Plant Physiol. (1990) 93, 1071–1077
0032-0889/90/93/1071/07/$01.00/0

Received for publication August 30, 1989
Accepted February 6, 1990

Boron Deficiency in Cultured Pine Cells[1]

Quantitative Studies of the Interaction with Ca and Mg

Robert Dixon Teasdale* and Dianne Katherine Richards[2]

Centre for Plant Biotechnology, School of Science and Technology, Bond University, Gold Coast, Qld 4229, Australia

ABSTRACT

A pronounced interaction between calcium, magnesium, and boron was found in growth studies with *Pinus radiata* cell cultures. Quantitative isoactivity data for the interaction was analyzed in terms of selected simple and plausible theoretical models. The data was found to be consistent with a model in which a critical acceptor molecule is activated only by binding both Ca and B at separate sites; Mg competitively displaces Ca to inactivate the acceptor. It was found that B is, surprisingly, not bound strongly (K_{diss} = 450 ± 80 micromolar) and that the affinity for Ca is two orders of magnitude stronger than for Mg. Therefore only a small proportion of the acceptor will be boronated under natural conditions. Moderate levels of mannitol were found to aggravate B deficiency due to its effective removal by direct chemical complexation. At higher concentrations of mannitol (or other sugars), where osmotic contribution is significant, little B was needed to overcome growth inhibition—a result consistent with B having a primary role in cell wall biosynthesis.

As noted in a review by Gupta (8), boron (B) deficiency is more extensive than deficiency of any other micronutrient, affecting many species in most parts of the world. B deficiency in stands of *Pinus radiata* trees is recognized as a major cost to forestry in Australia, causing dieback, stem malformation, and poor tree form (3).

Despite considerable research effort extending over half a century, the primary biochemical role of B remains unclear. It has been implicated in roles such as auxin function (7), DNA biosynthesis (11, 13), membrane function (6, 15), cellulose biosynthesis (5), lignin biosynthesis (12), and cell elongation (1). No B-macromolecule complex has yet been isolated, and it has not been possible to determine whether the observed effects are primary or secondary consequences of B deficiency.

In studies of the mineral nutrition of *Pinus taeda* cells growing in suspension culture, a surprisingly high requirement for B was found, whereas such a high B supply was subsequently found unnecessary for culture of *P. radiata* cells in a different (P6) medium (19). It was considered unlikely that there would be an intrinsic biological difference between the B requirements of these two systems, so that differences in the media formulations were considered. The most significant differences between the two basal media were a low level of Ca (0.15 mM) and high Mg (7.5 mM) in the L medium employed with *P. taeda* cells, compared with more standard levels of these ions found in the P6 medium.

It was unclear from the published literature whether these compositional characteristics of the L[3] medium account for the high B requirement. An interaction between Ca and B is recognized from field application studies, but this is a negative interaction with high Ca (as lime) inducing B deficiency (8). Gupta and Macleod (9) report that this effect is due to an increase in soil pH rather than elevated [Ca], with $CaSO_4$ not inducing B deficiency. As reported in Gupta's review (8), Wolf (20) found that Mg had a greater effect on lowering B concentrations in plants than did Ca. An analysis of nutrient levels in sugarcane leaf tissue (2) showed that B levels were correlated positively with Ca but negatively with Mg. Yamauchi (21) reported that B deficiency induced a decrease in the amount of Ca associated with pectin constituents of cell walls in tomato leaves. An interaction between Ca, Mg, and B therefore remained an open possibility. We report here exploration of this possibility using the growth of cell-suspension cultures of *P. radiata* in a manner reminiscent of earlier studies of copper nutrition (17). This approach has the benefit of using a precisely quantified growth measurement as a physiological response, thereby avoiding the difficulties of trace B estimation. More importantly, when B is growth limiting, such measurements provide an indirect means to assay the activity of the unknown B-requiring step that is metabolically essential through analysis of these growth data in a novel way, avoiding confusion with B esterified non-physiologically to various sugars and carbohydrates. Thus, it represents a different approach to the study of B and provides new insights into what is, after 60 years of research, an elusive process in plant nutrition. It was found that both Ca and B must be bound to the acceptor concurrently for activity and that B is bound surprisingly weakly (K_{diss} = 450 ± 80 μM), with Ca exhibiting binding two orders of magnitude more

[1] This research was supported by the Rural Credits Development Fund grant CAE/8663, Australian Research Grants Scheme award AO8515299, and The Pine Research Fund.

[2] Present address: School of Applied Science, Gippsland Institute of Advanced Education, Switchback Road, Churchill, Vic 3842, Australia.

[3] Abbreviations: L medium, Litvay medium; MS, Murashige and Skoog medium.

