Current Topics in Plant Biochemistry and Physiology
Volume 10:195-220, 1991

# INFLUENCE OF BORON ON NET PROTON RELEASE AND ITS RELATION TO OTHER METABOLIC PROCESSES

**Heiner E. Goldbach[1], Jürgen Blaser-Grill[2], Norbert Lindemann[3], Marion Porzelt[1], Christa Hörrmann[2], Brigitte Lupp[2] and Bernhard Gessner[4]**



## I. Introduction

The knowledge on boron's primary role in plant metabolism is scanty and to a larger part still a matter of speculation, although considerable efforts have been made to further elucidate the mechanisms envolved. There is a striking difference between monocots (esp. gramineaceous species) and dicots concerning the requirement for B (52). Whether the much higher B demand of dicots is just the consequence of a sequestration of B at "non-active" sites, e.g., in the cell wall, or whether there are some definite reactions which do require higher concentrations of B, is not yet clear. A summary of differences between the two groups of plants which might be relevant to the action of B is given in Table I. In any case, the target compounds for the interaction with B should bear hydroxylic groups (e.g. cis-diols, Figure 1). B may further be involved in the synthesis and/or hydrolysis of Schiff' bases or hydroxythiol-esters in a pH dependent manner (38,41,42). This information, however, is based only on results in the chemical literature. Unequivocal evidence for the occurrence of these reactions in the (plant) cell is still missing.

The highest concentrations of B are likely to be found in the apoplast (37,56), and there it may exert control over transport mechanisms (44) and cell wall growth (52). We thus looked for the

[1]Abtlg. Agarökologie, Universität Bayreuth, Postf. 101251, D-W 8580 Bayreuth, Germany
[2]LSt. Pflanzenernährung
[3]Gruppe Biophysik
[4]LSt. Landtechnik; 2 - 4: Techische Universität München-Weihenstephan,D-W 8050 Freising 12, Germany

**Table I.** Comparison of *Gramineae* and dicots with regard to cell wall formation and plasmalemma/apoplastic reactions as related to their differing B demands.

| | Gramineae | Dicots |
|---|---|---|
| PL-bound Reductases | - | + |
| Pectins + Ca | - | + |
| Walls Loosened by Chelators | - | + |
| Ion Uptake | Cat ■ An | Cat > An |
| Rhizosphere-Acidification | - | + |
| Xyloglucans | - | + |
| "Extensin" in Cell Walls | < 0.5% | 5% |
| Silica-Deposition* | + | - |
| B-Demand | Low | High |

* In Diatomeae, however, there is a high B-demand, possibly caused by the ordered deposition of silica on mannose-rich membranes (diester formation of borate with mannose and silica?); in *Gramineae*, silica is supposed to bind to (free?) phenolics

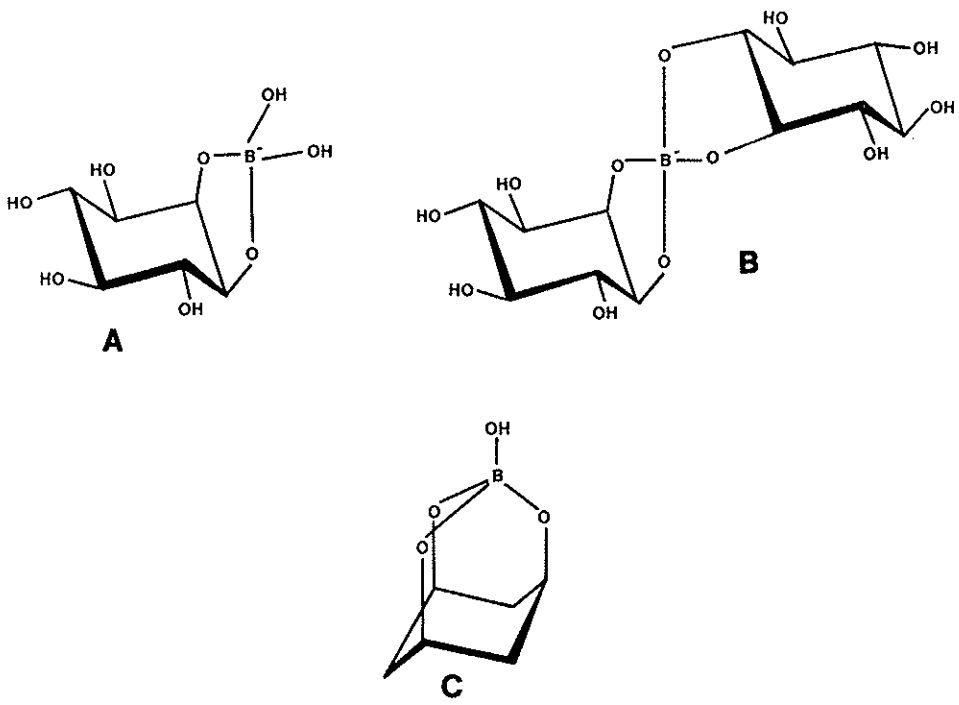



**Figure 1**. Possibilities of borate ester formation with inositol; A= monoester, B= diester, C= "tridentate" ester (after (1)).

participation of B in these processes.

Another point, only indirectly related to these subjects is the increasing demand for B with higher light intensities (36,52). Therefore, we also began investigations on the effects of B deficiency on PS II fluorescence quenching.

## II Influence of B Deficiency on Proton Release and Ferricyanide Reduction

One effect of B deficiency in *Daucus carota* cell cultures (and in several cases in tomato cell cultures as well) is a decrease in net proton release (Figure 2) as measured by changes in pH. It can be observed only when stimulating the proton release by withholding Fe or supplying ferricyanide, and can be inhibited by vanadate (Figure 2) and DCCD (Figure 3). B deficiency decreases proton release usually by over 50%.

Depending on the previously supplied B concentration, it takes between 30 to over 90

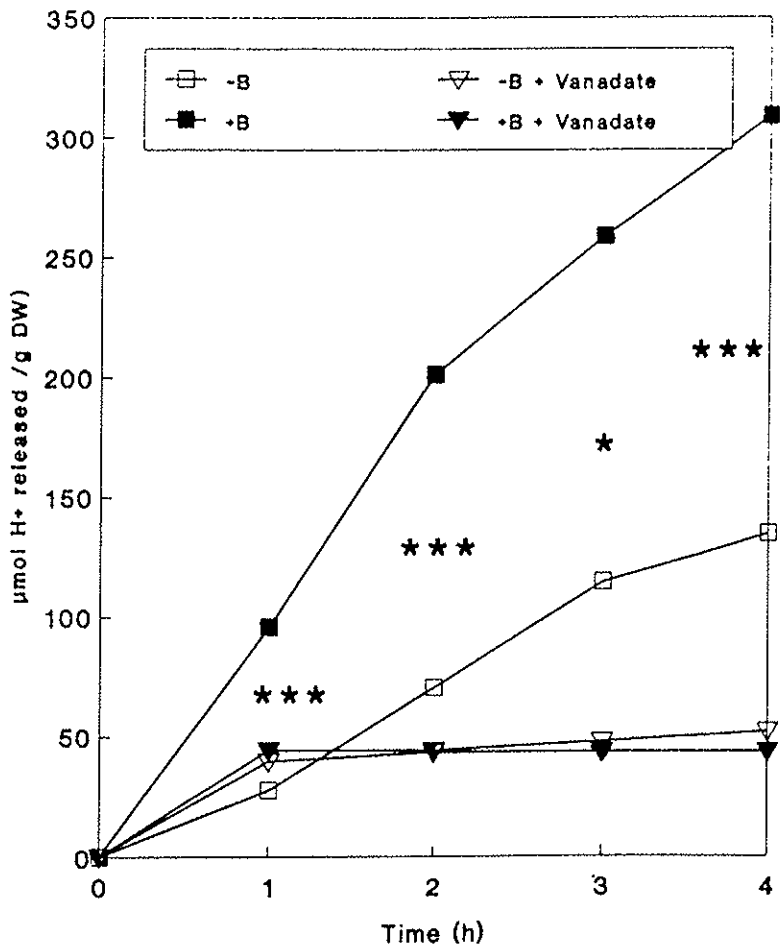



Figure 2. 1mM Ferricyanide induced net proton release of *Daucus carota* cell cultures with and without 100 $\mu$M $B(OH)_3$ and its inhibition by 100 $\mu$M $NaVO_3$ (16 h +/-B). *,*** indicates differences between +B and -B to be significant at the 5 and 1% level, respectively (for treatments without vanadate). From (19) with permission.

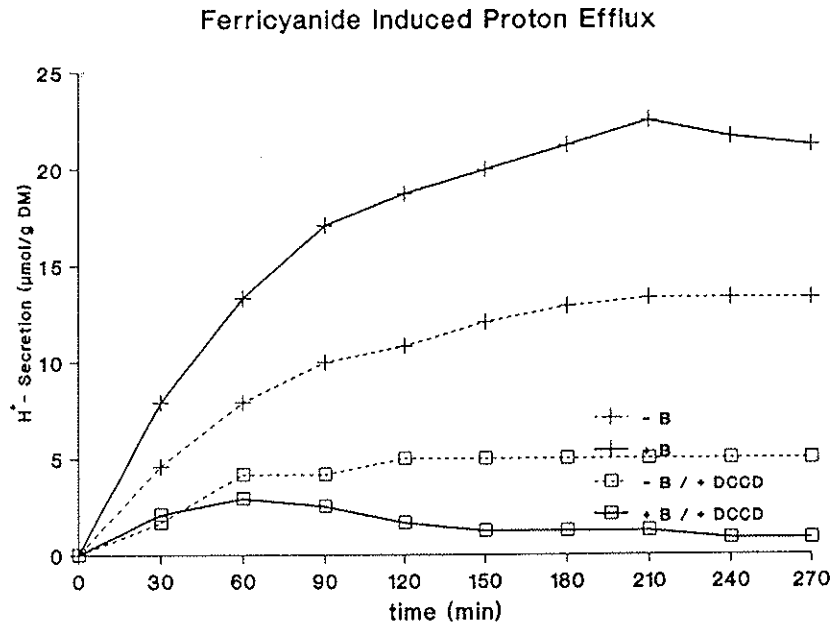



**Figure 3**. 1 mM Ferricyanide induced net proton release of *Daucus carota* cell cultures with and without 100 $\mu$M $B(OH)_3$ and its inhibition by DCCD (dicyclohexyl carbodiimide; 16 h +/-B).

minutes until differences between B treatments are to be seen. It is likely, that there is some kind of exchange of "bound" B from cell walls before a true deficiency situation occurs. It is very difficult, however, to find out whether or not there are sites which do bind B without being really relevant to growth and development (non active sites). Resupplying B at a concentration of 100 $\mu$M, restores the proton release to the normal rate within about 90 minutes (Figure 4).

There has been some controversy in the past whether or not B deficiency symptoms are caused by excessively high auxin concentrations (5,18,23,24,53). In our group, we usually observed only slight effects on the auxin concentrations in the tissues most affected by B deficiency, namely root and shoot tips (10).

The effects on net proton release were observed in auxin heterotrophic cell cultures. Omitting auxin (IAA and/or 2,4-D) from the medium, led to a strong depression of proton release and completely levelled off the differences between B treatments (Figure 5). In most cases, B deficient cell cultures even showed somewhat higher release rates than sufficient ones. Upon adding auxin, however, the $H^+$ release was rapidly restored to the "normal" level within 30 minutes, whereas in B deficient cultures, no such rapid recovery has been found (Figure 5).

