SOME EFFECTS OF BORON ON ROOT GROWTH

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Summary

The absence of boron in the root environment reduced the total linear growth of the radicles of the four dicotyledon and one monocotyledon species studied. After growth for 4 days in a boron-free medium, the growth rate of the maize radicle was comparable to that in a plus-boron medium, whilst the growth of the field bean radicle ceased. The minimum boron requirement for the unrestricted growth of the field bean (*Vicia faba* var. *minor*) radicle over 120 hr was 0·005 p.p.m. B. Each microgram of boron in this medium evoked a mean radicle elongation of 51 mm.

Chemical analysis and the growth response of beans upon transfer from plus-boron to boron-free solutions demonstrated that, despite a higher concentration of boron in the radicle tip (as in other young tissues), the reserves of boron in the radicle tip were sufficient to support only about 5 hr of growth at the plus-boron growth rate. Feeding experiments and seed analysis, coupled with growth studies, showed that there was little movement of boron from either the seed to the radicle or from the epicotyl to the radicle tip.

No regrowth occurs from the tips of radicles immersed for 72 hr or more in solutions lacking boron. An analogy is suggested between the effects of boron deficiency and X-irradiation on the bean root tip. The influence of boron deficiency on lignification and differentiation in the bean radicle is discussed.

I. Introduction

The growth of roots, either attached to the plant or when excised and grown in sterile culture (Neales 1959*b*), is severely restricted in the absence of boron in the growth medium. Whittington (1957, 1959) and Scholz (1959) have used the bean radicle for studies of the metabolic effects of boron deficiency. However, they did not investigate the boron requirement for the growth of this root: they used nutrient cultures either without boron or with a boron content (0·5 p.p.m.) in excess of that required for root growth.

This paper reports the results of experiments in which the minimal boron requirement for the growth of the bean and other radicles was examined. The bean radicle was also used in an examination of some physiological aspects of the growth inhibition effected by the absence of borate in the growth medium.

II. Methods and Materials

(*a*) *Plant Material*

The following species were used: field (or tick) bean (*Vicia faba* var. *minor*); broad bean (*Vicia faba* cv. Leviathan); garden pea (*Pisum sativum* cv. Greenfeast); maize (*Zea mays* cv. Hickory King); flax (*Linum usitatissimum* cv. Ventnor).

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(*b*) *Cultural Methods*

The seeds were sterilized in an ethanol–hydrogen peroxide mixture (1 : 1 v/v) for 5 min; they were then washed and soaked for 12 hr in boron-free water. The imbibed seeds were then allowed to germinate for 48 hr on moist vermiculite at 22°C in the dark. The seedlings were then transferred to the culture solutions, when their radicles were between 20 and 30 mm in length. The composition of the culture medium is given in Table 1. The micronutrients and iron were supplied from B.D.H. reagents, A.R. grade. The macronutrients and water were freed from boron contamination by methods described previously (Neales 1959*b*).

The seedlings were grown in 9-l. polythene containers, on each of which was placed a "Perspex" sheet in which holes 3 mm in diameter were drilled. The radicles passed through these holes into the nutrient solution. Up to 50 plants were grown in each container.

TABLE 1

COMPOSITION OF THE BASIC CULTURE MEDIUM

Boron was added, in varying amounts, as H_3BO_3

Macronutrients	Concn. (mg/l)	Microelements	Concn. (p.p.m.)	Microelements	Concn. (p.p.m.)
$Ca(NO_3)_2$	33·6	Fe(as Fe-EDTA*)	0·5	Cu(as $CuSO_4.5H_2O$)	0·0075
$MgSO_4.7H_2O$	4·2	Mn(as $MnSO_4.4H_2O$)	0·075	Zn(as $ZnSO_4.7H_2O$)	0·0075
KH_2PO_4	6·0	Mo(as $(NH_4)_6Mo_7O_{24}.4H_2O$)	0·0025	I(as KI)	0·0125

* Ethylenediaminetetra-acetic acid.

Radicle growth took place in a dark room at 22°C. The maximum growth period for any experiment was 170 hr. Radicle measurements were normally made each day by withdrawing the seedling and measuring the length of the radicle against a rectangle of clean, ruled, millimetre graph paper. From these measurements the daily increments of radicle growth were obtained. The radicle growth curves presented in this paper are a plot of the sum of the daily growth increments against time. This method of presentation reduces the variation due to the differences in radicle length at the beginning of each experiment.

(*c*) *Boron Analysis*

Boron was determined by a modification of the method of MacDougall and Biggs (1952). Root material was washed three times in boron-free water prior to drying at 95°C, weighing, and ashing. The plant ash was acidified and boiled for 2 min with hydrazine sulphate prior to filtration, thus reducing the nitrate in the ash which interferes with this estimation (Hewitt 1952, p. 196). Equivalent quantities of hydrazine sulphate were added to the reagent blanks. The optical density

(O.D.) of the quinalizarin–borate colour was measured at 600 mμ. A plot of O.D. against added boron was linear between 0–4 μg B.

(d) Translocation Studies using the Field Bean Seedling

Bean seedlings grown for 48 hr in plus-boron solutions were used, when the epicotyl was approximately 30 mm and the radicle 60 mm long. The epicotyl was then cut off 10 mm above the cotyledons and solutions fed via a glass capillary, which was pushed a distance of 5 mm into the stump of the epicotyl. Decapitation of the epicotyl did not affect radicle growth over 160 hr. Approximately 25 μl of

