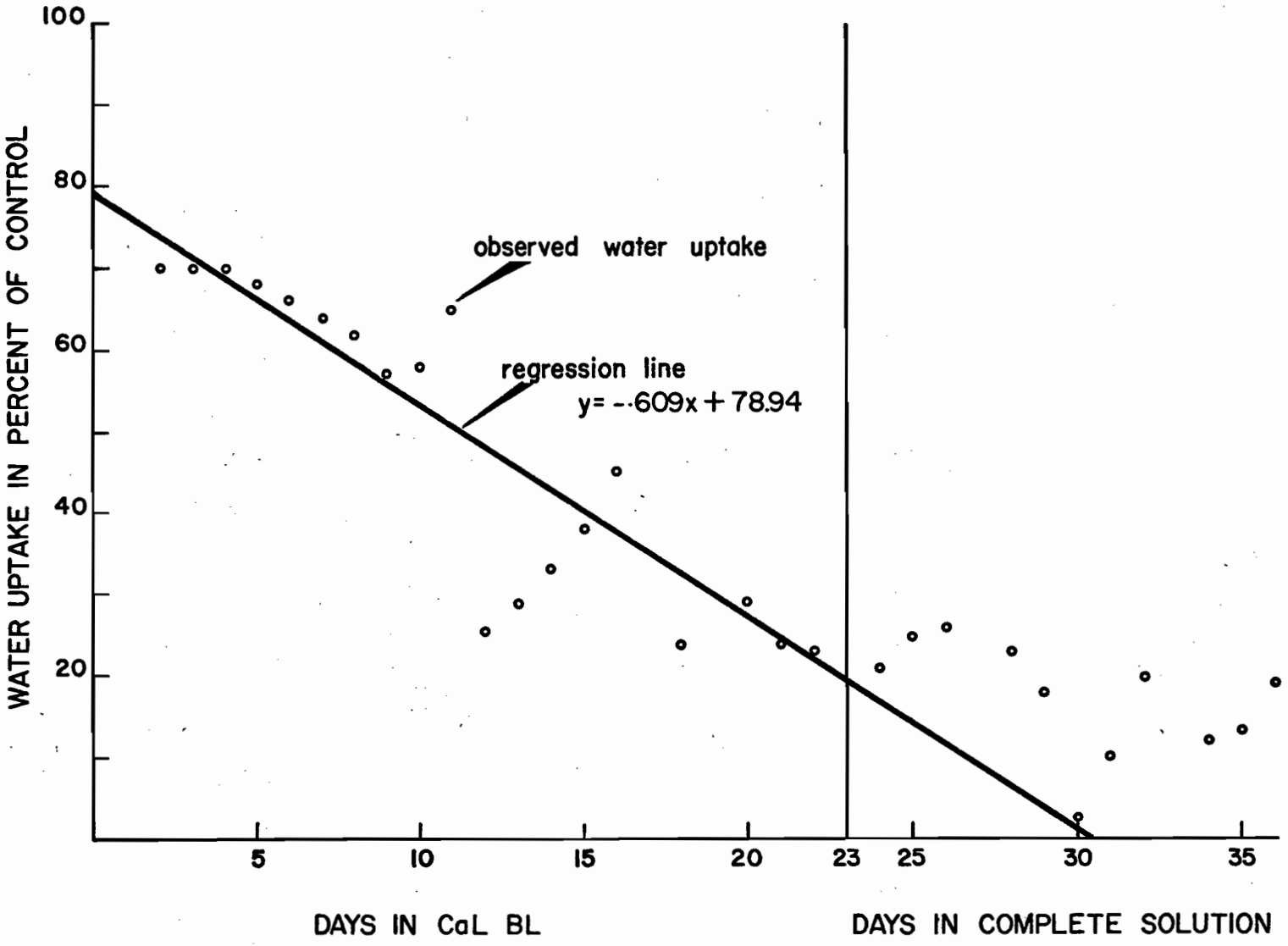
Treatments	Correlation coefficients				
	1 - 30th day	30 - 37th day	1 - 37th day	38 - 66th day	1 - 80th day
CaL BL 1/	-.89	-.53	-90	.78	.488
CaL BN	.31	-.53	-.03	.58	-.01
CaL BH	.14	-.94	-.45	.66	.16
CaN BL 1/	-.88	-.25	-.91	.82	.36
CaN BN	1.00	1.00	1.00	1.00	1.00
CaN BH	.08	-.39	-.06	.23	-.62
CaH BL 1/	-.84	-.59	-.84	.55	.48
CaH BN	.09	.04	-.19	.22	.23
CaH BH	.04	-.11	.14	.34	.61
	A = .64 B = .51	A = .87 B = .75	A = .56 B = .44	A = .50 B = .39	A = .324 B = 1248

1/ Plants exposed to boron deficiency for 30 days, were transferred to complete (CaN BN) solution for the duration of the experiment. Plants exposed to 0.5 ppm and 2.5 ppm boron were maintained in test solutions throughout.

A = Required for significance at 1% level.