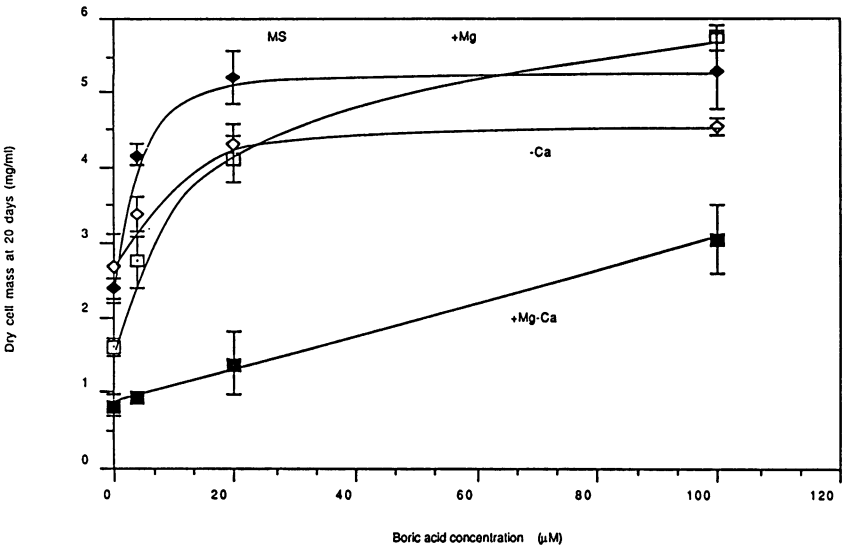

Figure 1. A plot of the dry cell mass of *Pinus radiata* cell cultures filter harvested and washed after 16 d of culture at 25°C in 1.0 mL volumes of MS base media modified in relation to the Mg, Ca, and B content. The first plot (♦) illustrates the response to varying [H_3BO_3] in basal MS medium. In the second curve (◇) is shown the effect of increasing [Mg] from 1.5 mM to 7.5 mM; curve (□) shows the effect when [Ca] is diminished from 3.0 mM to 0.2 mM. The fourth curve (■) shows the combined effect of both increasing [Mg] to 7.5 mM and reducing [Ca] to 0.2 mM. All treatments were made in quadruplicate and error bars indicate standard deviations.

strongly than Mg; studies of B deficiency in the presence of osmotic support gave results consistent with the primary role of B being in cell wall biosynthesis.

MATERIALS AND METHODS

Culture Initiation

Sustainable cell suspension cultures of *Pinus radiata* were obtained as described earlier (18, 19) by culture of an embryo excised from an elite seed (Australian plus-tree register 50048X 80055) in modified Schenk and Hildebrandt medium. The culture was subsequently maintained in P6 medium (19) supplemented with naphthalene acetic acid (2 $mg \cdot L^{-1}$).

Growth Experiments

Growth measurements were made using suspension cultures for which the microcalli colonies were first sheared by stirring on a 500 μm stainless steel sieve and viable colonies then collected on a 100 μm sieve. Cells were extensively washed with sterile water, then suspended in MS medium (14) appropriately modified to contain the minimal required concentrations of those nutrients for which concentration responses were sought. The suspensions were thoroughly agitated prior to withdrawing inocula of (typically) 950 μL and dispensing into wells of disposable trays (24 well, Costar No. 1324). The wells of these trays were preloaded (as appropriate) with 50 μL volumes of 20-fold concentrated stocks of the required nutrients. Cells were grown for 16 to 20 d (as appropriate) with shaking at 60 $orbit \cdot m^{-1}$ and 25°C. They were then filter harvested and water washed on pre-tared glass fiber discs (Whatman GF/A, 25 mm), and finally weighed to a precision of 0.01 mg on an electrobalance after drying at 65°C for 24 h. All treatments were replicated four times and error bars calculated as standard deviations.

RESULTS AND DISCUSSION

Studies of Effect of Ca, Mg, and B on Growth of Cell Cultures

The dry cell mass of cultured *Pinus radiata* cells (18) was used as a quantitative growth response, as described previously (17, 19). Growth experiments were undertaken in a MS-type medium (14) containing moderate levels of Ca (3 mM) and Mg (1.5 mM). Figure 1 shows the growth response of *P. radiata* cells under four different combinations of [Ca] and [Mg]. It is seen that when [Mg] is elevated fivefold (to 7.5 mM) the response to B is changed slightly toward greater requirement. A similar result was obtained when [Ca] was lowered to 0.2 mM. However, when both these changes were combined ([Ca] = 0.2 mM; [Mg] = 7.5 mM), a dramatic change in B requirement ensued.

Examination of the effect of Ca on B requirements showed (data not presented) that B deficiency could be obtained at any [Ca], but that at concentrations greater than 0.3 mM, B-limited growth required very low [B] (<10 μM), for which avoidance of contamination can be problematic and hence precise concentrations are difficult to control. Moreover, other preliminary studies showed that washing of cells with low [Ca] medium led to a considerable variability in response to B. This was probably due to Ca carryover by adsorption to the cells and subsequent slow release. Simple washing with distilled water before resuspension in medium of low [Ca] (0.2 mM) gave a more consistent response and this practice was adopted for all subsequent experiments.

