Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin[1]

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The responses of sunflower root cells to conditions of boron deficiency and to the addition of exogenous auxin were studied on the ultrastructural level. Although gross morphological effects such as inhibition of root elongation and a change in the direction of cell expansion from longitudinal to radial are similar in both auxin-treated and boron-deficient seedling roots, ultrastructural changes are different in the two treatments. An increase in cell wall thickness and a loss of membrane integrity are seen as early as 6 h in seedlings grown without boron. Auxin-treated root cells do not show this response. A role for boron may involve maintenance of membrane integrity rather than determining endogenous auxin levels.

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Les réactions des cellules racinaires de tournesol à des conditions de carence en bore et à l'addition d'auxine exogène ont été étudiées au niveau ultrastructural. Bien que les effets morphologiques, tels l'inhibition de l'élongation racinaire et un changement dans la direction de l'expansion cellulaire de longitudinale à radiale, soient semblables dans les racines des plantules traitées à l'auxine et dans celles des plantules carencées en bore, les changements ultrastructuraux sont différents dans les deux traitements. On constate une augmentation de l'épaisseur de la paroi cellulaire et une perte de l'intégrité membranaire après seulement 6 h chez les plantules cultivées en absence de bore. Les cellules racinaires traitées à l'auxine ne présentent pas cette réaction. Le bore pourrait avoir un rôle dans le maintien de l'intégrité de la membrane, plutôt que dans le contrôle des niveaux d'auxine endogène.

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Introduction

Plants require boron in minute quantities, ranging from 0.01 to 1.0 ppm for plants grown in water culture. Plants grown in the absence of boron show deficiency symptoms quickly. As early as 6 h after withholding boron, root elongation ceases (Neales 1960); thereafter, other deficiency symptoms occur and eventually the plant dies. Although the essentiality of boron for plant growth and development has been recognized since studies by Agulhon (1910*a*, 1910*b*), the primary role(s) that boron plays in the metabolism of the plant is still unknown.

Many of the early symptoms brought about by boron deficiency in root tips are suggestive of supraoptimal auxin concentration. These include inhibition of root elongation, a change in the direction of cell expansion from longitudinal to radial, browning of root tips, and proliferation of lateral roots (Albert and Wilson 1961; Kouchi and Kumazawa 1975*a*, 1975*b*, 1976). The manifestations of these symptoms have led some workers to suggest that the removal of boron from the culture medium leads to the formation of supraoptimal concentrations of indoleacetic acid (IAA) within the plant (Brandenburg 1949; Neales 1960). Reports of increased levels of auxin in root tissues (Coke and Whittington 1968; Jaweed and Scott 1967) have been published but are debated (Crisp *et al.* 1976; Smirnov *et al.* 1977). Bohnsack and Albert (1977) described an increase in auxin oxidase levels in boron-deficient squash roots which they attributed to an induction caused by increased auxin concentrations in turn caused by boron deficiency. Likewise, the finding that there is an increase in cytoplasmic peroxidase activity in *Vicia faba* roots grown under boron deficiency (Robertson and Loughman 1974) also supports the hypothesis that there are increased levels of endogenous auxins in −B root tips.

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Addition of exogenous auxin to seedlings grown in water culture has long been known to cause inhibition of root elongation (cf. Torrey 1956). Root inhibition in response to supraoptimal auxin concentration is accompanied by typical morphological responses including radial cell enlargement and root swelling near the root tip, precocious maturation of tissues near the root apex, including root hair development and xylem maturation, and stimulation of lateral root formation (Torrey 1965). Coke and Whittington (1968) interpreted some of these responses in boron-deficient plants as involving a boron–auxin interaction.

Recent studies of boron-deficient plants have described some of the ultrastructural symptoms of boron deficiency observed in root cells (Starck 1963; Kouchi and Kumazawa 1976) and also in leaf cells (Lee and Aronoff 1966; Hudak and Herich 1976). The present investigation was designed to explore the early ultrastructural changes which could be observed in sunflower seedling roots grown in the absence of boron compared with normal roots. A further comparison was made between normal and boron-deficient seedling roots treated with exogenous auxin levels adequate to elicit comparable inhibition of root elongation. The primary question was whether roots inhibited in elongation by supraoptimal auxin concentrations were structurally like roots inhibited by boron deficiency.

Materials and methods

Sunflower seeds (*Helianthus annuus* L., W. Atlee Burpee Co. cultivar Mammoth lot 15349) were germinated in sand watered with one-quarter strength Hoagland's solution (Hoagland and Arnon 1950) containing 0.5 ppm boron (B). Three days after germination, the seedlings were transferred to one-quarter strength Hoagland's solution with 0.5 ppm B and were grown hydroponically in plastic trays with roots continuously aerated. Seedlings were maintained in growth chambers provided with 16 h light (mixed cool-white fluorescent and incandescent lights) and 8 h dark at a constant temperature of 25°C. After 2 days in water culture, the seedlings were transferred to fresh medium containing macro- and micro-nutrient salts of Bonner–Devirian (Goforth and Torrey 1977) plus 5 ppm FeEDTA as the source of iron. Boron was provided at 0.3 ppm or was omitted. Roots were marked with India ink at 5 mm behind the root apex at the time of transfer and net root elongation was determined at selected time intervals by measuring the displacement of the India ink mark.

