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Early Effects of Excess Boron on Photosynthesis and Growth of *Cucurbita pepo*[1]

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ABSTRACT

Summer squash plants (*Cucurbita pepo* L., cv. 'Early Prolific Straightneck') hydroponically cultured under optimal boron nutrition (+B)[3] until age 5 d were transferred to hydroponic solutions supplemented with excess boron (++B)[3]. As boron accumulated in the leaves with time, leaf conductance to water vapour, $^{14}CO_2$ fixation, and chlorophyll content of the oldest leaf became significantly less than in the +B-control plants of the same age; shoot growth essentially ceased. Boron also accumulated in the roots; concomitant inhibition of root elongation and lateral root development resulted. These metabolic changes all occurred prior to the accumulation of sufficient boron to cause the appearance of any symptoms characteristic of boron toxicity.

Key words: Boron toxicity; Stomatal conductance; Photosynthesis; Transpiration; Shoot and root growth; *Cucurbita pepo*.

INTRODUCTION

Boron has been demonstrated to be an essential micronutrient for a large number of vascular plants (Sommer and Lipman, 1926). In spite of the diversity among higher plants, the range in boron concentration optimal for growth is very narrow; approximately 0·01 to 4·0 mg B l^{-1} (Wilcox, 1960). Levels of available boron that are only slightly above optimal are toxic to many plant species (Mengel and Kirby, 1978).

In vascular plants, boron is carried passively in the transpiration stream and accumulates where the transpiration stream ends (Kohl and Oertli, 1961). Because boron is relatively immobile in the phloem, very little of the accumulating boron moves out of these tissues (Oertli and Richardson, 1970; Raven, 1980). For these reasons, leaves usually exhibit the first visible symptoms of boron toxicity: yellowing of the leaf tip, with the chlorosis subsequently progressing along the leaf margin and then spreading into the blade. Necrosis of the chlorotic tissue follows, then leaf abscission (Eaton, 1944; Wilcox, 1960). Thus, boron toxicity results in a loss of plant productivity.

While there are many reports in the literature relating the development of leaf symptoms to elevated levels of boron in leaves, there is very little information regarding the actual manner in which boron is toxic to plants. In this communication, we report the relationship between

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[3] Abbreviations: +B, 0·1 mg boron l^{-1} hydroponic culture solution, optimal boron, boron sufficient; ++B (++B-20 or ++B-40, 20 or 40 mg boron l^{-1} hydroponic culture solution), excess boron, boron intoxicated.

the development of the visible symptoms of boron toxicity and changes in chlorophyll content, CO_2 fixation, leaf conductance to water vapour, and transpiration of the oldest leaf; shoot and root growth; and mineral nutrition status of summer squash plants (*Cucurbita pepo* L., cv. 'Early Prolific Straightneck').

MATERIALS AND METHODS

Chemicals

All radiolabelled chemicals were purchased from New England Nuclear. Handifluor liquid scintillation cocktail was purchased from Mallinckrodt, Inc. Mineral salts for Shrive's nutrient solution and all other chemicals were of analytical reagent quality and were purchased from Fisher Scientific Co.