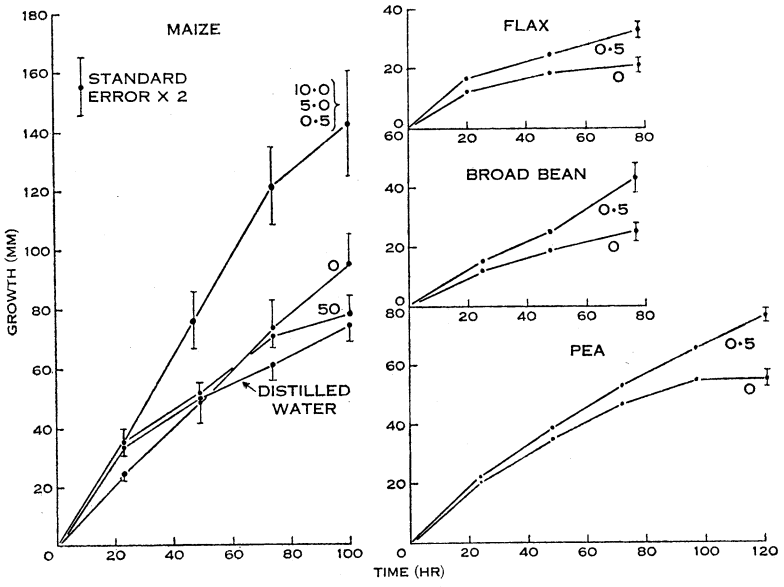

Fig. 1.—Effect of boron on the growth of the radicles of various species. The concentration of boron (in p.p.m.) in the culture medium is indicated near the respective growth curves.

solution was thus fed to each plant, and this was absorbed in 12 hr. Immediately after decapitation and the insertion of the capillary, the plants were transferred to a boron-free solution. The translocation of boron from the epicotyl to the radicle tip, a distance in excess of 70 mm, was assessed by the linear growth of the radicles of fed plants after transfer to the boron-free solution. The transfer of bean plants from a plus- to a minus-boron solution normally inhibits radicle growth after 48 hr (Table 6). After blotting the guttation drop which appeared on decapitation, there was no sign of external leakage of solution from the capillary. The possibility of leakage of boron from the upper part of the bean radicle into the culture solution, and thus supplying the radicle tip with boron, was minimized by renewing the minus-boron culture solution every 12 hr.

III. Results

Apart from an inhibition or reduction of radicle growth, the absence of boron in the radicle growth medium also induced a curling and swelling of the radicle tip (Plate 1), and differentiation of the stele almost to the tip of the radicle (Plate 1, Fig. 2). These observations accord with those of Warington (1923), Sommer and Sorokin (1928), and Whittington (1957, 1959).

(*a*) *Effect of Boron on the Growth of the Radicles of Maize, Pea, Broad Bean, and Flax*

The growth of the radicles of all the species studied was significantly reduced by the absence of boron in the growth medium (Figs. 1 and 2). Table 2 indicates the degree to which radicle growth rate was limited, over the last sampling period,

Table 2

Effect of the absence of boron in the root environment on the mean radicle growth rate over the last sampling period of each experiment

Plant	Period of Growth (hr after start of expt.)	Radicle Growth Rate (mm/24 hr)		Growth Rate in Minus-boron Medium as % of Plus-boron Growth Rate	Figure showing Growth Curves
		Plus Boron	Minus Boron		
Maize	74–99	20·5	20·2	98·5	Fig. 1
Field bean	68–95	27·8	0·1	0·4	Fig. 2(*a*)
Flax	48–78	6·2	3·0	48·4	Fig. 1
Broad bean	48–77	15·1	5·7	37·7	Fig. 1
Pea	97–121	11·1	0·4	3·6	Fig. 1

in boron-deficient media when compared with the growth rate in the presence of boron. It is apparent that the inhibitory effect of a lack of boron in the root environment is greatest in the field bean and least in maize. The lower boron requirement for the growth of the maize radicle, compared to that of the field bean, conforms to the generalization that monocotyledons have a lower boron requirement for growth than the dicotyledons (Bertrand and Silberstein 1941; Marsh 1942; Shkol'nik and Makarova 1949).

There was considerable variation in the radicle growth rate of the species studied. The mean growth over each experiment and the maximum daily growth rate are given in Table 3. The results of experiments with the field bean are included for comparison. Occasionally a growth of up to 70 mm in 24 hr was recorded for the maize radicle.

The growth of the radicle of both the field bean and maize was reduced in distilled water. However, the bean radicle was translucent and flaccid after 24 hr, whilst the maize radicle was slowly growing and of normal appearance after 72 hr in distilled water. It would seem that the maize radicle has a much lower ionic requirement for growth than that of the bean. A similar relationship exists between the boron requirement for radicle growth of the two species. The growth inhibition of the bean radicle by distilled water is identical to that reported by True (1914) for the radicle of *Lupinus albus*.

The radicle of the field bean has a high growth rate (approx. 30 mm per day), is very sensitive to the absence of boron, and grows without the development of lateral roots for 96 hr. This root is thus the most suitable of those examined for

TABLE 3

RADICLE GROWTH RATES IN PLUS-BORON SOLUTIONS

Plant	Duration of Experiment (hr)	Total Growth (mm)	Mean Growth Rate (mm/24 hr)	Period (hr)	Duration (hr)	Total Growth (mm)	Maximum Growth Rate (mm/24 hr)
Maize	72	129·6	43·2	24–48	24	50·1	50·1
Field bean	95	109·5	27·7	44–68	24	29·8	29·8
Broad bean	77	43·3	13·5	48–77	29	18·3	15·1
Flax	78	32·5	10·0	0–24	24	16·9	16·9
Pea	121	77·2	15·2	0–24	24	22·2	22·2

the study of boron requirement of roots. The remainder of this paper describes experiments in which the growth of the field bean radicle was studied in relation to the boron concentration in the external medium.

(*b*) *Boron Requirement for the Growth of the Field Bean Radicle*

The effect of the following boron concentrations on the growth of the field bean radicle was studied: 0, 0·5, 5·0, 10·0, and 50 p.p.m.; in addition, the growth of radicles in distilled water was investigated. The results of this experiment are given in Figure 2(*a*). The growth data for the 0·5, 5·0, and 10·0 p.p.m. boron treatments were statistically indistinguishable and were therefore pooled.