Under the cultural conditions we used, the rate of elongation of the primary root frequently decreased following germination and subsequent transfer to liquid medium. However, large numbers of second-order laterals were initiated early and developed under conditions of both boron deficiency and sufficiency. In order to study a large number of roots per plant, effects of the different nutrient conditions were studied and compared in these secondary lateral roots.

Indoleacetic acid (IAA) (Calbiochem) was diluted from a freshly made stock solution to give a final concentration of either 5×10^{-7} or 5×10^{-6} *M*; 2,4-dichlorophenoxyacetic acid (2,4-D) (Nutritional Biochemicals Corp.) was used at 5×10^{-7} *M*. The auxins were added to both +B and −B media. Roots were marked as indicated above and measured at selected time intervals.

For electron microscope studies, the roots were fixed for 30–60 min either in 3% glutaraldehyde in 0.1 *M* phosphate buffer, pH 6.8, and rinsed with 0.1 *M* phosphate buffer, pH 6.8, or in 2.7% glutaraldehyde, 1.5% paraformaldehyde in 0.05 *M* cacodylate buffer, pH 7.4, and rinsed with 0.1 *M* cacodylate buffer of the same pH. Both fixatives gave satisfactory results. After rinsing, the root tips were postfixed in 2% aqueous osmium tetroxide, rinsed, dehydrated through acetone, and embedded in Spurr's (1969) resin. Silver sections were cut with a diamond knife, collected on copper grids, and after conventional electron microscope staining, were examined with a Phillips 300 electron microscope at 60 kV. Cortical cells within 1 mm of the root apex were examined from both longitudinal and transverse sections. These cells showed many meristematic features, namely small vacuoles, thin cell walls, and dense cytoplasm. Because of frequent cell divisions transverse to the long axis of the root, longitudinal walls were less variable in thickness and were chosen for comparisons among different treatments.

Sections for light microscopic study were made at 1 or 2 μm using glass knives. Staining of thick sections for polysaccharides was with the periodic acid – Schiff's reaction outlined by Feder and O'Brien (1968), pretreated with a saturated solution of dimedone to block aldehydes from the fixation procedure.

Results

Root morphology

Sunflower seedlings were very sensitive to the removal of boron from the culture medium. Root elongation of sunflower seedlings was inhibited within 3–6 h after being placed in −B medium. In agreement with Kouchi and Kumazawa (1975*a*) we found that a difference in length between control and boron-deficient roots could be detected often as early as 3 h after removal of boron from the medium. Adding IAA at either 5×10^{-7} *M* or 5×10^{-6} *M* or 2,4-D at 5×10^{-7} *M* also inhibited root elongation. The degree of inhibition was greater for treatments with added auxin than for roots grown under −B conditions alone (Fig. 1). Auxin-treated roots of seedlings grown in either +B or −B nutrient medium were extremely swollen in the elongation zone of the root and root hair growth was stimulated.

Cell wall thickening

Another symptom which often was observed as early as 6 h after the removal of boron was an increase in thickness of the cell wall. Previous observations of increased wall thickness were reported at both the light and electron microscope level (Spurr 1957*a*, 1957*b*; Starck 1963; Kouchi and Kumazawa 1975*b*, 1976). Unfortunately, this response is not always easily detectable at the light microscope level in the early hours of treatment.

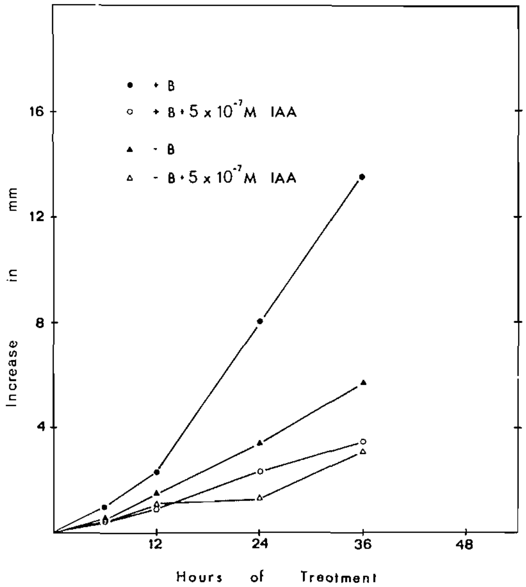

FIG. 1. Increase in length in millimetres of roots grown in various media for 36 h. Boron (B) provided at 0.3 ppm.

According to O'Brien and Thimann (1967), periodic acid – Schiff's reaction does not always stain cellulose. Our observations were in agreement with theirs and we found no reliable method at the light microscope level to determine if cell wall thickness had changed at 6 h. However, with the electron microscope, one could detect an increase in the thickness and (or) irregularity of the cell wall in −B roots at 6 h (Fig. 3). Increased cell wall thickness was unevenly distributed over the periphery of the cell and was in contrast to cell walls of +B plants (Fig. 2).

Cell wall thickening in −B roots was associated with increased vesicle formation at the wall–plasmalemma interface as compared with +B roots. Aggregations of lamellae were seen within the cortical cell appressed to the cell wall, especially in areas which were adjacent to intercellular spaces (Fig. 3). In some roots, extensive arrays of vesicles were seen within the cell wall and could be observed either in transverse sections of the wall (Fig. 16) or in oblique sections cut adjacent to the wall (Fig. 17). These lamellar and vesicular arrays are most similar to paramural bodies (Marchant and Robards 1968), sometimes called lomasomes or plasmalemmasomes, and are thought to be associated with cell wall synthesis (Cox and Juniper 1973).

