

## On the Possible Envolvement of Boron in the Cell Wall Stiffening Process

### Introduction:

Boron is known as an essential trace element to higher plants since 1910, and it is likely to be required by man and animals, too, although in much lower amounts. Its participation in definite physiological reactions, however, still remains to be elucidated.

Frequently observed B deficiency symptoms are the brittleness of leaves and stem, the occurrence of cracks in the tissues, and the accumulation of brown to black-coloured substances (polyphenols), esp. in younger plant parts. Another effect of B deficiency is the rapid cessation of extension growth, especially in roots and pollen tubes. Thus we started to look into the possible participation of B in the regulation of cell wall extension and phenol oxidation, stimulated by the publications of Dr.Fry on the importance of diphenol bridges in the cell wall stiffening process.

### Results and Discussion:

#### POD-/IAA-Oxidase Activities:

Some years ago, we observed an increase in peroxidase activity in cell cultures of *Daucus carota* after transferring those cells to a B deficient medium.

#### fig.1

The POD activity rose significantly after about 24 to 40 h, but to a much higher extend in the extracellular fraction than in the cells. Additionally, we happened to observe a very early increase in extracellular POD activity within the very first hours of B deficiency. This transitory raise of POD could not be suppressed by cycloheximide, and thus was a liberation of, probably, cell wall bound enzyme activity. Although we have made this observation at that time very

frequently, we were unable to repeat this early rise of extracellular activity in the experiments of this year. So we are cautious about generalising this effect.

A more detailed fractionation of the cell's POD activity is still under way. Some preliminary results show, that the increased POD activity is located to a larger extent in the

**fig.2**

wall fraction, liberated by 100 mM CaCl<sub>2</sub> and pectinase treatments, whereas the cytosolic (=buffer extractable) activity is less affected. This is also in agreement with the isoenzymic pattern revealed by IEF,

**fig.3**

where we got an increase of mainly cathodic peroxidase isoenzymes. This fraction also showed a higher IAA oxidase activity by a staining reaction on the gels with curcumin. This method might not be too reliable, but we also observed an increased IAA oxidation of <sup>3</sup>H-labelled IAA in B deficient cell cultures

**fig.4**

and lower auxin concentrations in the B deficient tissues of sunflower and tomato.

**fig.5**

A higher POD activity and lower concentrations of auxins might thus be responsible, in part, for the growth inhibition and the polyphenol accumulation observed under B deficiency.

**Proton Secretion:**

It has to be considered, though, that the increase in POD activity is a general stress reaction, observed as a consequence of many types of stress and not very specific for B deficiency. It is also true, that this effect is a rather late one, which can not be accounted for by the relatively rapid reactions occurring upon transfer of plants or cells to B deficient conditions. In cell cultures of tomato and

carrot we observed an inhibition of the Fe-deficiency- or ferricyanide-induced proton secretion within 6 to 16 h by always over 50%.

**fig.6**

According to recent experiments with intact squash plants, this effect seems to exist in the root tissue, too, especially in the subapical root zone, where most of the extension growth extension growth is taking place.

It is interesting to note, that the differences in ferricyanide-induced proton secretion of plant cells between the B treatments depend strictly on the presence of auxins in culture solution. Without auxins, the proton secretion is much lower and no differences between +B and -B are being observed. There is even a tendency towards a somewhat higher proton release of the -B cells (although in most cases these differences were not statistically significant).

**fig.7**

It may thus be assumed, that the action of auxin requires the presence of B to take place. B may act either by modifying the plasmalemma or by changing the plasmalemma-near cytoplasmic pH. Experiments are under way to test either possibility.

**Cell Wall-bound Phenols:**

As the before-mentioned effects of B deficiency: enhanced peroxidase activity, reduced auxin concentrations, and hampered auxin action, should also lead to a higher degree of phenol oxidation in the cell walls, we started recently to

extract wall-bound phenolic substances from different species. We have tested different methods of cell wall hydrolysis, and finally got the most reproducible results by extracting subsequently with buffer and acetone in order to remove free phenolic substances, (in some cases followed by a pectinase treatment), and hydrolysis by 0.2 and 2 M TFA. It was to be seen that the amount of total phenolic substances (determined by the sum of absorption at 280 nm over all peaks in HPLC-runnings) increased in the B deficient tissues as expected.

**fig.8**

The most prominent differences occurred in the 0.2M TFA hydrolysis fractions in leaves as well as in roots. Among the phenolic substances which increased under B deficiency, a peak co-chromatographing with authentic diferulic acid and almost equal spectral properties (as measured by a HP diode-array detector).

**fig.9**

Additionally, it has to be considered that B may also affect the enzymatic oxidation of phenolic substances by forming borate or boric acid esters with o-dihydroxy compounds. In own still unpublished *in vitro* experiments, we found that the oxidation of different phenolic substrates by peroxidase is inhibited by the addition of boric acid or borate by up to almost 60%. As there was a higher degree of inhibition at a pH of 7.5 than at 6.5, borate can be assumed as the responsible compound. Considering the relatively low pH in the apoplastic compartment, however, it is uncertain whether this effect should also play a role *in vivo*, too.