A study was made of the effect on the growth of cell cultures of varying [Mg] in the presence of varying [B], the results are shown in Figure 2. Under the low [Ca] conditions employed a very marked affect of increasing [Mg] was evident—with sufficiently high [Mg] B deficiency could be induced even when [B] was 500 μM. On most of these curves a small region is found, at low Mg concentrations, where growth is limited by some Mg-requiring function rather than the B-requiring step of interest here.

A simple explanation for the observed interaction involving B is that growth is limited by a critical metabolic step, wherein an acceptor molecule is activated by the joint binding of both Ca and B at separate sites, and that Ca is competitively displaced by Mg. Quantitative descriptions can be obtained for various models of such an interaction, but developing a correspondence between these models and experimental data is more hazardous than perhaps might be expected. The pattern of cell growth, irrespective of whether it be of a simple exponential type or some more complex behavior, may change not just quantitatively but also in character when growth is limited by a particular metabolic process. Moreover, a correspondence between growth rate (under supposedly B-limiting conditions) and activity of the acceptor is complicated by the appearance of maximum growth not being indicative of a point of B saturation, but rather the encountering of other growth-limiting factors.

Analysis Using Isoactivity Approach

One novel approach which circumvents essentially all of the above-mentioned difficulties is to determine a set of various concentrations of the interacting nutrients which all result in the same level of acceptor activity (Φ) and consequently result in identical growth responses within a single experiment; that is, combinations of nutrient concentrations which result in "isoactivity." The relationships between the different nutrient concentrations necessary to attain a set of isoactivity conditions can therefore be quantitatively interpreted in terms of the relevant binding processes and constants, assuming equilibrium occurs. This approach may be applied to various conceptual models of the interaction. In the "Appendix" are presented fairly complete mathematical descriptions of two models having particular relevance to the present study.

From the perspective of data analysis, the simplest of the two models under consideration is that where the binding of Ca occurs only after B has first bound and is therefore termed the sequential model. It is predicted (Eq. 13) that with constant [Ca] a set of various paired [Mg] and [B] values resulting in the same yield after any defined period of monotonic growth will provide a linear plot of [Mg] *versus* 1/[B].

The data of Figure 2 was analyzed in accordance with this isoactivity theory. A set of various conditions yielding the same amount of cell growth was obtained by drawing a horizontal line at a selected ordinate position so as to intersect the different response curves and provide, with interpolation as necessary, a set of [Mg] values and corresponding [B] values which represent alternative {[Mg], [B]} conditions for attaining the same endogenous concentration of the growth-limiting species *P*BCa. The position of the line (Φ_1 yielding 3.21 mg·mL^{-1}) was selected in order to intersect all the curves in a responsive region and close to primary data points so as to minimize interpolation errors. This data set was used to construct a plot of [Mg] *versus* 1/[B], in accordance with Equation 13 for the sequential model, as shown in Figure 3. This plot is markedly nonlinear, so that the sequential model was rejected as clearly inconsistent with the experimental data of Figure 2.

The second model differs from the sequential model in that the two sites for binding of Ca and B are considered separate and independent. For this model, the correspondence between such pairs of [Mg] and [B] values will be as described by Equation 6 so that a linear plot of [Mg] *versus* [B]/(K_1 + [B]) can be obtained numerically with estimation of K_1, the binding constant for attachment of B.

The data of line Φ_1 were therefore analyzed in accordance with Equation 6 for the independent site model. A plot of [Mg] *versus* [B]/(K_1 + [B]) was constructed by trialling values of K_1 to obtain the most linear plot. Too low a value results in a curve which is concave upwards, whereas too large a value produces a convex curve. The best K_1 value was therefore selected to give the minimum residual sums-of-squares for a regression fit. In Figure 4 is shown the plot obtained with the K_1 value of 480 μM which yielded a line of gradient 9.69 mM with $r^2 = 1.000$ and $T = 244$. To obtain an indication of the error involved in the K_1 estimate, two additional data