It is apparent from these data that a wide range of boron concentrations (0·5–10·0 p.p.m. B) supports a similar and high radicle growth rate. 50 p.p.m. B restricts, but does not inhibit, the growth of the bean and maize radicle. The growth of the bean radicle is inhibited after 48 hr in a growth medium lacking boron; this is similar to Whittington's (1957, 1959) results.

In a second experiment the growth of the bean radicle in media containing 0, 0·0005, 0·0025, 0·005, 0·05, and 0·5 p.p.m. B was studied. The results are given in Figure 2(*b*).

It is evident that the growth of the radicle over 100 hr is not restricted by lack of boron in concentrations above 0·005 p.p.m. At the end of the experiment all radicles growing in solutions of boron concentration of 0·0025 p.p.m. and below had the characteristic visual symptoms of boron deficiency (Plate 1). This was also true of a proportion of those radicles in 0·005 p.p.m. B. The growth of the radicles in 0·005 p.p.m. B from 120–168 hr was significantly less ($P<0·05$) than those in 0·5 p.p.m. B. It appeared, therefore, that the total boron supplied to

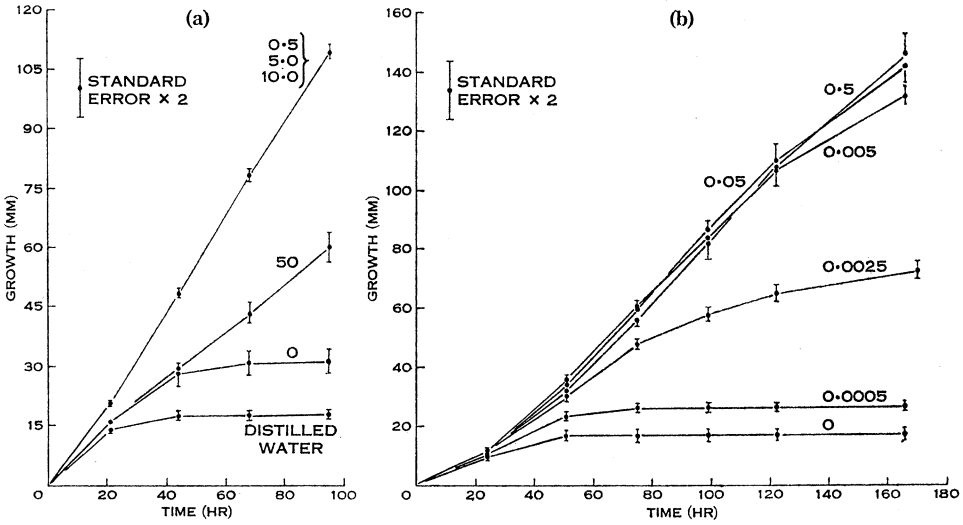

Fig. 2.—(*a*) Effect of 0–50 p.p.m. boron, and (*b*) of 0–0·5 p.p.m. boron on the growth of the field bean radicle.

the plants in the 0·005 p.p.m. boron treatment (45 μg) was just sufficient to support the growth of 20 plants for 168 hr. From these data it is possible to calculate the root growth response to the amount of boron supplied to the culture medium (Table 4).

From this table it is apparent that the maximum root growth response per unit amount of boron in the culture medium (51·0 mm growth per μg B supplied) occurs in the 0·005 p.p.m. B treatment. Similar root growth increments per μg B supplied are obtained in nutrient cultures of boron concentration lower than 0·005 p.p.m.; although, due to a lower boron content, root growth ceased before 168 hr (Fig. 2(*b*)). In the solutions of 0·05 and 0·5 p.p.m. B the root growth response (5·3 and 0·6 mm per μg B supplied) is smaller, indicating an incomplete utilization of the excess boron present in the medium.

Assuming a root growth extension response of 51·0 mm per μg B supplied, the mean extension growth of 17·6 mm in 0 p.p.m. B solution (Table 4) represents

a boron requirement of 0·35 μg B per plant. If there was no boron contamination in this medium, this 0·35 μg B is presumably derived from the bean cotyledons.

TABLE 4

BORON REQUIREMENT FOR THE GROWTH OF THE FIELD BEAN RADICLE

Concentrations of Boron in Nutrient Medium (p.p.m.)	Boron Added to 9 Litres of Solution (μg)	No. of Plants per Treatment	Mean Growth per Plant (mm)	Mean Increase in Root Length minus Increase in 0 p.p.m. Boron (mm)	Increase in Root Length per μg Boron Supplied (mm)
0	0	20	17·6	—	—
0·0005	4·5	20	26·6	9·0	39·1
0·0025	22·5	20	72·5	54·9	48·6
0·005	45	20	132·3	114·7	51·0
0·05	450	19	142·5	124·9	5·3
0·5	4500	20	146·6	129·0	0·6

Chemical analysis of a sample of imbibed beans and also of the testas of 5-day-old bean seedlings (Table 5) indicated that the boron content of the testa (0·9

TABLE 5

BORON CONTENT OF THE FIELD BEAN SEED

Stage of Development	Portion of Seed Analysed	Number	Dry Wt. (g)	Total Boron Content (μg)	Boron Content per Seed (μg)	Boron Concn. (p.p.m.)
Ungerminated but imbibed seed	Cotyledons, plus plumule and radicle	50	18·278	65·00	1·30	3·56
	Testa	50	2·900	43·75	0·88	15·09
	Complete seed	50	21·178	108·75	2·18	5·14
5-day-old seedlings	Testa	25	1·520	23·52	0·94	15·47

μg B per seed) is not available to the seedling during 5 days of growth. Thus the maximum amount of boron from the seed available for seedling growth is repre-

sented by that present in the cotyledons and embryo (1·3 μg B per seed). These values for the boron content of beans are similar to those reported by Owen, Snow, and Thom (1945), but differ from those of McLean and Hughes (1936) who used a much older analytical technique.