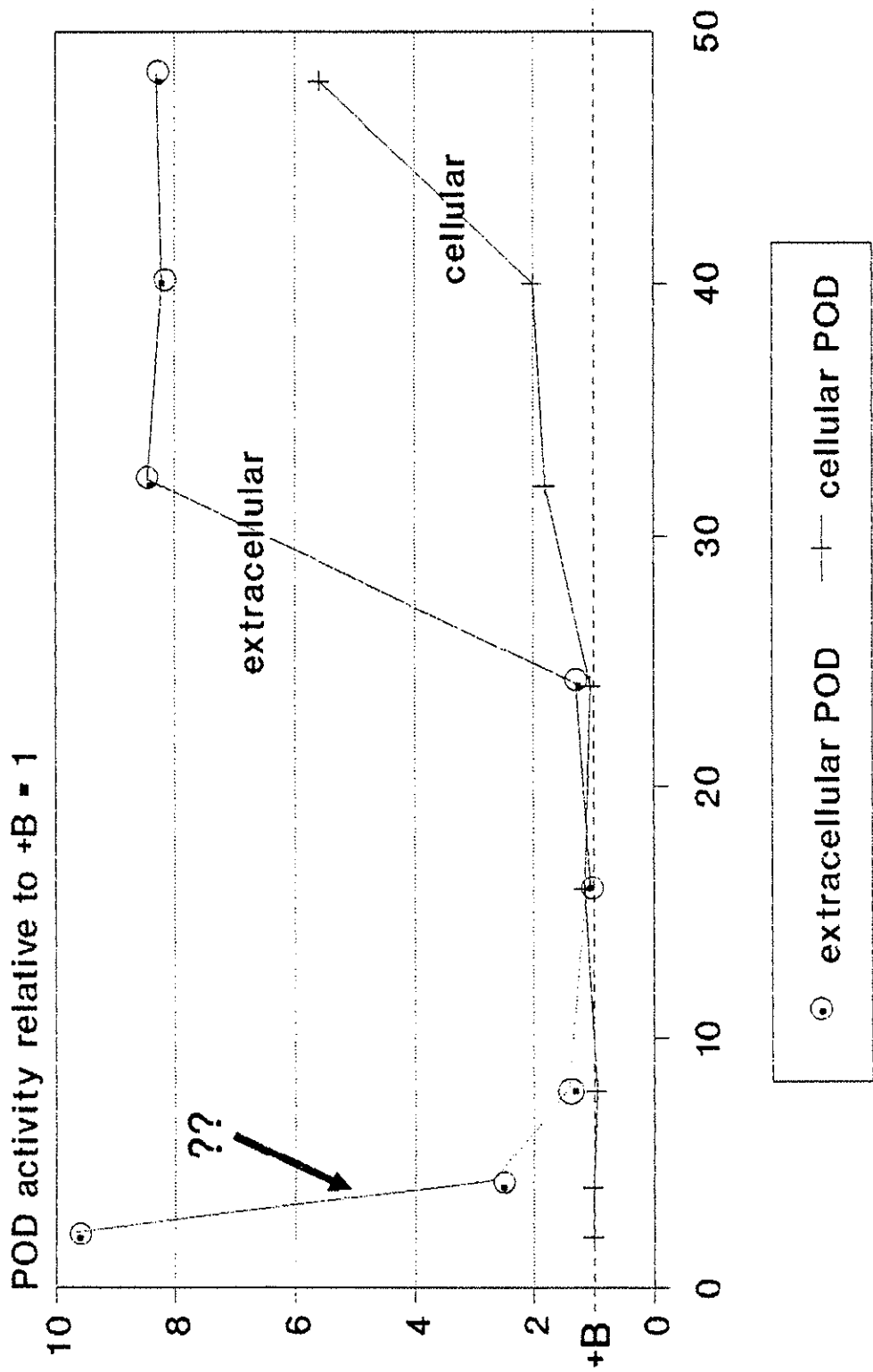
Summary and Conclusions:

B deficiency may affect the process of cell wall extension in a number of ways as listed in

**fig.10.**

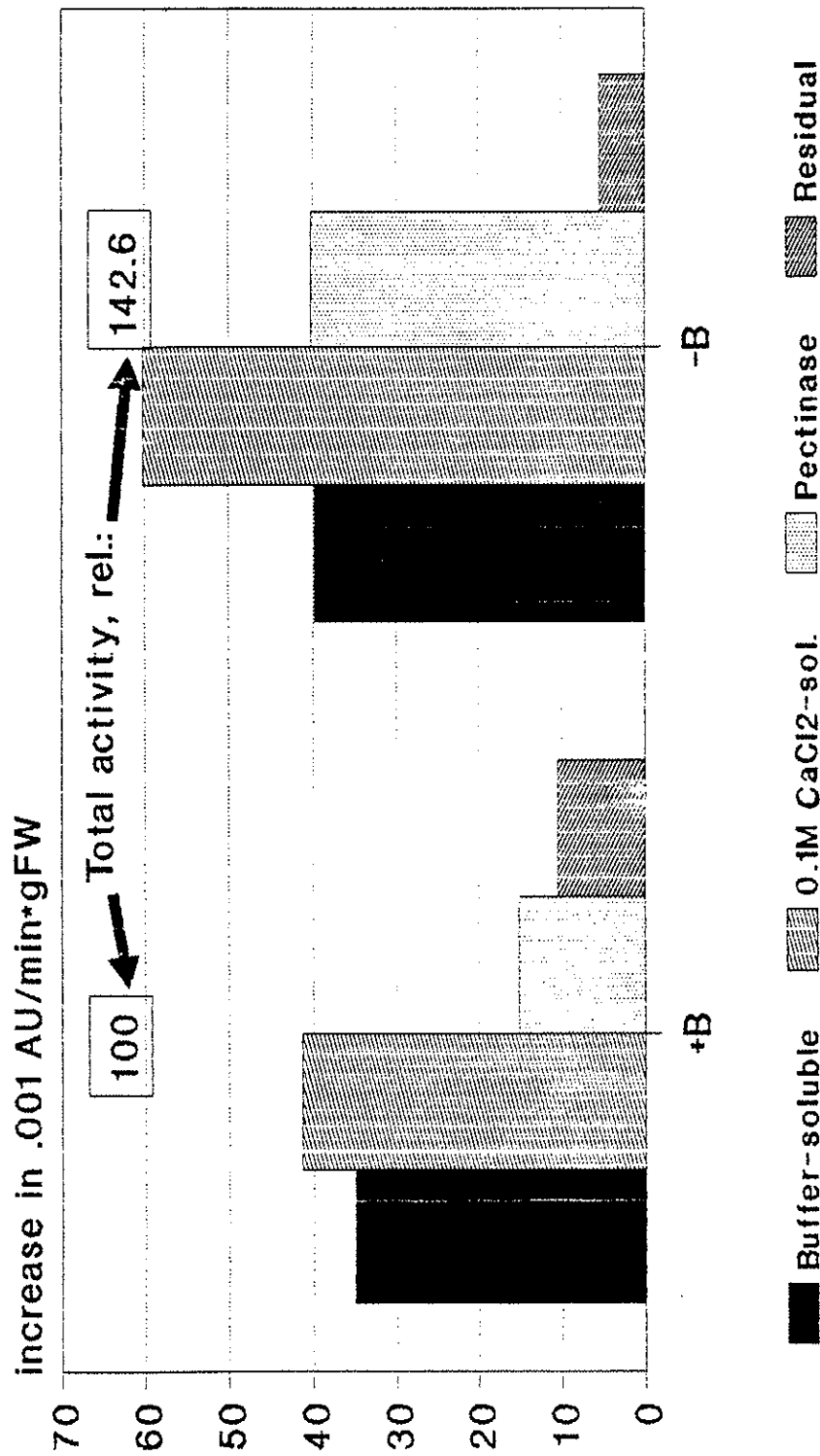
The importance of an adequate supply of B to growing tissues might thus to a large extent be explained by its functions in processes related to cell wall extension and growth. The differences in the composition of the cell walls and the different auxin requirements of monocots and dicots might also explain their differences in B requirement.