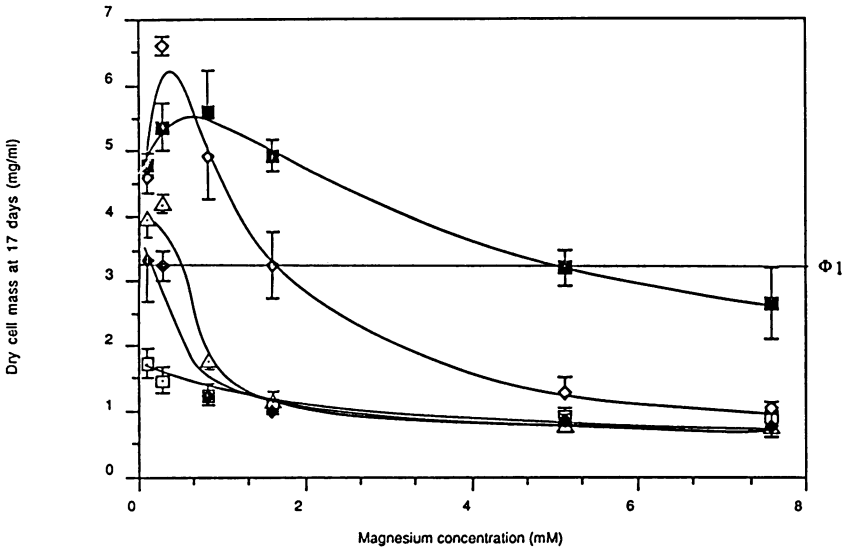

Figure 2. The effect of varying [Mg] on the growth of *Pinus radiata* cells cultured in 1.0 mL volumes of MS medium modified to have low [Ca] (0.2 mM) and with a set of different [H_3BO_3] {0 (□), 10 μM (♦), 30 μM (△), 100 μM (◇), and 500 μM (■)}. The ordinate axis shows the dry cell mass of filter-harvested cells cultured as described in the caption to Figure 1. The horizontal line at ordinate position 3.21 mg·mL^{-1} and labelled Φ_1 provides intercepts with each of the curves from which a set of isoactivity points were obtained for use in Figures 3 and 4.

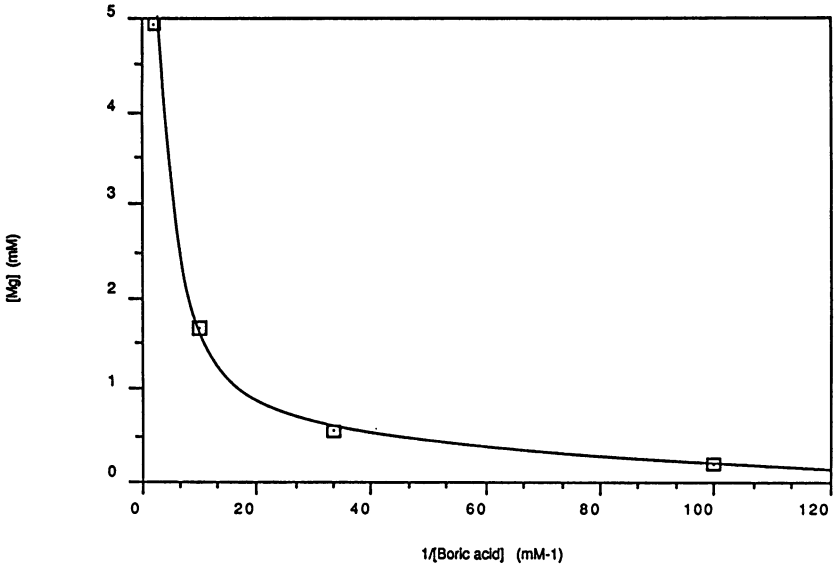

Figure 3. A plot of [Mg] *versus* 1/[B] obtained by first interpolating the data of Figure 2 to obtain a set of [Mg] and corresponding [B] isoactivity points (designated as Φ_1, all yielding a cell mass of 3.21 mg·mL^{-1}). Such data provide a test for the sequential model, yielding a linear plot if the model is valid.

sets were obtained from Figure 2, in the less reliable domains above (Φ_2 = 4.0 mg·mL^{-1}) and below (Φ_3 = 2.66 mg·mL^{-1}) the Φ_1 data set. The respective estimates were K_1 = 960 μM for Φ_2 (n = 4, T = 51.1) and K_1 = 275 μM for Φ_3 (n = 3, T = 271). The mean estimate of K_1 = 450 ± 80 μM was calculated by weighing each of the individual K_1 values by their $n \times T$ product. The gradient of 9.69 mM for the plot in Figure 4, in combination with the known value [Ca] = 0.2 mM and the arbitrarily assigned value of Φ = 0.5, provides the estimate K_3/K_2 = 24.5, which is in the generally encountered range of relative binding affinities for Ca and Mg.

Effects of High Mannitol

It can be predicted that the high concentrations of sugars (0.6 M) used in protoplast culture will make available B an order of magnitude lower than the total supplied, and therefore border on deficiency even with media containing the MS levels of Mg and Ca. It was therefore of interest to determine whether B deficiency may be inadvertently induced with such levels of mannitol. B deficiency was made accessible by use of low [Ca] (0.2 mM) and high [Mg] (5 mM) and growth responses to B were obtained in the presence of two concentrations of mannitol (0.044 M and 0.132 M), as shown in Figure 5. The sucrose concentration was halved to 0.044 M when mannitol was added, so that at the lowest mannitol concentration the medium had the same osmolarity as the control (MS) medium. Control experiments (data not shown) indicated that the lowering of sucrose did not of itself affect growth under the experimental conditions employed, which included harvesting before cell density had approached half the likely maximum of 15 mg·mL^{-1}. It is seen for the curve with [mannitol] = 0.044 M that the deficiency is aggravated, which is the expected result due to the lowering of free B through complexation with mannitol. Contrary to expectations, a further threefold increase in the mannitol concentration to 0.132 M alleviated to a considerable extent the growth inhibition caused by B deficiency. An almost identical result was obtained with sorbitol (data not shown) which indicated that this amelioration is not a specific effect of mannitol. Simple calculation according to binding equilibria also showed that this could not be due to B contamination of the added mannitol.