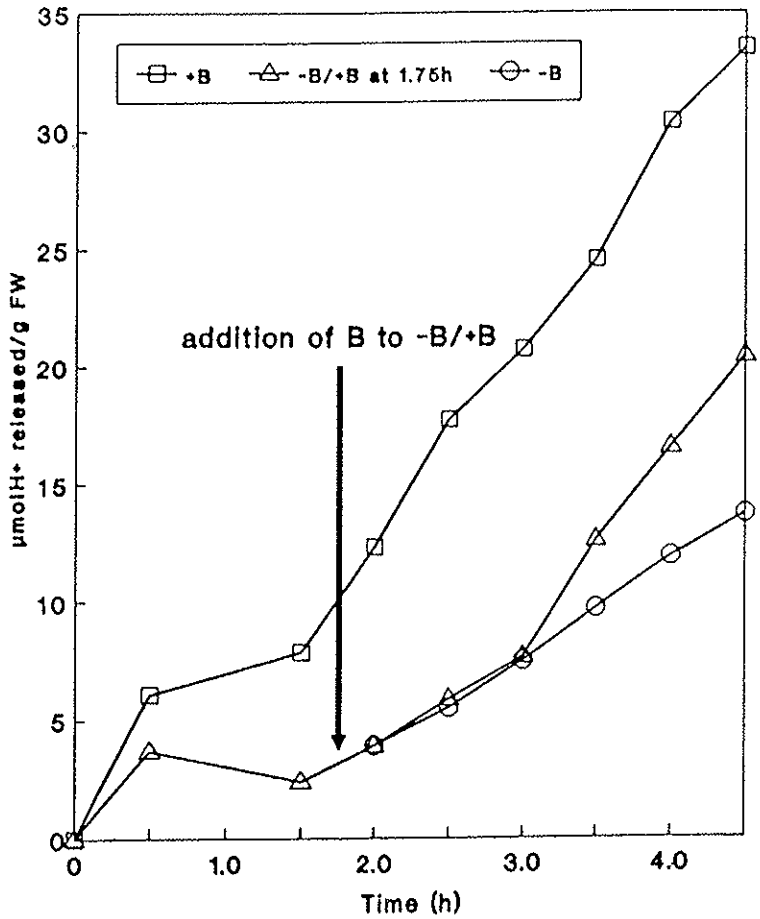



**Figure 4.** 1 mM ferricyanide induced net proton release of B deficient *Daucus carota* cells and its reversal by the addition of 100 $\mu$M $B(OH)_3$ (pretreatment 16 h +/-B). From (19) with permission.

Addition of the antiauxin TIBA (2,3,5-triiodobenzoic acid) completely suppressed the proton release (Figure 6), as did orthovanadate and DCCD, whereas naphtylphtalamic acid (NPA) affected the proton release only in relatively high concentrations (100 $\mu$M) (Figure 7). This strongly suggests that a) the action of auxins on the ferricyanide induced proton release is mediated by external binding sites, b) that B either affects the binding of auxin to the external receptor(s) or that it interferes somehow with the auxin response, e.g., by influencing secondary messengers and/or protein synthesis, and c) that the proton release is linked to the activity of ATPases. Suppression of Fe stress responses by antiauxins has been shown by Landsberg (32,33), and some, however unknown, effect on DNA/RNA and protein synthesis has been discussed repeatedly (23,24,30,39), even in rats (61).

In this context, from the chemical standpoint the most likely sites for $B(OH)_3/B(OH)_4^-$ to interfere with protein synthesis are the different types of RNA, as they do possess cis-diol groups at

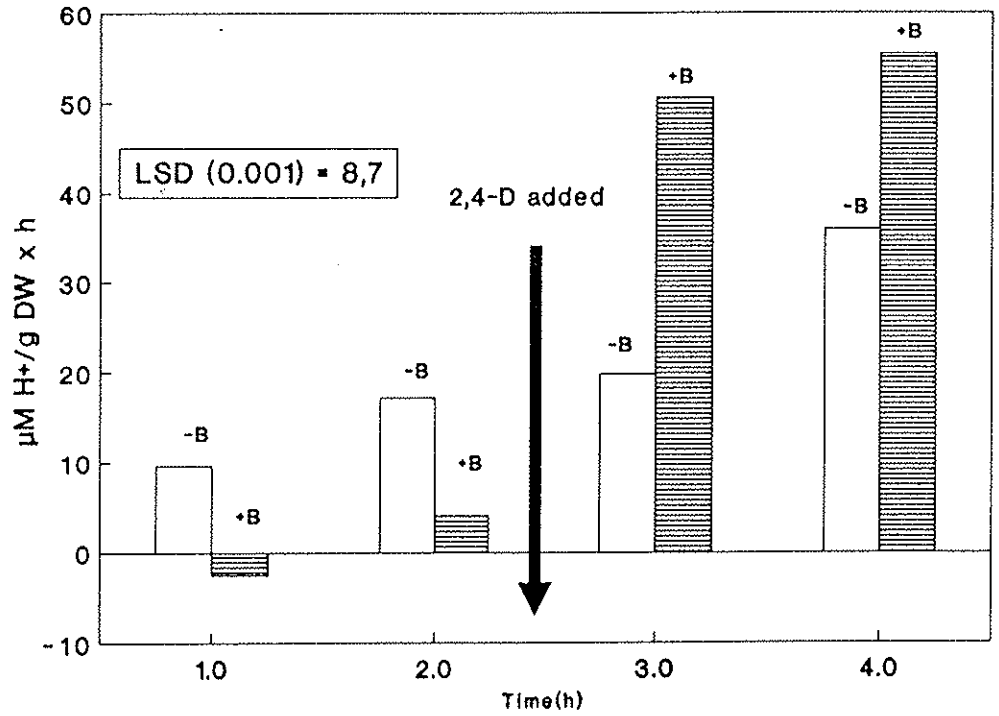



**Figure 5.** Ferricyanide induced net proton release of auxin free precultured *Lycopersicon esculentum* cells with or without addition of B (100 $\mu$M $B(OH)_3$ for 16 h); influence of 0.1 $\mu$M 2,4-D added at t = 150 min. From (19) with permission.

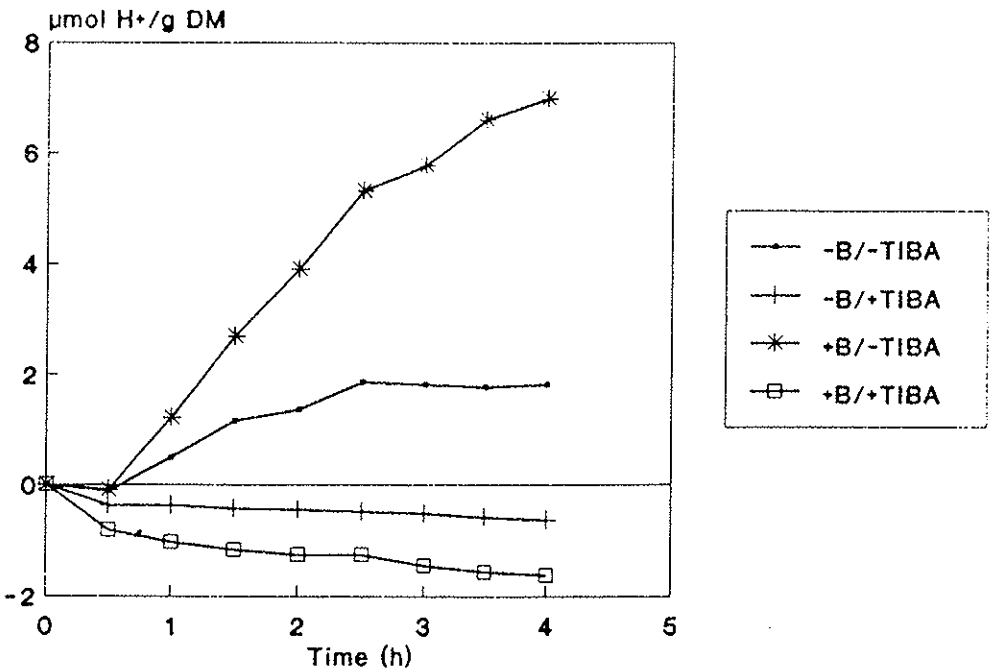



**Figure 6.** Influence of 50 $\mu$M TIBA and B supply on the ferricyanide induced net proton release in *Daucus carota* cells.

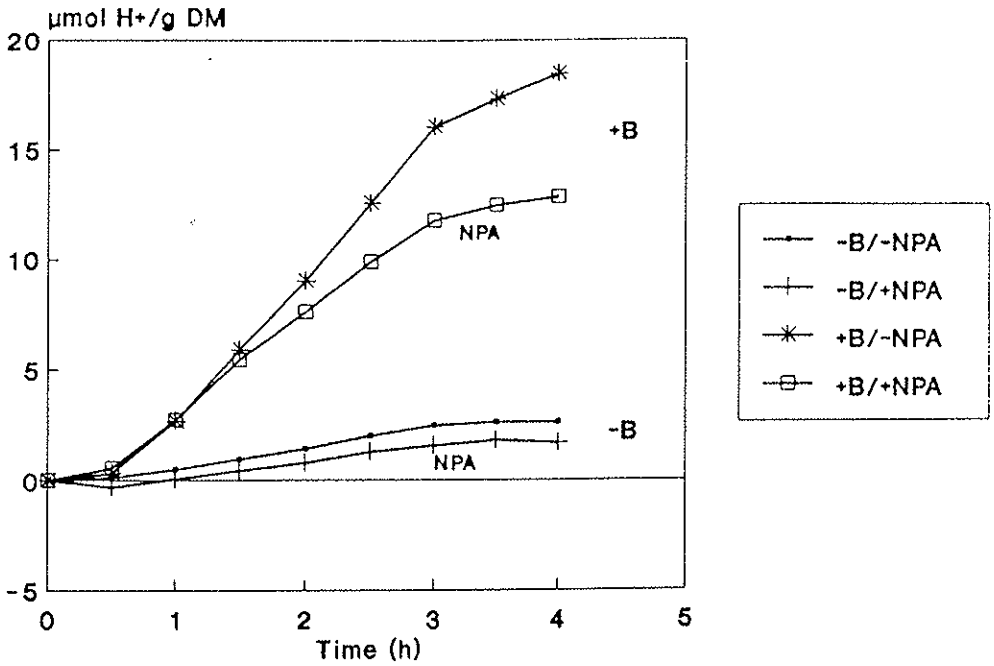



**Figure 7.** Influence of 100 $\mu$M NPA and B supply on the ferricyanide induced net proton release in *Daucus carota* cells.

the ribose moiety. To give an example: a small modification (2' ribosylation) at adenosine 64 of t-$RNA_{iMet}$ discriminates between peptide elongation or initiation of transcription (26). If B were to participate in the regulation of this process, only catalytic amounts of B would exert relatively strong effects on the total metabolic activity of a cell.

To date it is not yet clear whether the ferricyanide induced proton release is directly or indirectly coupled to the activity of reductases. Generally, we observed an inhibition of ferricyanide reduction under B deficiency as well. However, there seems to be no direct coupling between the effects of B on proton release and on extracellular/plasmalemma bound reductase activity, as both effects were differently affected by the addition of various metabolic inhibitors/effectors (unpublished results). In roots of intact zucchini plants, we even found higher reduction capacities under B deficiency as seen by Fe(III)-EDTA reduction/Fe(II)-BPDS staining (Figure 8). This effect might be caused by an increased oxidation of phenolic substrates (see section V).