# Cellular and Extracellular POD-Activity in B deficient Daucus carota Cells

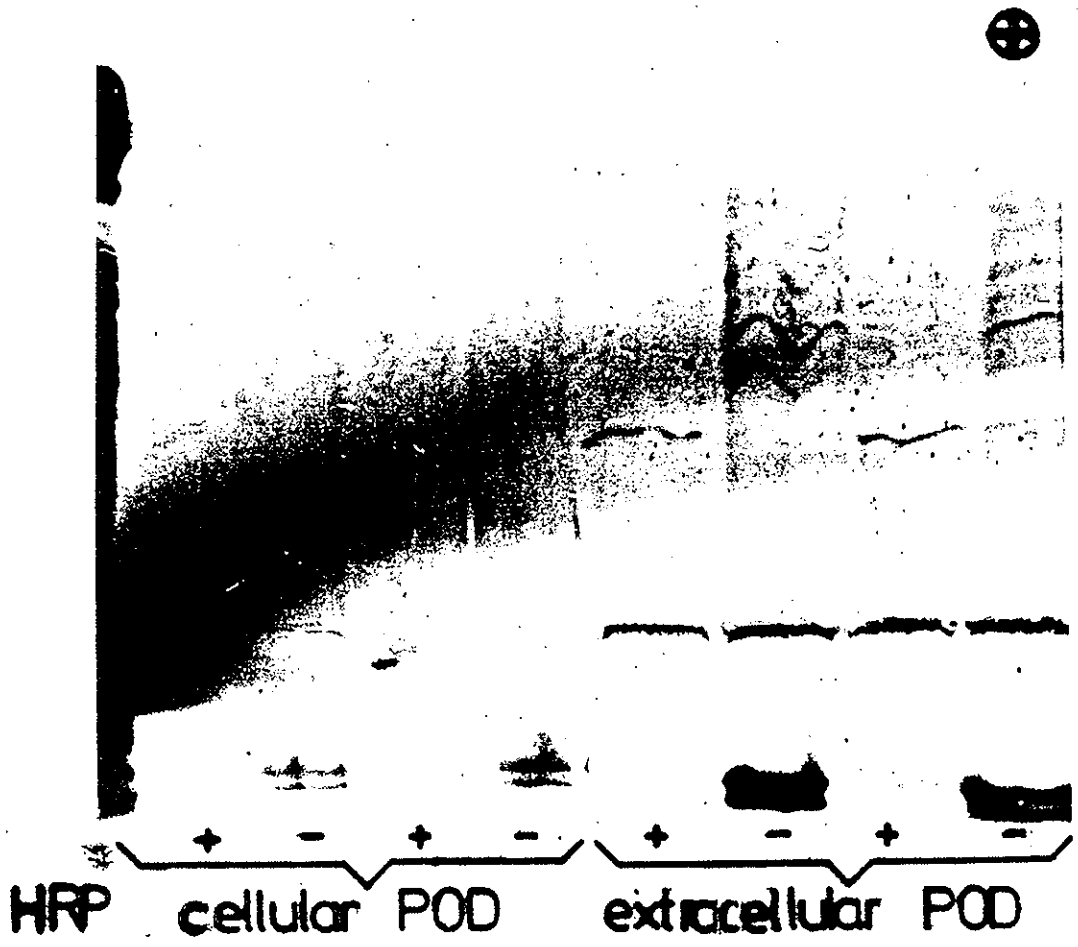


POD activity related to total protein  
(acc. to Lowry et al. 1950)

# Peroxidase Activity in Cell Cultures of *Daucus carota* under Boron Deficiency



preliminary results, treatment: 43.5 h  
 (activity determined with guajacole as  
 substrate, 20 mM P-buffer, pH 6.5)