Roots grown in +B+IAA medium for 6 h showed no significant structural differences from the control roots although root elongation was inhibited (Fig. 1). Examination of the cortical cell walls showed little or no change in wall thickness or in cell wall irregularity (Fig. 4). Few paramural bodies were seen. Vacuoles accumulated a dark-staining deposit not usually observed in control roots.

In roots grown in the absence of B but with IAA added, typical boron deficiency symptoms were observed at 6 h, that is, the walls were heavily thickened all along the periphery of the cell (Fig. 5). Irregularities of the plasma membrane along the cell wall were also observed. Dark-staining deposits were especially evident in the vacuoles of cortical cells of roots grown in −B+IAA. Thus, the combined effects of this treatment were apparent.

Response at 20 h

In Figs. 6–9 we show comparable sections of cortical cells from treated and untreated roots after 20 h when the symptoms apparent at 6 h become even more evident. Longitudinal cell walls of −B roots were thicker and more irregular (compare Fig. 6 with Fig. 7) and a greater accumulation of paramural bodies outside the plasma membrane was seen as compared with +B. This response was evident whether IAA was present or not (compare Fig. 8 with Fig. 9).

In addition, striking changes were apparent in mitochondrial structure induced by boron deficiency. In −B roots with or without added auxin, mitochondrial cristae were hypertrophied and more electron dense (Figs. 11, 13) than roots provided boron in the medium. Figures 10–13 show sections of cortical cells of roots from all four treatments after 20 h photographed at the same magnification. In the +B roots (Figs. 10, 12) one sees polysomes strikingly apparent and largely lacking in −B treated roots (Figs. 11, 13), which showed uniform and dense distribution of the ribo-

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ABBREVIATIONS USED IN FIGURES: *cw*, cell wall; *g*, Golgi body; *m*, mitochondrion; *mt*, microtubules; *p*, proplastid; *pd*, plasmodesmata; *pmb*, paramural bodies; *rer*, rough endoplasmic reticulum; *v*, vacuole.

FIGS. 2–5. Transverse sections of cortical cells of sunflower roots after 6 h of treatment. All × 24 030. Fig. 2. +B. Longitudinal cell wall. Normal features of a relatively meristematic cortical cell are evident. Part of the cell wall adjacent to the intercellular space appears thickened owing to slightly oblique section. Note longitudinal arrangement of microtubules in this region. Fig. 3. −B. Longitudinal and transverse walls. Unlabelled arrows indicate lamellar aggregations (paramural bodies) external to the plasma membrane. Fig. 4. +B+IAA. Longitudinal cell wall. Fig. 5. −B+IAA. Note early thickening of the longitudinal cell wall.