## III Influence of B on Membrane Potential and Impedance

If B would directly participate in the regulation of plasmalemma-bound proton pumps, then deficiency should affect membrane potential and impedance. In *Elodea densa*, we found a decrease in membrane potential and a concommitant increase in membrane impedance when changing to a B-free nutrient solution (Figure 9a,b). When switching to a B-containing solution again, potential and

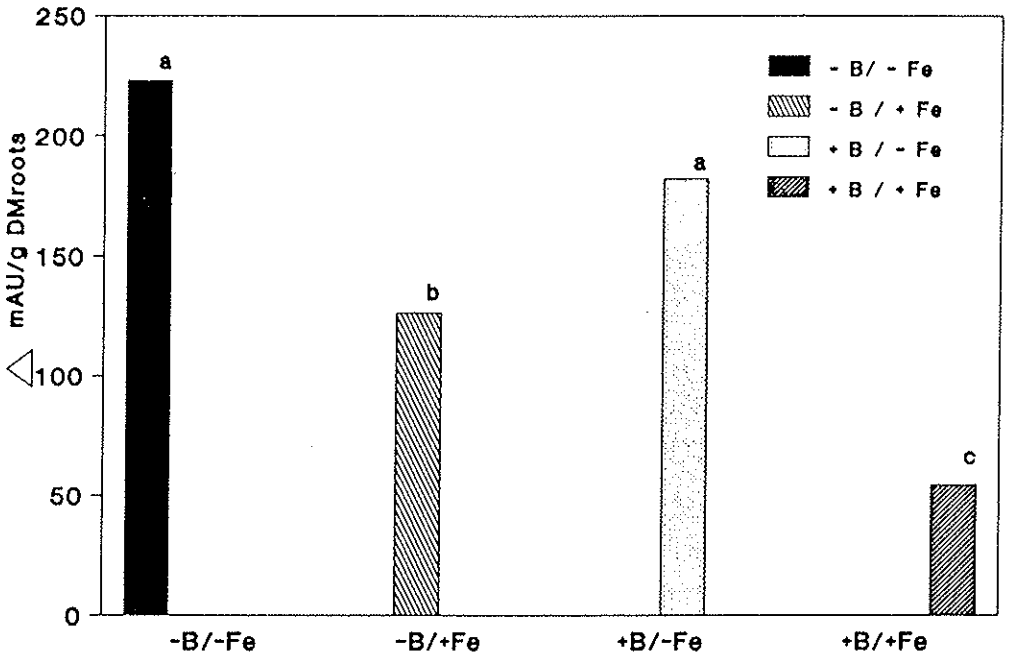


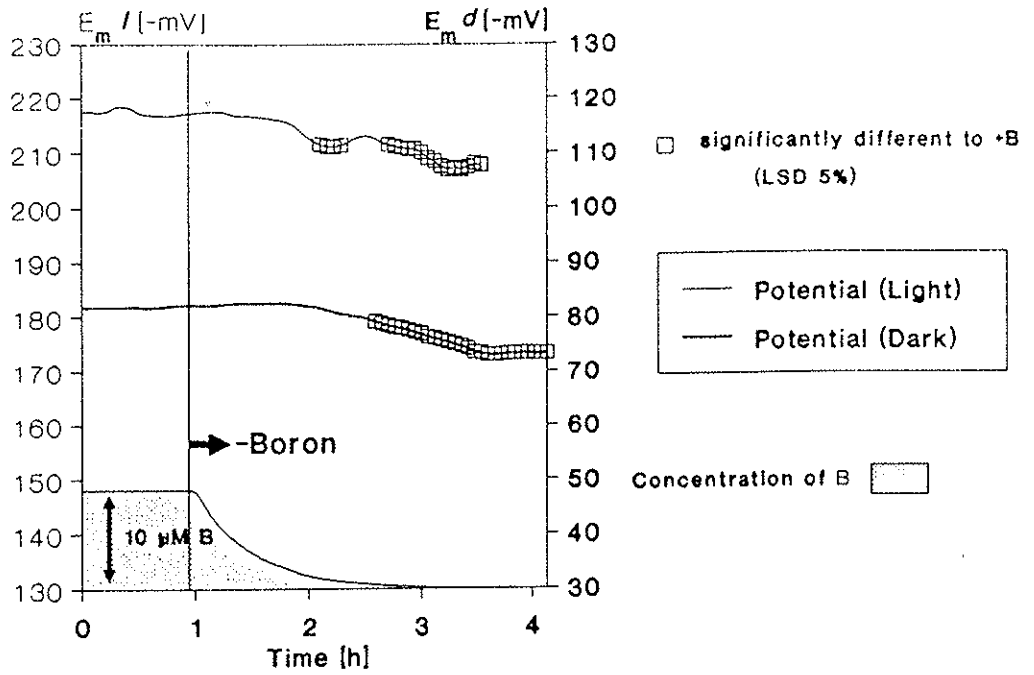

**Figure 8.** Fe(III)-EDTA reduction by intact *Cucurbita pepo* roots as influenced by B and Fe supply (Fe(III) reduction measured as Fe-BPDS staining)

impedance recovered to the original values (Figures 10 a,b).

Due to technical constraints at the time we undertook these experiments, we were only able to change the B concentration slowly (shaded area indicates B conc.). Thus, very rapid effects could not be registered. The time course of the changes observed is within the range we also reported for effects on proton release and reduction (i.e. changes turned up within about 20 - 90 minutes). Relatively shortly after transfer to -B solutions, the light-induced hyperpolarization of *Elodea densa* cells was reduced, and depolarization after turning the lights off was decelerated as well (Figure 11; for an explanation see Section IV).

A completely different design of the cuvette used in the subsequent experiments with intact zucchini plants enabled us to change the flow of nutrient solution in the range of seconds. When impaling root cells (about 2nd to 4th layer beneath the rhizodermis) from the elongation zone with the electrode, a different effect was to be observed. Upon supplying B to B deficient roots (20 h -B), a short hyperpolarization was followed by a transient decrease in potential which slowly tended to return to the original response (Figure 12). Changing to a B free solution resulted in the opposite (Figure 12, $t > 25$ min). Membrane impedance usually was inversely correlated to the potential. It was observed that elongation growth of the roots and root hairs was retarded within less than two hours after switching to the B deficient solution. Elongation growth is mainly controlled by the epidermal

A.




B.

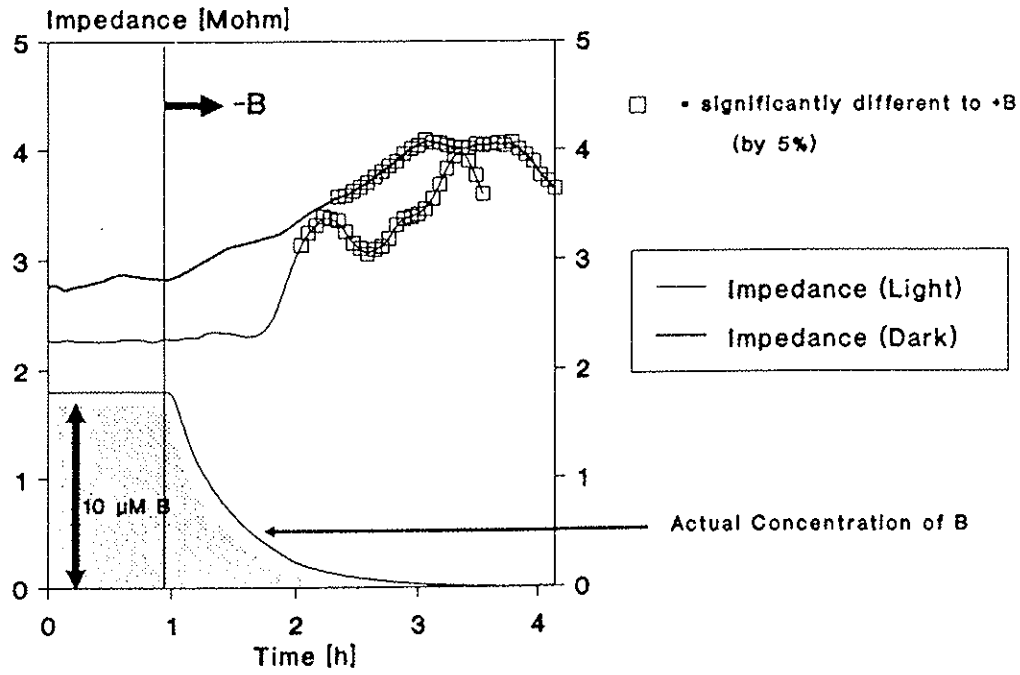
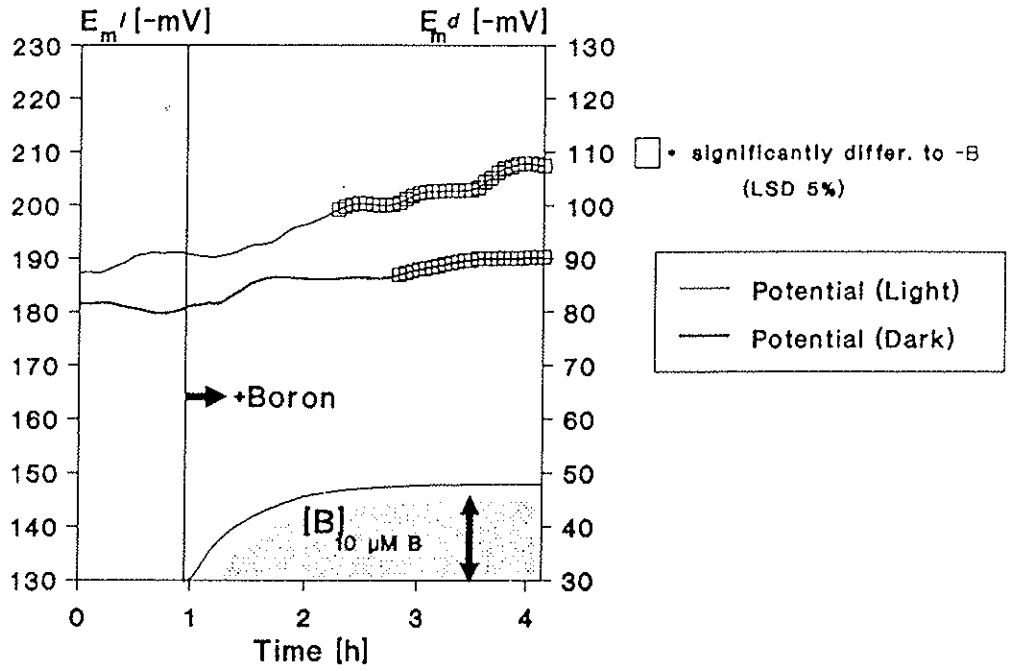



Figure 9. A) Membrane potential and B) impedance of *Elodea densa* after transfer to B-free conditions; boxed areas in the curves indicate significant differences from the (initial) +B level by >5%.

A.




B.