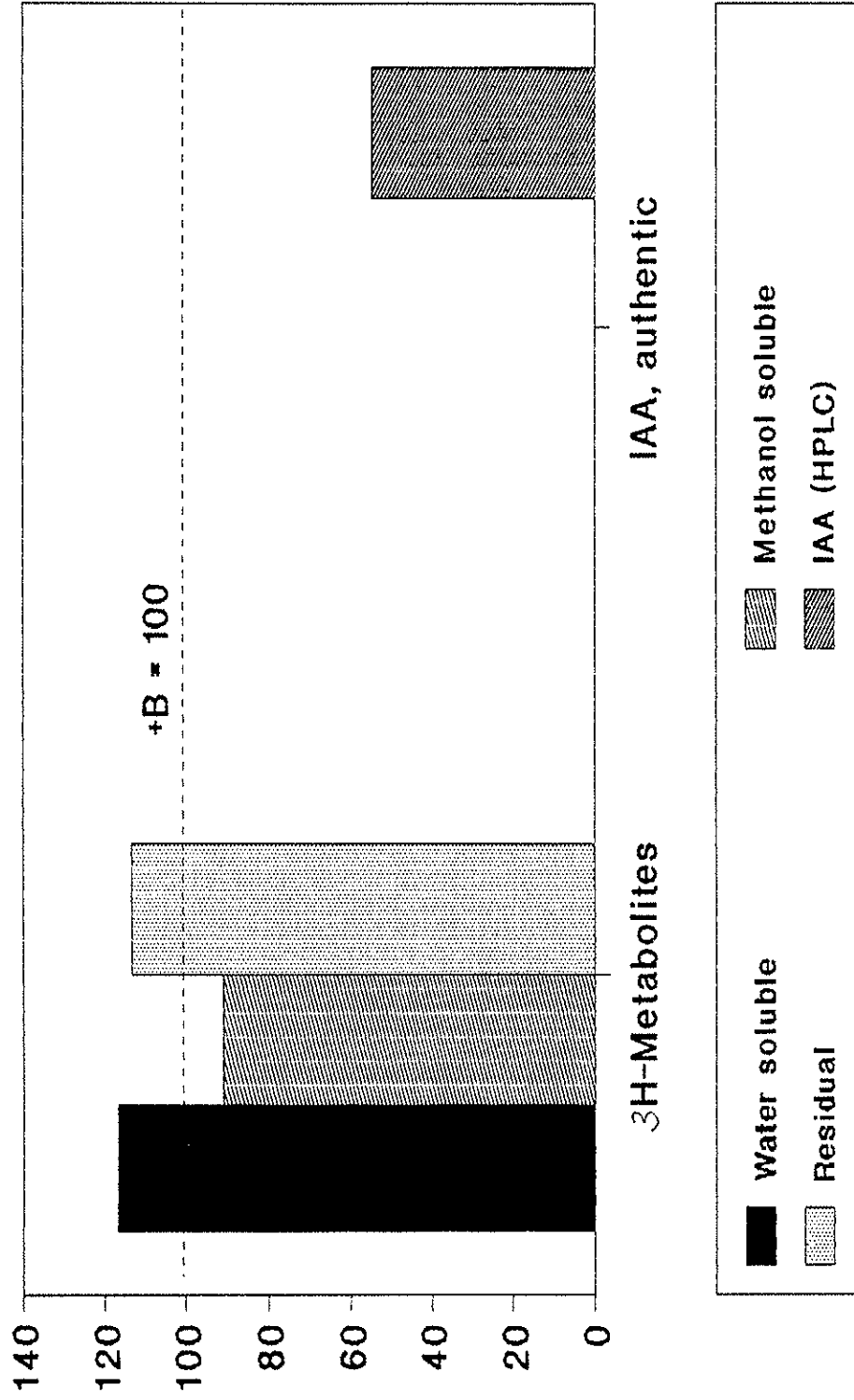


HRP    +    -    +    -    +    -    +    -  
cellular POD    extracellular POD

③



# Effect of B Deficiency on 3H-IAA Metabolization by *Daucus carota* cells



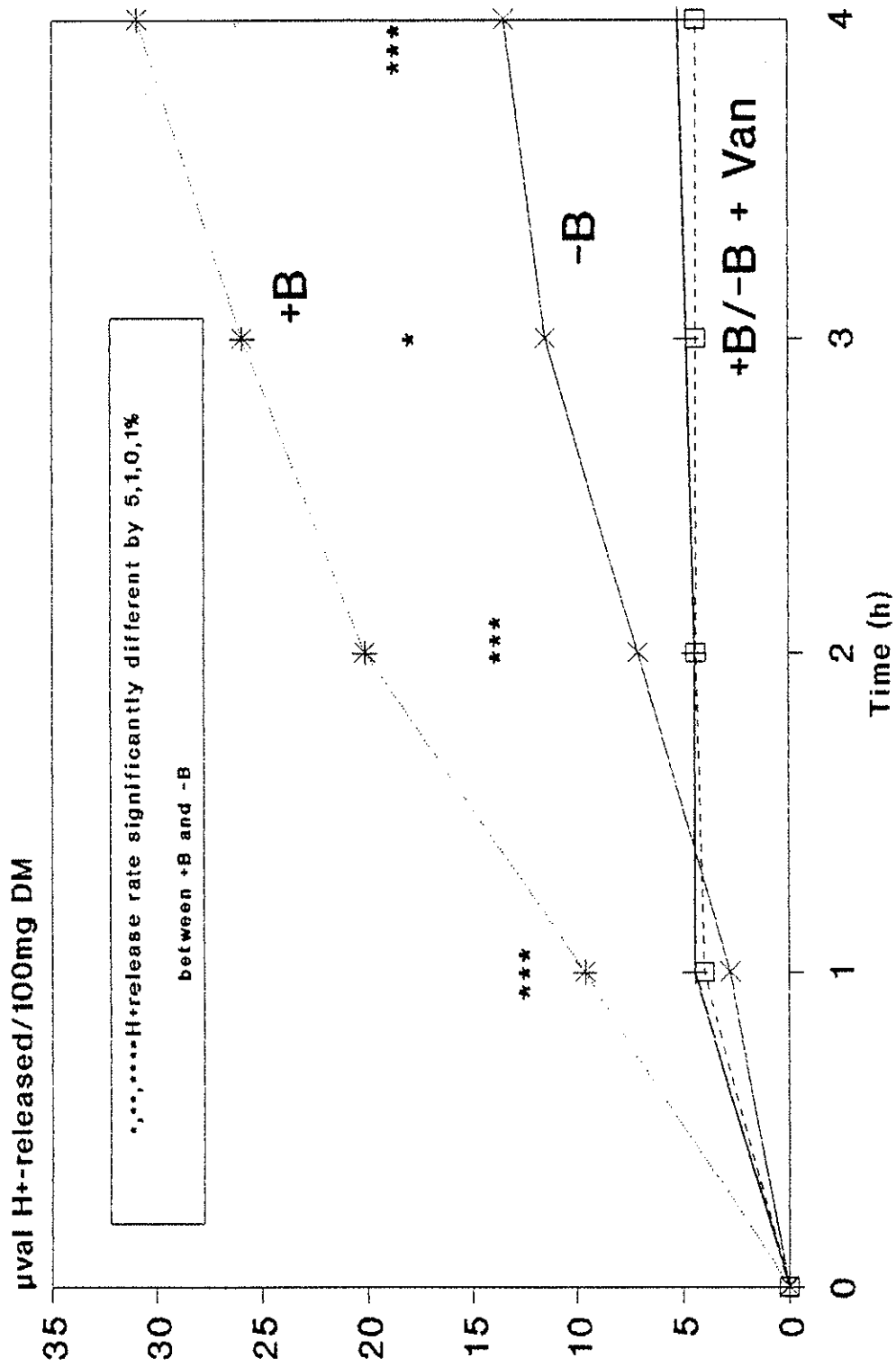
relative to +B = 100; 5d treatment  
(Goldbach & Amberger 1986)