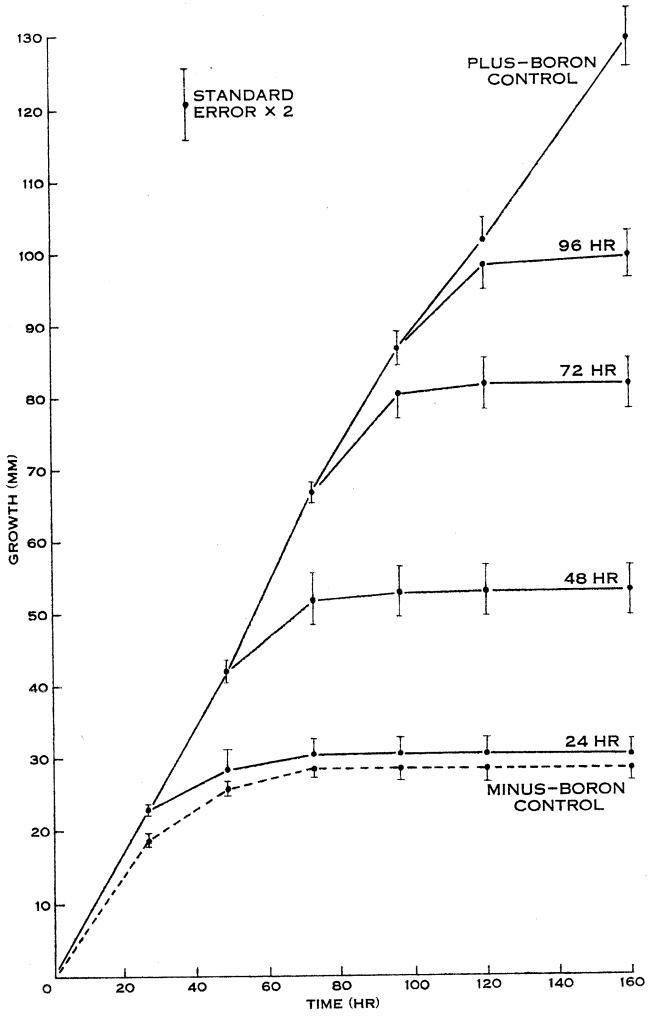

Fig. 3.—Effect of transfer from plus-boron (0·5 p.p.m.) to minus-boron solutions on the growth of the field bean radicle. Times at which plants were transferred are indicated.

(c) *Effect on Radicle Growth of Transferring Beans from Plus-boron to Minus-boron Solutions and vice versa*

A large sample of beans was grown in either 0·5 p.p.m. or 0 p.p.m. B solutions. At varying times from the beginning of the experiments beans supplied with boron in the culture medium were transferred, after washing the radicles in water,

to minus-boron cultures. The converse of this experiment was also done. Radicle lengths were measured periodically before and after the transfer.

(i) *Effect of Transferring Beans from* 0·5 *to* 0 *p.p.m. B Solutions.*—The results of this experiment are given in Figure 3. Despite the fact that the boron content of the medium was approximately 100 times that necessary for growth, it is apparent that the removal of boron from the root environment causes a very rapid inhibition of growth, and this is independent of the time at which the transfer is made (Table 6). It is apparent therefore that, under these conditions, the radicle is unable to maintain a physiologically active internal supply of boron. The increments of radicle growth in the 48 hr after transfer to minus-boron solutions are given in Table 6.

TABLE 6

RADICLE GROWTH IN THE FIRST AND SECOND 24-HR PERIOD AFTER TRANSFERRING BEAN SEEDLINGS FROM PLUS- TO MINUS-BORON SOLUTIONS

Time of Transfer	No. of Beans	Mean Growth (mm) after Transfer to Minus-boron Solutions		No. of Beans	Mean Growth (mm) of Beans not Transferred	
		First 24 Hours	Second 24 Hours		First 24 Hours	Second 24 Hours
At 24 hr	10	6·4	1·7	48	19·1	24·9
At 48 hr	10	8·8	0·5	38	24·9	19·9
At 72 hr	9	6·6	1·4	29	19·9	15·0
At 96 hr	10	4·7	1·3	19	15·0	14·5

Radicles transferred after growth for 72 hr in a 0·5 p.p.m. B solution grew a further 8·0 mm in the minus-boron solution. This growth corresponds to a "carry over" of 0·16 μg of physiologically active boron, assuming that 1 μg B evokes a radicle elongation response of 51·0 mm (Table 4). This estimate of the amount of boron in the radicle tip was compared with that found by chemical analysis of the radicles of beans grown for 72 hr in nutrient solutions containing 0·5 p.p.m. B (Table 7). It was found that the boron present in each radicle tip was approximately 0·04 μg, and that each whole radicle contained approximately 0·1 μg B. These results are further considered in Section IV.

(ii) *Effect of Transferring Beans from a Minus- to a Plus-boron* (0·5 *p.p.m.*) *Solution.*—The results of this experiment are given in Figure 4. These data indicate that the radicle apical meristem is irreversibly damaged if immersed for 72 hr or more in a minus-boron medium. Radicles grown for 24 hr in a minus-boron medium and then transferred to a plus-boron medium have a subsequent growth indistinguishable from the plus-boron control treatment. The effect of transfer from

a minus- to a plus-boron medium at 48 hr has an effect intermediate between the results of transfer at 24 and 72 hr.