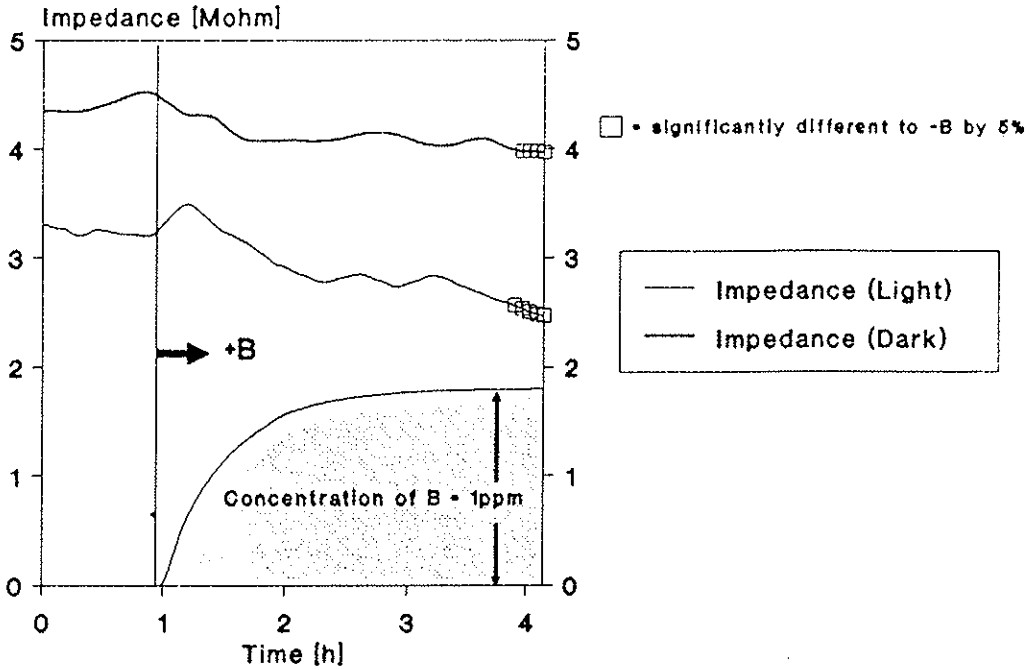



**Figure 10.** A) Membrane potential and B) impedance of *Elodea densa* after transfer from 20 without B to 10 $\mu$M B; boxed areas in the curves indicate significant differences from the (initial) +B level by >5%.

cell layer and B may affect cell wall elasticity (see also section V).

Thus a premature inhibition of the elongation of the epidermal cell layer should lead to an increased solute concentration and consequently a higher potential of the inner cells. To test this possibility, monitoring membrane potential and impedance as well as cell wall elasticity would be necessary, though it is very difficult to get stable potential readings in the epidermal cell layer for the required length of time. An increased permeability for $K^+$ after the addition of B to B deficient sunflower root tip cells has recently been reported (48), suggesting a more or less direct effect of B on proton pumps or $K^+$-channels.

## IV Effect of B deficiency on Chlorophyll (PS II) Fluorescence

It has been observed that B deficiency symptoms are aggravated under higher light intensities (36,55). Chlorosis and chloroplast deformation might develop as one of the first B deficiency symptoms in leaves and a significant proportion of B was supposed to be bound in isolated chloroplasts (4). Considering this and the inhibition noticed in the light-induced hyperpolarization of *Elodea densa* cells (see III), we initiated some experiments to look into the possible involvement of B in the photosynthetic process.

The first visible B deficiency symptom in the shoots of spinach plants was a slight chlorosis. It developed even before there were distinct root symptoms, which usually show up first (27). Analysis at the time of chloroplast isolation (two weeks treatment) showed lower chlorophyll concentrations and less extractable chloroplasts (Table II). Surprisingly, the intactness of the isolated chloroplasts (as measured by the FeCN-reduction before and after osmotic shock) generally tended to be higher in the B deficient chloroplasts (data not shown).

Isolated, osmotically broken chloroplasts reduced more FeCN per unit of chlorophyll and time when isolated from B deficient leaves (Table III). Addition of 1 $\mu$g/ml B as $B(OH)_3$ to the various steps of chloroplast isolation from plants grown on normal B levels resulted in a slight but usually significant stimulation of the FeCN reduction (51.6 vs. 48.6 $\mu$mol $O_2$/mg Chl h, significant at the 1% level for $n=12$).

This stimulation was observed in the chloroplasts of B supplied plants up to a concentration of 100 mM B, whereas the -B chloroplasts seemed to be inhibited by concentrations above 10 mM (Figure 13). This strongly suggests that the stimulation of the $O_2$ evolution (PS II activity) under B deficiency is not directly affected by B (e.g. via boric acid ester or borate ester formation). The observed differences are rather indirect effects of other disturbances in cell metabolism. The addition of boric acid to B sufficient chloroplasts might have had some kind of "uncoupling" effect, as it could interfere with the buildup of a proton gradient, due to its nature as a

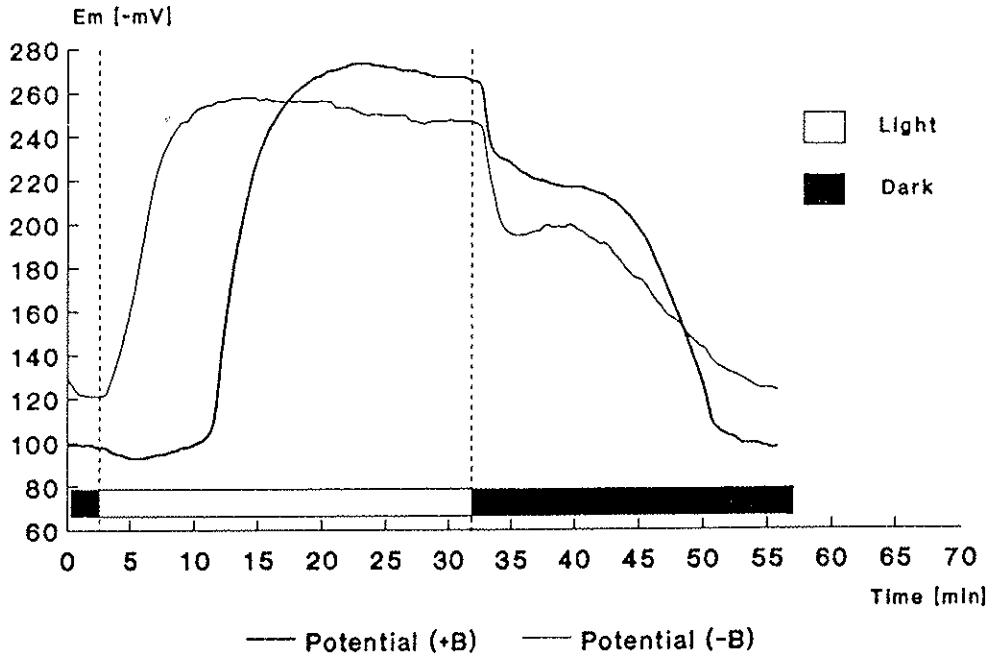



**Figure 11.** Membrane potential of *Elodea densa* under light-/dark changes as related to B supply.

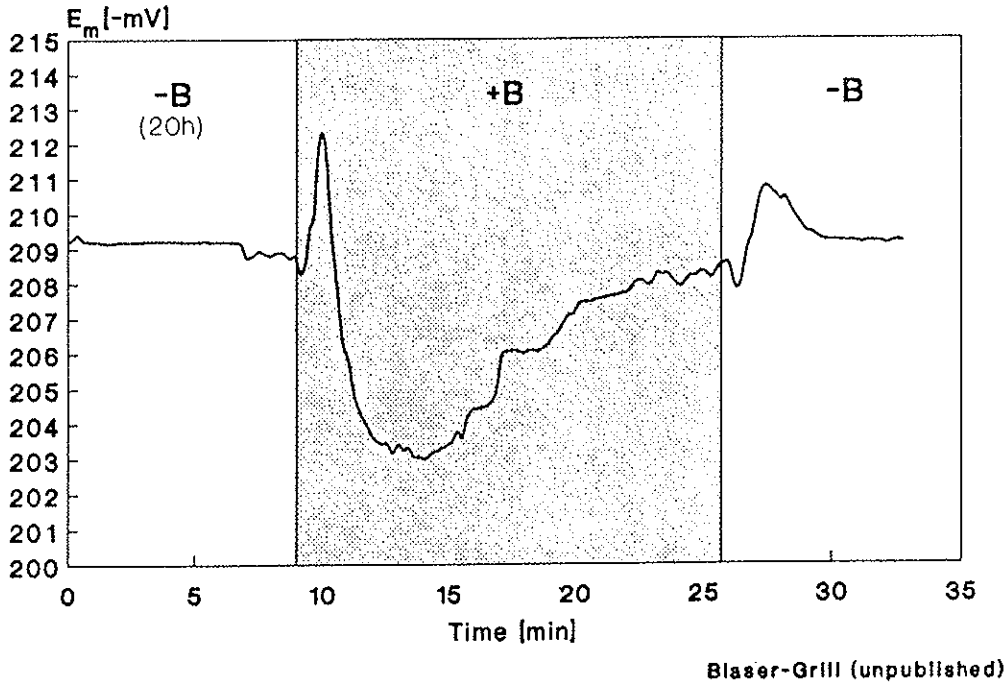



**Figure 12.** Membrane potential of *C. pepo* root cells from intact plants as influenced by the B supply; plants were precultivated for 20 h in the absence of B. (Blaser-Grill, Knoppik, Amberger and Goldbach, unpublished)

**Table II.** Influence of B deficiency (2 weeks) on dry matter (%), chlorophyll content in leaves and isolated intact chloroplasts of *Spinacea oleracea L.* (cv. "Matador"). From (35) with permission.

| | -B | +B |
|---|---|---|
| Leaf DM (%) | 10.60 | 11.30 |
| Chlorophyll in leaf DM (%) | 1.20 | 1.50 |
| mg Chlorophyll $ml^{-1}$ chloroplast suspension | 0.67 | 0.77 |

**Table III.** $O_2$-evolution ($\mu$mol $O_2$ (mg Chl·h)$^{-1}$) of intact and osmotically broken chloroplasts of *Spinacea oleracea* in the presence of $K_3Fe(CN)_6$ as influenced by 14 d with or without B ; ***= significantly different by p = 0.1 % (from (35) with permission)

| | +B | -B |
|---|---|---|
| Intact chloroplasts | 11.11 ± 0.66 | 5.24 ± 0.46*** |
| Osmot. broken chloroplasts | 55.89 ± 2.04 | 70.87 ± 2.20*** |

weak acid with a high mobility in a lipophilic phase. We have been unable as yet to test the ATP formation of B deficient and B sufficient plants which could explain whether there is some kind of uncoupling or not. It has been reported that the addition of B stimulates oxidative and photophosphorylation (9,25).