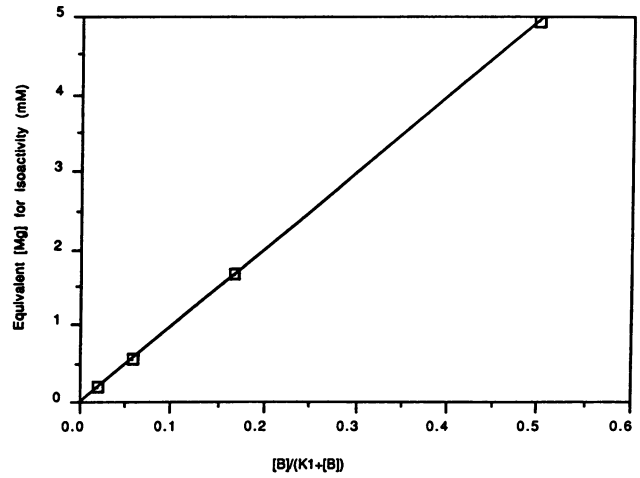

Figure 4. A plot of [Mg] and corresponding values of the term [B]/(K_1 + [B]) obtained from the same set of ([Mg], [B]) isoactivity points (Φ_1) used in Figure 3 above, then trialling various values of K_1 in accordance with Equation 6 to obtain the best statistical fit. The optimum fit shown was obtained with K_1 = 480 μM.

One plausible interpretation is that the total osmolality of the medium (0.274 osm) is sufficient to significantly support a weakened cell wall—a recognized effect of B deficiency. Consistent with this interpretation is the observation that suspension-cultured cells used in this study were extensively autolysed after culture under B deficiency conditions. While the osmotic support should protect against immediate cell lysis, there is serious risk that the culture regime will inhibit the regeneration of new cell walls so that inadvertent B deficiency may indeed contribute to failure in protoplast culture, particularly where [Ca] is also low.

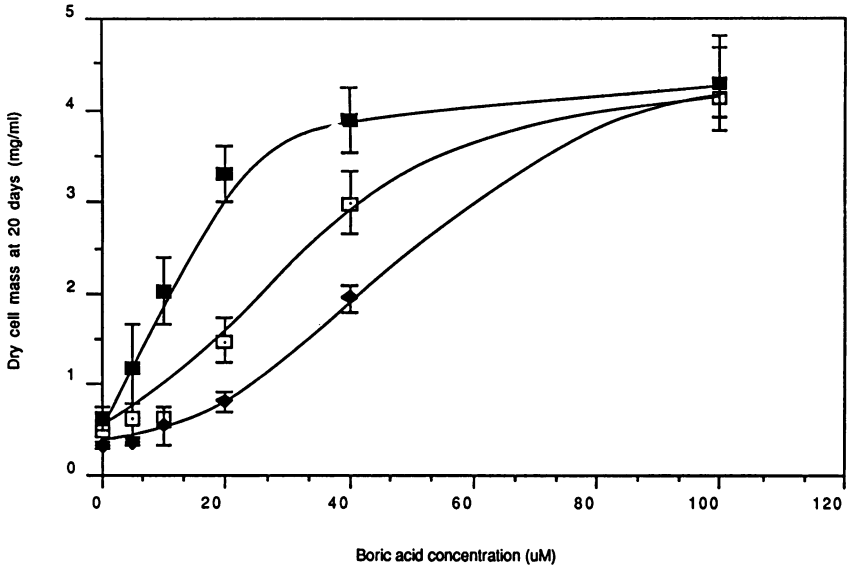

Figure 5. The effect of added mannitol on the response to B supply of *Pinus radiata* cells grown as described for Figures 1 and 2, but with [Ca] = 0.2 mM and [Mg] = 5.0 mM, which are conditions conducive to B deficiency. The [sucrose] is 0.088 M in the control curve (□) lacking mannitol, but diminished to 0.044 M for the data sets where mannitol is added at 0.044 M (♦) and 0.132 M (■). The error bars indicate standard deviations obtained from quadruplicate experimental determinations.

CONCLUSIONS

It is important that the existence of interactions such as that between B, Ca, and Mg are recognized by workers in plant nutrition and tissue culture, otherwise B deficiency may be incurred unwittingly (particularly during protoplast regeneration). The indication of a common role for Ca and B may well also prove useful in furthering understanding of Ca function, particularly the role of Ca in 1,3-β-glucan synthesis (10). In the practical sense, the interactive phenomena can be employed to make controlled B deficiency more readily accessible for experimental study.