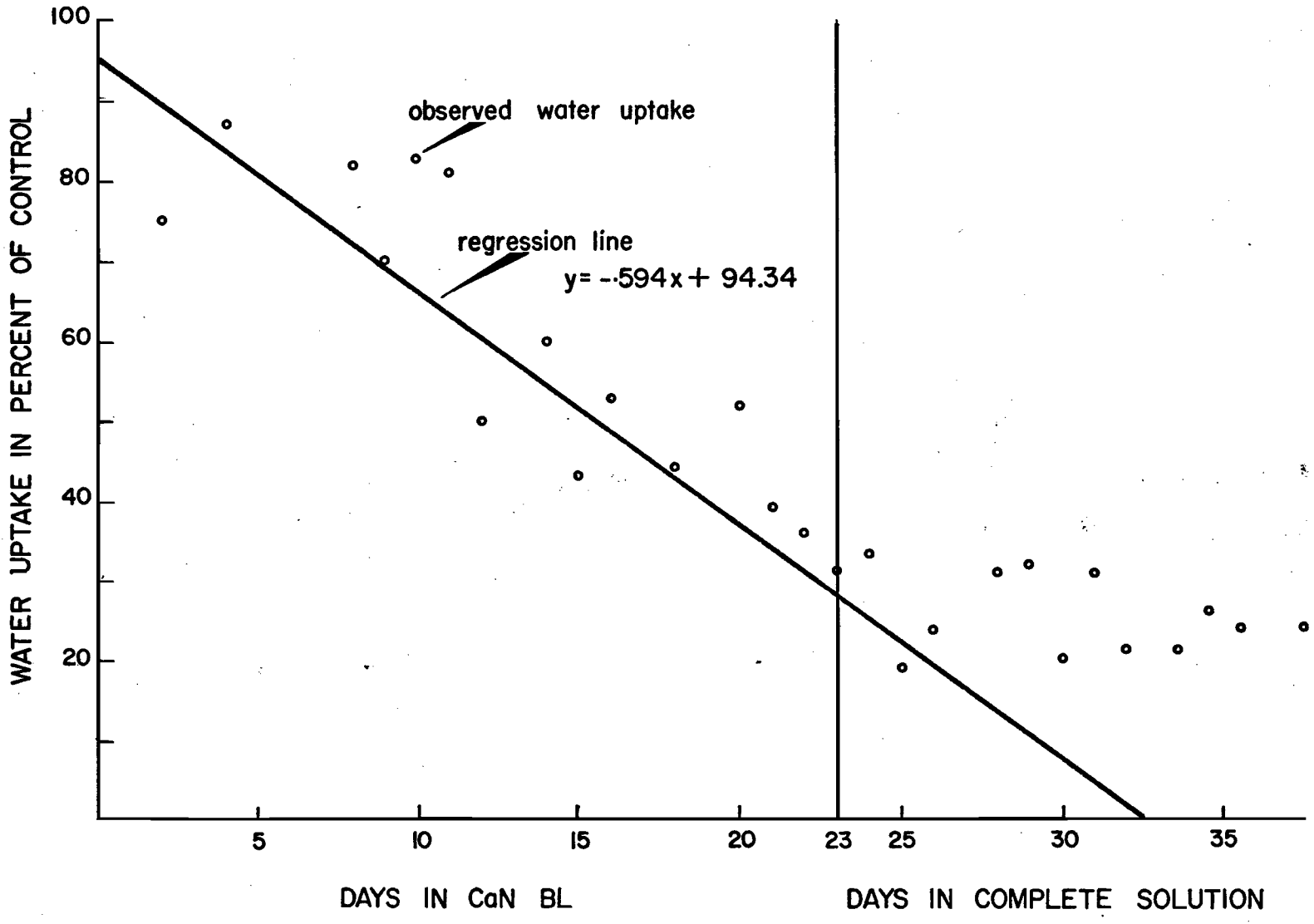
B = Required for significance at 5% level.

Fig. 58. Observed data of water uptake and regression equation based on y = mx b.