Chlorophyll fluorescence was followed by a Waltz-PAM-system, consisting of a PAM 101 processor, 101 ED emitter detector unit, 101 F fiber optics, PAM 102 controller with PAM 102 L light source, PAM 103 controller with a FL 103 light source for producing saturating light flashes (1 $\mu$s), to obtain $F_m$. Fluorescence was determined in dark adapted leaves of intact, about 4 weeks old, zucchini plants at 20°C in temperature controlled cuvettes (designed at the University of Bayreuth's Biophysics Department). A 3-channel multiplexer developed by N.L. was used for simultaneous measurements in 3 separate cuvettes and data transfer to a PC-based data retreival system. Details for evaluating fluorescence kinetics are described by Schreiber and coworkers (49,50,51). The time course for fluorescence measurements which was used in our experiments is shown by a typical fluorescence curve (Figure 14). It was first: low intensity measuring light on (light pulses at 100 kHz, integrated intensity about 1 $\mu$mol quanta·$m^{-2}$·$s^{-1}$, which should leave all reaction centers open), then one saturating flash (1 s; FL 103-settings: 0.5, slit 5) ($=F_{md}$-$F_{od}$), 4 min pause, actinic light on ($=t_0$, intensity 45 $\mu$mol quanta·$m^{-2}$·$s^{-1}$), after 4 s one saturating flash, for further 4 min

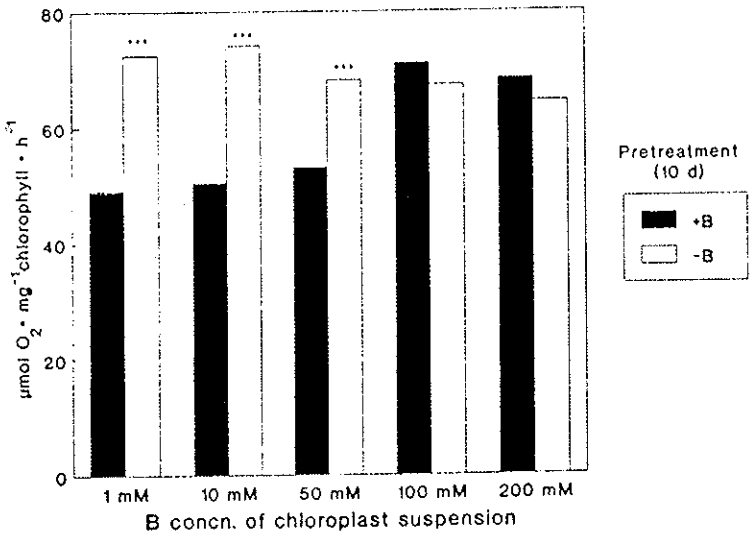



**Figure 13.** Influence of B concentration on $O_2$ evolution of intact spinach (*Spinacea oleracea*) chloroplasts from plants precultured for 10 d with (solid columns) or without (solid columns) 20 $\mu$M B. From (35) with permission.

one saturating flash every 30 s, thereafter saturating flashes every 200 s. The quenching coefficients were calculated on basis of the respective fluorescence values, where $qQ = 1 - [F_v/F_{vm}]$ (photochemic quenching) describes basically the rate of linear e-transport. It is imperative to consider time dependent changes in $F_o$ as in our experiments, as sometimes changes of $F_o$ have been observed over time. The non-photochemic quenching coefficient is calculated as $qN = 1 - [F_{vm}\cdot(F_{md} - F_{od})^{-1}]$, where ($F_{md}$, $F_{od}$) are the respective fluorescence values of the dark adapted plant (12 h in our experiments). As transfer from the growth chamber to the laboratory might affect the quenching behavior, plants were kept in the laboratory at least 24 h prior to the beginning of the dark adaptation. Fluorescence was determined in leaves of three developmental stages: just unfolded (y), younger leaves during exponential growth phase (e), and almost fully developed leaves (m). Care was taken to select leaves of a comparable developmental stage in both treatments.

Photochemic quench (qQ) reached equilibrium conditions much faster in B deficient leaves than in sufficient ones, irrespective of leaf insertion (Figure 15a,b). As the B deficient plants had lower chlorophyll contents (Table II), this might be due to different activities of the Calvin cycle. Having a higher proportion of functional photosystems, there has to be a higher degree of $CO_2$ assimilation and thus more electrons need to be transferred, which consequently needs a longer period of time in +B leaves (Figure 15c). These differences are to be seen, too, when comparing leaves of different ages, i.e., the mature leaves do have higher chlorophyll contents than the younger ones (data not shown). In healthy plants, qQ reaches a definite value under steady state conditions, irrespective

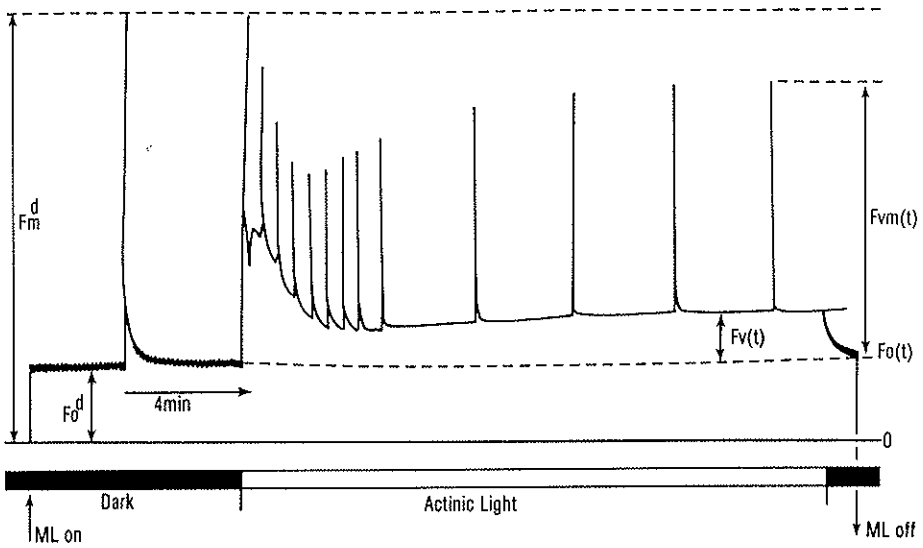


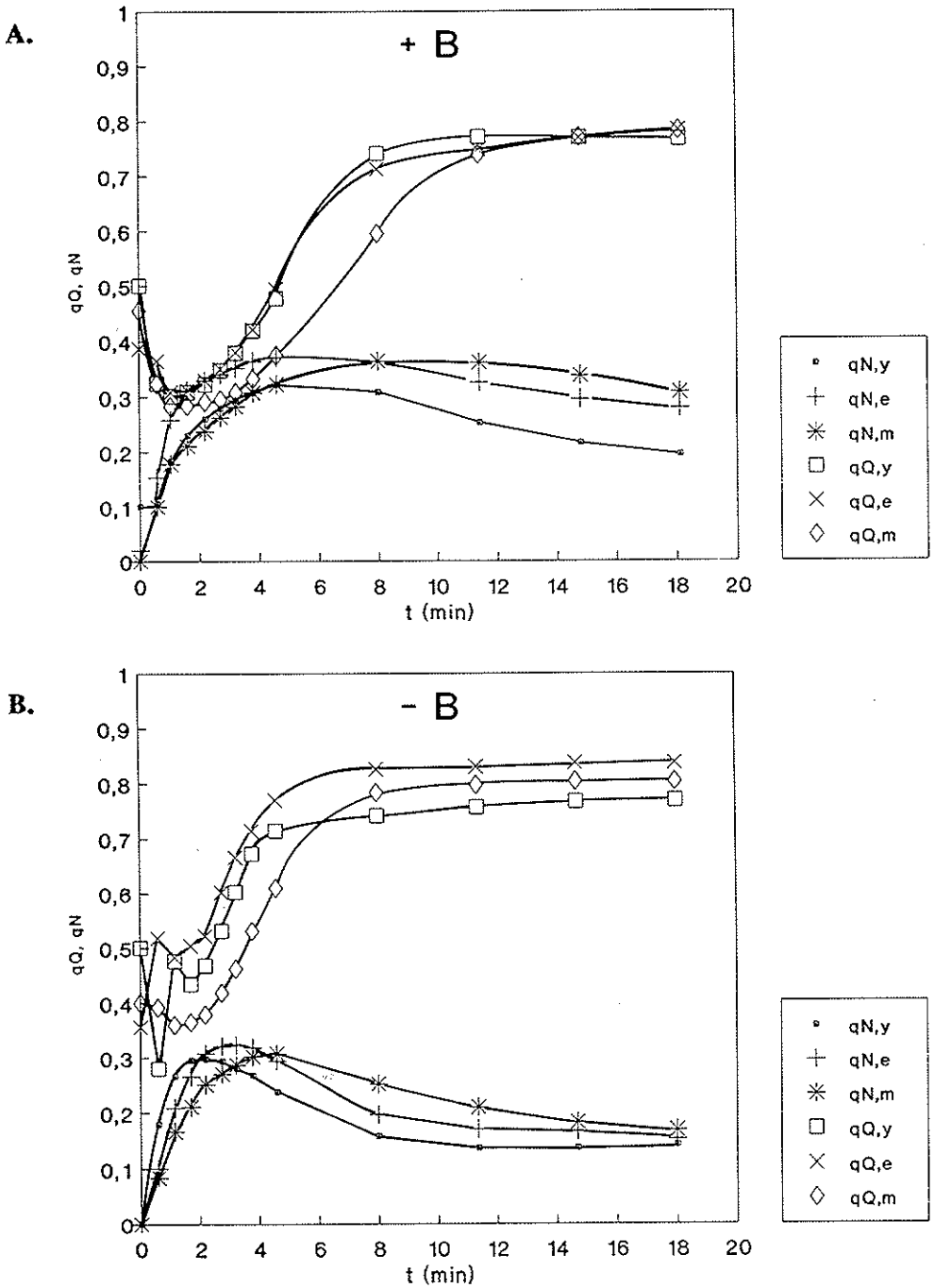

**Figure 14.** Cycle for PSII chlorophyll fluorescence determination as used for Figures 15 and 16. From (35) with permission.

of leaf age (Figure 15a). This quench is mainly caused by the linear electron transport.

An increase in exciton densities as a consequence of higher chlorophyll content (or higher light intensities) causes an increase in qN which prevents an overreduction of PQ and excessive exciton densities in PS II. Most of the qN is caused by an energy dependent quench qE (as we observed only the rapidly reversible components), and is highly correlated with an increase of the proton gradient across the thylakoid membranes (28). This gradient in turn will control ATP synthesis and consequently determine the degree of phosphorylation of the LHC's. They will then be able to transfer excitons to PS I, thus optimizing the energy distribution between the two photosystems.

In all B deficient plants a limitation of qN below the values of the control plants has been observed, whereas qQ was always higher (Figure 15b). It seems sound to assume that there is a reduced energy transfer to PS I under B deficiency. This is apparently compensated for by higher qQ's, which would also explain the stimulated $O_2$-production and FeCN reduction described above.

There was a further indication for an inhibited energy transfer from PS II to PS I: at a higher resolution we found a typical delay in the decay of the fluorescence after saturating flashes (Figure 16a). After the rapid phase of the decay ($t < 50$ ms) there were still higher fluorescence values in B deficient leaves towards steady state conditions. This higher "variable fluorescence" (Figure 16b) is likely to be the consequence of a blocked exciton transfer from PS II to PS I, such that the PQ pool has to be reoxidized mainly by direct electron transfer from PS I. It is also striking that the fluorescence of B deficient leaves apparently oscillates before reaching the equilibrium value $F_v(t)$ (Figure 16a, dashed line) after light saturating flashes (Figure 16a, upper part).