The model proposed is the simplest conceivable one to account for the qualitative character of the observed cell-growth behavior. The ability to quantitatively fit the data with excellent correlation strongly supports the validity of this model. It is further noted that the novel approach of using isoactivity data for interpreting such an interactive phenomena is in fact quite general and not confined to micronutrients or use of cell-culture growth responses. Thus, in principle, measurements such as root or shoot extension may be used in connection with any set of interacting chemical (or physical) parameters.

The value of $K_{diss} = 450 \pm 80$ μM for the B dissociation constant is surprisingly high for a micronutrient. A direct consequence of this low affinity for B is that, at the concentrations of B generally encountered in nutrient media (typically 100 μM for tissue-culture media, lower for hydroponic solutions) less than 20% of the acceptor species will be in the active form with B bound to it. This would not necessarily be the case with the (rejected) sequential model where high [Ca] could pull the equilibria through to make the active form the dominant one. It also may not reflect the situation for intact plants where local concentration of B may occur, such as in the needles of *P. radiata* trees (3), so that the acceptor may be closer to being saturated with B. Nevertheless, the B concentrations used in tissue culture are usually not growth limiting, so it seems that only a small proportion of the total amount of acceptor need be active. The acceptor may serve other biological functions in its non-boronated form, or the process may simply be inefficient. That is, such a low binding affinity may be an intrinsic feature of the metabolic role of the acceptor, or may in fact be a biological imperfection reflecting what is possibly the most recently evolved biological binding site for an inorganic compound.

It is a clear assumption of this analysis that equilibrium in the binding processes has been reached. If the acceptor is located intracellularly, then either the concentrations of B, Ca, and Mg in the cytoplasm (or organelles) are identical to those in the extracellular medium, or defined concentration gradients exist across the various cell membranes. In the latter case the estimates for binding constants will be proportionately changed, which possibly could account for the high dissociation constant for B.

If the B acceptor is located in the cell wall, as is indeed the view of the present authors, there is little doubt that effective equilibrium will be reached in the time scales involved in cell growth. However, the chemical environment the acceptor faces will not simply be that of the external medium. Fixed anionic charges on cell wall polymers will lead, through electrostatic attraction and specific binding, to a local accumulation of cations significantly above those in the external environment (16). Thus, the levels of ionic Mg and Ca in the cell wall region (in addition to bound Ca and Mg) will be markedly elevated above those specified in the culture medium. The analysis above has not attempted to estimate the individual constants for binding of Ca and Mg to the B acceptor, but merely their ratios to one another using the arbitrarily assumed value $\Phi = 0.5$, and this only as a matter of secondary importance. With regard to local distortion in [B], this nutrient exists at all physiological pH values as the unionized boric acid (H_3BO_3) species and consequently the local concentration of free B within the cell wall is not expected to be affected by ionic groups on cell wall polymers, but will equal that in the bulk medium. Thus, such consid-

erations of cell wall binding are not expected to affect the primary conclusions of this study.

No assumptions have been made about the size or composition of the acceptor molecule. This biomolecule could therefore be a critical metabolite or a macromolecule such as a structural carbohydrate rather than an enzyme or other protein. The only size requirement is that it should be large enough to accommodate separate binding sites for Ca and B. The weak binding of B also has implications for any attempts to isolate the B-acceptor complex. B deficiency symptoms are evident in cultures of pine cells after 36 h, so that when free B is removed, the B-acceptor complex is unlikely to be kinetically stable for 24 h. Accordingly, the complex will dissociate if attempts are made to purify it in the absence of B. However, the concentration of acceptor in any cell extract is unlikely to reach the level (>450 μM) necessary to ensure most of the acceptor exists as B-complex, and consequently the high background of B will prohibit identification of a B-rich fraction. In the absence of an alternative detection system, the main prospect for purification seems to be in the complex having sufficient kinetic stability so that integrity is retained in the absence of B for the duration of fractionation steps, followed by analysis for B-containing fractions. The presence of high [Ca] throughout purification is also advised in light of this study.

The indication that B deficiency effects may be countered by provision of osmotic support is consistent with the primary role of B being in cell wall biosynthesis. It is more difficult to envisage how osmotic support can offset defects in membrane integrity or DNA biosynthesis, so that the effects of B deficiency on these processes are likely to be secondary. Reports of roles of B in cellulose biosynthesis (4, 5) and in association with cell wall pectins (21) are therefore particularly pertinent to the results of this study. It is tempting to speculate that B plays a key role in contributing to cell wall strength during the expansionary phase of cell growth by participating in reversible formation of a carbohydrate gel surrounding the cellulose fibrils which would allow progressive change in cell wall shape and size without the energetically expensive need to break and reform covalent bonds. Nevertheless, the role of B in higher plants currently remains unknown and all efforts at elucidating this role and its detailed nature are to be encouraged; it is hoped that the findings presented here will assist progress toward this goal.