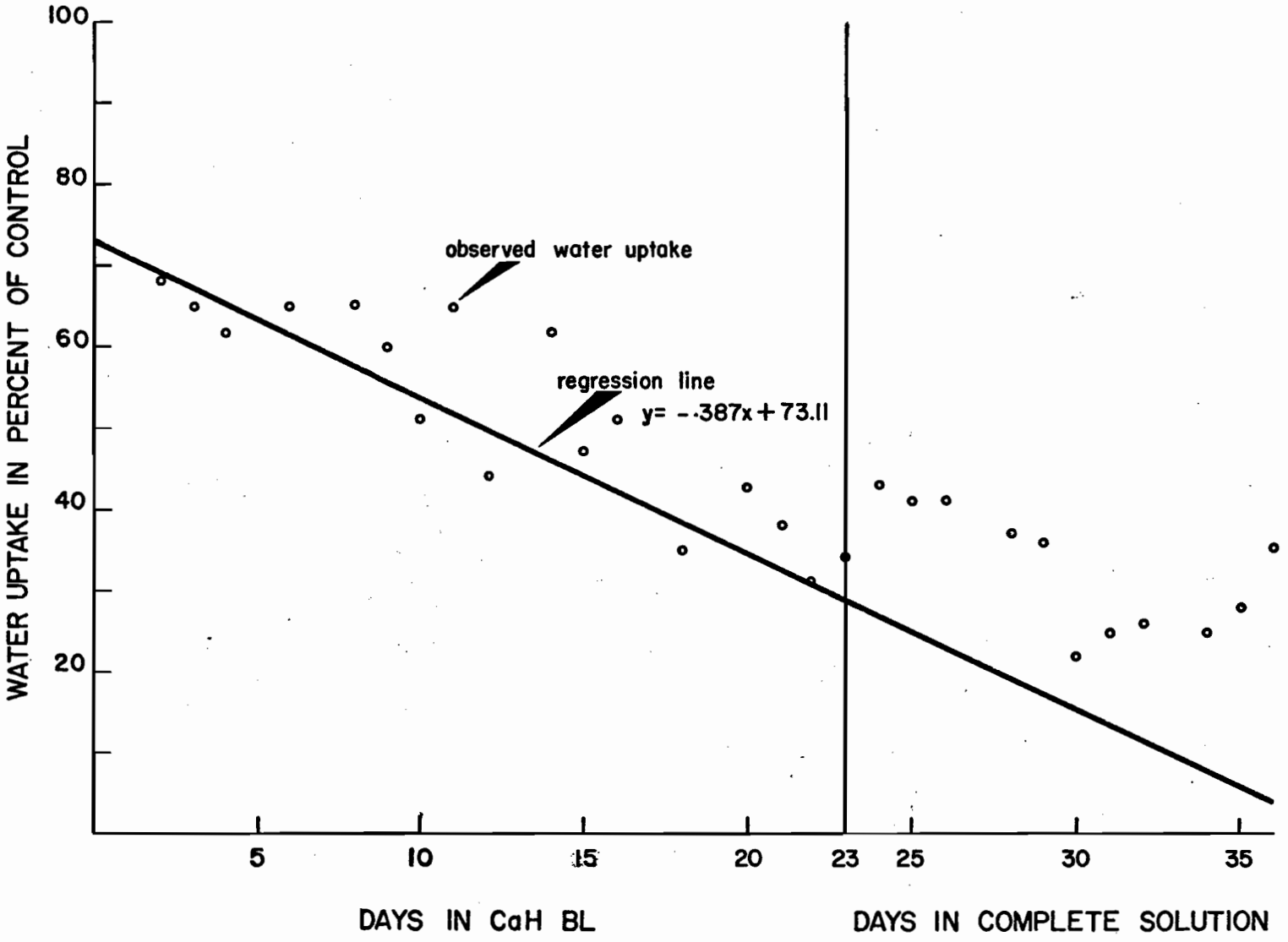
Day 23 = 30th of CaL BL treatment.

Fig. 59. Observed data of water uptake and regression line based on y = mx b.



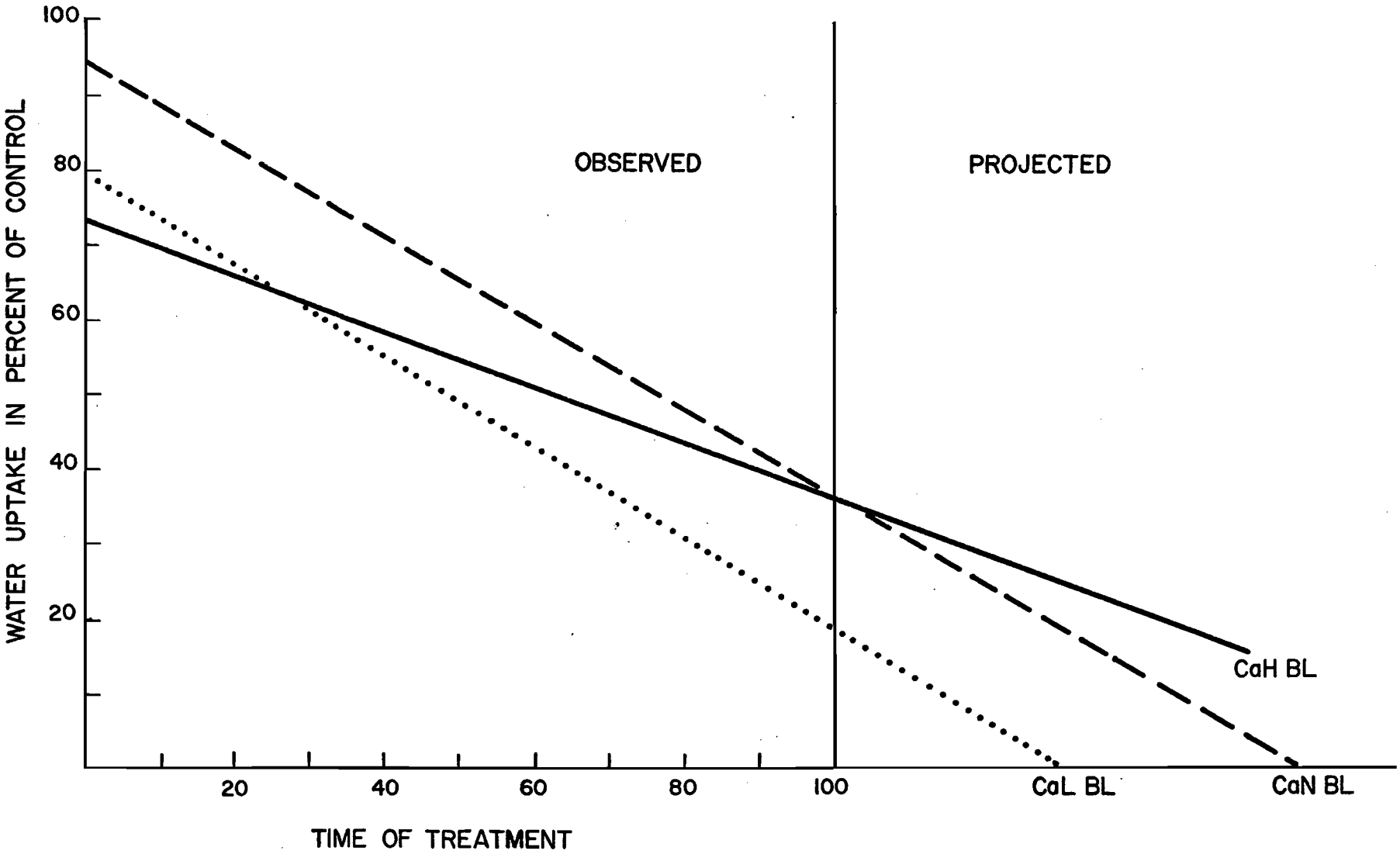
Day 23 = 30th day of CaN BL treatments.