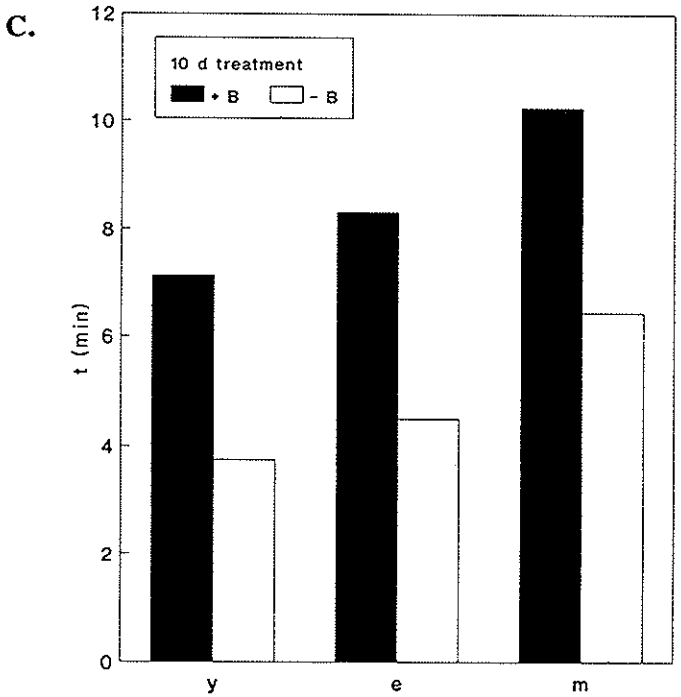




**Figure 15.** Photochemic (qQ) and non-photochemic (qN) PSII chlorophyll fluorescence quench coefficients of *Cucurbita pepo* leaves of a) B sufficient (+B) and b) deficient (-B) plants; y= young, e= expanding, m= mature (almost fully expanded) leaves. c) Time required to reach 90% of the steady state fluorescence values (data from Figure 15 a, b); solid columns = B sufficient, open columns = B deficient leaves. From (35) with permission.

The molecular basis for the alterations caused by B deficiency is not yet clear. In heterocysts of the cyanobacterium, *Anabaena PCC 7119*, B is apparently needed to protect the nitrogenase from $O_2$ (3,16). It is assumed that B acts by stabilizing a glycolipid layer via the formation of borate esters. Although chloroplasts contain most of the glycolipids in the cells (up to 80%), the relatively low concentration of B in the isolated but functional chloroplasts, and the failure to change the chloroplast reaction to the +B level by adding boric acid to the suspensions, make a similar role for B in higher plant cells less likely. Addition of B to +B chloroplasts even raises the $O_2$ production to the -B level (Figure 13). As there seems to be an inhibition of the phosphorylation, the ATP synthesis could also be impaired by lower $P_i$ concentrations. Very low cytosolic $P_i$ concentrations have been found by the NMR technique (Loughman, personal communication and this volume). Whether this is the real cause for the observed effects of B deficiency on phyotosynthesis, or whether other mechanisms (radical attack as a consequence of phenol oxidation) or some kind of uncoupling mechanism are causative for the damages caused by B deficiency, can not be decided yet. An impairment of chloroplast activity, however, has been reported to affect plasmalemma bound redox- and proton pumping activities (33,43).

## V. Influence of B deficiency on Peroxidases and Cell Wall Phenolics

An increased peroxidase (POD) and/or polyphenoloxidase (PPhO) has been reported repeatedly as a consequence of B deficiency (2,18,52). Our results point to a two step reaction, at least in *Daucus* cell cultures: first, a liberation of ionically (cell wall) bound peroxidases (Figure 17), later followed by an increase in total peroxidase activity, apparently due to *de novo* synthesis, as seen by the higher cytosolic (buffer extractable) enzyme activity. The latter may be interpreted as a general stress phenomenon, not specific for B deficiency, whereas the former could be caused by a liberation of wall bound isozymes.

This is possibly be caused by a change in variable wall charges and/or a liberation of peroxidases which might be linked from their (mannose rich) sugar moieties via boric acid/borate ester bridges to cell wall polymers. Possibly even more important is, that the oxidation of phenolic substrates might be retarded by a reaction with boric acid and/or borate.

Free and bound phenolic substances occur in the cell walls of most higher plants, where they form part of the defense mechanisms against pathogens. Bridging cell wall polymers (hydroxyproline rich glycoproteins = HPRG's and arabinoxylans) via di- or oligophenolic bridges is supposed to play a major role in regulating and limiting extension growth (11,12,13,14,15) and as a defense mechanism against pathogens. Beside an enhanced phenol synthesis which has been postulated by several authors (52,60), B deficiency may thus have multiple effects on membrane integrity (damage

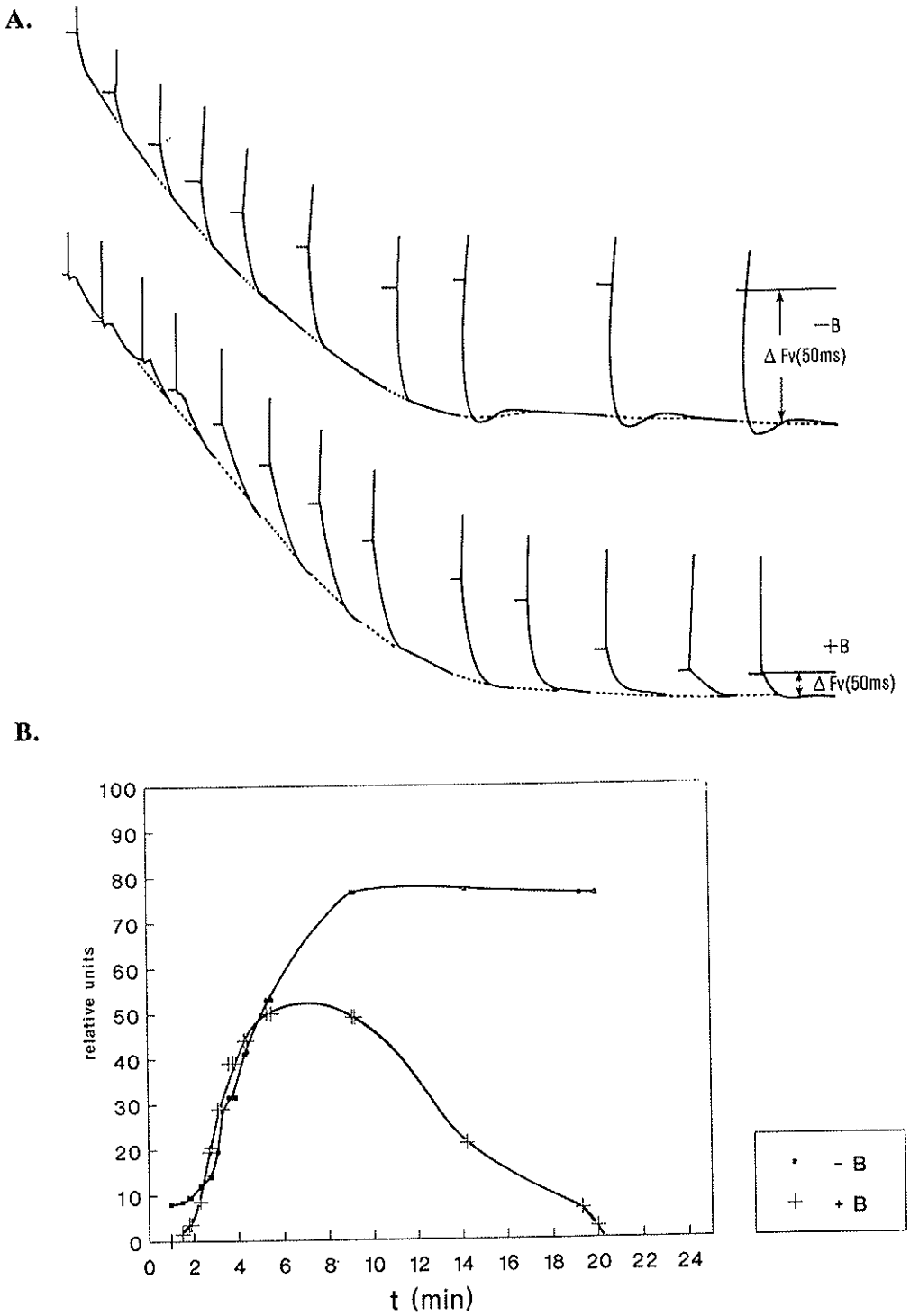



**Figure 16.** A) PS II fluorescence decay after saturating light flashes in leaves of *C. pepo* precultured for 7 d with (+) or without B (-); curves were taken in the period of slow fluorescence decay after switching the actinic light on; $\Delta F_v$(50ms) indicates the fluorescence level 50 ms after giving a saturating light flash minus the "actinic" fluorescence level (broken line). B) $\Delta F_v$(50ms) (relative to maximum fluorescence) of *C. pepo* leaves from Figure 16a; t=0: actinic light on; permanent high values of $\Delta F_v$(50ms) under B deficiency are indicative for a disturbance of exciton transfer from PS II to PS I. From (35) with permission.

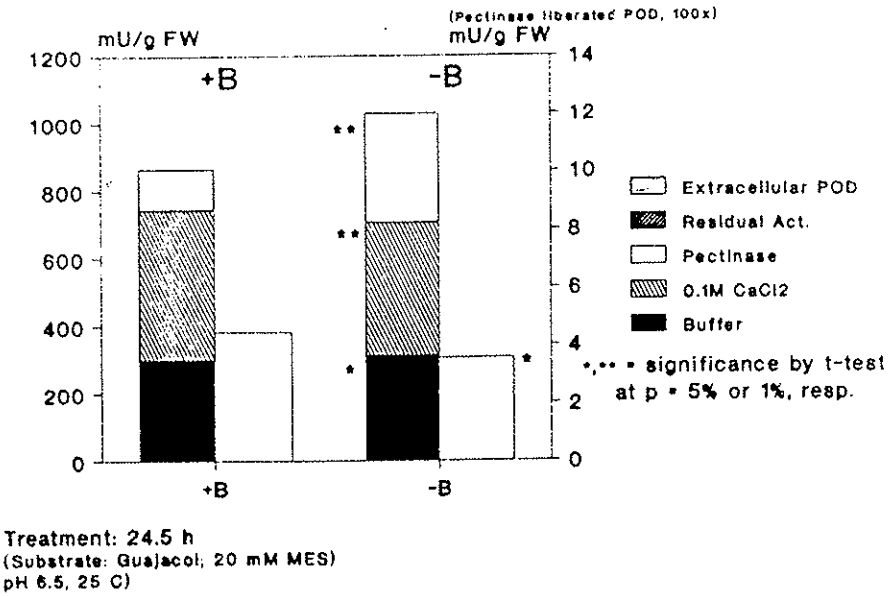


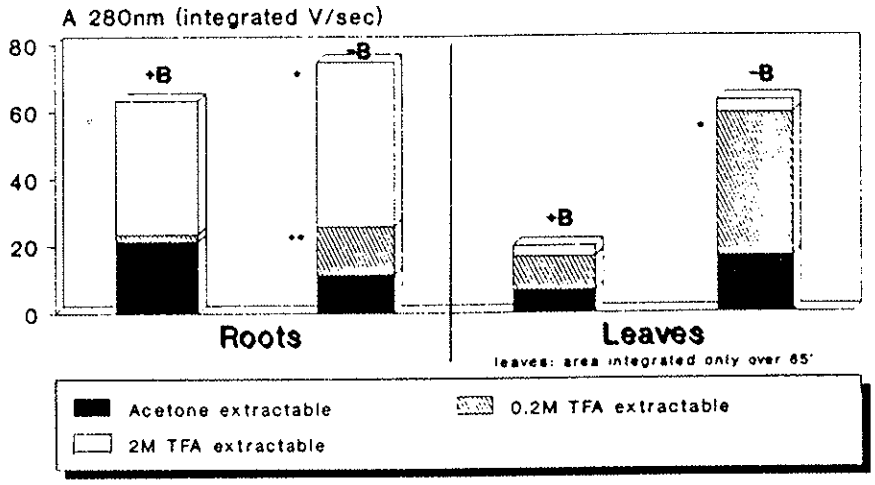

**Figure 17.** Activity of different peroxidase fractions from cell cultures of *Daucus carota* 24.5 h after transfer to a B free medium.

by free radical attack caused by an enhanced oxidation of apoplastic phenols) and wall structure (via the premature formation of phenolic cross links). Alterations and deformations in cell walls have been reported frequently as one early B deficiency symptom (21,29,54). Apart from probably being directly involved in cell wall polymer synthesis (7,17) or bridging (between hydroxylic groups of sugar polymers, esp. of galactose, mannose, and arabinose, whose esters with boric acid do have higher stability constants), B might also regulate the orderly formation of the phenolic cross links. An enhanced phenol oxidation might also be the cause for the higher amounts of phenolics in 0.2 and 2 M trifluoroacetic acid hydrolyzable cell wall fractions (Figure 18).