Plant material

Summer squash seeds (*Cucurbita pepo* L., cv. 'Early Prolific Straightneck'), supplied through the courtesy of the Joseph Harris Seed Co., Inc., were imbibed in distilled H_2O for 24 h at room temperature. They were then spread evenly between two sheets of paper towelling placed in a plastic box (33 cm × 33 cm × 9·5 cm) and moistened with 7·0 ml H_2O. The covered box was placed in an incubator where the seeds germinated in the dark for 48 h at 30 °C. After germination, the terminal 5·0 mm of the primary root was pinched off to promote lateral root formation, and the seedlings were inserted into holes in the lid of a polyurethane breadbox (28 cm × 14 cm × 13 cm, 4·5 l) covered with aluminum foil and containing Shive's nutrient solution [5·0 mM $Ca(NO_3)_2$, 2·0 mM $MgSO_4$, 2·0 mM K_2SO_4, 1·0 mM KH_2PO_4, 1·0 mg Fe l^{-1}, 0·13 mg Cl l^{-1}, 1·0 mg Mn l^{-1}, 0·1 mg Zn l^{-1}, 0·1 mg B l^{-1}, 0·01 mg Cu l^{-1}, and 0·01 mg Na l^{-1}] adjusted to pH 4·7 with KOH. The box was transferred to a growth chamber where the plants were hydroponically cultured in an aerated solution at 30 °C under continuous illumination of 310 μE m^{-2} s^{-1}. After 3 d, plants of uniform appearance were selected for treatment. The lateral roots of several plants were marked with India ink 10 mm from the tip to determine the rate of root elongation. The shoots of several plants were marked with India ink just below the plumule to determine the rate of shoot growth. The plants were then transferred to soft glass (boron-free) jars (1·8 or 3·7 l) containing either complete Shive's nutrient solution or modified Shive's nutrient solution containing increasing concentrations of boron. The plants were transferred to a growth chamber (the time of transfer is designated as T_0) and allowed to grow for specified periods in aerated solutions at 30 °C under continuous illumination of 310 μE m^{-2} s^{-1}. At the end of the treatment period, the plants were harvested and the following determined: (i) root elongation; (ii) increase in root and shoot dry weight; (iii) leaf chlorophyll content; (iv) leaf conductance to water vapour; (v) transpiration; (vi) photosynthesis; and (vii) mineral nutrient content of roots and shoots.

Chlorophyll content determination

Weighed samples of intact leaves (pieces of leaf tissue were not used since chlorosis is not uniform over the leaf from a + + B plant), enough to give at least 20 mg fresh weight ml^{-1}, were homogenized in 3–10 ml 80% acetone with a Polytron homogenizer (PCU-2, Brinkman Instruments). Chlorophyll content was determined spectrophotometrically (Bausch and Lomb Spectronic 2000) at 663 nm and 645 nm on appropriately diluted samples (OD < 1·0) (Bruinsma, 1963).

Gas exchange measurements

Gas exchange was determined using a portable tritium and carbon-14 double isotope porometer (Johnson, Rowlands, and Ting, 1979). A small area in the centre of the leaf was exposed for 20 s to an airstream containing known concentrations of ^3HOH vapour and $^{14}CO_2$. A leaf disc (0·385 cm^2) was excised from within the exposed area with a cork borer and immersed in 2·0 ml 80% methanol. Chlorophyll was bleached from the methanol extract by illuminating the samples with photography lamps overnight. The samples were diluted with 10 ml Handifluor liquid scintillation cocktail, and the content of ^3H and ^{14}C determined by using a Beckman LS-9000 liquid scintillation spectrometer. The rates of ^3HOH and $^{14}CO_2$ uptake were used to compute transpiration (g H_2O dm^{-2} h^{-1}), CO_2 fixation (mg CO_2 dm^{-2} h^{-1}), and leaf conductance to water vapour (cm^2 s^{-1}), according to the methods of Hanscom and Ting (1977). In addition, leaf temperature, PAR, and chamber humidity were determined for each sample just prior to the use of the double isotope porometer by using a steady state diffusion porometer (Model LI-1600, Lambda-LICOR Instrument Co., Inc., Lincoln, NB). *C. pepo* plants were transferred to treatments of 0·1, 20, and 40 mg B l^{-1} at 5 d of age and cultured hydroponically at 30 °C and 310 μE m^{-2} s^{-1} under a 12 h photoperiod. Photosynthetic measurements

were taken, starting 48, 72, 96, and 120 h after transfer, every hour from 1100 h to 1600 h, using the oldest leaf of each of three plants for each hourly measurement.