Fig. 60. Observed data of water uptake and regression line based on y = mx b.



Day 23 = 30 day of CaH BL treatment.

Fig. 61. Regression and projected lines based on water uptake by low boron treatments.



Complete time of treatments. CaL BL meet time axe at 127, CaN BL at 163 and CaH BL at 197.

V. DISCUSSION

A. BORON DEFICIENCY

From the experiments reported here, many questions are raised but remain unanswered. Flax seedlings under boron deficient conditions cannot grow more than 21 days without the symptoms of boron deficiency occurring. Such seedlings may appear healthy before the symptoms developed, but for how many days were they actually suffering from a lack of boron, unable to get the necessary boron from the seeds? This has not been discovered. However, when the plants were grown for one month in a complete nutrient solution, the symptoms of boron deficiency occurred in 18, 20, and 22 days when low boron was combined with low, high and normal levels of calcium respectively. Therefore, the plant which grew for one month in a complete nutrient solution, had not stored more boron than is originally supplied in the seeds. When the seedlings were started in boron deficient condition, there is no difference between calcium levels, the symptoms occurring in 21 days, but after one month's normal growth, the boron deficiency symptoms are different in their manifestation and in time. However, it was observed that plants under a higher level of calcium treatment showed boron deficiency symptoms two days later than when low boron was combined with low level of calcium; while plants in the combined low boron and normal calcium treatment showed symptoms

later. If the quantity of boron used up in 18 to 22 days from the one month normal growth plant is the same as the amount removed from the seeds in 20 days, and the boron-calcium relationship is the same in both cases, what can be the reason for the delay in the occurrence of the symptoms?

When flax seedlings were started right at the beginning in boron deficient conditions, the symptoms of deficiency occurred at the same time whatever the calcium levels, while a difference was observed when flax plants were grown first for a month under normal nutrient conditions. It thus seems that the reserves in calcium in the seeds were quite uniform. This suggests that it might be worthwhile experimenting with seeds produced by flax plants grown under different calcium treatments, to find out if there is a difference in calcium content, and if the symptoms of boron deficiency would occur sooner in one of the treatments than in the others.

Rapid examination of Figs. 18 and 47 might suggest that these plants are suffering from poor growth conditions. Closer examination will show that plants growing in CaL BL for 18 days, (Fig. 18) after one month's normal growth, were showing the bent tip just as the calcium deficient plants (Fig. 47). This might be confusing at first, but they are different, because in calcium deficient plant, buds were developing in the leaf axils near the bending point of the stem, while no buds were growing on the boron deficient plants (Fig. 18). Even if this is known, how is it that

the low level of boron with low level of calcium induced the bending over of the stem tip, while the low level of calcium combined with normal or high level of boron treatment did the same, but 10 days later? This is another of these as yet unanswered questions raised by the work of this thesis.

B. BORON EXCESS

It was found that symptoms of boron excess (yellowing of the leaf from the tip to the base of leaf) manifested itself first on the cotyledons, and progressively began to invade the bottom leaves. The symptoms did not go higher than half way up the plant. However, this does not agree with the findings of Millikan (1951), who found that the leaves in the middle portion of the stem were first affected by a greyish-green transparent discoloration, commencing at the tips of the leaves and gradually extending downwards until the whole leaf was dead. It is a peculiar difference. These symptoms in the experiments were not mixed with other symptoms, i.e., the symptoms of calcium excess only developed about 25 days later. Millikan's results could be explained by the use of higher boron concentrations, as he used about 12 ppm boron in his solutions.

