THE ROLE OF BORON IN THE TRANSLOCATION OF SUCROSE[1]

HUGH G. GAUCH AND W. M. DUGGER, JR.
Department of Botany, University of Maryland, College Park, Maryland

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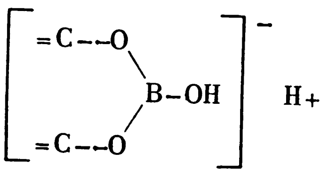
Introduction

The essentiality of boron for higher plants was demonstrated (**1**) in 1910 and extensively verified in the two succeeding decades. Since then a voluminous literature has accrued concerning the anatomical, morphological, and biochemical alterations of boron-deficient plants. Inasmuch as they are not pertinent to the following report, many of the specific or general roles that have been postulated for boron will not be discussed. By their omission the authors do not mean to imply that boron has no role or roles other than the one presented since, for example, the inverse relationship between boron level and per cent. moisture in plant tissues has been firmly established by Minarik and Shive (**15**) and others. To date, however, no one has demonstrated the exact role or mode of action of boron in higher plants. It is the purpose of this report to offer evidence for one role of boron.

In view of the well-known reaction of the borate ion with OH-rich compounds and its recent application in the borating of sugars in order that they may be separated on ion-exchange columns (**14**, **18**), the authors conceived and tested an hypothesis as to one essential role of boron in plants. The hypothesis is that boron combines with sugar to form a sugar-borate complex (ionizable) which is translocated with greater facility than are non-borated, non-ionized sugar molecules. Inasmuch as most studies have indicated that sucrose does not move readily through cellular membranes, the work reported herein was limited to sucrose and the effect of a few (5 to 10) parts per million of boron on its translocation.

According to Zittle (**26**) the borate ion reacts most strongly with OH-rich compounds in which there are two OH groups in the cis position. Although the structure of sucrose does not fulfill this requirement, there is ample evidence that there is a reaction with sucrose and that it involves the trans-1,2-diol groups. Further (**26**), it is suggested that the following structure is formed with sucrose



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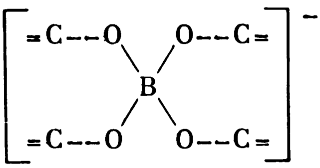

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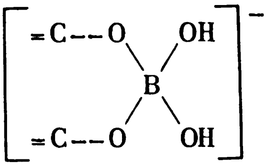

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as would be formed with certain sugars and other carbohydrates, the form or forms varying with different conditions such as changes in concentration and pH.

Methods and results

The first experiments to test the hypothesis involved the Warburg apparatus with which the effect of boron on the rate of entry and translocation of the substrate (sucrose) could be studied. With this apparatus it was possible to measure the O_2 uptake of excised root tips. Some of these received, in addition to the base nutrient salts, only sucrose and others received sucrose plus 5 p.p.m. of boron as boric acid. Boron alone, at this concentration, had no effect on the respiration of the root tips used in these studies.

In certain cases the root tips did not respond to added sucrose owing to an internal source of carbohydrate (starch). Therefore, in order to get a response to added sucrose it was sometimes necessary to excise the root tips from the germinating pea or lima bean seeds and to culture the tips in an oxygenated base nutrient solution for 18 hours to effect a comparatively low level of endogenous respiration. In all cases in which the root tips did respond to an exogenous source of sucrose, without exception a greater respiration (O_2 uptake) occurred when 5 p.p.m. of boron was present in the sucrose and base nutrient solution. Increases in respiration of 50 to 85% were observed after 10 hours (fig. 1 A, B, C and D) with greater percentage increases in the earlier part of certain experiments.

It should be borne in mind that these responses were obtained with root tips that were presumably adequately supplied with boron from the cotyledons. In various experiments the increases in respiration in the presence of boron did not occur with the same concentration of sucrose. It is suggested that the metabolic status of the root tips at the time they were used caused this variation with respect to the particular level of sucrose in which the boron effect occurred (fig. 1 A) or was most striking (fig. 1 D). It would appear from the results that factors other than sucrose were limiting in certain of the experiments. When this situation exists, the boron effect with the higher level of sugar cannot possibly show up as an increase in respiration (fig. 1 A and D). Recognizing the possibility that a factor other than sucrose might limit the respiration, O_2 uptake could not be relied upon as an accurate measure of the translocation of all concentrations of sucrose (or its hydrolytic products) to the cells in the root tips.