Mineral nutrient analysis

Leaves or roots were washed in Joy detergent, forced-draught oven dried at 60 °C, ground in liquid nitrogen using a mortar and pestle, and stored in glass vials. Nitrogen was determined by the micro-Kjeldahl method, phosphorus by the molybdovanadate colorimetric method using a Bausch and Lomb Spectronic 100 spectrophotometer (Kitson and Mellon, 1944), boron by the colour reaction with carmine (Hatcher and Wilcox, 1950), also with a Bausch and Lomb Spectronic 100 spectrophotometer. A Perkin–Elmer 460 Atomic Absorption Spectrophotometer was used to determine potassium, calcium, magnesium, sodium, iron, manganese, zinc, and copper (Labanauskas, Stolzy, and Luxmoore, 1975). Analyses were performed on pooled samples of the oldest leaf or total roots from more than 50 plants for each treatment. Standards for each assay were run simultaneously.

RESULTS

Visible symptoms of boron toxicity

Five-day-old summer squash plants cultured under optimal conditions of boron nutrition (0·1 mg B l^{-1}), were transferred to solutions containing 0·1, 1·0, 10, 20, or 40 mg B l^{-1}. At the time of transfer, the first true leaf (subsequently always referred to as the oldest leaf) was still folded lengthwise along the mid-rib and was about 5·0 mm long.

Symptoms characteristic of boron toxicity—yellowing of the leaf apex and margin—were just visible when the level of accumulated boron reached 750 μg B g^{-1} dry weight in the oldest leaf, where the effects of boron toxicity would be expected to appear first (Kohl and Oertli, 1961). In our system, boron toxicity proved to be a function of the concentration of available boron and the length of exposure. This result is consistent with the passive uptake of boron in the transpiration stream and with the view that, given enough time, a leaf should show symptoms of boron toxicity even when grown in the presence of a concentration of boron considered optimal (Kohl and Oertli, 1961).

In this investigation, boron toxicity was induced in hydroponically cultured squash plants in a relatively short period by adding higher concentrations of boron to the hydroponic nutrient solution than normally would be encountered in the field. Boron concentrations that are toxic to a particular species or cultivar have proved to be nearly constant regardless of the stage of growth (El-Sheikh, Ulrich, Awad, and Mawardy, 1971). Chlorosis of the leaf tip and leaf margin was first evident only 96 h after 5-d-old squash plants were transferred to nutrient solution containing 40 mg B l^{-1} or after 120 h of culture in solution containing 20 mg B l^{-1}. In both treatments, the level of boron in the oldest leaf was 750 μg B g^{-1} dry weight or greater. Our results compared favourably with an earlier report in the literature also employing *Cucurbita pepo* (El-Sheikh *et al.*, 1971). Mineral nutrient analysis of the leaves and roots of the hydroponically cultured squash plants also confirmed that inducing boron toxicity in this manner did not cause differences between the + B and + + B plants with regard to the levels of any of the other nutrients essential to plants (Table 1).

Chlorophyll content

Leaf chlorophyll content of healthy *C. pepo* seedlings increased with time under conditions of optimal boron nutrition (Table 2). Additional boron prevented this normal increase in chlorophyll content and an actual loss in existing chlorophyll content was observed in plants hydroponically cultured for 96 h at 40 mg B l^{-1}. The symptoms of boron toxicity were first visible at this time.

TABLE 1. *Mineral nutrient analysis of the oldest leaf and total roots from +B and ++B* C. pepo *plants*