The large variability in the mean length of the radicles transferred at 48 hr may be attributed to the fact that one radicle failed to grow after transfer to the

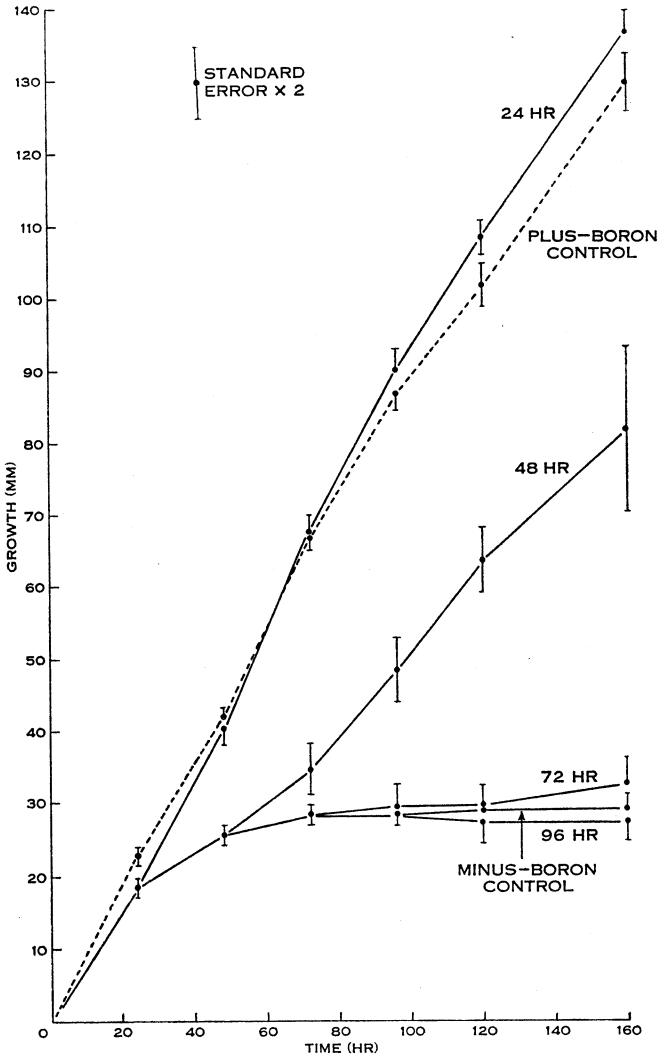

Fig. 4.—Effect of transfer from minus-boron to plus-boron (0·5 p.p.m.) solutions on the growth of the field bean radicle. Times at which plants were transferred are indicated.

plus-boron solution; the remainder recovered and from day 5 to day 7 grew as rapidly as the plus-boron controls. The regrowth of radicles after 72 hr in a solution lacking boron was apparent only in four radicles, and was evident only in the last day of the experiment. Examination showed that this regrowth proceeded from a

lateral root initial, which can be seen to be present almost to the tip of an old boron-deficient root (Plate 1, Fig. 4). After 48 hr in a minus-boron solution, the regrowth of roots was apparently terminal, but a constriction behind the meristem

TABLE 7

BORON CONTENT OF THE RADICLE TIPS OF BEANS GROWN FOR 72 HR IN A NUTRIENT SOLUTION CONTAINING 0·5 p.p.m. BORON

Portion of Radicle	No. in Sample	Fresh Weight (mg)	Dry Weight (mg)	Boron Content per Radicle (μg)	Boron Concentration (p.p.m.)		
					Fresh Weight Basis	Dry Weight Basis	Root Volume Basis
Experiment 1							
20-mm tip	47	709·4	43·9	0·035	2·29	37·1	2·75
Remainder of radicle	57	8330·4	498·3	0·056	0·38	6·4	—
Total				0·091			
Experiment 2							
15-mm tip	70	836·1	54·9	0·044	3·71	56·5	4·61
Remainder of radicle	60	9582·7	552·7	0·056	0·35	6·1	—
Total				0·100			

was initially apparent (Plate 1, Fig. 4). A similar feature has been illustrated in the boron-deficient pea root tip (Sommer and Sorokin 1928, plate VII, fig. 2),

TABLE 8

EFFECT OF FEEDING SUCROSE AND BORIC ACID THROUGH THE EPICOTYL ON THE GROWTH OF BEAN ROOTS

Boron Content of Growth Medium (p.p.m.)	Solution (vol. 25 μl) Fed via Epicotyl	Root Growth after 74 Hr (mm)
0·5	—	75·5±3·7
0	Water	13·0±2·6
0	50 μg boron	37·8±3·5
0	3000 μg sucrose	13·8±1·3
0	50 μg boron + 3000 μg sucrose	32·7±4·1

and also in the tip of the bean root after irradiation with X-rays (Gray and Scholes 1951). Thus, it is most probable that the bean root terminal meristem is damaged if boron is absent from the root environment for periods above 24 hr.

(d) Translocation of Boron from the Epicotyl to the Radicles of the Bean

Since the lack of boron has such a marked inhibition on the growth of the bean radicle, and also because the site of this inhibition resides in the radicle meristem, the bean seedling is a most suitable object for the study of boron translocation. The methods used in this experiment have been described earlier.

Eight plants were allocated to each of five treatments (Table 8). At zero time the plants were fed with various solutions through their epicotyls, the radicles were washed in boron-free water, and they were then transferred to nutrient media

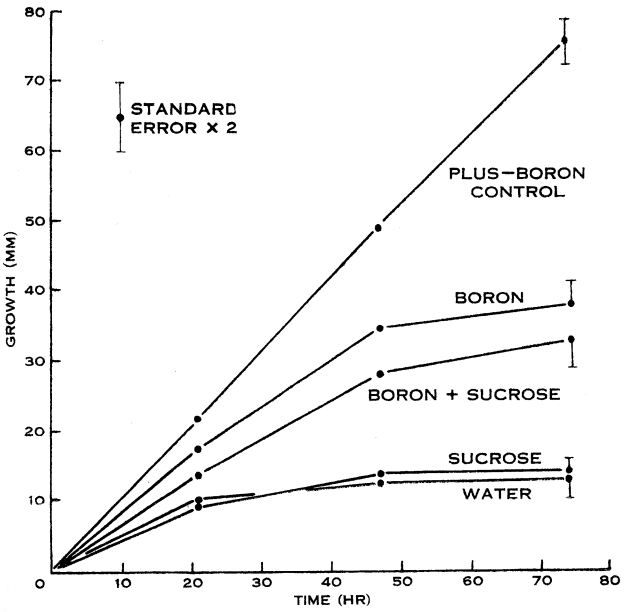

Fig. 5.—Effect of feeding sucrose and boron (as H_3BO_3) via the epicotyl on the growth of the field bean radicle in a boron-deficient medium. The plus-boron control plants were growing in a medium containing 0·5 p.p.m. boron (see Table 8).

lacking boron. The radicle growth of each plant was then individually followed for 74 hr. The radicle growth curves are given in Figure 5 and accumulated radicle growth of each treatment in Table 8.