C. FASCIATION

The most unexpected result of the boron work was the development of fasciated shoots on boron deficient plants. As far as it is possible to determine, there is no mention in the literature of

boron deficiency conditions followed by normal growth conditions, being responsible for fasciation. Danilova and Luzina (1962) observed fasciation on Vicia Ervilla, Wild. after a period of unfavorable weather with high precipitation and low temperature, followed by normal growth conditions. It is known that boron is much less available to plants when there is a period of rain or a period of drought (Ouellette 1963), and that in some cases, such weather can cause boron deficiency. It might be possible that the boron deficiency solution created a situation very similar to those of Danilova and Luzina (1962). Therefore, it is possible that boron deficiency may be the cause of fasciation in both cases. However, is it a direct or an indirect effect of boron deficiency conditions, followed rapidly by normal or favorable conditions, that induce the fasciation on the shoots?

It is very difficult to give a definition of fasciation based on the cause of fasciation; how is it that a given bud in leaf axil does develop that way? On boron deficient roots, there were numerous root primordia, some very close together, that developed for a short period and then turned necrotic. The boron deficient stem showed numerous bolting shoots, which grew for a certain time, and then stopped developing. If there is a certain relationship between those observations, it would be reasonable to expect fasciation on the new roots as well, but none was observed. However, on boron deficient roots, 2 days after the transfer to normal condition, healthy root primordia were observed (Fig. 28).

Assuming that two or more shoots or buds were initiated very close to the others on the stem, and that the transfer happened just before the boron deficiency affected the cell division in the bud meristem, it could be expected that all buds would keep growing normally, but because of the lack of space, a partial fusion of those buds could occur. This would explain the bumps observed on the fasciated stems (Fig. 29).

On the other hand, is it possible, that a bud once having started to develop under boron deficient conditions, might have had trouble in cell division long enough to make a single growing point divide in such a way as to give fasciation.

To find out the exact mechanism that induces fasciation on the flax plants, could be as difficult as to find out what causes a shoot to develop normally. This would require histological and biochemical studies of normal and fasciated stem tips.

These attempts to explain fasciation are indications of the difficulties that such work can present. The exact cause of the development of fasciation has not been found. However, in all the experiments conducted, fasciation occurred on one fifth of the flax plants which suffered boron deficiency and then returned to normal conditions. Therefore, since a relatively simple means of producing fasciation is knon now, the first step has been taken.

D. SEED PRODUCTION

Since higher seed production was observed on plants growing

in CaH BN treatment, and a lower production in CaN BH treatment, the question arises: is the calcium level assumed to be normal, really normal, and is the concentration of boron considered normal, really so? As reported by Hewitt (1966), previous workers had used a calcium level of 160-200 ppm as normal, and boron at 0.5 ppm. However, Neales (1959a) found that 0.05 ppm boron was an adequate level for flax seedlings up to 3 weeks. Therefore, it can be assumed that 0.05 ppm boron might be sufficient for at least the first three weeks of the life of the plant. Under such conditions, the optimum level of boron in the solution would be situated in between 0.05 and 0.5 ppm boron.

The importance of boron nutrition for a higher seed yield has been demonstrated previously (Gauch and Dugger 1954). Montgomery (1951) found that in Alsike clover, the optimum concentration for seed production was 0.5 to 1.00 ppm boron. This concentration was higher than the concentration that gives optimum vegetative growth, 0.1 to 0.25 ppm boron. Shustova (1961) reported that on buckwheat the most important stage for the boron nutrition was at flowering time.

According to Jones and Scarseth (1944), Reeve and Shive (1944) as well as Ouellette (1963), there is a certain calcium-boron ratio that is optimum for each species. In sugar beet, for example, the optimum is 100 units of calcium for 1 of boron, 500 of calcium for 1 of boron in soya plants, and a healthy alfalfa plant has 1200 parts of calcium for one of boron. However, as

each of those elements is absorbed by the plant according to a ratio based on the concentration of each in the medium, one can be stimulant as well as inhibitor to the other. The calcium and boron content of a plant is therefore, dependant on the concentration of both elements in the nutrient media, soil or nutrient solution (Jones and Scarseth 1944).

In the experiments performed, a calcium-boron ratio of 40 to 0, 200 to 0, as well as 480 to 0 in the nutrient solution, induced boron deficient conditions. When the ratio was 40 to 0.5 and 40 to 2.5, calcium was deficient. From the yield results, the ratio 480 to 0.5 was the best, and 200 to 2.5 the worst; while 480 to 2.5 and 200 to 0.5 were very close in their yields. This was the ratio of the nutrient solution, not necessarily the ration of the internal content of the plant. However, boron toxicity symptoms were observed in all cases with a boron content of 2.5 ppm. It therefore, seems that 40 ppm calcium was enough to permit the absorption of a toxic concentration of boron, and that a calcium boron ration from 200 to 0.5 to 480 to 0.5 could be optimum in nutrient solution.