APPENDIX

Theory of Nutrient-Limited Growth

Consider a simple system in which growth is limited by deficiency of the active form of a critical acceptor molecule which requires binding of both Ca and B for activation. Mg competitively binds at the same site as Ca but yields an inactive complex. Two models will be considered. In the first, the independent site model, the sites for binding of B (denoted by B, although boric acid is considered to be the active species) and for Ca are assumed to be independent and reversible. In the second model, the sequential model, Ca is only bound following binding of B.

The Independent Site Model

If the acceptor molecule is denoted by P, the equilibria can be described by the following equations.

$$P + \mathrm{B} \rightleftharpoons P\mathrm{B}; \quad K_1 = [P][\mathrm{B}]/[P\mathrm{B}] \tag{1}$$

$$P + \mathrm{Ca} \rightleftharpoons P\mathrm{Ca}; \quad K_2 = [P][\mathrm{Ca}]/[P\mathrm{Ca}] \tag{2}$$

$$P + \mathrm{Mg} \rightleftharpoons P\mathrm{Mg}; \quad K_3 = [P][\mathrm{Mg}]/[P\mathrm{Mg}]. \tag{3}$$

The six acceptor species co-existing in solution have a constant total (cellular) concentration $[\bar{P}]$.

$$\begin{aligned}[\bar{P}] &= /P] + [P\mathrm{B}] + [P\mathrm{Ca}] + [P\mathrm{Mg}] \\ &\quad + [P\mathrm{BMg}] + [P\mathrm{BCa}] \\ [\bar{P}] &= [P]\{1 + [\mathrm{B}]/K_1 + [\mathrm{Ca}]/K_2 \\ &\quad + [\mathrm{Mg}]/K_3 + [\mathrm{B}][\mathrm{Mg}]/K_1K_3 \\ &\quad + [\mathrm{B}][\mathrm{Ca}]/K_1K_2\}.\end{aligned} \tag{4}$$

The proportion of the total acceptor that exists as the active form (PBCa) can be represented by the symbol Φ, such that,

$$\Phi = [P\mathrm{BCa}]/[\bar{P}] \tag{5a}$$

$$\Phi = \frac{[\mathrm{B}][\mathrm{Ca}]}{K_1K_2(1 + [\mathrm{B}]/K_1)(1 + [\mathrm{Ca}]/K_2 + [\mathrm{Mg}]/K_3)}. \tag{5b}$$

The pattern of cell growth, irrespective of whether it be simple exponential or some more complex behavior, may change not just quantitatively but also in character when growth is limited by a particular metabolic process. Moreover, a correspondence between growth rate (under B-limiting conditions) and Φ is complicated by the appearance of maximum growth not being indicative of a point of B saturation, but rather the encountering of other growth-limiting factors. One approach which circumvents these difficulties is to determine different combinations of the concentrations of the interacting nutrients which achieve identical growth responses. The relationships between the different nutrient concentrations necessary to attain a set of isoactivity conditions will therefore correspond to the relevant binding processes and constants.

By rearrangement of Equation 5b, we obtain the expression,

$$[\mathrm{Mg}] = K_3[\mathrm{Ca}][\mathrm{B}]/K_2\Phi(K_1 + [\mathrm{B}]) - K_3(1 + [\mathrm{Ca}]/K_2) \tag{6}$$

Thus, with [Ca] held constant, a set of different [Mg] and [B] concentrations can be determined which result in identical growth behavior. In accordance with Equation 6, a linear plot of [Mg] *versus* $[\mathrm{B}]/(K_1 + [\mathrm{B}])$ can be numerically obtained to yield an estimate of K_1. This plot will have a gradient of $K_3\,[\mathrm{Ca}]/K_2\Phi$, so that an estimate of K_3/K_2 requires knowledge of Φ.

Equation 5b can be rearranged in an alternative way to yield Equation 7,

$$\begin{aligned}1/[\mathrm{Ca}] = {} & K_3[\mathrm{B}]/K_2\Phi(K_1 + [\mathrm{B}])(K_3 \\ & + [\mathrm{Mg}]) - K_3/K_2(K_3 + [\mathrm{Mg}]).\end{aligned} \tag{7}$$

This equation indicates that by holding [Mg] constant, K_1 can be numerically estimated by linearizing a plot of 1/[Ca] *versus* $[\mathrm{B}]/(K_1 + [\mathrm{B}])$. The gradient and intercept of this plot

can then be simply combined to estimate Φ. By designing the experiment to provide a plot of [Mg] *versus* [B]/(K_1 + [B]) for the same Φ value, then K_3/K_2 may be calculated from the gradient of this latter plot in accordance with Equation 6.

In principle, this approach can be extended and the value of the ordinate intercept of a [Mg] *versus* [B]/(K_1 + [B]) plot used with the preceding data to subsequently estimate the individual values of K_2 and K_3. The errors incurred with these serial calculations will compound so that it is potentially hazardous to manipulate data to the extent needed for such parameter estimates.