These data demonstrate that the inhibition of radicle growth in minus-boron solutions is not alleviated by feeding sucrose through the epicotyl. The radicle of beans in the 0·5 p.p.m. B solutions (in which 45 μg B was available to each plant) had a uniform and normal growth rate for 74 hr (Fig. 5), whereas 50 μg boron fed through the epicotyl of each plant growing in minus-boron solutions did not prevent either a large decrease in radicle growth rate or the eventual appearance of boron deficiency symptoms in the radicle tips. It is apparent that there is a very limited movement of boron from the epicotyl to the radicles.

IV. Discussion and Conclusions

(a) *Boron Concentration Necessary for Root Growth*

The results of the first experiments reported in this paper consist of a quantitative description of the degree to which the radicle growth of several species is dependent upon an external supply of boron. In the field this potential restriction of root growth is rarely manifest because most soils contain adequate available boron for plant growth (Swaine 1955). Haynes and Robbins (1948) have reported that both calcium and boron in the root environment are indispensable for root growth.

The smallest concentration of boron in the root environment that supported the normal growth of the bean radicle over 168 hr was 0·005 p.p.m. boron (Fig. 2). This is 200 times less than the boron concentration used by Whittington (1957, 1959) in his "plus boron" series, and is similar in magnitude to the minimal boron requirement for the growth of broad beans (Brenchley and Warington 1927) and flax (Neales 1959*a*).

(b) *Effects of Boron Deficiency on Root Differentiation*

The significance of the advance of lignification and stelar differentiation almost to the tip of the boron-deficient radicle tip (Plate 1, Fig. 2) may be interpreted in at least two ways: (1) Whittington (1957) has demonstrated the inhibition of cell division in the bean radicle tip by lack of boron in the growth medium. If is it assumed that the normal differentiation of the primary stelar structure is not altered by boron-deficiency, then it is possible to envisage the formation of a lignified stele almost to the tip of the radicle, as indeed is the case in boron-deficient radicles. (2) On the other hand, it has been established that boron-deficient plants contain a higher peroxidase (Nason, Oldewurtle, and Propst 1952; Odhnoff 1957) and polyphenol oxidase (Reed 1947; MacVicar and Burris 1948; Klein 1951) activity than normal plants. Furthermore, boron-deficient plants have been shown to accumulate caffeic and chlorogenic acids (Perkins and Aronoff 1956), the former of which has been shown (McCalla and Neish 1959) to be a precursor of lignin. Thus it is possible that lignin formation is actually *enhanced* in boron-deficient bean roots. This possibility also reopens the question of the effect of boron deficiency on the auxin status of plants (Eaton 1940). Jensen (1955) has demonstrated that lignification and peroxidase activity in the bean root could be increased by treatment with indolylacetic acid (IAA), and Torrey (1953) recorded similar effects of IAA on lignification in isolated pea root tips. Thus one possible explanation of this effect of boron deficiency on lignification and peroxidase activity is that boron-deficient roots contain high concentration of an auxin identical, or similar in action, to IAA. This hypothesis is the opposite of that of Eaton (loc. cit.) who attempted to reverse the effects of boron deficiency by supplying plants with exogenous IAA.

If one of the functions of boron in plants concerns the orderly regulation of differentiation and lignification (as discussed above), then the observation of Brown, Wright, and Neish (1959), that monocotyledons possess a lignin biosynthesis pathway not possessed by dicotyledons, may afford a possible reason for the much lower boron requirement for the growth of the maize radicle compared to that of

the dicotyledonous species (Table 2). It is apparent that a study of the effects of boron deficiency on the phenolic acid pool in plants (McCalla and Neish 1959) is most desirable.

(c) *Mobility of Boron in the Bean Seedling*

Vascular plants can grow normally in media of very low boron content. It is apparent therefore that the initial distribution of boron, from the external medium to all parts of the plant, must be accomplished without restriction. However, under boron-deficient conditions there is good evidence (Skok 1957*a*) that redistribution of boron after absorption into sunflower seedlings is either lacking or of small extent. The data given in this paper for the bean seedling support this conclusion. The linear radicle growth of bean seedlings, planted into a medium deficient in boron when their radicles were 20 mm long, was 17·6 mm after which growth ceased (Fig. 2). This is equivalent (Table 4) to the radicle growth-promoting effect of 0·35 μg B, whereas the cotyledons of each bean contained 1·30 μg B (Table 5). Thus only 27 per cent. of the cotyledonary boron is available for radicle growth. The limited movement of the boron from the cotyledons to the roots of bean plants has also been reported by McLean and Hughes (1936). The experiments reported above on the degree to which excess boron fed to the hypocotyls moves to the radicles of bean seedlings also demonstrated that boron did not move readily from epicotyl to radicle tip (Table 8; Fig. 5). In fact, the stimulation caused by feeding 50 μg B (with or without sucrose) was equivalent to the movement to the radicle tip of a "growth activity" equivalent to only 0·4 μg B. A similar restricted growth response to boron solutions injected into plants is recorded by Maier (1941).