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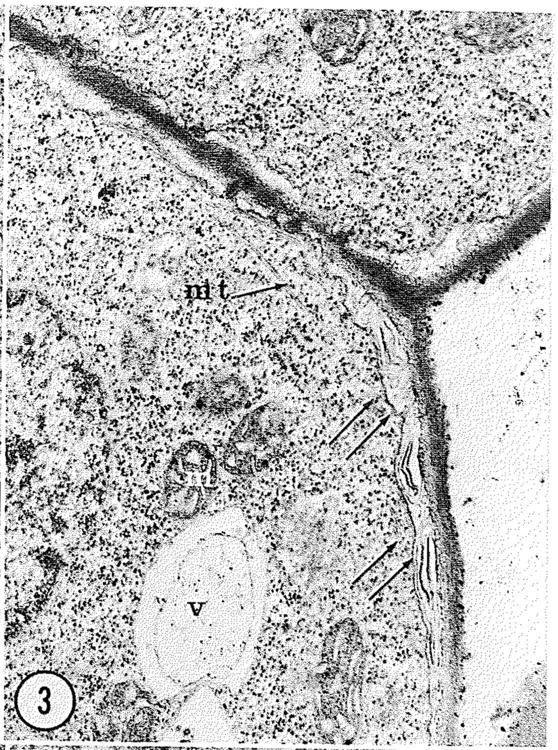
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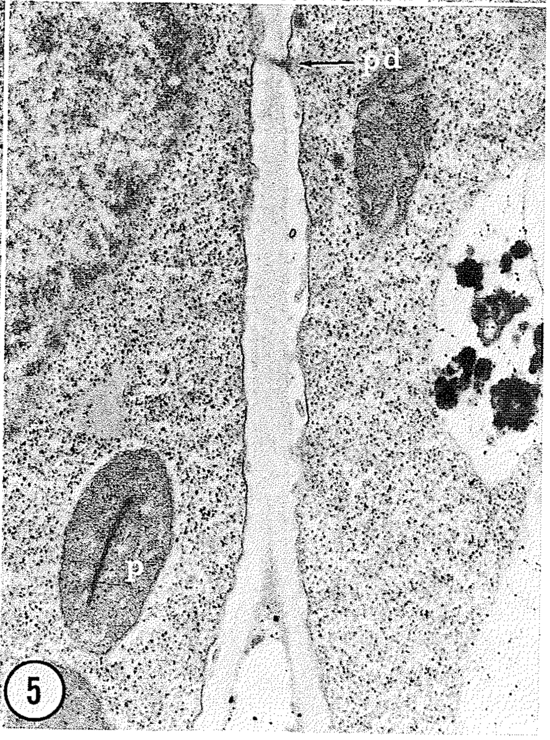