Influence of B-deficiency on IAA and ABA concentrations in tomato apices (about 5 mm long) (cv. «Hild's Hellfrucht, Frühstamm»).

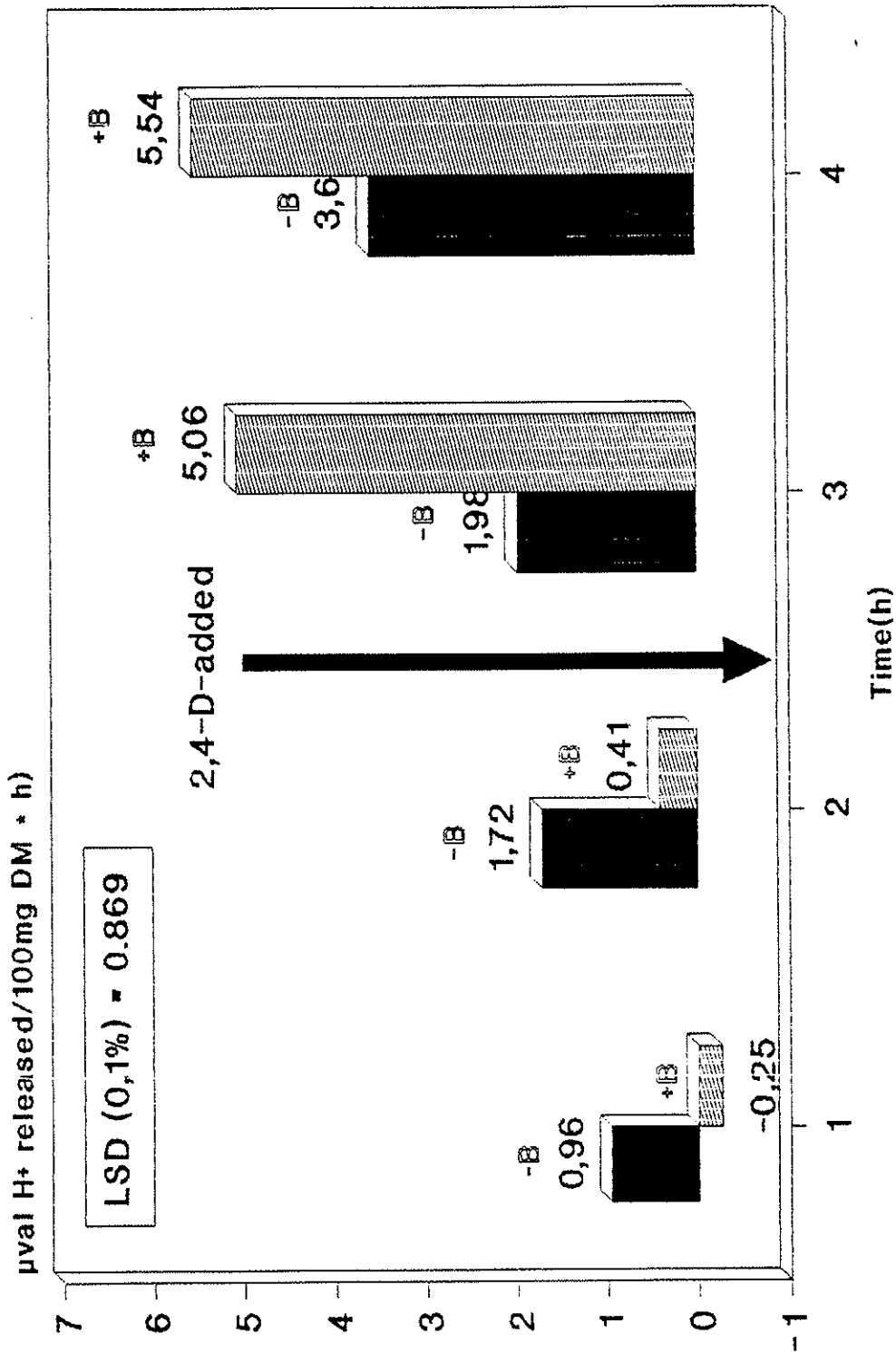
Days of B-deficiency treatment	Treatment	IAA		free ABA		bound ABA	
		(pmol/g FW)	(rel.)	(pmol/g FW)	(rel.)	(pmol/g FW)	(rel.)
2.5	+B	1417	(100)	203	(100)	675	(100)
	-B	1170	(83)	154	(76)	433	(64)
12.5	+B	522	(100)	130	(100)	628	(100)
	-B	311	(60)	14	(11)	220	(35)

+B = Nutrient solution with  $10^{-5}$  M B.

