A RELATIONSHIP BETWEEN BORON & AUXIN IN C^{14} TRANSLOCATION IN BEAN PLANTS [1, 2]

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The essentiality of boron for higher plants is well established although no definitive role proposed for this element has remained undisputed. So many processes are affected indirectly by boron that the primary effect(s) have remained obscure (9). Gauch and Dugger (9) suggest that a relatively specific role in carbohydrate translocation is indicated. Sucrose translocation is considerably reduced in boron deficient plants before morphological symptoms are evident. Gauch and Dugger (8) maintain that subsequent symptoms are simply the expression of carbohydrate deficiency resulting from the impaired translocation system. They suggest that a negatively charged sugar-boron complex might more easily traverse cell membranes than non-borated sugar molecules or that boron might be a constituent of the membrane site across which the sugar moves. Skok (20, 21, 22) points out that it is also possible for the relationship of boron to translocation to be indirect. He proposes that the boron effect on translocation is the result of the elements' essentiality to the metabolic activity of meristematic regions which have large substrate requirements. The effect of this particular metabolic activity including that associated with cell enlargement can be called sink effect; its relationship to translocation is now well established (3, 12, 23). If Skok is correct, cessation of growth would be the cause rather than the result of impaired carbohydrate translocation in boron deficient plants.

Dugger et al. (4) proposed that boron affects the rate of translocation through its effect on the sugar-starch balance of the leaves. Scott (18) presents a similar hypothesis: "It appears that boron performs a protective function in plants in that it prevents excessive polymerisation of sugars at sites of sugar synthesis". It would appear, however, that boron must have an additional role in the metabolism of meristematic regions, since these last mentioned theories deal primarily with the availability of carbohydrates for translocation; applications of sugars do not prevent the death of terminal buds of boron deficient plants (19, 20).

Prior to breakdown of phloem tissue in the more advanced stages of deficiency, the capacity for translocation should exist in boron-deficient plants although this capacity is not normally expressed. If metabolic activity or growth can be promoted in boron deficient plants by some means such as auxin applications, then translocation should increase if boron is not directly essential to the process. Our experiments designed to test this proposition will be described here.

MATERIALS & METHODS

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) were used in all experiments in this study. Seeds were soaked for 2 hours in distilled water and then transferred to washed, fine quartz sand in hard rubber trays for germination. After 7 days, plants were transferred to nutrient solutions in black-painted, aluminum foil-wrapped 1-quart Mason jars. The aerated mineral solution consisted of the following salts: 5×10^{-3} M $Ca(NO_3)_2$; 5×10^{-3} M KNO_3; 2×10^{-3} M $MgSO_4$; 1×10^{-3} M KH_2PO_4; 1×10^{-4} M EDTA Na-Fe (5 ppm Fe); 9.2×10^{-6} M $MnCl_2 \cdot 4H_2O$; 5.7×10^{-7} M Na_2MoO_4; 5.1×10^{-7} M $ZnCl_2$; 2.9×10^{-7} M $CuCl_2 \cdot 2H_2O$. All glassware was either boron-free or low-boron soft glass.

Germination and subsequent growth took place in a controlled environment room. The light intensity supplied by cool white fluorescent tubes (96 T8) and 60 w tungsten bulbs (10: 1 wattage ratio) was approximately 1,000 ft-c at the base of the plant. Temperatures were maintained at 24 ± 2 C during the 16 hour light period and 18 ± 2 C during the 8 hour dark period. Under these conditions boron deficiency symptoms were visible in the terminal region approximately 14 days after seed soaking.

To half the plants boron, as H_3BO_3, was added to give a concentration of 4.6×10^{-5} M. Naphthaleneacetic acid (NAA) was used for auxin treated plants because of its greater biological stability when compared to IAA. Application of NAA to the terminal bud consisted of placing a 10 lambda drop of 5 ppm

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[1] Received March 13, 1961.

[2] Contribution No. 643, Department of Botany and Plant Pathology, Ohio State University, Columbus. The work reported here was part of a dissertation submitted by the senior author to the graduate school of the Ohio State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The authors gratefully acknowledge the assistance of a grant from the Rockefeller Foundation made to Dr. C. A. Swanson.

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NAA in a 0.02 % Tween-80 (polyoxyethylene sorbitan monooleate) solution directly on the bud. Application of NAA to the root system was accomplished by additions of 1 ml aliquots of a 9.5 ppm NAA stock solution per quart jar. The first addition provided a concentration of 0.01 ppm in the mineral culture solution.