The analysis of the oil content of seeds produced by flax plants under the treatments combining normal and high calcium with normal and high boron levels, indicated that the treatment combining normal levels of boron showed a higher content in unsaturated fatty acid, and high levels of both elements, the highest oil content. Synthesis of lipids by plants depends on numerous factors (Butt and Beevers 1966), among the most important,

are the products of the photosynthesis. In Skol'nik (1962) it is reported that boron, by improving photosynthesis, improves the synthesis and the transfer of carbohydrates, mainly saccharose towards the fruiting parts of the plants. Shive (1941) and Marsh and Shive (1944) reported that the absence of boron interfered with the production of fats. Gauch and Dugger (1954) reported that Schropp in 1940 found that fat content of soyabeans was higher in plants receiving additional boron. Therefore, it seems to be logical to expect a higher oil content in seeds from plants grown in a higher boron treatment, in as much as more boron is absorbed and used by the plant (Reeve and Shive 1944). According to this hypothesis, a higher oil content is to be expected in seeds from flax grown in a solution giving the optimal calcium-boron ratio content for the plant.

E. WATER UTILIZATION

Flax plants grown under boron deficient conditions showed a steady decrease in water uptake when compared with the control. Since water uptake by plants is a function of more than one factor (Mayer and Anderson 1959), one or more of these factors could be implicated in that difference in water utilization.

Plants under boron deficient conditions did not develop for long before the growing point of the stem died, and the changes occurred on the roots. Because the plants were smaller than the control, and had less leaves, absorption was less, and

there was a consequent reduction in the water loss due to evaporation and transpiration.

According to Kramer (1956), there is little water uptake through the meristematic region of the root, because of the high resistance to water movement offered by the compactly arranged cells of that region, and because of the absence of xylem elements to conduct water to the shoot. But according to Neales (1960), the roots of flax ceased to elongate after 48 hours under boron deficient conditions. The longitudinal sections showed that necrosis set in, and many secondary and tertiary roots were initiated along the roots already formed before the boron deficient treatment started. This induced a great deal of meristematic tissue on these roots, and it is therefore not surprising that these roots with the abundant meristems did not take as much water as normal ones.

Lachance (1940) found that boron deficient roots of cruciferss showed a thickening of the cell wall of the epidermis. According to Kramer (1959), the thickness of cell wall is an important factor limiting water absorption by the roots. It was observed in the present work, that the boron deficient flax roots had thicker cell walls, than healthy ones.

Baker, Gauch and Dugger (1956) summarized the effects of boron on the water relations as follows: the transpiration rate of boron deficient bean plant is lower than that of normal plant because of at least 3 factors. First there is a higher sugar and colloid

concentration in boron deficient leaves. Second, decreased rate of water absorption is reported, and thirdly, the transpiration is affected by an abnormal leaf morphology including a high percentage of non functional stomata.

In further experiments, cotyledons could be removed from the seedlings in order to find out if there is a certain amount of boron and calcium stored in that part of the plant, and for how long a cotyledonless plant can grow in deficient nutrient solutions.

Still other experiments might be conducted to study the effects of short periods of boron and of calcium deficiency, i.e. 5, 10, 15 days, using plants at different stages of development. This might suggest the best stage for boron fertilization to give highest seed production.

In still other experiments, lower boron levels such as 0.1 and 0.25 ppm could be used, in order to find out which would permit optimum growth and best yield.

At the same time it would be worthwhile finding out exactly how long flax plants could grow under boron and calcium deficiency conditions without developing irreversible deficiency symptoms.

VI SUMMARY

1.- "Marine" flax was grown in nutrient solutions containing all combinations of excess, normal and deficient levels of boron and calcium.

2- When flax seedlings were started in boron deficient solutions, the symptoms of boron deficiency occurred after 20 days, whatever the calcium level. On the stem, the symptoms were a yellowing of the leaves of the stem tip, and death of the growing point, while buds in the leaf axils developed into shoots all along the stem. Longitudinal sections of the deficient growing tips showed that all cells were disorganized, cell wall had disappeared, and the leaf primordia were necrotic. Microscopic examination of longitudinal sections of root tips showed that the tips were desintegrating, and that numerous secondary root primordia were either developing, or aborted, or had turned necrotic.

3 - Flax plants suffering boron deficiency for one month and transferred to a complete nutrient solution, grew both healthy and fasciated shoots, and new roots were formed from the deficient ones.

4 - Fasciated shoots developed an enlarged, flattened stem, that in most cases coiled at the stem tip. The fasciated stems developed flower that failed to set seed. The meristem region of the fasciated shoots contained more leaf primordia than in a normal plant.

5 - Symptoms of boron toxicity occurred after 15 days of treatment. These consisted of yellowing of the leaf tip, which then extended down the midrib until the whole leaf was yellow. The leaf then died. No symptoms were observed higher than half way up the stem, the first symptoms occurring on the cotyledons and first leaves.

6 - In nutrient solutions containing a low level of calcium, (40 ppm) flax plants showed "withertop" symptoms or bending over of the tip after 42 days. At the same time, shoots developed in the leaf axils, and then withered. In longitudinal sections it could be seen that in the meristematic region of calcium deficient plants, buds were developing very early in the leaf axils, and that there were bud primordia on the buds.