-B = without B supply (results are means of two replicates, each consisting of 12 plants).



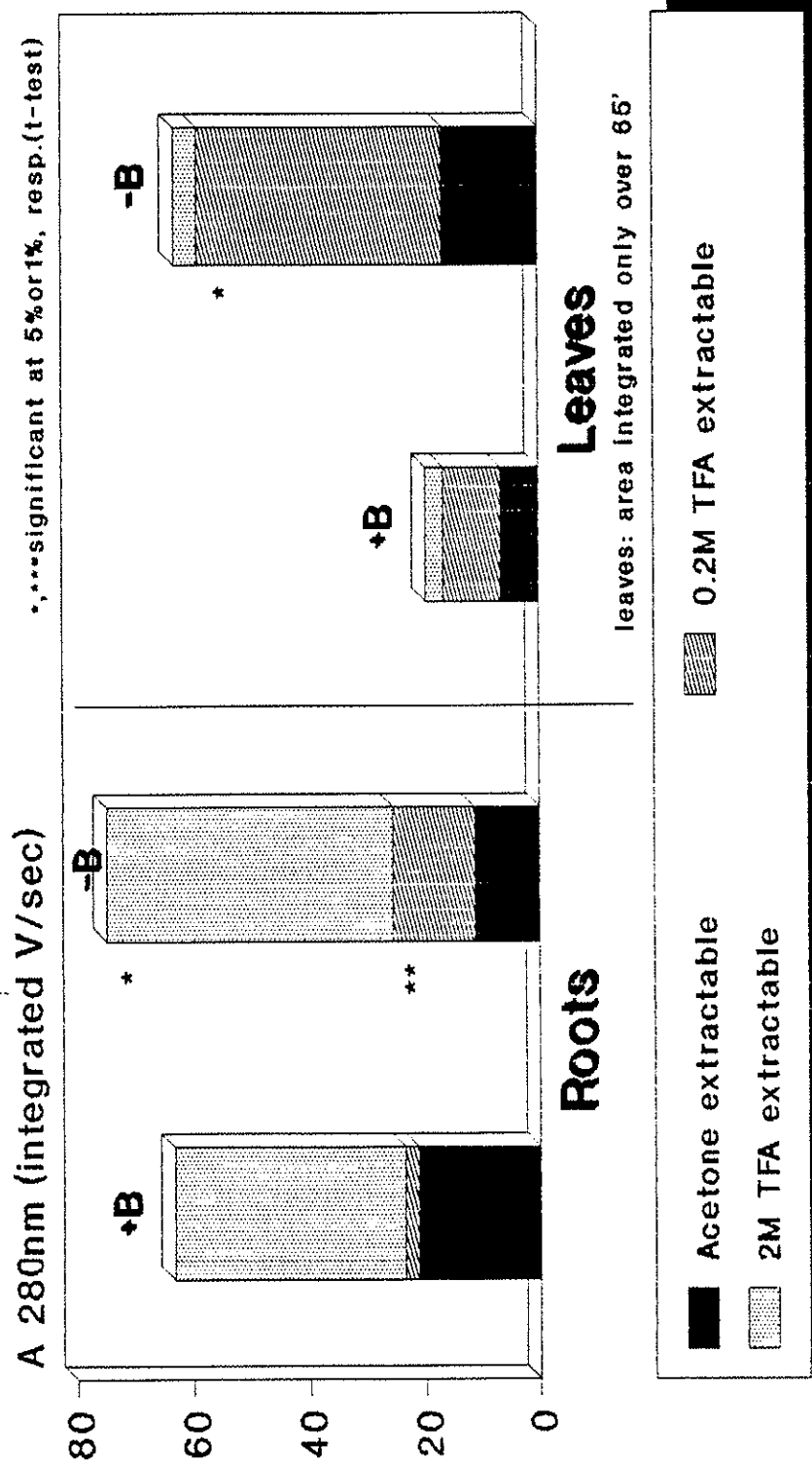
Influence of 16 h B-deficiency on the ferricyanide-induced proton secretion and its inhibition by 400µM vanadate