Treatment period (h)	Treatment (mg B l^{-1})	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	B
		(%)						($\mu g\ g^{-1}$ dry wt.)				
Leaf												
72	0·1	7·0	1·5	5·0	0·86	0·36	0·1	117	56	70	30	64
	20·0	6·7	1·5	5·3	0·85	0·41	0·1	159	56	84	30	652
96	0·1	6·4	1·3	5·7	0·99	0·41	0·1	105	56	84	27	77
	20·0	6·3	1·4	6·1	1·01	0·43	0·1	139	56	86	26	719
120	0·1	5·8	1·2	5·7	1·61	0·46	0·1	126	56	112	21	93
	20·0	6·0	1·3	6·0	1·10	0·42	0·1	119	56	99	27	750
Root												
72	0·1	4·4	1·5	7·0	0·83	0·24	0·1	1300	56	83	100	60
	20·0	4·5	1·5	5·5	0·82	0·17	0·1	1172	56	104	137	370
96	0·1	4·4	1·5	6·2	0·92	0·23	0·1	1172	56	159	102	67
	20·0	4·1	1·5	5·1	1·14	0·18	0·1	1261	56	108	107	290
120	0·1	4·0	1·4	5·8	0·93	0·20	0·1	1119	56	156	99	65
	20·0	4·1	1·4	4·1	0·89	0·16	0·1	1216	56	114	116	433

TABLE 2. *Influence of accumulating boron on the chlorophyll content of the oldest leaf of* C. pepo

Treatment conditions (h)	μg Chlorophyll g^{-1} fr. wt. ± s.d. (n = number of experiments)	
	0·1 mg B l^{-1}	40 mg B l^{-1}
48	551 ± 75 (3) (48 h control)	319 ± 38 (3) (58% of control)
72	998 ± 183 (3) (72 h control)	449 ± 59 (3) (45% of control)
96	1094 ± 65 (3) (96 h control)	322 ± 70 (3) (29% of control)
120	1346 ± 167 (4) (120 h control)	227 ± 65 (4) (17% of control)

Gas exchange

For each gas exchange parameter measured, there were no significant time-dependent differences (at the 5% level, Duncan's Multiple Range Test) within a treatment during the measurement period. Results are reported as an average of all measurements made between 1100 h and 1600 h.

In the + B-control plants, CO_2 fixation did not increase with time in a manner that simply parallelled the increase in chlorophyll content; it appeared to follow a 48 h cycle (Fig. 1a). This pattern was also observed in the + + B plants, but the rate of photosynthesis was depressed. The mg $^{14}CO_2$ fixed $dm^{-2}\ h^{-1}$ was about 30% less in the oldest leaf of plants grown in the presence of 40 mg B l^{-1} for 72 h or longer compared to the rate of photosynthesis in the oldest leaf of the + B-control plants of the same age (significant at the 5% level by Duncan's Multiple Range Test). There was no significant difference at the 5% level in rates of $^{14}CO_2$ fixation between the control plants and those grown at 20 mg B l^{-1} for any of the treatment

periods. Thus, only in the + + B-40 plants was photosynthesis consistently reduced prior to the onset of the visible symptoms of boron toxicity.

Leaf conductance exhibited the same 48 h cycle as $^{14}CO_2$ fixation in both the + B and + + B plants (compare Fig. 1a and b). For the oldest leaf of the + + B-40 plants, leaf conductance to water vapour was 30% less than that of the same leaf in the control plants at the 5% level (Duncan's Multiple Range Test). Transpiration followed the same 48 h cycle as leaf conductance and was reduced by excess boron but not significantly at the 5% level (Fig. 1c).

Plant growth

Shoot and root growth were measured as a function of accumulating boron to determine when a loss in plant productivity occurred relative to the time at which leaf conductance and