A leaf-cup method modified from Aronoff (2) was used to supply one of the primary leaves with 10 μc of $C^{14}O_2$ in a 5 % atmosphere of CO_2. In this modification, the CO_2 is generated in the leaf cup after it is sealed to the underside of the leaf with petrolatum. This method has the advantage that reproducible quantities of C^{14} as Na_2CO_3 can be pipetted into the leaf cup prior to its being affixed to the leaf. The CO_2 is released by injecting 1 N HCl through a serum vial stopper by means of a hypodermic syringe. Application of $C^{14}O_2$ took place in an illuminated fume hood. The light intensity was of the same magnitude as in the controlled environment room but supplied from a water-filtered tungsten lamp source. A fixation and translocation period of 2 hours was allowed prior to plant harvest. Plants were harvested by excising the terminal bud and root systems and placing the excised parts in boiling 80 % ethanol for extraction on a water bath at 60 C for 1 hour. The extracts were decanted directly into planchets and the extraction process repeated. Extracted tissues were dried at 50 C for 48 hours and weighed. Relative counting rates of samples were determined by use of a 1.4 mg/cm^2 end-window G.M. tube encased in a lead shield.

Radioactivity assays expressed as counts/minute · mg dry weight were analyzed by means of the Kruskal-Wallis Test (11), a non-parametric analogue of the analysis of variance test, to determine over-all significant differences. The non-parametric test was used since the extreme non-homogeneity of variance among the samples violated the assumptions of the more commonly used F test. The Mann-Whitney U test (14) was used as a non-parametric analogue of the t-test to determine specific differences between pairs of groups.

Results

Bud Application of NAA. NAA (10 λ of 5 ppm solution) was applied to two groups of eight plants each; one group was supplied with boron (B+), the other group was boron deficient (B−). Applications were made to each plant 24, 12, and 1 hour(s) before $C^{14}O_2$ administration. The first application was made at the time of incipient boron deficiency symptoms in the B− plants after about seven days in nutrient solution (table I). The amount of ethanol-soluble C^{14} appearing in the terminal region after a 2-hour translocation period in the B− plants is significantly less than in the B+ plants. Boron-deficient plants treated with NAA did not differ significantly from the B+ plants with respect to activity translocated to the buds. Translocation in B+ plants treated with NAA was inhibited when compared to the control B+ plants. It would appear from these data that meristems of boron sufficient plants contain an optimum concentration of auxin while boron deficient plants are sub-optimum in auxin concentration.

Table I

Differences in C^{14}-Translocation to Terminal Bud of Boron-Deficient & Boron-Sufficient Bean Plants†

Group	B−	B−, NAA	B+	B+, NAA
B−	(23)††	**	**	**
B−, NAA		(158)	...	...
B+			(164)	*
B+, NAA				(109)

† As influenced by applications of NAA to the terminal bud region.
†† Parentheses enclose mean c/minute mg for treatment groups of eight plants each.
* Significant at the 0.05 level of confidence.
** Significant beyond the 0.01 level of confidence.

Root Application of NAA. NAA was added to the solution cultures of B− and B+ plants in quantities of 0.0095 mg per quart jar; the first application provided a concentration of 0.01 ppm. Separate applications were made 48, 24, and 1 hour(s) before $C^{14}O_2$ administration. The first application was made at the time of appearance of incipient boron deficiency symptoms in the B− plants after about seven days in nutrient solution (tables II & III). Data from the harvested apical buds (table II) are essentially the same as obtained by direct application of auxin to the meristems. The inhibition of translocation by treatment of auxin to B+ plants was not detected, however. Continued applications of NAA to either the buds or the roots resulted in improved shoot growth of the B− plants.