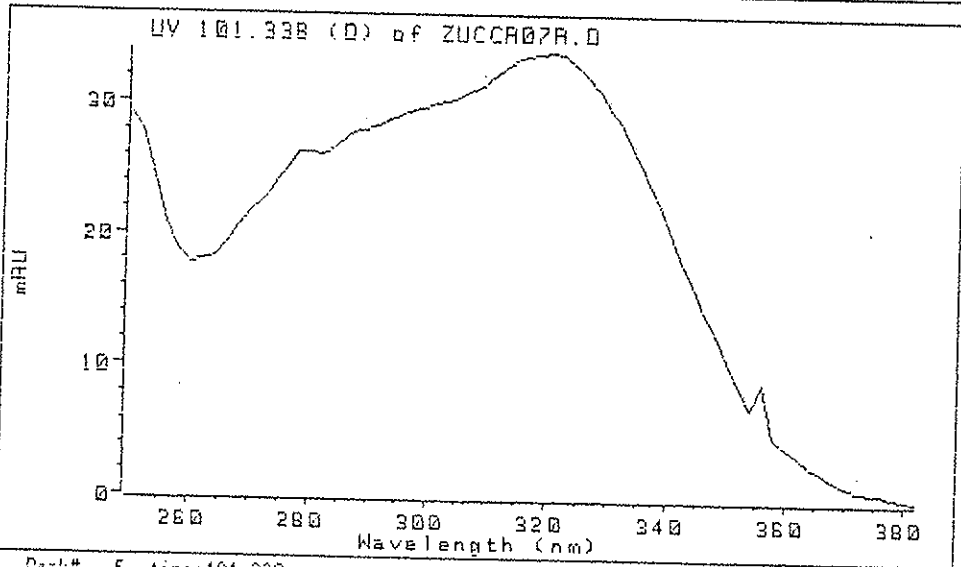
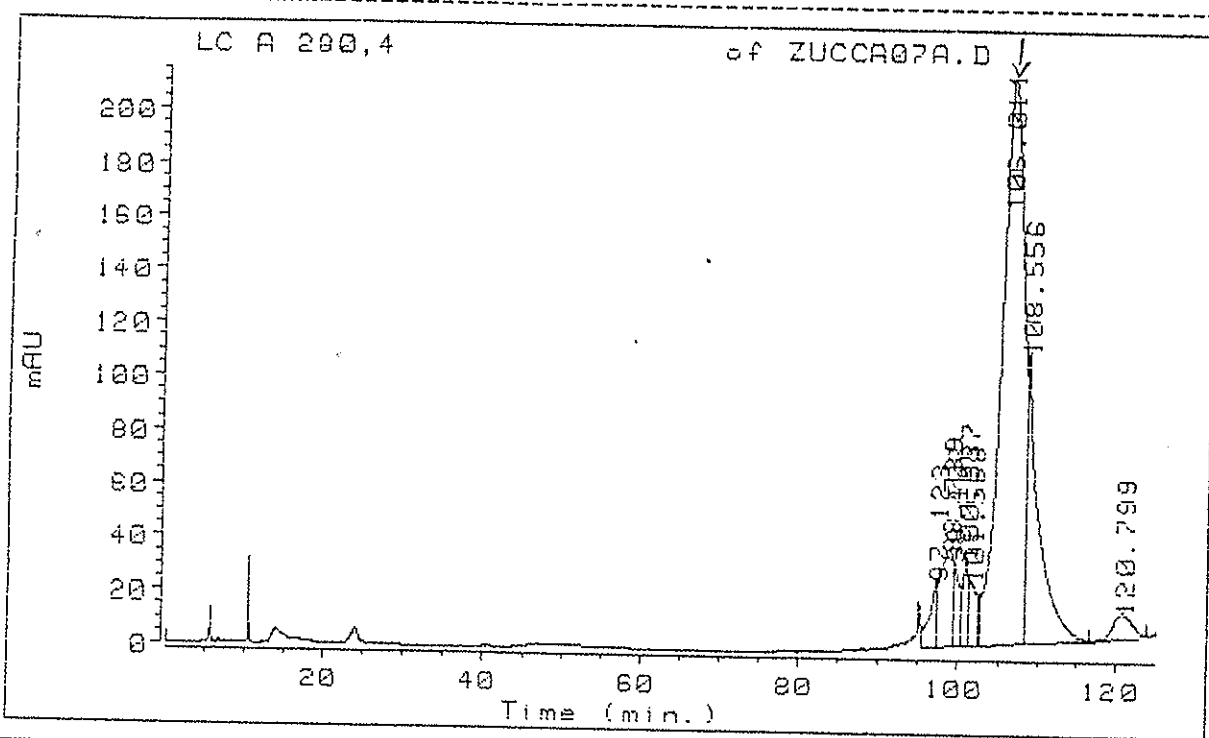
Effect of 2,4-D on net proton release of tomato cell suspension cultures, pretreated 16 h +/-B and without 2,4-D

# B-Deficiency: Squash Cell Wall Phenoles

Sum of Absorbance at 280 nm (HPLC-Runs) #  
in Different Cell Wall Fractions

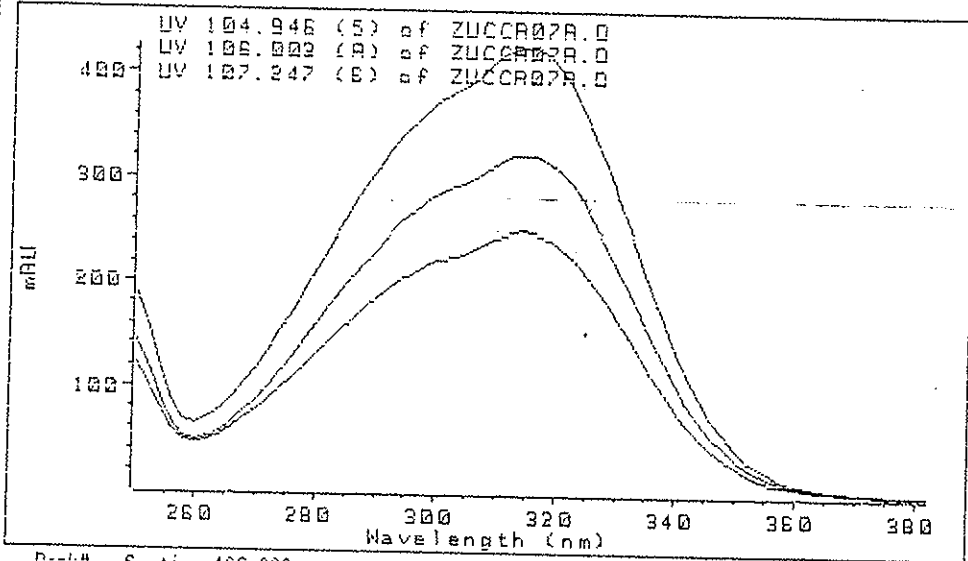


# Sum peak areas over 75' (-blanc); elution AcN/50mM Acetate, pH 4.0, from 0.5 to 70% (concave profile); Spherisorb ODS II 25cm



Peak# 5 time:101.339  
 Peak spectra search....  
 T: UV 101.338 (D) of ZUCCRO7A  
 Z: LC A 280,4  
 Y: LC A 280,4  
 X: UV 101.338 (D) of ZUCCRO7A

5 101.57 ??? 960 143.