In a preliminary experiment at Dr. Fry's lab we have tested the incorporation of the plasmalemma impermeable $^{3}H$ and $^{3}H/^{14}C$ labelled feruloylated arabinobiose in *Daucus carota* cells. Under low B supply (we did not really get a true B deficiency during these experiments) an enhanced labelling was found in the following fractions (Figure 19):

- Ethanol precipitable from culture medium (= oligomers and cell wall material < 0.2 $\mu$m, which has been sloughed off during culture);
- phenol acetic acid soluble material (non-covalently bound wall proteins and oligomeric substances);
- total cell wall.

Although these results need further confirmation, they strongly suggest that B may control cell extension growth by regulating the formation of phenolic cross links in the primary (and secondary?)




#Sum peak areas over 75'(-blanc);elution AcN/50mM Acetate,pH4.0,from 0.5 to 70% (concave profile);Spherisorb ODS II 25cm

**Figure 18.** Total phenolics (measured as sum of absorbance at 280 nm from HPLC runs) of different fractions from root and leaf extracts. *,**=significant at 5% or 1% respectively (t-test).

cell walls. Considering that one important group of cell wall polymers assumed to control cell wall extension, i.e. HPRG's ("extensin"), are present in dicot walls 5- to 10-fold the amount found in monocotyledons (14), this could even explain part of their differences in B requirement. Differences in the composition of cell wall phenolics between the individual species might generally be responsible for different reactions to B deficiency. As B deficiency interferes with the regulation of auxin activity as well (2,18,22,53), this could inhibit cell extension growth even further.

Pollen tube growth is known to be strongly inhibited without B, even *in vitro* (45,46,47). Usually, floral tissues contain higher amounts of phenolic substances. One of probably several functions of B might be to retard the phenol oxidation during pollen tube growth to enable the latter to reach the ovary. Further research, especially on pollen tube growth, should lead to further informations on vital functions of B in cell elongation growth.

## VI Conclusions

On basis of literature data and the results presented above, the following consequences of B deficiency are suggested (Figure 20):

- an increased oxidation of free and bound o-dihydroxy phenols leads to free radical attack on membrane compounds such as reductases and/or ATPases and unsaturated fatty acids; an enhanced and probably unordered formation of phenolic cross links results in a disturbance of

cell wall structure;

- there is probably an increased demand for electrons, which are required to reduce toxic chinons and thus compete with other redox-reactions at the plasmalemma;
- peroxidase release, and at a later stage of B deficiency, higher POD and/or PPhO activity will further contribute to the oxidative damages under B deficiency; POD's with auxin oxidase activity may lead to a local decrease in (external) auxin concentration; IAA oxidation might even further stimulate phenol oxidation by peroxidases; depending on the sites most affected (probably the exodermis of root cells), this effect may hardly be seen at the tissue level, as it could be restricted to a relatively small compartment; when proton and/or electron pumps are inhibited by B deficiency, the transport and distribution over different cell and tissue compartments of the weakly acidic auxins may be different as well, thus influencing the tissue's reaction to

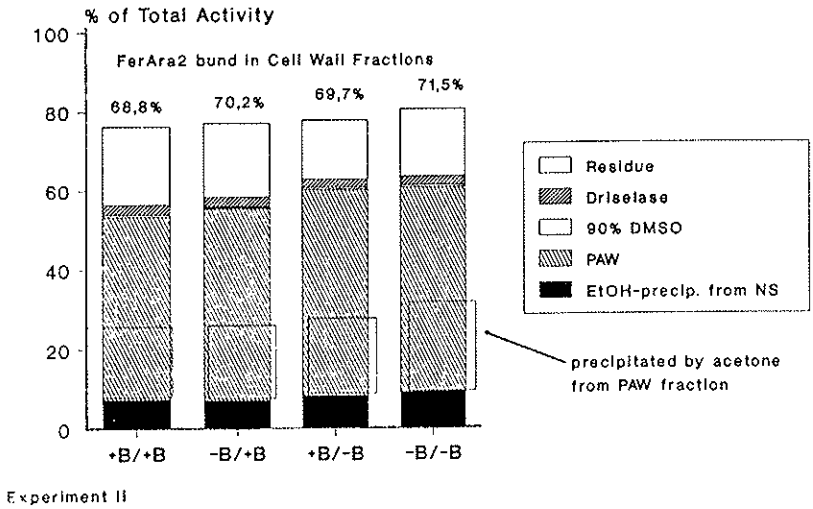



**Figure 19.** Effect of B supply on the incorporation of $^{3}$H-feruloylated arabinobiose into different cell wall fractions of cultured *Daucus carota* cells (EtOH precipitated= precipitable by 80% Ethanol from filtered nutrient solution after harvesting cells; extractable by PAW=80% phenol:acetic acid, 4:1 v/v, extracts mainly non covalently bound proteins; 90% DMSO=extractable by 90% dimethyl sulfoxide; Driselase= extractable by incubation overnight with 2mg/l of purified driselase preparation).

internal as well as to externally applied auxins; membrane alterations might also inhibit the normal binding of auxins to their external receptor sites; the transport and distribution of Ca in plant tissues, which is thought to be affected by B deficiency as well, could either be caused by changes in auxin distribution, but could also be affected by changes in the charges of the apoplast; boric acid esters and/or borate esters with polyhydroxy compounds of the

cell wall could be binding sites for $Ca^{2+}$ and borate esters with polyhydroxycarboxylates are known from the chemical literature to be effective chelators for $Ca^{2+}$;

- lower cytosolic $P_i$ concentrations as a consequence of lower ATPase activities may also affect the photophosphorylation (lack of $P_i$), apart from a direct influence of B on phosphorylation reactions (9,57,58); under higher light intensities, this may lead to a suboptimal energy distribution between photosystems I and II and thus will further contribute to the oxidative damage under B deficiency; low cytosolic $P_i$ concentrations will also reduce the triose exchange between chloroplasts and cytosol; this may explain the higher starch contents in chloroplasts of B deficient leaves;
- the degree to which B is directly affecting protein synthesis, is still unknown; as the ribose moieties of the different RNA's show the appropriate *cis-diol* configuration to react to a significant degree with borate and boric acid, a direct influence can not be excluded, although the B concentrations in the inner part of the cells are reported to be low; however, it is very difficult to confirm either possibility in cases where only catalytic amounts are needed;
- there are further possibilities for B to participate either as structural and/or functional element in

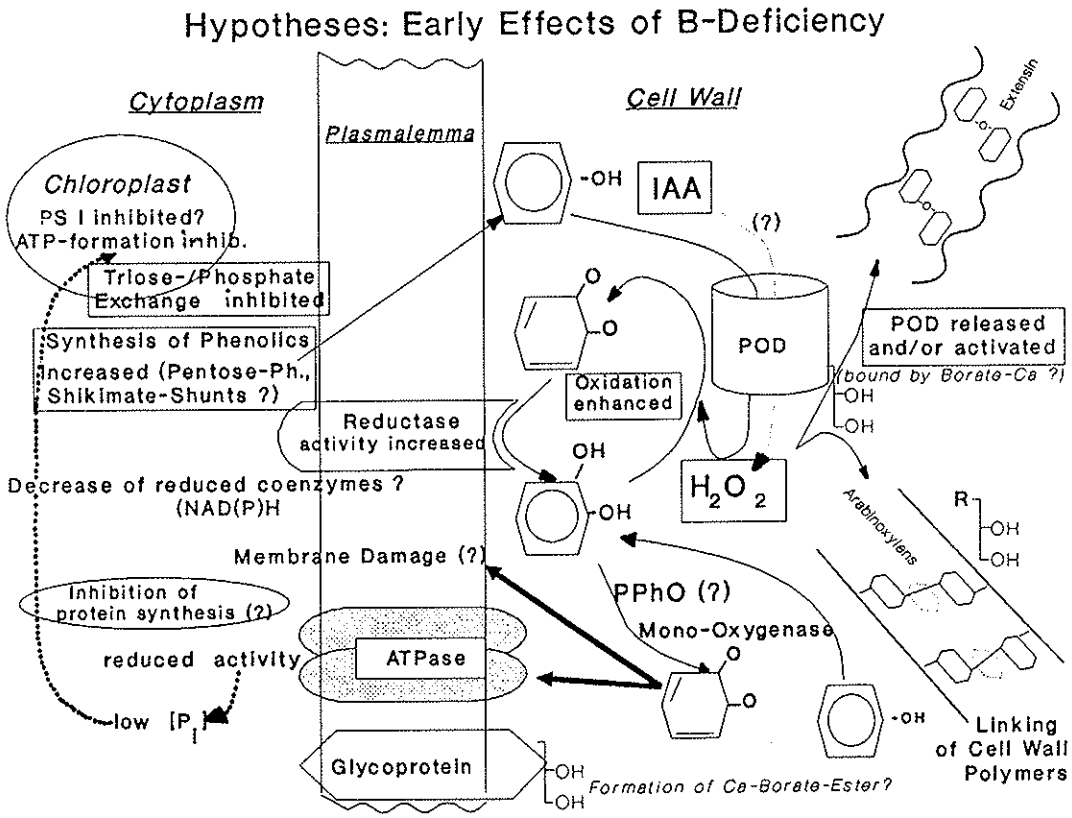



**Figure 20.** Scheme for some of the possible effects of B deficiency (for an explanation see section V).

the plant cell by reacting with hydroxylic groups of quite a number of different compounds, e.g. by linking polysaccharide chains in the cell wall or by shifting the synthesis and/or hydrolysis rates of Schiff'bases; as it will only shift the equilibrium of the reaction more to one or the other site, the true targets of B reactions are very hard to find out; as equilibria between the bound and free forms of B esters are reached very rapidly, it seems to be useless trying to extract B containing compounds of plant cells.

Much research is still needed to further elucidate B primary functions in plants and animals. It has to be kept in mind that there are most likely several mechanisms which may be influenced simultaneously by boric acid/borate ester formation.


## Acknowledgements

Most part of the work presented above has been carried out at the Department of Plant Nutrition of the Technical University Munich-Weihenstephan; the encouragement as well as the helpful comments of Prof.em. Dr. A. Amberger are greatfully acknowledged; generous financial support has been provided by the Deutsche Forschungsgemeinschaft; we are especially indebted to Prof. Dr. D. Knoppik for his collaboration in the biophysical experiments and his steady support; the authors are greatful for the excellent technical assistance by Mrs. Susanne Vorhammer and the help in preparing this manuscript by Mrs. Brigitte Menzel.


## Literature Cited


1. Bell, C.F., Beauchamp, R.D. and E.L.Short. 1986. A study of the complexes of borate ions and some cyclitols using B - n.m.r. spectroscopy. Carbohyd. Res. 147:191-203.

2. Bohnsack, C.W. and L.S. Albert. 1977. Early effects of boron deficiency on indoleacetic acid oxidase levels of squash root tips. Plant Physiol. 59:1047-1050.