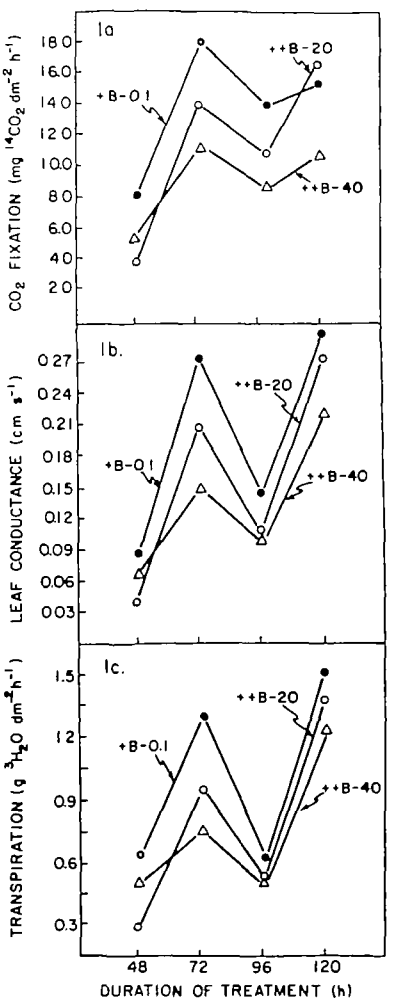

FIG. 1. CO_2 fixation (a), leaf conductance to water vapour (b), and transpiration (c) in 5-d-old *C. pepo* plants transferred to + B nutrient solution (0·1 mg B l^{-1}) (●) and to + + B nutrient solutions (20 mg B l^{-1}) (○); (40 mg B l^{-1}) (△). CO_2 fixation and leaf conductance in the + + B-40 plants were significantly different from the + B-control plants at the 5% level (Duncan's Multiple Range Test) for all treatment periods 72 h or longer. There was no significant difference at the 5% level (Duncan's Multiple Range Test) between the + B-control and + + B-20 plants for any treatment period.