7 - Calcium deficient flax plants recovered when transferred to complete nutrient solution. They grew further shoots in the leaf axils, the plants took on a bushy appearance, and some of the new shoots withered again.

8 - Calcium excess (480 ppm) did not induce marked symptoms on flax plants. After 40 days of treatment, a yellowing of the bottom leaves was observed. This yellowing extended very slowly, and when the plants were mature, only about half the leaves were dead.

9 - Flax plants grown under CaH BN gave a significantly higher number of seeds than those in CaN BH. The percentage of good seeds was also significantly higher. Plants grown under CaN BN

and CaH BH gave about the same yield which was not significantly different from the others.

10 - The gas chromatography analysis of oil revealed a higher oleic acid content in CaN BN and CaH BN treatments, than in CaH BH and CaN BH. Although differences were small, linoleic and linolenic acid content appeared to be higher in CaN BH and CaH BH series than in the others.

11 - Compared with the control, plants under low boron treatments showed a highly significant negative decrease in water uptake during the time of treatment. The water uptake was still decreasing one week after transfer to complete nutrient solution. A projected regression line based on data of water uptake during 30 days can be used to predict when the plants would stop to absorb water.

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APPENDIX

TABLE OF ANALYSIS OF VARIANCE

Appendix table 1. Per cent good seeds per plant

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	51	51	4.2	10.13
Treatments	3	912	304	25.3	9.28
a	1	9	9	0.7	10.13
b	1	658	658	54.0	10.13
ab	1	245	245	20.0	10.13
Error	3	37	12		
Total	7	1000			

Appendix table 2. Number of good seeds per plant

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	4380	4380	2.81	10.13
Treatments	3	17069	5687	3.65	9.28
a	1	2093	2093	1.34	10.13
b	1	6864	6864	4.41	10.13
ab	1	8112	8112	5.22	10.13
Error	3	4663	4663		
Total	7	26112			

Appendix table 3. Weight of 1,000 seeds

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	0.0	0.0	0	10.13
Treatments	3	0.17	0.056	0.032	9.28
a	1	0.005	0.005	0.0028	10.13
b	1	0.09	0.09	0.056	10.13
ab	1	0.075	0.075	0.043	10.13
Error	3	0.52	0.173		
Total	7	0.69			

Appendix table 4. Seeds per boll

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	0.66	0.66	6.77	10.13
Treatments	3	0.61	0.20	2.22	9.28
a	1	0.04	0.04	0.44	10.13
b	1	0.20	0.20	2.22	10.13
ab	1	0.37	0.37	4.11	10.13
Error	3	0.28	0.09		
Total	7	1.55			

Appendix table 5. Bolls per plant

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	1.04	1.04	0.13	10.13
Treatments	3	18.37	6.06	0.78	9.28
a	1	15.60	15.60	2.02	10.13
b	1	0.30	0.30	0.03	10.13
ab	1	2.47	2.47	0.31	10.13
Error	3	23.17	7.72		
Total	7	42.58			

Appendix table 6. Per cent ripe bolls

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	15.96	15.96	0.14	10.13
Treatments	3	77.40	25.80	0.22	9.28
a	1	6.50	6.50	0.05	10.13
b	1	4.62	4.62	0.04	10.13
ab	1	66.28	66.28	0.59	10.13
Error	3	336.97	112.32		
Total	7	430.33			

Appendix table 7. Ripe bolls per plant

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	12	12	0.20	10.13
Treatments	3	29	7.6	0.12	9.28
a	1	18	18	0.30	10.13
b	1	0.4	0.4	0.06	10.13
ab	1	10.6	10.6	0.17	10.13
Error	3	178	59		
Total	7	219			

Appendix table 8. Total seeds per plant

Sources of variation	df	Sum of squares	Mean square	F	5%
Replicates	1	3124	3124	1.17	10.13
Treatments	3	4455	1485	0.55	9.28
a	1	1706	1706	0.64	10.13
b	1	607	607	0.22	10.13
ab	1	2142	2142	0.20	10.13
Error	3	7991	2663		
Total	7	15570			

Appendix table 9. Water uptake for 1-30th day of treatments

Treatments	Sum X	SQQX	Ave X	S. Dev	Corr. Coef.	O.b.	m
Time	891.6	64090	59.44	27.19	--	0.0	1.0000
CaL BL	641.0	32555	42.73	18.55	-.89**	78.94	-.60923
CaL BN	1306.0	115016	87.06	9.33	.31	80.70	.10698
CaL BH	1085.0	79273	72.33	7.26	.14	70.03	.03869
CaN BL	888.0	57614	59.20	18.33	-.88**	94.62	-.59606
CaN BN	1500.0	150000	100.00	0.00	1.00	100.00	0.000
CaN BH	1341.0	1207614	89.40	7.79	.08	88.00	02341
CaH BL	751.0	39929	50.06	12.46	-.84**	73.09	.38735
CaH BN	1314.0	116868	87.60	10.83	.09	85.46	.03595
CaH BH	1131.0	86491	75.40	8.99	.04	74.49	.01524