It is recognized that many interpretations might be offered for this effect of boron, including the possibility that it is directly affecting some phase of the respiratory mechanism. In view of the fact that such a role has not been established, this possibility is minimized. An alternative possibility is that the enhanced respiration is effected by an increase in the rate of entry and movement of the substrate to the respiring cells in the root tips. The Warburg technique yielded indirect evidence supporting this concept.

In order to supplement the respiration studies, a tracer technique involving labeled sucrose was employed. This latter technique provided a direct measure of the movement of sucrose (or its hydrolytic products) and, in contrast with the Warburg technique, did measure the full effect of boron on translocation. A single leaf of an intact plant (**24**) was immersed into a solution of labeled sucrose. After various intervals of treatment (4 to 24 hours) the plants were fractionated, the samples oven-dried, and the intensity of radioactivity in the various portions was determined. Mitchell and Linder's (**17**) technique was employed for preparing the samples of dried and ground plant material prior to counting with a windowless flow counter.

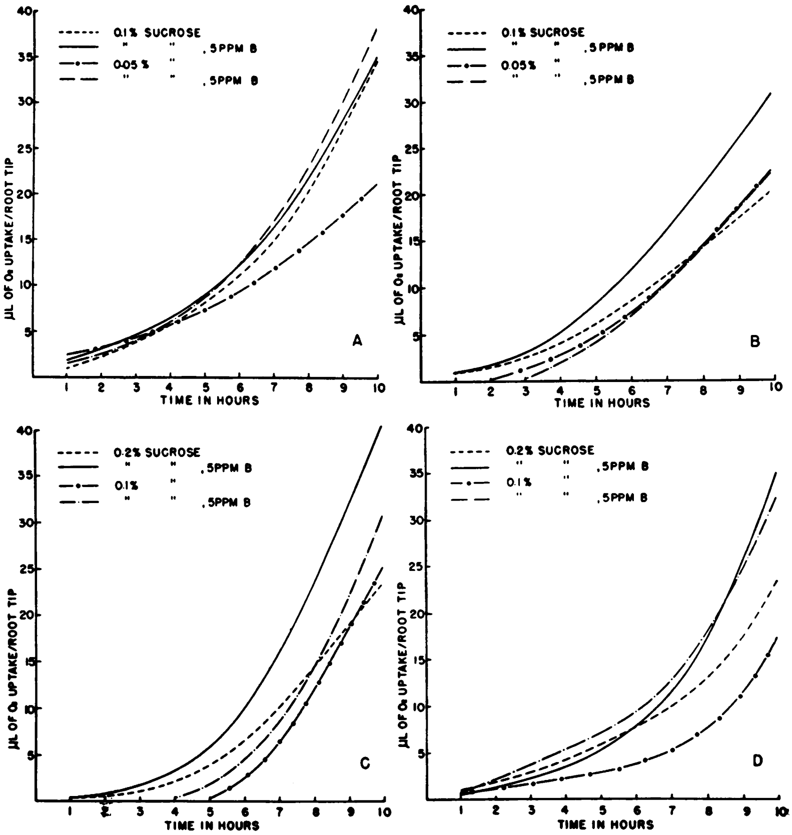

Fig. 1. The respiratory utilization of sucrose by root tips as affected by 5 p.p.m. of boron. Endogenous respiration of controls without added substrate has been subtracted. A, B: Five lima bean root tips per vessel; C, D: Eight pea root tips per vessel. All treatments were triplicated.

With tomatoes which had been grown in sand under a low level of mineral nutrition receiving no boron and depleted of carbohydrate by a 48-hour period of darkness (**24**), the uptake of sucrose by an immersed leaf and its distribution throughout the plant were very rapid and striking when