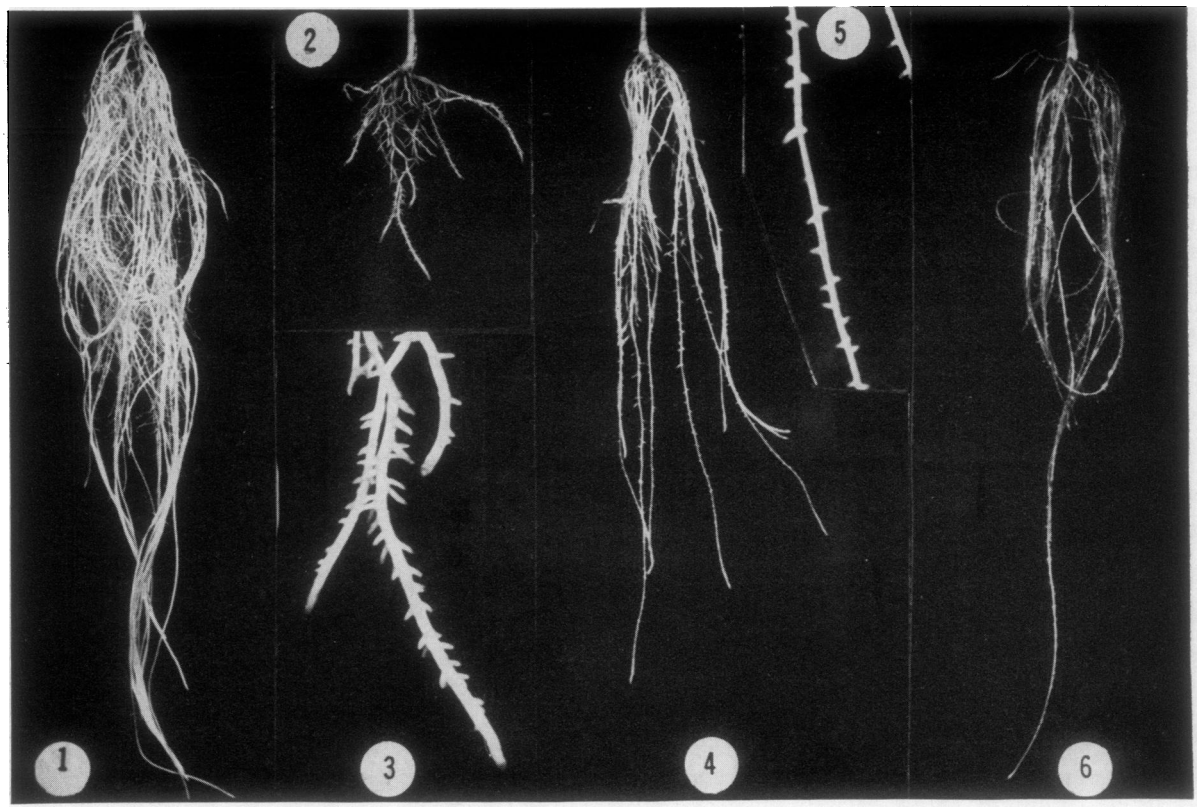
The apparent anomaly of these results can perhaps be explained with the help of morphological observations after continuing the treatment conditions for a week (figs 1–6). During early stages of boron deficiency the terminal root tip ceases growing and ultimately dies. With the corresponding loss of apical

Table II

Differences in C^{14}-Translocation to Terminal Bud of Boron-Deficient & Boron-Sufficient Bean Plants†

Group	B−	B−, NAA	B+	B+, NAA
B−	(80)††	**	**	**
B−, NAA		(324)	...	...
B+			(165)	...
B+, NAA				(169)

† As influenced by applications of NAA to the roots.
†† Parentheses enclose means c/minute mg for treatment groups of eight plants each.
** Significant beyond the 0.01 level of confidence.



FIGS. 1-6. Root systems after 1 week's treatment. Whole root systems are to the same scale. Figure 1. B+; figure 2. B—; figure 3. B— enlarged; figure 4. B+, NAA to root system; figure 5. B+, NAA to root system enlarged; figure 6. B—, NAA to root system.

dominance, numerous short, bulbous, lateral roots are formed but fail to elongate (figs 2–3). Similar lateral roots are formed with the application of NAA to B+ root systems (figs 4–5). The increase in translocation in these plants can be explained by the effect of these numerous additional sinks in the root system.

The data from the root systems (table III) are quite surprising. More activity (c/min · mg) was translocated to the roots of boron-deficient plants than to those of plants supplied with boron. NAA applications increased translocation to the root system in both B+ and B— plants.

TABLE III

DIFFERENCES IN C^{14}-TRANSLOCATION TO ROOTS OF BORON-DEFICIENT & BORON-SUFFICIENT BEAN PLANTS†

GROUP	B—	B—, NAA	B+	B+, NAA
B—	(81)††	**	**	...
B—, NAA		(334)	**	**
B+			(32)	**
B+, NAA				(81)

† As influenced by applications of NAA to the roots.
†† Parentheses enclose mean c/minute mg for treatment groups of eight plants each.
** Significant beyond the 0.01 level of confidence.

Application of NAA to B— roots resulted in increased elongation and associated increase in translocation to the root system of these plants when compared to B— plants without the auxin treatment. According to the classical view of the auxin relations of roots (1), the auxin content of intact roots is well over the optimum level, and as a rule, only inhibitions of longitudinal growth have been reported as a result of adding auxin. The increase in elongation and the associated increased translocation we observed with auxin treatment to boron deficient roots indicate that these root systems are below optimum in auxin concentration.