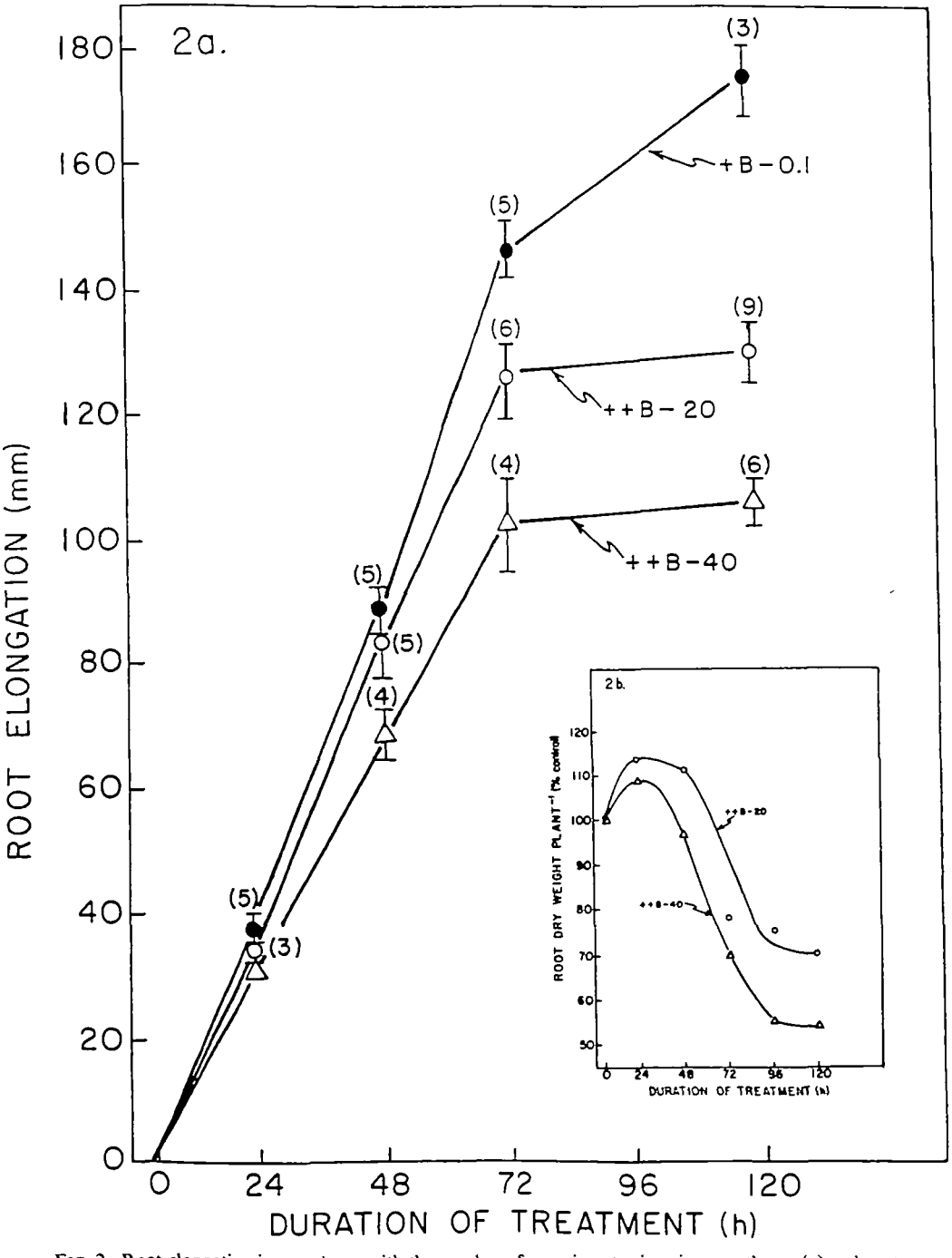

FIG. 2. Root elongation in mm ± s.e. with the number of experiments given in parentheses (a) and root dry weight as a percent of the + B-control plants (b) in 5-d-old *C. pepo* plants transferred to + B nutrient solution (0·1 mg B l^{-1}) (●) and to + + B nutrient solutions (20 mg B l^{-1}) (○); (40 mg B l^{-1}) (△).

$^{14}CO_2$ fixation decreased under + + B conditions and in relation to the appearance of early symptoms of boron toxicity in the oldest leaf.

One of the earliest effects of boron toxicity was a reduction in the rate of root elongation and total root production (Fig. 2a). When 5-d-old squash plants, were transferred to media

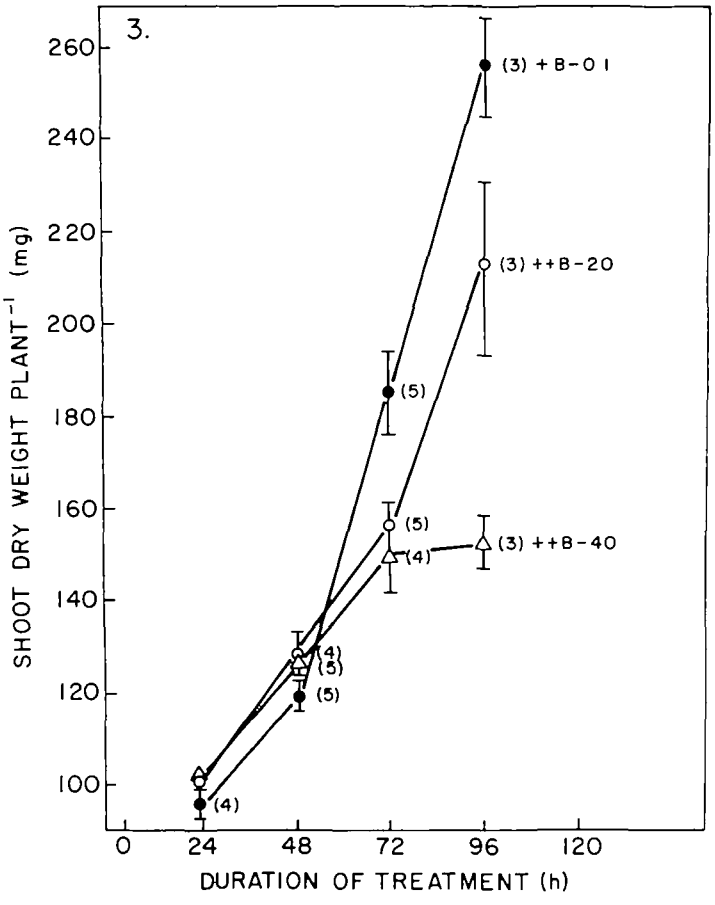

FIG. 3. Shoot dry weight in mg ± s.e. with the number of experiments given in parentheses of 5-d-old *C. pepo* plants transferred to + B nutrient solution (0·1 mg B l^{-1}) (●) and + + B nutrient solutions (20 mg B l^{-1}) (○); (40 mg B l^{-1}) (△).