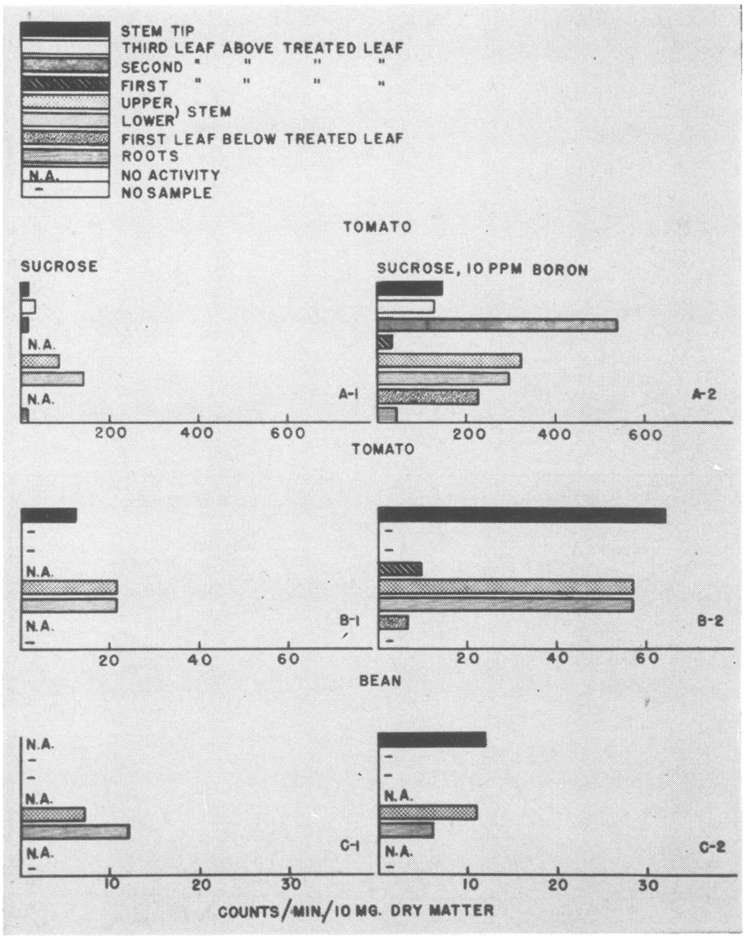

FIG. 2. The intensity of radioactivity (C^{14}) in various plant parts as a result of the movement of labeled sucrose (or its hydrolytic products) from a lower leaf immersed for 24 hours in a solution of labeled sucrose or labeled sucrose plus boron. All treatments were triplicated. A. Tomatoes cultured in sand, receiving no boron in the substrate, and subjected to 48-hour dark period prior to and during treatment; B. Soil-grown tomatoes receiving complete nutrition and kept in the light; C. Soil-grown snap bean plants receiving complete nutrition and subjected to a 48-hour dark period prior to and during treatment.

10 p.p.m. of boron was supplied with the sugar (fig. 2, A-2). In the absence of additional boron the sucrose entered in small amounts and only into the stem near the point of attachment of the immersed leaf (fig. 2, A-1). There was no directional mobilization of sucrose to the stem tip and to the young, developing leaves such as occurred in the presence of boron (fig. 2, A-2).

These data show the striking magnitude of difference between the translocation of sucrose (or its hydrolytic products) in plants deficient in boron and those adequately supplied with boron.

Under a deficiency of boron the root tips die sooner than the stem tips (**8**) even though the root tips are the first to come in contact with the boron that is available. However, if boron deficiency symptoms are caused by a deficiency of sugar, then it is understandable that the stem tips which are nearer the source of production of carbohydrates would be in a more favorable position and would live longer than the root tips. These observations are interpreted as evidence for the hypothesis that boron deficiency is actually a matter of sugar deficiency. With a low level of carbohydrate in the plant the tops have been reported by **Hoagland** (**12**) and others to utilize the available carbohydrate to the exclusion of the root system, resulting in higher top/root ratios.

In an experiment with vigorously growing, young greenhouse tomatoes the presence of 10 p.p.m. of boron in the sucrose solution enhanced the entry and movement of sucrose into the stem and stem tip (fig. 2, B-2 compared with B-1), even though the plants were receiving boron from the soil and they were not depleted of carbohydrate by a period of darkness. This is consistent with the hypothesis proposed herein. The enhanced movement of sucrose could conceivably proceed even beyond the capacity of the plant to utilize all of the sugar as fast as it is being translocated. **Eaton** (**11**) observed that boron-toxicity symptoms and maximal growth overlapped in many of the species of plants which he studied.

In an experiment with snap bean plants grown in soil and adequately supplied with boron but depleted of carbohydrate by a 48-hour period of darkness, the effect of boron on the mobilization of sucrose by the stem tip is shown (fig. 2, C-2 compared with C-1).