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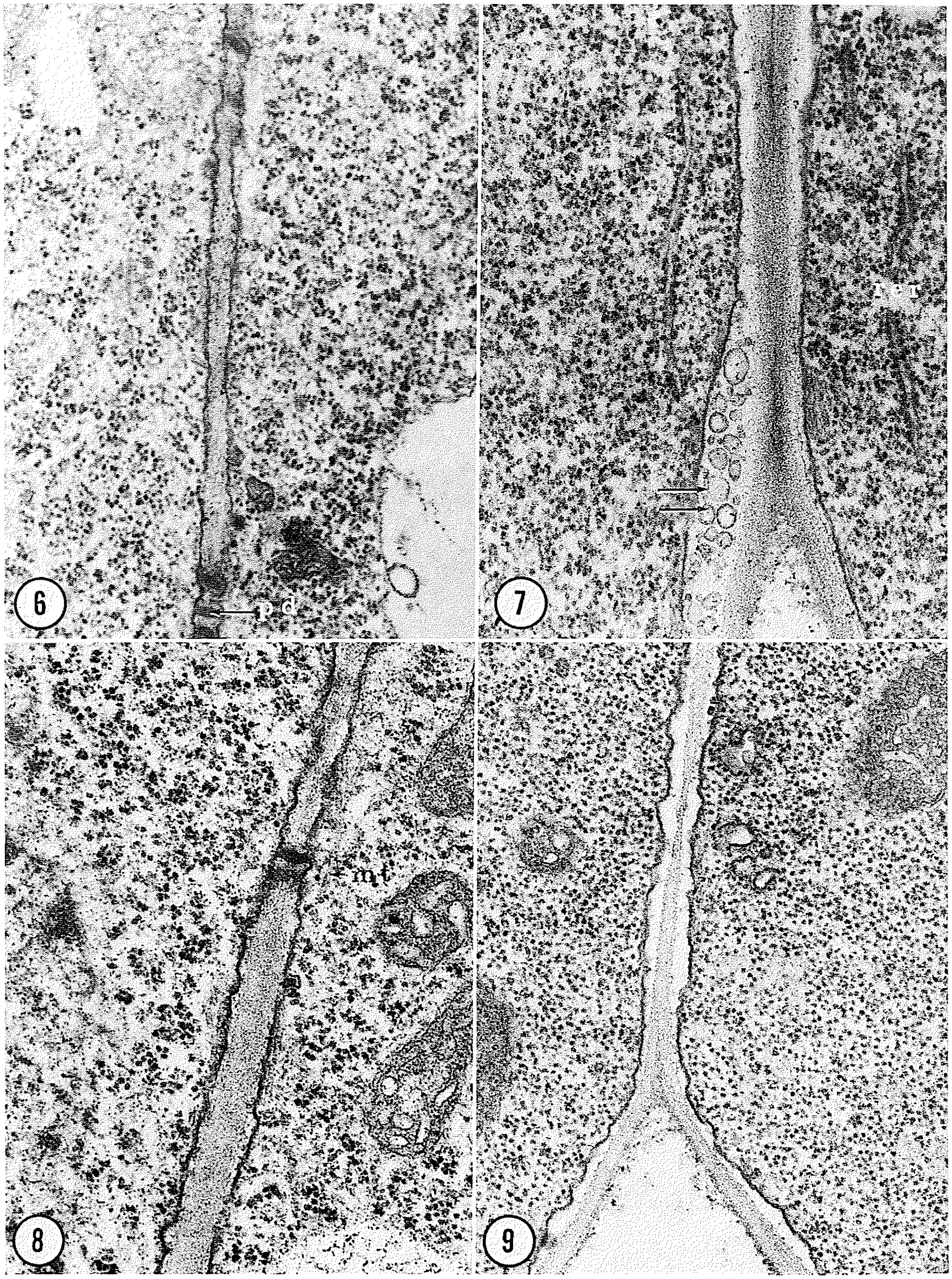
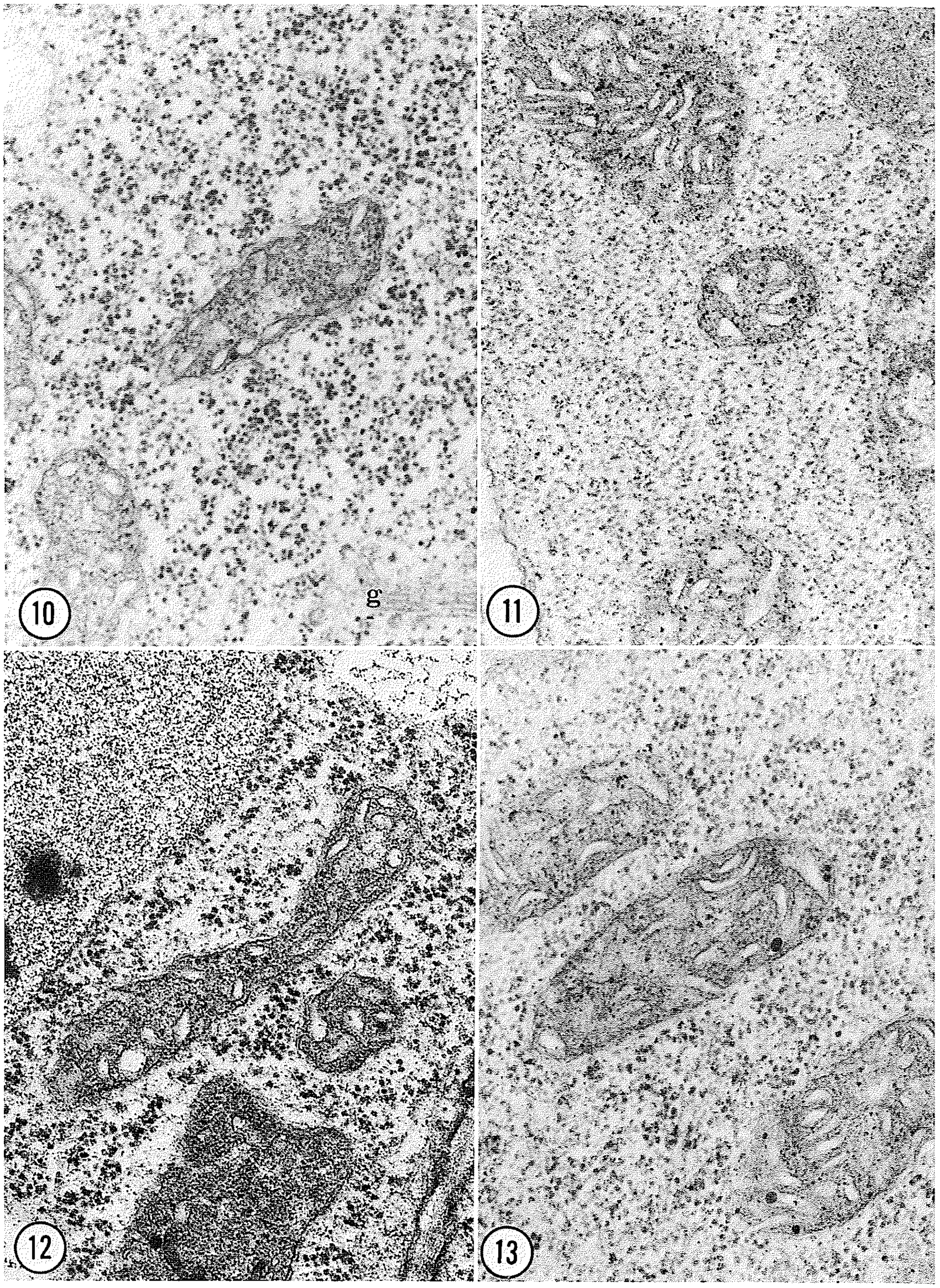
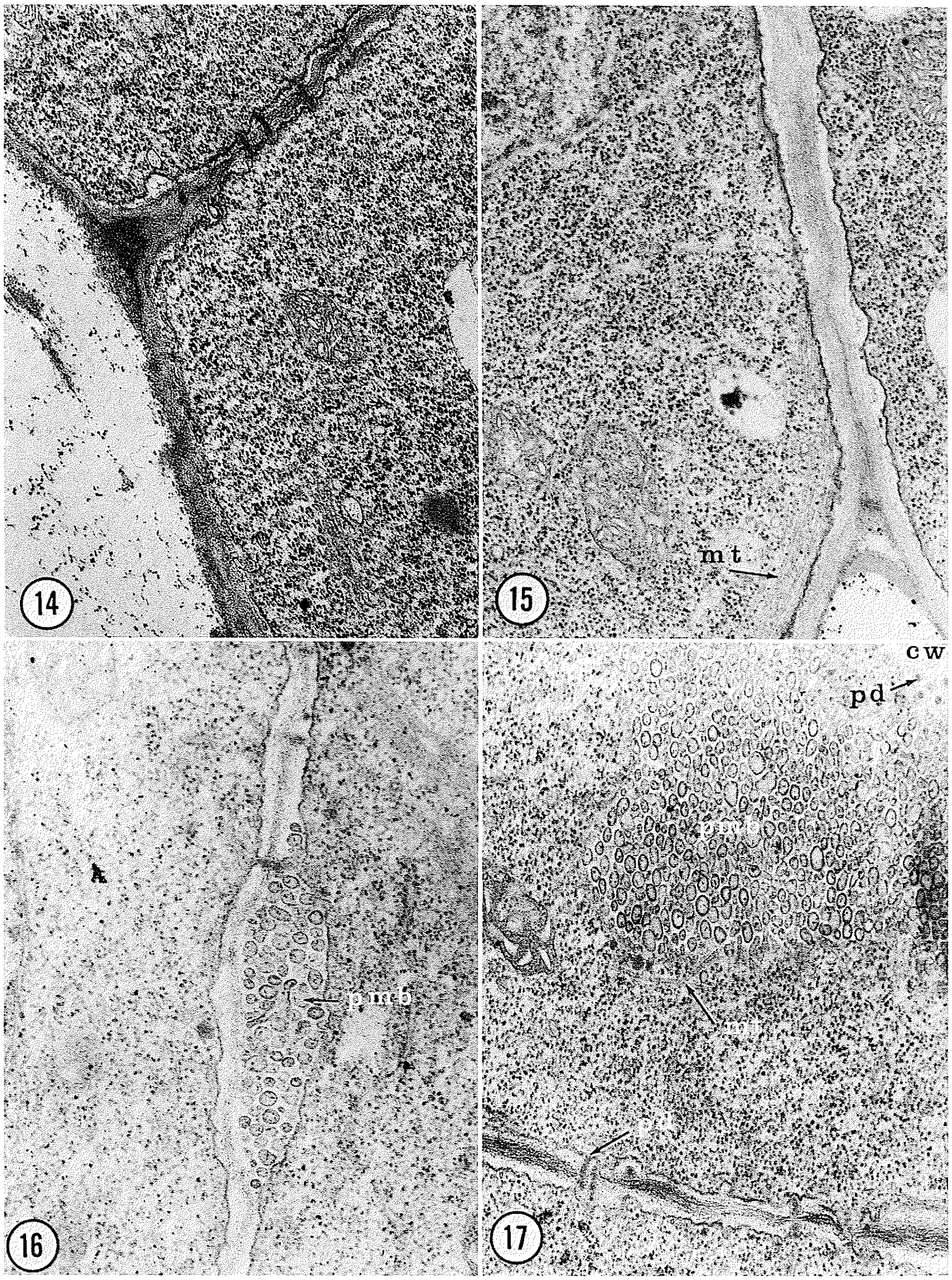