DISCUSSION & CONCLUSION

The primary object of this work, as stated in the introduction, was to test the hypothesis proposed by Skok that the role of boron in translocation is "indirect and related to cellular activity and growth rather than directly to the formation of a boron-sugar complex", as proposed by Gauch and Dugger. Although this latter hypothesis has stimulated much research in this area it now seems to be untenable.

With the onset of boron deficiency, both sink activity and translocation to the region decline and ultimately stop with the death of the meristem. Proposals such as Scott's (18) that boron affects

sugar-starch balances in regions of sugar synthesis, could potentially be used to explain reduced translocation in boron deficient plants, however, it is difficult to account for the subsequent death of the meristems on the basis of sugar deficiency. Applications of sugar (19, 20) or citric acid (20) to the terminal buds of boron deficient plants do not result in increased growth or a delay in the time of onset of deficiency symptoms. C^{14}-labelled sucrose is absorbed by excised flax roots whose growth is inhibited by lack of boron (17). Sugar concentrations in boron-deficient meristems do not appear to be limiting (16).

NAA applications to the meristems of boron-deficient plants in the absence of added boron, as reported in this paper, do promote increased translocation, presumably through the auxin's effect on the metabolic activity of the meristem. It is therefore concluded that the boron deficient plant prior to phloem necrosis possesses a translocation system capable of functioning and that it functions at much reduced capacity because substrates are not being utilized.

Gauch and Dugger (9) state, "There is also considerable evidence that there is a relationship between boron and plant hormones". Many other workers have also noted this relationship. We consequently find it surprising that much of the recent work on boron metabolism and its effects on plants is centered on in vitro carbohydrate metabolism (4, 5, 18) with no experimental efforts being directed toward elucidating the observed relationship between boron and plant hormones.

Eaton (6) was the first to report that "Some of the symptoms of plants deficient in boron are sufficiently similar to those expected in plants deficient in auxin as to suggest that the role of boron in plant nutrition is closely associated with the formation of auxin and possibly of other plant hormones". He reported a partial replacement of boron by auxin in growth at low light intensities. Subsequent workers (13, 15) failing to duplicate Eaton's experimental conditions, did not reproduce his results. As a consequence, Eaton's idea has largely been ignored. It is now recognized that failure to react to auxin may be due, among other things, to: A: exposure to unfavorable conditions of light or temperature; B: limitation by some other growth factor, such as adenine or kinetin (7).

The data provided by this study are perhaps best systematized by the hypothesis that boron plays an essential role in the biosynthesis of auxins in the meristems of the plant, translocation occurring as a result of growth rather than the reverse. This, of course, would not preclude boron having other effects on plant metabolism. The auxin synthesis hypothesis is strengthened by observations of the effects of ionizing radiation on plants. A major radio-sensitive process in plants is the synthesis of indoleacetic acid (10). Presumably the enzyme which converts indoleacetaldehyde to indoleacetic acid is destroyed by ionizing radiation. The increase in radioresistance which accompanies withdrawal of boron (21) indicates that boron might either be a component of this enzyme or otherwise necessary for its synthesis.

There are several possible ways of testing this hypothesis. The most direct would be to investigate the boron requirement for the in vitro synthesis of IAA. It will be more difficult to evaluate the boron requirement for the synthesis of the enzymes responsible for IAA formation. Preliminary attempts were made to obtain an enzyme extract from boron deficient bean plants which would synthesize IAA. We obtained very low yields with our preparations, however, adding boron to the reaction mixture did double the yield in two out of four cases. There was no effect on the other two. These data are mentioned only to strengthen our case for research in this area and are not presented as conclusive evidence.

Pertinent comparative cytological observations could also be made on boron deficient meristems and those made auxin deficient by ionizing radiation. Gross morphology of the boron deficient plants used in this study and those treated by low levels of ionizing radiation (23) were similar enough to be confused. Young trifoliate leaves were observed to abscise in both cases, a further link to auxin metabolism.

Summary

Bean plants, *Phaseolus vulgaris* L. var. Black Valentine, were used in experiments designed to test the hypothesis that the role of boron in translocation is indirect, the boron exerting its influence on this process through effect on metabolic activity at sites of utilization. NAA applications to the meristems of boron-deficient plants in the absence of added boron, promoted increased translocation of photosynthetically incorporated C^{14}. It was therefore concluded that the boron deficient plant, prior to phloem necrosis, possesses a translocation system capable of functioning and that it functions at a much reduced capacity because substrates are not being utilized. It is consequently quite clear that boron deficient plants are not limited in growth by sugar deficiency and that boron is not necessary to sugar translocation per se.

The relationship between boron and auxin is considered and the suggestion revived that boron is essential to auxin metabolism, possibly synthesis.

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