**Required for significance at 1% level: .641
Required for significance at 5% level: .514

Appendix table 10. Water uptake for 30 - 37th day of treatments

Treatments	Sum X	SQQX	Ave X	S. Dev	Corr. Coef.	O.b.	m
Time	398	28383	56.92	28.52	--	.00	1.000
CaL BL 1/	133	2923	19.00	7.52	-.53	27.04	-.14129
CaL BN	627	57203	89.57	12.19	-.53	102.50	-.22721
CaL BH	424	26546	60.57	11.10	-.94**	81.55	-.36864
CaN BL 1/	190	5372	27.14	5.54	-.25	30.43	-.05790
CaN BN	700	70000	100.00	0.00	1.00	100.00	0.00000
CaN BH	618	55280	88.28	10.13	-.39	96.19	-.13886
CaH BL 1/	254	9516	36.28	6.54	-.59	44.02	-.13596
CaH BN	605	53247	86.42	11.69	.04	85.39	.01811
CaH BH	555	44437	79.28	7.86	-.11	81.14	-.03260

** Required for significance at 1% level: 0.874
Required for significance at 5% level: 0.754

1/ Plants exposed to boron deficiency for 30 days, were transferred to complete (CaN BN) solution for the duration of the experiment. Plants exposed to 0.5 ppm and 2.5 ppm boron were maintained in test solutions throughout.

Appendix table 11. Water uptake for 1 to 37th day of treatments

Treatments	Sum X	SQQX	Ave X	S. Dev	Corr. Coep.	O.b.	m
Time	1222	86439	58.21	29.95	--	0.00	1.000
CaL BL 1/	756	35154	36.0	19.44	-.90**	73.70	-.64912
CaL BN	1793	156819.0	85.38	13.32	-.03	86.34	-.01654
CaL BH	1438	100778	68.47	10.48	-.45	78.76	-.17674
CaN BL 1/	1043	61651	49.66	21.65	-.91**	92.65	-.73837
CaN BN	2000	200000	100	0.00	1.00	100	0.000
CaN BH	1868	167796	88.95	8.81	-.06	90.09	-.01970
CaH BL 1/	972	48376	46.28	12.69	-.84**	69.46	-.39810
CaH BN	1799	158155	85.66	13.87	-.19	91.49	-.10009
CaH BH	1604	124204	76.38	8.96	.14	73.50	.04942

** Required for significance for 1% level: .56
Required for significance for 5% level: .44

1/ Plants exposed to boron deficiency for 30 days, were transferred to complete (CaN BN) solution for the duration of the experiment. Plants exposed to 0.5 ppm and 2.5 ppm boron were maintained in test solutions throughout.

Appendix table 12. Water uptake for 37th to 66th day of treatments

Treatments	Sum X	SQQX	Ave X	S. Dev	Corr. Coef.	O.b.	m m
Time	1332	92572	53.31	29.33	--	0.00	1.00000
CaL BL 1/	683	22029	27.32	11.60	.78**	10.75	.31066
CaL BN	1856	142916	74.24	14.32	.58**	59.06	.28464
CaL BH	1446	85810	57.84	9.32	.66**	46.63	.21014
CaN BL 1/	866	33850	34.64	12.41	.82**	15.97	.35005
CaN BN	25000	250000	100.00	0,00	1.00	100.00	0.00000
CaN BH	1996	160082	79.84	5.37	-.23	82.16	-.04369
CaH BL 1/	936	36612	37.44	7.92	.55**	29.47	.14933
CaH BN	2151	187787	86.04	10.42	.22	81.68	.08164
CaH BH	2194	192476	87.76	14.04	.34	78.95	.16519

** Required for significance at 1% level: 0.505
Required for significance at 5% level: 0.396

1/ Plants exposed to boron deficiency for 30 days, were transferred to complete (CaN BN) solution for the duration of the experiment. Plants exposed to 0.5 ppm and 2.5 ppm boron were maintained in test solutions throughout.

Appendix table 13. Water uptake for time of treatments

Treatments	Sum X	Ave X	SSQX	S. Dev	Corr	O.b.	m m
Date	3446	58.85	232635	27.11	--		1.000
CaL BL 1/	2753	43.01	160803	25.73	0.4880**	18.02	46408
CaL BN	5215	81.48	439525	15.09	-0.019	82.07	-.01102
CaL BH	4211	65.79	290115	14.27	0.164	61.12	.08680
CaN BL 1/	3342	52.21	218476	26.20	0.3660**	33.11	.35470
CaN BN	6400	100.00	640000	0.00		100.00	0.00000
CaN BH	5097	79.64	417851	13.64	-0.602**	95.98	-.30355
CaH BL 1/	3428	53.56	236260	28.68	0.480**	26.18	.50831
CaH BN	5731	89.54	527801	15.10	0.237	82.40	.13253
CaH BH	5626	87.90	513378	17.14	0.612**	67.04	.38737

**Required for significance at 1% level: 0.324
Required for significance at 5% level: 0.248

1/ Plants exposed to boron deficiency for 30 days, were transferred to complete (CaN BN) solution for the duration of the experiment. Plants exposed to 0.5 ppm and 2.5 ppm boron were maintained in test solutions throughout.