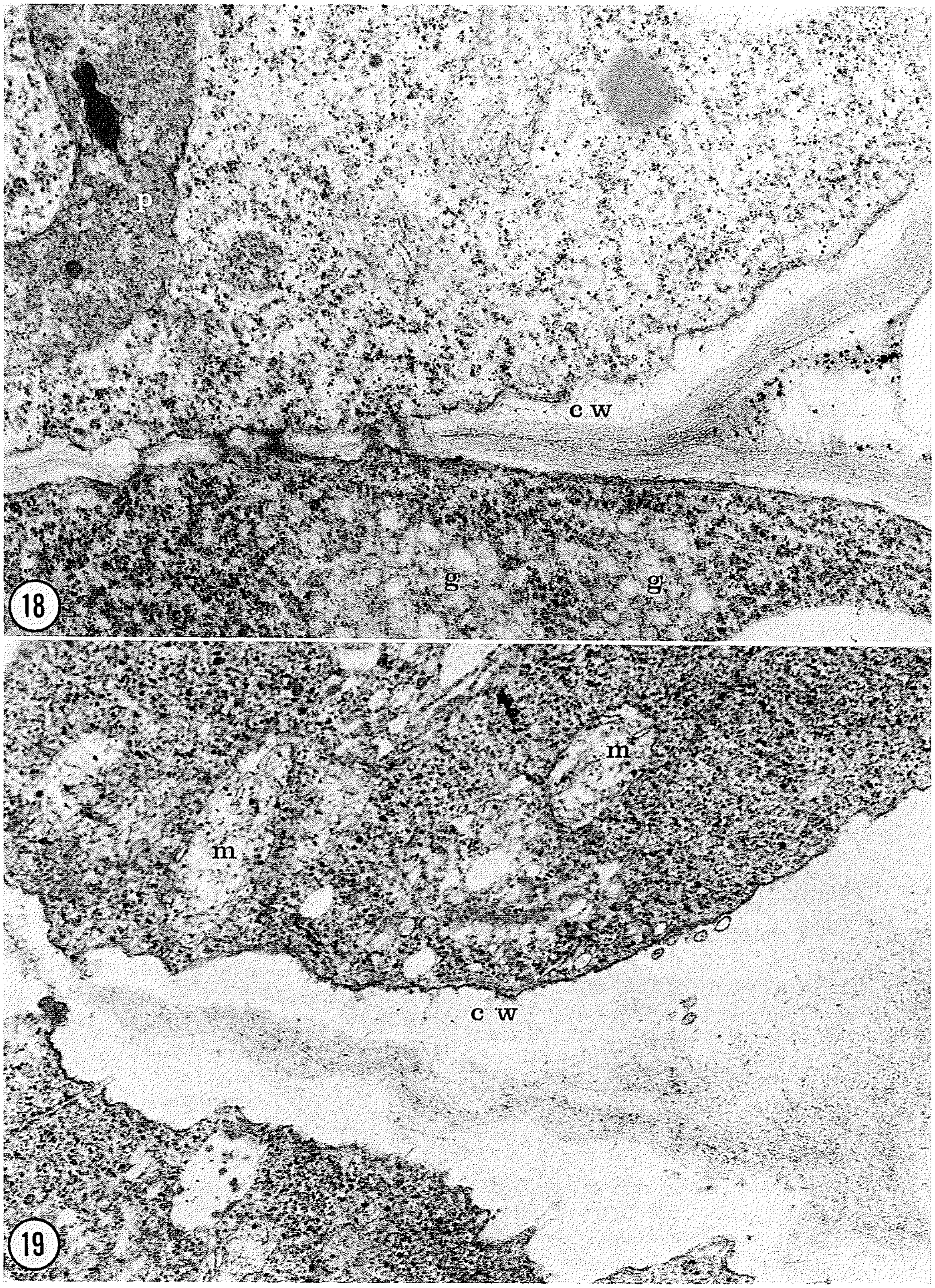
FIGS. 6–9. Transverse sections of cortical cells of sunflower roots 20 h after treatment. All × 46 527. Fig. 6. +B. Longitudinal cell wall. Fig. 7. −B. Longitudinal cell wall. Unlabelled arrows indicate vesicular aggregations (paramural bodies) adjacent to plasma membrane. Note endoplasmic reticulum (*rer*). Fig. 8. +B+IAA. Section through cell wall is slightly oblique. Fig. 9. −B+IAA.