Discussion

In a biochemical study of boron-deficient and sufficient plants, **Johnston** and **Dore** (**13**) in 1928 reported that the leaves of boron-deficient plants have comparatively high concentrations of sugars and starch, whereas the concentration of sugars in the stems is low. Their data indicated that in the absence of adequate boron the translocation of sugars from the leaves to the stems is impaired, but the authors attributed this to a breakdown of the conducting tissues, particularly the phloem. **Askew** *et al.* (**3**) reported an increase in the per cent. of sugar in raspberry fruits by a soil application of boron to certain field-grown plants. Likewise, in a very exhaustive study on the Eastern Shore of Maryland, **Stark** and **Matthews** (**22**) found that the per cent. of soluble solids (mostly sugar) in cantaloupe fruits could be increased from around 8% to higher than 10% by spraying the leaves with either borax or magnesium sulphate solutions. It would appear that increases in the concentration of sugars in fruits of plants supplied with supplemental boron must be the result of an increase in translocation of sugar rather than an effect on photosynthesis, since the leaves of boron-deficient plants are consistently, abnormally high in sugars and starch. Inasmuch as SHEAR *et al.* (**21**) have discussed evidence that the boron level in plants may be affected by the magnesium level, it seems likely that the response to magnesium was an indirect effect so far as the translocation of sugar in cantaloupe is concerned (**22**), the causal relationship in translocation being between boron and sugar. The fact that the application of magnesium and boron in combination did not result in a greater effect than with boron alone would also indicate that the enhanced sugar translocation was strictly a boron effect. Calcium could also affect the translocation of sugar indirectly through its well-established relationship to the boron level in plants (**6**).

The hypothesis presented and substantiated by the experimental data, presented herein, would account for boron deficiency symptoms. It is almost universally reported that root and stem tips die and that flowers and fruits abort and abscise. These are all regions of high metabolic activity, therefore requiring large amounts of sugar to sustain their respiration and to permit their characteristically rapid rates of growth. WHITE-STEVENS (**25**) found that carbohydrates are deficient in the meristems and roots of boron-deficient plants, even though the leaves have an excess of carbohydrate in all forms. It would appear certain then that, in the absence of adequate boron, certain parts of the plant die owing to a lack of sugar. It is submitted that boron deficiency symptoms are an expression of sugar deficiency. WENT and CARTER (**24**) have shown by the spraying of sucrose solution on plants that the abscission of tomato flowers under high night temperatures is caused by an inadequacy of sugar, and that sugar (not auxin) is the limiting factor. Flowers also abscise under boron deficiency. In the light of WENT and CARTER'S (**24**) findings and the research presented herein, an explanation is now available for the abortion and abscission of flowers associated with a deficiency of boron.

It is interesting to project this concept into a consideration of the formation and translocation of hormones. The observations by ALEXANDER (**2**) and CHANDLER (**8**) that boron-deficient plants lose their capacity to respond to gravity would indicate that there is a relationship between boron and either the production or the translocation of hormones, or both. With 2,4-dichlorophenoxyacetic acid applied to bean leaves, MITCHELL and BROWN (**16**) observed that the movement of the growth regulator was associated with the movement of carbohydrate from the treated leaves. BRENCHLEY and THORNTON (**5**) reported that in the absence of boron a vascular connection was poorly if at all established between the nodules of legumes and the vascular tissues of the root. This differentiation may be controlled by growth regulators which also normally move in association with sugar, but both of which would fail to move in the absence of adequate boron. The growth regulators may actuate and control the differentiation, and carbohydrate would certainly be required for the syntheses associated with differentiation (*e.g.*, cell wall thickening). It was not determined whether the increased fruit set of apparently healthy Anjou pear trees sprayed with boron (**4**) was caused by an increased translocation of sugar, growth regulators, or both to the flower buds. However, BATJER and THOMPSON (**4**) report that Degman (unpublished work) could not increase the fruit set of Anjou pears by applying various growth-regulating chemicals. This latter work would indicate, as WENT and CARTER (**24**) maintained, that not auxin but sugar is the controlling factor. However, the research by MITCHELL and BROWN (**16**) which first showed the relationship between the movement of growth regulators and carbohydrates must be kept in mind when evaluating the results obtained by sucrose applications alone. Hormone assay would appear to be necessary for a reliable interpretation of such data. PILAND *et al.* (**19**) were able to increase the yields of alfalfa hay about 10% by an application of boron in the field while, at the same time, the yield of seed was enhanced by as much as 600%. SCOTT (**20**) reported an improved set of grapes by the application of boron at certain locations in the field even though the plants showed no evidence whatsoever of boron deficiency.