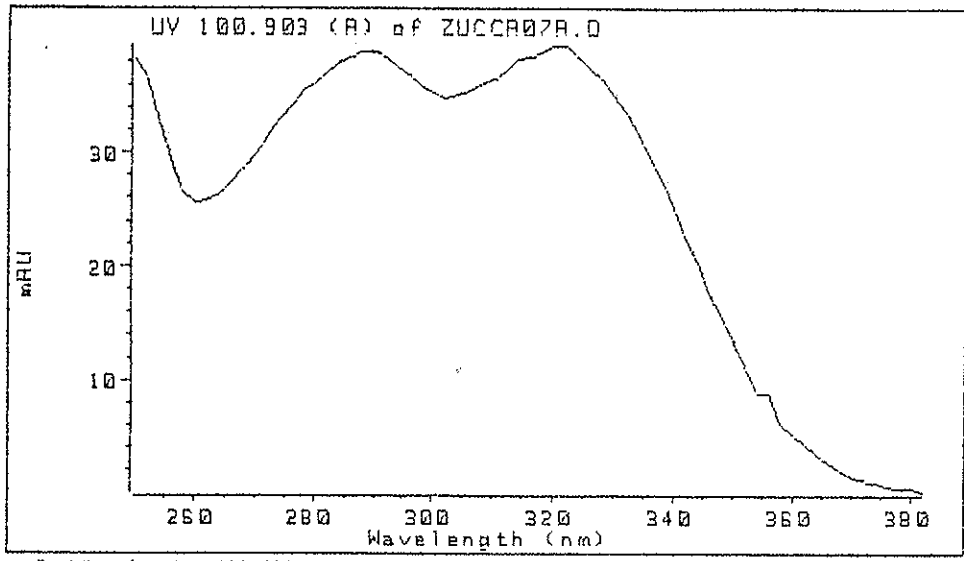


Peak# 6 time:106.003  
 Peak spectra search....  
 T: null.  
 Z: LC A 280,4  
 Y: LC A 280,4  
 X: Set of 3 UV

Treutter & Goldbach, 1983  
 (unpubl.)

9a

6 106.01 998 780 134.95 1 Diferulasaure

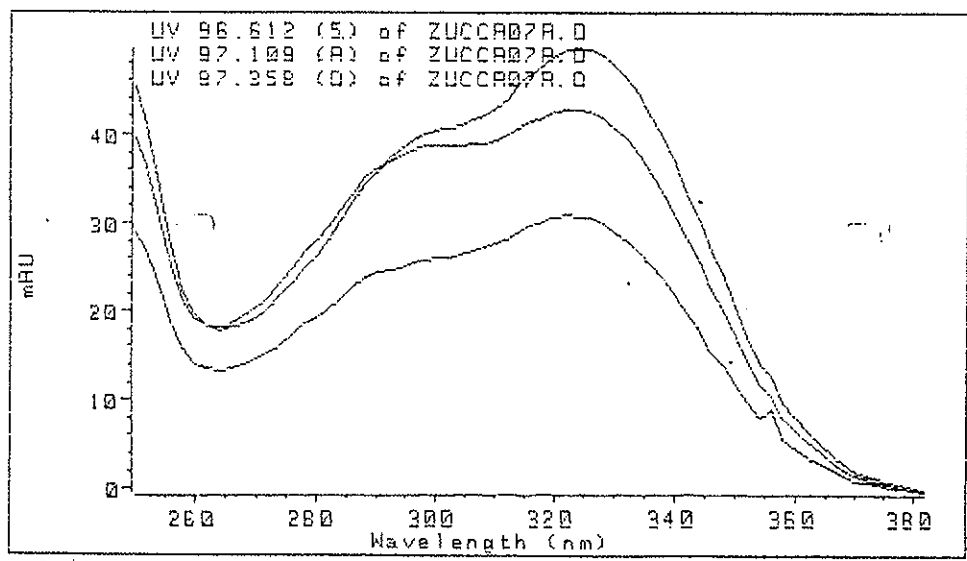


Peak# 4 times:100.903  
 Peak spectra search.....  
 T: null.  
 Z: LC A 280,4  
 Y: LC A 280,4  
 X: UV 100.903 (A) of ZUCCAD7A



4 100.94 777 919 143.29 2 Diferulasaeure

----- Sample ----- Match ----- Library -----  
 Peak# Time Purity Time Entry# Name



Peak# 1 times: 97.109  
 Peak spectra search.....  
 T: null.  
 Z: LC A 280,4  
 Y: LC A 280,4  
 X: Set of 3 UV



1 97.12 980 966 134.95 1 Diferulasaeure

CONCLUSIONS: On the Possible Influence of  
Boron Deficiency on the Cell Wall Extension

- Inhibition of auxin-enhanced proton secretion
- (reduced  $\beta$ -1,4 glucan synthesis)
- enhanced peroxidase activity, esp. wall-bound fraction
- lowered auxin concentration
- increased phenol oxidation due to:
  - higher POD activity
  - no complexation between o-dihydroxy-phenols and boric acid/borate (?)
- (still unknown interactions with Ca uptake and transport)