FIGS. 10–13. Mitochondria from root cortical cells after 20 h of treatment. All × 46 257. Fig. 10. +B. Fig. 11. −B. Fig. 12. +B+IAA. Fig. 13. −B+IAA.



FIGS. 14 and 15. Transverse sections of cortical cells of sunflower roots 34 h after treatment. Both × 38 448. Fig. 14. +B. Longitudinal and transverse walls. Fig. 15. −B. Longitudinal wall. FIGS. 16 and 17. Cortical cells of sunflower roots 6 h after −B treatment, both × 31 018. Fig. 16. Transverse section of cell wall and adjacent paramural bodies. Fig. 17. Oblique section showing extensive array of paramural bodies and their orientation in relation to microtubules and the cell wall. Lower cell wall is transversely sectioned.



FIGS. 18 and 19. Transverse sections of cortical cells of sunflower roots 72 h after treatment. × 38 448. Fig. 18. +B. Longitudinal cell wall. Fig. 19. −B. Longitudinal cell wall. Mitochondrial membrane integrity is lost and the cell appears senescent.

TABLE 1. Timetable of early boron deficiency symptoms reported in the literature

Hours from onset of deficiency	Reported symptom	Plant	Reference
0–4	IAA levels presumed to increase	—	Brandenburg 1949; Neales 1960
3	Root elongation inhibited	*Cucurbita pepo* L.	Bohnsack and Albert 1977
4	Increased incorporation of ^{32}P into nucleic acids	*Vicia faba* L. var. *minor*	Cory *et al.* 1966
6	Increased incorporation of [^{14}C]uridine into RNA	*Phaseolus aureus* L.	Chapman and Jackson 1974
	Root elongation ceases	*Lycopersicon esculentum* Mill.	Albert and Wilson 1961
		Helianthus annuus L.	Kouchi and Kumazawa 1975*a*
		Cucurbita pepo L.	Cohen 1972
	Mitosis ceases	*Cucurbita pepo* L.	Cohen and Albert 1974
	Increase in Golgi and rough endoplasmic reticulum; accumulation of deposits in vacuoles	*Lycopersicon esculentum* Mill.	Kouchi and Kumazawa 1976
	IAA oxidase increases	*Cucurbita pepo* L.	Bohnsack and Albert 1977
8	Cell walls thicken	*Lycopersicon esculentum* Mill.	Kouchi and Kumazawa 1976
20–24	DNA synthesis ceases	*Cucurbita pepo* L.	Cohen and Albert 1974
	RNA content decreased by 50%	*Lycopersicon esculentum* Mill.	Albert 1965
72	Radicle apical meristem irreversibly damaged	*Vicia faba* L. var. *minor*	Neales 1960

somes. Occasionally, −B roots were sectioned after 20 h treatment in which the cytoplasm already showed signs of degradation, an appearance which progressed with further periods of B deficiency. In these roots, electron-dense fibrillar material as well as aggregations of dark-stained deposits were commonly seen in the vacuoles. Thereafter, much of the distinction between cytoplasm and organelles was lost, presumably due to progressive breakdown of cellular membranes. These long-term boron-deficient cells showed many of the characteristics of senescing cells (Butler and Simon 1971).

Cortical cells of roots grown in +B medium do not show these symptoms. Although paramural bodies are seen in +B cells, the numbers seen in −B roots are much greater, and in addition few of the other characteristics symptomatic of boron deficiency were observed. Mitochondria were normal, cell walls were thin, and few deposits occurred within the vacuoles.

Treatment of roots grown in +B medium to which IAA was added did not produce symptoms characteristic or in any way mimicking boron deficiency. Cell walls were normal (compare Fig. 6 with Fig. 8; Fig. 8 appears thicker because the wall is sectioned slightly obliquely) and mitochondria were normal (compare Fig. 10 with Fig. 12). The only apparent change in auxin-treated root cells was an increase in electron-dense material within the vacuoles. Thus, it seems clear that, although boron deficiency and auxin treatment both elicit inhibition of root elongation, these treatments in no way resemble each other in their effects on cell ultrastructure.

Later responses to boron deficiency

At 34 h, boron-deficient roots showed further progression in cortical cell wall thickening (Fig. 15) and by 72 h cortical cell wall thickness was quite striking (Fig. 19) and evident signs of cytoplasmic disintegration were visible. Membrane integrity had disappeared and the roots were dying. In comparable roots provided boron, root cortical cell structure appeared fairly normal (Figs. 14, 18). In the later stages of root growth, some cell wall thickening had occurred (Fig. 18).