It is recognized that at least two possibilities exist for the manner in which boron accentuates the translocation of sucrose or its hydrolytic products. First, the borate ion could react with sucrose (or glucose or fructose), the sugar then passing through the cellular membranes as the ionized sugar-borate complex until such time as a cell utilizes this complex and liberates the borate ion. Secondly, it is possible that the borate ion is associated with the cellular membranes, that it there reacts chemically with the sugar molecule facilitating its passage through the membrane, and that the sugar is freed on the inside of the cell by a second reaction. In either event these two hypotheses are consistent with the well-established fact that at any given time in the life of the plant a small amount of boron suffices. However, plants must continue to absorb boron throughout their life cycle, indicating that, as new cell membranes are formed, more boron is required which would imply some degree of immobility of boron to new cells and tissues and, indeed, the evidence indicates that boron is relatively immobile in plants (**11**). The evidence thus favors the concept that boron is associated with the membranes.

The authors recognize that factors other than boron may be involved in the absorption and, perhaps, in the translocation of sucrose. DORMER and STREET (**9**) and STREET and LOWE (**23**) have postulated that sucrose is phosphorylated prior to its entry into tomato roots, and DOUDOROFF (**10**) has obtained from *Pseudomonas saccharophila* an extract containing a phosphorylase specific for sucrose. DORMER and STREET'S (**9**) report concerning the rapid absorption of sucrose by tomato roots is in direct conflict with the report by WENT and CARTER (**24**) to the effect that sucrose does not enter through the roots of tomato plants. The extensive use of sucrose as a solute for inducing and maintaining plasmolysis would indicate that sucrose phosphorylase must indeed be of limited distribution in plant tissues, since the plasmolyzed cells do not recover.

DORMER and STREET (**9**) hypothesize a phosphorylation of sucrose at the surface of the protoplast, passage of the sugar through the membrane in the form of fructose-1-6-diphosphate, and a dephosphorylation reaction inside the cell. Their findings concerning the non-permeability of tomato roots to simple sugars would thus appear to make mandatory a re-synthesis of sucrose prior to the movement of sugar into an adjoining cell. Although the phosphorylation of sucrose may be of importance prior to the entry of this sugar into certain cells or organs, the authors are inclined to the view, supported by evidence presented in this paper, that boron is the dominant factor in the movement of sucrose (or its hydrolytic products) from cell to cell within the plant. Brown (7) has proposed that a transport mechanism is involved in the movement of sugar across a diffusion barrier such as a membrane, and it is hereby suggested that boron may be involved in or associated with the hypothetical reactive points in the membrane.

Summary

An hypothesis is presented and supported with experimental evidence to account for one essential role of boron in plants. The data suggest that one role involves a reaction of boron with sugar to form a sugar-borate complex (ionizable) which moves through cellular membranes more readily than non-borated, non-ionized sugar molecules.

Studies employing the Warburg apparatus were indicative of greater O_2 uptake by lima bean and pea root tips in the presence of sucrose plus 5 p.p.m. of boron, as compared with sucrose alone. A tentative interpretation of the increased (50 to 85%) respiration was that it was the result of an increase in the movement of sucrose (or its hydrolytic products) to the respiring cells. Five p.p.m. of boron had no effect on the endogenous respiration.

By employing uniformly labeled sucrose both alone and in the presence of 10 p.p.m. of boron, it was shown that there was a striking dependence of translocation of sugar on the presence of boron. When a leaf of a tomato plant was immersed in a labeled sucrose solution containing 10 p.p.m. of boron, the stem tip of the plant had 550% more radioactivity than if the solution contained no boron.

Data are presented which may be interpreted as evidence for the fact that sugar does not move readily through cellular membranes unless the sugar is borated. It is submitted, therefore, that boron deficiency symptoms are an expression of sugar deficiency in the cambia, stem tips, root tips, and flowers or fruits.

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