(d) *Boron Utilization and Storage by the Radicle Tip*

There was a rapid inhibition of radicle growth upon the transfer of bean seedlings from a plus- to a minus-boron culture medium (Fig. 3). This inhibition indicated that there was a rapid conversion of the physiologically active boron (Skok and McIlrath (1958) demonstrated this to be the dialysable fraction of the boron in plants) to the inactive form, and hence the need for a continuous supply of this element for unrestricted root growth. This indicated that either boron could not be accumulated in any quantity by the radicle tip (where it exerts its growth-promoting effects (Whittington 1957)) or the rate of utilization is so high that the tip becomes rapidly deficient in "active" boron. It was found (Table 7) that the mean boron content of 15 mm of radicle tips of plants growing for 72 hr in 0·5 p.p.m. B was 0·044 μg B. This quantity of boron has a growth potential (Table 4) of approximately 2·5 mm. However, the growth over 24 hr of these radicles after washing and transfer to minus-boron solutions was 8·0 mm (Table 6). It would appear, therefore, that the limited radicle growth after transfer from the 0·5 p.p.m. B solution is in excess of that to be expected from the boron content of the radicle tip. This "excess" growth, which equals $8{\cdot}0-2{\cdot}5 = 5{\cdot}5$ mm, is equivalent to approximately 0·1 μg B (Table 4), and may be attributed either to a slight carry-over of boron from the plus-boron solution or to the limited translocation of boron from the upper portion of the radicle to the radicle tip. Thus

despite the fact the roots were growing in a solution whose boron content (0·5 p.p.m. B) was 100 times in excess of the minimal requirement, the radicle *tips* did not contain enough boron (assuming it to be all available for growth) to support normal growth for more than a few hours. In terms of root volume the radicle tip concentration of boron is only 5·5 times that of the external medium. These results point to the fact that in the growing radicle tip there is an extremely rapid absorption and utilization of boron, with a very low internal reserve indeed. It is of note that the boron concentration in 15 mm of radicle tips (Table 7) is approximately 10 times that in the remainder of the radicle. This is consistent with the observations of Bertrand and Silberstein (1944, and previous publications listed therein) and McLean and Hughes (1936), who reported higher concentrations of boron in meristematic and embryonic, compared to older, differentiated plant tissues.

(*e*) *Boron and Cell Division*

Whittington (1957) has recorded that in the absence of boron mitotic division in the bean radicle meristem ceases. Many authors (see, for instance, Warington 1926; Sommer and Sorokin 1928; Jolivette and Walker 1943; and Vial, Carlton, and Strang 1957) have suggested that boron is necessary for cell division in both primary and secondary meristems in many different plants. Experiments reported in this paper (Fig. 4) indicate that in radicles grown for 72 hr in a minus-boron medium, no revival of growth and cell division takes place upon transfer to plus-boron solutions. The regrowth of the root after 48 hours in a minus-boron medium (Plate 1, Fig. 4) is similar in appearance to the type of regrowth obtained after X-irradiation of bean meristems (Gray and Scholes 1951), which Clowes (1959) has suggested are chimerical in nature. This chimera, Clowes suggests, is due to the commencement of division by cells of the pro-meristem that were previously quiescent. This indication that it is the dividing cells that are most damaged by a lack of boron is consistent with the conclusions of Skok (1957*b*) who demonstrated that X-irradiation damage to sunflower seedlings is reduced if boron is removed from the root environment of these plants for a period prior to irradiation.

Although it is improbable that the absorption of X-irradiation by plant tissues will be related to their boron content, it is interesting to note (Conger and Giles 1950; Locksley and Sweet 1954) the direct relationship between radiation damage to living tissues by thermal neutrons and the boron content of these tissues. This is due to the fact that slow neutrons cause the naturally occurring isotopes ^{10}B and ^{11}B to emit ionizing radiation. A high proportion of the damage done to *Tradescantia* anthers can be attributed to the neutrons absorbed by the boron in this tissue, despite the fact that the boron content of this tissue was 2·9 p.p.m. (fresh weight basis) (Conger and Giles 1950). Thus since bean radicle tips contain more boron than the rest of the radicle (Table 7), irradiation by slow neutrons will engender damaging radiation especially in the vicinity of those cells (the region of cell division and early differentiation) which are most sensitive to such irradiation (Gray and Scholes 1951; Clowes 1959). These considerations give some radiobiological importance to the distribution of boron in plants.

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VI. References

Bertrand, G., and Silberstein, L. (1941).—Sur la repartition du bore parmi les espèces vegetales. *C.R. Acad. Agric. Fr.* **27**: 24.

Bertrand, G., and Silberstein, L. (1944).—Sur la repartition du bore dans les diverses partes de la graine. *Ann. Agron., Paris* **14**: 257.

Brenchley, W. E., and Warington, K. (1927).—The role of boron in the growth of plants. *Ann. Bot., Lond.* **41**: 167.

Brown, S. A., Wright, D., and Neish, A. C. (1959).—Studies in lignin biosynthesis using isotopic carbon. VII. *Canad. J. Biochem. Physiol.* **37**: 25.

Clowes, F. A. L. (1959).—Reorganization of root apices after irradiation. *Ann. Bot., Lond.* (N.S.) **23**: 1.

Conger, A. D., and Giles, N. H. (1950).—The cytogenetic effect of slow neutrons. *Genetics* **35**: 397.

Eaton, F. M. (1940).—Interrelations of the effects of boron and IAA on plant growth. *Bot. Gaz.* **101**: 700.

Gray, L. H., and Scholes, M. E. (1951).—The effect of ionizing radiation on the broad bean root. VIII. Growth rate studies and histological analysis. *Brit. J. Radiol.* (N.S.) **24**: 82–92, 176–80, 228–36, 285–91, 348–52.

Haynes, J. L., and Robbins, W. R. (1948).—Calcium and boron as essential factors in the root environment. *J. Amer. Soc. Agron.* **40**: 795.

Hewitt, E. J. (1952).—Sand and water culture methods used in the study of plant nutrition. Tech. Commun. Bur. Hort., E. Malling. No. 22.

Jensen, W. A. (1955).—The histochemical localization of peroxidase in roots and its induction by IAA. *Plant Physiol.* **30**: 426.