containing excess boron, the rate of root elongation decreased with increasing length of exposure to boron and increasing concentrations of boron. By 72 h of boron excess, root elongation had essentially ceased in both + + B treatments (20 or 40 mg B l^{-1}). It is not known whether inhibition of root growth was due to the concentration of boron in the total roots (370 μg g^{-1} dry weight) or the amount of boron in the oldest leaf (650 μg g^{-1} dry weight). In either case, shoot growth was less sensitive to excess boron than root growth since inhibition of shoot growth occurred only in plants grown for 72 h at 40 mg B l^{-1} (Fig. 3). Inhibition of both root and shoot growth occurred prior to the appearance of any visible symptoms of boron toxicity in the leaves.

DISCUSSION

Research to determine the actual manner by which boron is toxic to plants has been minimal. It has been assumed that the chlorosis, and subsequent necrosis, of leaf tissue resulting from the accumulation of boron to a toxic level results in a loss in photosynthetic capacity that accounts for the eventual reduction in plant productivity. The results of the present study are consistent with this hypothesis. One of the earliest effects of boron excess was the failure of chlorophyll to accumulate at a normal rate in the developing leaves of + + B-40 plants. Within 48 h after transferring 5-d-old + B squash plants to solutions containing 40 mg B l^{-1},

the chlorophyll content of the oldest leaf was 40% less than that of the + B-control plants of the same age. This observation would probably be more marked in newer leaves of + + B-40 plants since their total development would occur after transfer to + + B conditions. The appearance of visible symptoms characteristic of boron toxicity occurred after a treatment of 96 h at 40 mg B l^{-1} and simultaneously with the actual destruction of existing chlorophyll.

Photosynthesis was reduced under conditions of excess boron. Changes in the rates of CO_2 fixation seemed less dramatic than changes in leaf chlorophyll content. A 30% reduction in CO_2 fixation occurred after treatment for 48 h at 40 mg B l^{-1}, well before the appearance of any visible symptoms of boron toxicity. However, as chlorophyll content of the leaf dropped to a level that was only 17% of the +B-control plants; over this same period, CO_2 fixation rates remained, on the average, 30% lower than that of the control plants. This result suggests that major perturbations in chlorophyll content were occurring in the cells of the leaf tip and margin. Cells in the centre of the leaf blade, only 3–5 mm from the leaf margin, appeared to be less affected by the loss in chlorophyll. The influence of accumulating boron on CO_2 fixation in these cells paralleled the reduction in leaf conductance accompanying boron intoxication.

The results of the present study suggest that a loss in plant productivity may occur before the appearance of any symptoms characteristic of boron toxicity due to lowered rates of CO_2 fixation and a loss in leaf area resulting from a reduction in shoot growth. Inhibition of root and shoot growth occurred in plants grown in the presence of 40 mg B l^{-1} for 72 h or longer. It is not known from the present study whether the inhibition of root growth is due to the concentration of boron observed in the leaves or in the roots themselves. Our results suggest that roots may accumulate boron to a level that is toxic to their metabolism before sufficient boron has accumulated in the leaves to result in the appearance of boron toxicity symptoms. This information may be important to recognizing and understanding the effects of boron toxicity in the field.

The concurrent reductions in plant growth and CO_2 fixation suggest the possibility that the reduced rate of root and shoot growth might be due to limited availability of photosynthate. Because of the observed sensitivity of plant growth to excess boron, we have not ruled out the possibility that reduced growth in either tissue may be due to inhibition of some other essential metabolic process. These two possibilities deserve examination in future work seeking to determine the mechanisms of boron toxicity in plants.

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