Discussion

In attempts to come to an understanding of the role of boron in higher plants, a number of researchers have studied the very early symptoms of boron deficiency in a diversity of plants. A summary of the early effects of boron deficiency is presented in Table 1, together with the appropriate reference citations.

Clearly, roots and especially root tips are the most sensitive site of boron deficiency, showing evident morphological responses within 3–6 h. Possibly earlier signs of deficiency could be measured using biochemical marker studies. In agreement with other studies, we observed inhibition of root elongation under conditions of boron deficiency as early as 3 h. The most striking and earliest ultrastructural response observed was root cell wall thickening which was evident at 6 h.

The cell wall thickening in −B roots was characterized by the irregular deposition of vesicular aggregations along the primary cell wall outside the plasmalemma appressed to the wall. In some roots at 6 h cell wall thickness had already shown increases from incorporation of new wall material intermixed with lamellar membranous fragments (Fig. 3, 5). At later stages (Figs. 7, 15, and 19) the thickening especially along longitudinal walls had increased to a highly abnormal degree.

Kouchi and Kumazawa (1976) reported an increase in cell wall thickness in cells of roots grown in −B medium. They also reported an increase in the number of dictyosomes in association with cell wall thickening, an observation we were unable to confirm. The origin of the paramural bodies is inexplicable from our studies.

Auxin-treated roots provided boron did not show the abnormal cell wall thickening at 6 h. Paramural bodies are present but are increased over the control only in the −B+IAA treatment. Only at much later stages were there signs of cell wall changes associated with +B+IAA treatment. Roots exposed to 5 × 10^{-7} *M* IAA for periods longer than 20 h showed thickening, but this thickening is probably not substantially different from control roots. In isolated pea root cortical explants grown on complete medium plus 2,4-D at 5 × 10^{-6} *M* and IAA at 10^{-6} *M*, Bowes and Torrey (1976) found that auxin was a factor in causing root cortical cell walls to undergo thickening by 72 h. Here, too, lomasome-like invaginations occurred at the plasmalemma – primary cell wall interface.

The effects of boron deficiency on mitochondrial structure were also clear. Roots grown in −B medium showed mitochondria with swollen cristae as early as 6 h; in later stages, mitochondrial cristae disintegrated and mitochondria senesced. A similar response was described by Kouchi and Kumazawa (1976) in boron-deficient tomato roots. They occasionally observed altered mitochondria in the extension zone of normal roots and expressed the view that changes in mitochondrial structure were more a consequence of cell aging rather than specifically owing to boron deficiency.

Kouchi and Kumazawa (1976) reported an increase in the frequency of vacuoles containing dark-stained "lipid-like" deposits in −B roots. However, they did not perform any histochemical tests to verify the identity of these deposits. Smirnov *et al.* (1977) and other workers reported an increase in phenolic compounds in boron-deficient plants and believed the occurrence of phenols was a primary response to lack of boron. Whether the electron-dense deposits seen by Kouchi and Kumazawa (1976) in vacuoles of tomato root cells or the dark product contained within the cytoplasm and the degenerating chloroplasts of 72 h −B sunflower mesophyll cells (Lee and Aronoff 1966) are phenolics is difficult to ascertain.

Sections of auxin-treated sunflower roots showed accumulation of electron-dense deposits in the vacuoles at an early stage and these deposits were a consistent feature. Such deposits appeared also in −B roots, especially at the later stages examined. We performed various histochemical tests to identify these products but the results were inconclusive. Two different reactions intended to indicate peroxidase activity did not show a significant difference between −B and +B sunflower roots. These data are in contrast to the results obtained by Robertson and Loughman (1974) who found that −B *Vicia faba* roots had higher peroxidase activity. Tests to histochemically localize tannins gave negative results. A slightly positive result obtained with Sudan IV which stains total lipids did not differentiate between −B and +B roots. Because the electron-dense deposits often have a fibrillar as well as amorphous appearance and because the fibrillar products react positively in certain histochemical tests for polysaccharides, it is reasonable to speculate that some of the vacuoles contain cell wall material. Auxin-treatment does result in an eventual increase in cell wall thickness which the vacuolar product may foreshadow. However, if the dark-staining deposits are indeed phenolics, this may reflect the fact that the −B and auxin-treated root cells respond to their respective conditions by exhibiting premature cell senescence.

From these studies we conclude that the effects of boron deficiency are not completely duplicated by exogenous treatment with auxin and it therefore seems unlikely that boron deficiency acts through an effect leading to supraoptimal auxin levels in the root. Rather one must find some other explanation.

The common feature in early symptoms of boron deficiency in sunflower roots observable at the ultrastructural level involves changes in the integrity of membranes; the plasmalemma in relation to cell wall formation, paramural body or lomasome formation, the mitochondrial cristae which lose their integrity, and vacuolar membranes, early associated with accumulation of electron-dense product and later with senescent phenomena. One common role for boron in these would be the maintenance of membrane integrity and (or) replication. With membrane instability, normal cell functioning soon ceases. With increased numbers of paramural bodies and other organelles, there is a concomitant increase in the thickness of the cell wall. Other workers have also suggested that boron may play a role in membrane interactions (Tanada 1975; Pollard *et al.* 1977). Perhaps it is to some type of membrane-mediated response that a unique role for boron can be assigned.

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