Jolivette, J. P., and Walker, J. C. (1943).—Effect of boron deficiency on the histology of garden beet and cabbage. *J. Agric. Res. Wash.* **66**: 167.

Klein, R. M. (1951).—The relation of gas exchange and tyrosinase activity of tomato tissues to the level of boron nutrition of the plants. *Arch. Biochem.* **30**: 207.

Locksley, H. B., and Sweet, W. H. (1954).—Tissue distribution of boron compounds in relation to neutron-capture therapy of cancer. *Proc. Soc. Exp. Biol., N.Y.* **86**: 56.

MacDougall, D., and Biggs, D. A. (1952).—Estimation of boron in plant tissues. *Anal. Chem.* **24**: 566.

MacVicar, R., and Burris, R. H. (1948).—Relation of boron to certain plant oxidases. *Arch. Biochem.* **17**: 31.

Maier, W. (1941).—Untersuchungen über die Notwendigkeit des Bors in der Pflanze. *Landw. Jb.* **90**: 105. (*Biol. Abstr.* **24**: 30974 (1950).)

Marsh, R. P. (1942).—Comparative study of the Ca/B metabolism of representatives of dicotyledons and monocotyledons. *Soil Sci.* **53**: 75.

McCalla, D. R., and Neish, A. C. (1959).—Metabolism of phenylpropanoid compounds in *Salvia*. II. Biosynthesis of phenolic cinnamic acids. *Canad. J. Biochem. Physiol.* **37**: 537.

McLean, R. C., and Hughes, W. L. (1936).—The quantitative distribution of boron in *Vicia faba* and *Gossypium herbaceum*. *Ann. Appl. Biol.* **23**: 231.

Nason, A., Oldewurtle, H. A., and Propst, L. M. (1952).—Role of micronutrients in the metabolism of higher plants. I. Changes in oxidative enzyme constitution of tomato leaves deficient in micronutrient elements. *Arch. Biochem. Biophys.* **38**: 1.

NEALES, T. F. (1959*a*).—Effect of boron supply on the sugars, soluble in 80% ethanol, in flax seedlings. *Nature* **183**: 483.

NEALES, T. F. (1959*b*).—The boron requirement of flax roots grown in sterile culture. *J. Exp. Bot.* **10**: 426.

ODHNOFF, C. (1957).—Boron deficiency and growth. *Physiol. Plant.* **10**: 984.

OWEN, E. C., SNOW, D., and THOM, C. L. (1945).—The effect of borax on the growth and yield of field beans. *J. Agric. Sci.* **35**: 119.

PERKINS, H. J., and ARONOFF, S. (1956).—Identification of the blue fluorescent compounds in boron deficient plants. *Arch. Biochem. Biophys.* **64**: 506.

REED, H. S. (1947).—A study of boron deficiency in plants. *Hilgardia* **17**: 377.

SCHOLZ, G. (1959).—Über die physiologische Wirkung des Bors auf Keimwurzeln von *Vicia faba*. *Flora* **148**: 295.

SHKOL'NIK, J. J., and MAKAROVA, N. A. (1949).—Possible reasons for different boron requirements in mono- and dicotyledonous plants. *Dokl. Akad. Nauk. S.S.S.R.* **68**: 613. (*Chem. Abstr.* **44**: 710 (1950).)

SKOK, J. (1957*a*).—The substitution of complexing substances for boron in plants. *Plant Physiol.* **32**: 308.

SKOK, J. (1957*b*).—Relationship of boron nutrition to radiosensitivity of sunflower plants. *Plant Physiol.* **32**: 648.

SKOK, J., and MCILRATH, W. J. (1958).—Distribution of boron in cells of dicotyledonous plants. *Plant Physiol.* **33**: 428.

SOMMER, A. L., and SOROKIN, H. (1928).—Effects of the absence of boron and some other essential elements on cell and tissue structure of the root tips of *Pisum sativum*. *Plant Physiol.* **3**: 237.

SWAINE, D. J. (1955).—The trace-element content of soils. Comm. Bur. Soil Sci. Tech. Commun. No. 48.

TORREY, J. G. (1953).—The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots. *Amer. J. Bot.* **40**: 525.

TRUE, R. H. (1914).—The harmful effect of distilled water. *Amer. J. Bot.* **1**: 255.

VAIL, J. W., CARLTON, W. E., and STRANG, R. M. (1957).—Dieback in wattle—a boron deficiency. *E. Afr. Agric. J.* **23**: 100.

WARINGTON, K. (1923).—Effect of boric acid and borax on the broad bean and certain other plants. *Ann. Bot., Lond.* **37**: 629.

WARINGTON, K. (1926).—The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. *Ann. Bot., Lond.* **40**: 27.

WHITTINGTON, W. J. (1957).—The role of boron in plant growth. I. *J. Exp. Bot.* **8**: 353.

WHITTINGTON, W. J. (1959).—The role of boron in plant growth. II. The effect on growth of the radicle. *J. Exp. Bot.* **10**: 93.

EXPLANATION OF PLATE 1

Fig. 1.—Bean seedlings grown for 76 hr in the presence (*B*) or absence (*A*) of boron. ×0·25.

Fig. 2.—Bean radicles grown for 120 hr in the presence (*B*) or absence (*A*) of boron, and subsequently cleared and stained with acid phloroglucinol. Note the extent of lignification in *A* compared with *B*. ×0·4.

Fig. 3.—Pea root tips after 72 hours' growth in the presence (*B*) or absence (*A*) of boron. Note the swollen root and enlarged root cap of *A*. ×6.

Fig. 4.—The tips of bean roots grown for 96 hr in the presence (*B*) or absence (*A*) of boron. *C* is a bean root tip grown for 48 hr in a minus-boron solution, followed by 48 hr in a solution containing 0·5 p.p.m. boron. ×1·75.

EFFECTS OF BORON ON ROOT GROWTH