The Sequential Model

This model differs from the independent site model in that Ca and Mg bind only to species *P*B, and not to *P*, with the equilibria described by the set of equations below.

$$P + B \rightleftharpoons PB; \quad K_1 = [P][B]/[PB] \tag{8}$$

$$PB + Ca \rightleftharpoons PBCa; \quad K_2 = [PB][Ca]/[PBCa] \tag{9}$$

$$PB + Mg \rightleftharpoons PBMg; \quad K_3 = [PB][Mg]/[PBMg] \tag{10}$$

Accordingly, there are only four acceptor species in solution so that the analogous expression for $[\bar{P}]$ is

$$[\bar{P}] = [P] + [PB] + [PBMg] + [PBCa]$$

$$[\bar{P}] = [P](1 + [B]/K_1 + [B][Ca]/K_1K_2 + [B][Mg]/K_1K_2). \tag{11}$$

The corresponding proportion of acceptor in the active form is given by

$$\Phi' = [B][Ca]/(K_1K_2 + K_2[B] \cdot (1 + [Ca]/K_2 + [Mg]/K_3). \tag{12}$$

Equation 12 can be readily rearranged to yield the expression

$$[Mg] = (K_3[Ca](1 - \Phi')/K_2\Phi') - K_3 - K_1K_3/[B]. \tag{13}$$

Thus, with [Ca] held constant, a plot of [Mg] *versus* 1/[B] will be linear and the negative gradient will directly yield the product K_1K_3. Such a plot will not be linear for a system behaving in accordance with the independent site model, and provides an explicit means of distinguishing between the two models.

LITERATURE CITED

1. **Birnbaum EH, Beasley CA, Dugger WM** (1974) Boron deficiency in unfertilized cotton (*Gossypium hirsutum*) ovules cultured *in vitro*. Plant Physiol **59:** 1034–1038
2. **Bowen JE** (1981) Micro-element nutrition of sugar-cane. II-Interactions in micro-element accumulation. Trop Agric **58:** 215–220
3. **Crane W, Borough C** (1987) Boron—a micronutrient of importance to forest growers. Aust For Grower **10:** 24–26
4. **Dugger WM, Palmer RL** (1980) Effect of boron on the incorporation of glucose from UDP-glucose into cotton fibres grown *in vitro*. Plant Physiol **65:** 266–273
5. **Dugger WM, Palmer RL** (1985) Effect of boron on the incorporation of glucose by cotton fibres grown *in vitro*. J Plant Nutr **8:** 311–325
6. **Goldbach H** (1985) Influence of boron nutrition on net uptake and efflux of ^{32}P and ^{14}C glucose in *Helianthus annuns* roots and cell cultures of *Daucus carota*. J Plant Physiol **118:** 431–438
7. **Goldbach H, Amberger A** (1986) Influence of boron deficiency on [^{3}H]IAA uptake and efflux in cell cultures of *Daucus carota* L. Plant Growth Regul **4:** 81–86
8. **Gupta UC** (1979) Boron nutrition of crops. Adv Agron **31:** 273–307
9. **Gupta UC, Macleod JA** (1981) Plant and soil boron as influenced by soil pH and calcium sources on podzol soils. Soil Sci **131:** 20–25
10. **Kaus H** (1987) Some aspects of calcium-dependent regulation in plant metabolism. Annu Rev Plant Physiol **38:** 47–72
11. **Krueger RW, Lovatt CJ, Albert LS** (1987) Metabolic requirement of *Cucurbita pepo* for boron. Plant Physiol **83:** 254–258
12. **Lewis DH** (1980) Boron, lignification and the origin of vascular plants—a unified hypothesis. New Phytol **84:** 209–229
13. **Moore HM, Hirsch AM** (1983) Effects of boron deficiency on mitosis and incorporation of tritiated thymidine into nucleii of sunflower root tips. Am J Bot **70:** 165–172
14. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497
15. **Pollard AS, Parr AJ, Loughman BC** (1977) Boron in relation to membrane function in higher plants. J Exp Bot **28:** 831–841
16. **Sentenac H, Grignon C** (1981) A model for predicting ionic equilibrium concentrations in cell walls. Plant Physiol **68:** 415–419
17. **Teasdale RD** (1984) Application of growth analysis to trace-element nutrition: study of copper-uptake with a loblolly pine (*Pinus taeda*) cell suspension culture. J Exp Bot **35:** 1496–1506
18. **Teasdale RD** (1986) Generation of a sustainable *Pinus radiata* cell suspension culture and studies of cellular nitrogen nutrition. NZ J For Sci **16:** 377–386
19. **Teasdale RD, Dawson PA, Woolhouse HW** (1986) Mineral nutrient requirements of a loblolly pine (*Pinus taeda*) cell suspension culture. Plant Physiol **82:** 942–945
20. **Wolf B** (1940) Plant and soil boron as influenced by soil pH and calcium sources on podzol soils. Soil Sci **50:** 209–217
21. **Yamauchi T, Hara T, Sonoda Y** (1986) Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant Cell Physiol **27:** 729–732