3. Bonilla, I., Garcia-Gonzales, M. and P. Mateo. 1990. Boron requirement in cyanobacteria -its possible role in the early evolution of photosynthetic organisms. J. Plant Physiol. 94:1554-1560.

4. Bussler, W. and H.W. Döring. 1979. Verteilung und Löslichkeit von Bor in *Helianthus annuus* in Abhängigkeit vom Bor-Angebot. Z. Pflanzenern. u. Bodenkde. 142:719-730.

5. Coke, L. and W.J. Whittington. 1968. The role of boron in plant growth. IV. Interrelationships between boron and indolyl-3-acetic acid in the metabolism of bean radicles. J. Exp. Bot. 19:295-308.

6. Dugger, W.M.. 1973. Functional aspects of boron in plants. Adv. Chem. Ser. 123:112-129.

7. Dugger, W.M.. 1983. Boron in plant metabolism. In: **Inorganic Plant Nutrition (Encyclopedia of Plant Physiology)**; A. Läuchli and R.L.Bieleski (eds.), Springer Heidelberg, New York, pp. 626-650.

8. Ehlers, H.. 1951. Untersuchungen zur Ernährungsphysiologie der Pollenschläuche. Bio. Zentr. 70:432-451

9. Eichhorn, V.M. and H. Augsten. 1974. Influence of boron on the populations of *Wolffia arrhiza* (L.). Wimm. of different ages cultivated in chemostat. Biochem. Physiol. Pflanz. (Jena) 165:371-385.

10. Fackler, U., Goldbach, H., Weiler, E.W. and A. Amberger. 1985. Influence of boron-deficiency on indol-3yl-acetic acid and abscisic acid in root and shoot tips. J. Plant Physiol. 119:295-299.

11. Fry, S.C.. 1982. Isodityrosine, a new cross-linking amino acid from plant cell-wall glycoprotein. Biochem. J. 204:449-455.

12. Fry, S.C.. 1982. Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharide. Biochem. J. 203:493-504.

13. Fry, S.C.. 1983. Feruloylated pectins from the primary cell wall: their structure and possible functions. Planta 157:111-123.

14. Fry, S.C.. 1988. **The Growing Plant Cell Wall: Chemical and Metabolic Analysis**. Longman Scientific and Technical, Harlow, U.K. 333 pp.

15. Fry, S.C. and J.G. Miller. 1987. $H_2O_2$-dependent cross-linking of feruloyl-pectins *in vivo*. Food Hydrocolloids, 5/6:395-397.

16. García-Gonzalez, M., Mateo P., and I. Bonilla. 1988. Boron protection for $O_2$ diffusion in heterocysts of *Anabaena sp. PCC 7119*. Plant Physiol. 87:785-789.

17. Goldbach, H.E. and A. Amberger. 1986a. Influence of boron deficiency on cell wall polysaccharides in cell cultures of *Daucus carota*. J. Plant Physiol. 123:236-239.

18. Goldbach, H.E. and A. Amberger. 1986b. Influence of boron deficiency on $^3H$ indole-3yl-acetic acid uptake and efflux in cell cultures of *Daucus carota L.* Plant Growth Regulation 4:81-86.

19. Goldbach, H.E., D. Hartmann and T. Rötzer. 1990. Boron is required for the stimulation of the ferricyanide-induced proton release by auxins in suspension-cultured cells of *Daucus carota* and *Lycopersicon esculentum*. Physiol. Plant. 80:114-118.

20. Hewitt, E.J.. 1963. The essential nutrient elements: requirements and interactions in plants. In: **Plant Physiol.**, F.C. Steward (ed.)

21. Hirsch, A.M. and J.G. Torrey. 1980. Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. Can. J. Bot. 58:856-866.

22. Hirsch, A.M., W.L. Pengelly and J.G. Torrey. 1982. Endogenous IAA levels in boron deficient and control root tips of sunflower. Bot. Gaz. 143:15-19.

23. Hundt, I., W. Bergmann and G. Schilling. 1970. Untersuchungen über den Einfluß des Mikronährstoffes B auf den Nucleinsäurestoffwechsel und die Gewebestruktur von *Helianthus annuus L.* Albr. Thaer-Archiv 14:725-737.

24. Jaweed, M.M. and E.G. Scott. 1967. Effect of boron on ribonucleic acid and indoleacetic acid metabolism in the apical meristems of sunflower plants. Proc.West Va. Acad. Sci. 39:186-193.

25. Kibalenko, A.P.. 1966. The importance of boron for the structure and the function of the plant cell. In: **Proc. Intl. Symp. on Stimulation of Plants**, Sophia. J. Papov (ed), pp. 122-128.

26. Kiesewetter, S., G. Ott and M. Sprinzl. 1990. The role of modified purine 64 in initiator/elongator discrimination of $tRNA_{i met}$ from yeast and wheat germ. Nucleic Acids Research 18(16):4677-4682.

27. Kouchi, H. and K. Kumazawa. 1975. Anatomical responses of root tips to boron deficiency. I. Effects of boron deficiency on the elongation of root tips and their morphological characteristics. Soil Sci. Plant Nutr. 21:218-228.

28. Krause, G.H. and E. Weis. 1988. The photosynthetic apparatus and chlorophyll fluorescence. An introduction. In: **Applications of Chlorophyll Fluorescence**, H.K. Lichtenthaler (ed.), Kluwer Academic Publishers, Dordrecht, pp. 3-11.

29. Krosing, M.. 1973. Wirkung von Bormangel auf Gewebe und Zellen intakter Pflanzen und auf isoliert wachsendes Gewebe. Doctoral Thesis, TU Berlin, Nr. 34.

30. Krueger, R.W., C.J. Lovatt and L.S. Albert. 1987. Metabolic requirement of *Curcubita pepo* for boron. Plant Physiol. 83:254-258.

31. Landsberg, E.C. 1980. Fe-deficiency stress induced development of transfer cells in the epidermis of red pepper roots. Plant Physiol. 65, Supp. 83.

32. Landsberg, E.C. 1981. Energy driven $H^+$ efflux pump in sunflower roots - activated by Fe deficiency stress. Plant Physiol 67, Supp. 702.

33. Lass, B., G. Thiel and C.J. Ullrich-Eberius. 1986. Electron transport across the plasmalemma of *Lemna gibba*.G1. Planta 169:251-259.

34. Leegood, R.C. and D.A. Walker. 1983. Chloroplasts. In: **Isolation of Membranes and Organelles from Plant Cells**. J.L.Hall and A.L.Moore (eds.) Academic Press Inc., London, New York, pp 185-210.

35. Lindemann, N., D. Knoppik, B. Gessner and H.E. Goldbach. 1991. Effect of boron deficiency on Hill reaction in chloroplasts of *Spinacea oleracea* and chlorophyll fluorescence in *Cucurbita pepo* L. Physiol. Plant., submitted.

36. Mac Innes, C.B. and L.S. Albert. 1969. Effect of light intensity and plant size on the rate of development of early boron deficiency symptoms in tomato root tips. Plant Physiol. 44:965-967.

37. Martini. F. and M. Thellier. 1980. Use of (n, α) nuclear reaction to study the long-distance transport of boron in *Trifolium repens* after foliar application. Planta 150:197-205.

38. Matsuda, H., H. Nagamatsu, T. Okyama and T. Fueno. 1984. Influence of boric acid on the hydrolysis of a hydroxy Schiff base. Bull. Chem. Soc. Jpn. 57:500-505.

39. Moore, H.M. and A.M. Hirsch. 1983. Effects of boron deficiency on mitosis and incorporation of tritiated thymidine into nuclei of sunflower root tips. Am. J. Bot. 70:165-172.

40. Mourioux, G. and R. Douce. 1981. Slow passive diffusion of orthophosphate between intact isolated chloroplasts and suspending medium. Plant Physiol. 67:470-473.

41. Nagamatsu, H., T. Okyama and T. Fueno. 1984. Hydrolysis of N-salicylidene-2-methoxyethylamine. Intramolecular general base catalysis and specific effects of boric acid. Bull. Chem. Soc. Jpn. 57:2502-2507.

42. Nagamatsu, H., T. Okyama and T. Fueno. 1984. Effects of boric acid on the rate and equilibrium of Schiff base formation from salicylaldehyde. Bull. Chem. Soc. Jpn. 57:2508-2514.

43. Novak, V.A., N.G. Ivankina, E.A. Morokova and A.I. Miclashevich. 1988. The link between the electrogenic and redox functions of the plasmalemma and the energy metabolism of the cell. Physiol. Plant 73:165-169.

44. Parr, A.J. and B.C. Loughman. 1983. Boron and membrane function in plants. In: **Metals and Micronutriens: Uptake and Utilization by Plants.** D.A. Robb and W.S. Pierpoint (eds.) Acad. Press. London, pp. 87-107.

45. Schmucker, T.. 1932. Bor als physiologisch entscheidendes Element. Naturwiss. 20:839.

46. Schmucker, T. 1933. Zur Blütenbiologie tropischer *Nymphaea*-Arten. II Bor als entscheidenster Faktor. Planta 18:641-650.

47. Schmucker, T. 1934. Über den Einfluβ von Borsäure auf Pflanzen, insbesondere keimende Pollenkörner. Planta 23 (1/2):264-283.

48. Schon, M.K., A. Novacky and D.G. Blevins. 1990. Boron induces hyperpolarization of sunflower root cell membranes and increases membrane permeability to $K^+$. Plant Physiol. 93:566-571.

49. Schreiber, U. 1983. Chlorophyll fluorescence yield changes as a tool in plant physiology. I. The measuring system. Phot. Res.4:361-373.

50. Schreiber, U. 1986. Detection of rapid induction kinetics with a new type of high frequency modulated chlorophyll fluorometer. Phot. Res. 9:261-272.

51. Schreiber, U., U. Schliwa and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Phot. Res. 10:51-62.

52. Shkolnik, M.Y. 1984. **Trace Elements in Plants.** Elsevier, Amsterdam, Oxford, New York, Tokyo.

53. Smirnov, Y.S., T.A. Krupnikova and M.Y. Shkolnik. 1977. Content of IAA in plants with different sensitivity to boron deficits. Fiziol. Rast. 24:270-276.

54. Spurr, A.R. 1957. Boron in morphogenesis of plant cell walls. Science 126:78-80.

55. Tanaka, H. 1966. Response of *Lemna paucicostata* to boron as affected by light intensity. Plant Soil 25:425-434.

56. Thellier , M., Y. Duval and M. Demarty. 1979. Borate exchanges of *Lemna minor* L. as studied with the help of the enriched stable isotope and of a (n, α)-nuclear reaction. Plant Physiol. 63:283-288.

57. Timashov, N.D. 1967. Photosynthetic phosphorylation in chloroplasts isolated from boron-deficient sunflower and barley leaves. Doklady Akademii Nauk SSSR 175:240-242.

58. Timashov, N.D. 1968. Effect of boron on oxidative phosphorylation and other biochemical properties of sunflower and barley mitochondria. Fiziol Ras. 15:597-604.

59. Walker, D.A. 1980. Regulation of starch synthesis in leaves - the role of orthophosphate. Proc. 15th Coll. Int. Potash Inst. Bern, 195-207.

60. Watanabe, R., W. Chorney, J. Shok and S.H. Wender. 1964. Effect of boron deficiency on polyphenol production in the sunflower. Phytochem. 3:391-394.

61. Weser, U. 1968. Einfluβ des Borats und Germanats auf die RNA-Biosynthese . Hope-Seylers Z. Physiol. Chem. 349:989-994.