THE REGULATION OF ROOT DEVELOPMENT BY ALUMINIUM

IN <u>Zea</u> <u>mays</u> L.

<u>VOLUME 2</u>

by

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LIST OF FIGURES AND APPENDIX
(VOLUME 2)

Figure 1   Roots of <u>Zea mays</u> (cv. TX 24) grown for 28 days in a complete
nutrient <u>solution</u> containing 8,0 mg dm^{-3} Al, to illustrate the
effect of Al on root morphology (Section 1.2.1).

Note the thickened, stubby appearance of the roots and the
initiation of lateral roots along the root axis, almost to
the root apex.   Many lateral roots failed to develop and
finely branched roots are absent.

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Figure 2   Diagram of the principal tissue regions of the root apex of
           Zea mays indicating the location of the proximal meristem (PM),
           cap meristem (DM) and quiescent centre (QC)(Section 1.2.3.1).
           The proximal meristem functions as the ultimate source of cells
           for cortex (C) and stele (S) comprising the axial structure of
           the root.   The cap meristem contributes cells to the root
           cap (RC) in the direction indicated (unlabelled arrows).
           Roots of Zea are characterized by a distinctive boundary
           between the cap and distal region of the root (CB).   Data
           derived from Ellmore (1982) and Feldman (1984b).

C

S

PM

C B

QC

DM

RC
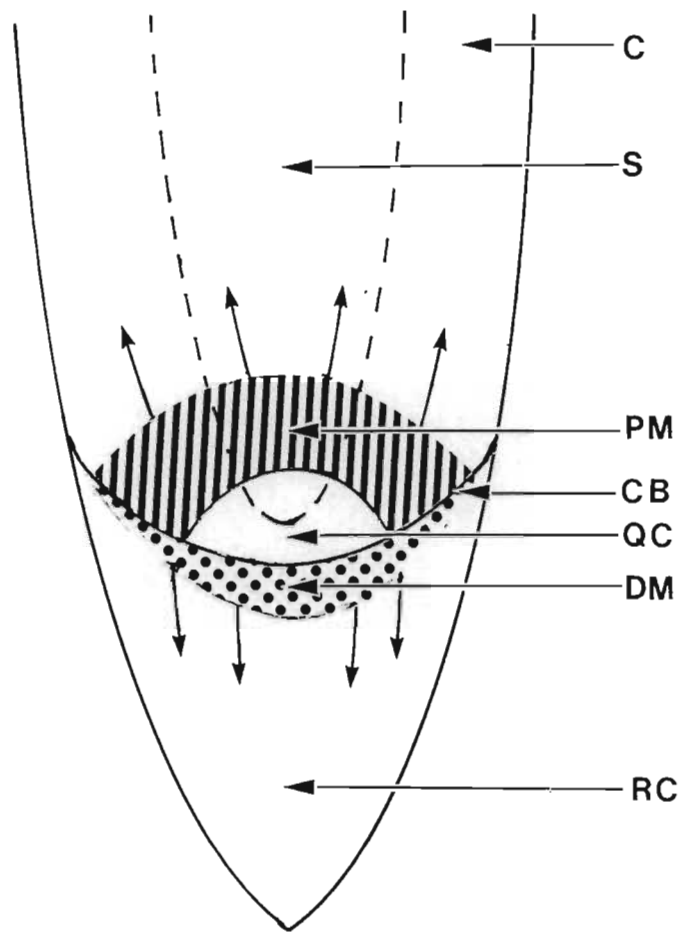
Figure 3   Nutrient solution pH changes observed over a 15 day growth
            period for Zea mays (cvs. TX 24 and HL 1), grown in full-strength
            nutrient solutions incorporating Al treatments of
            0 (control); 0,5; 1,0; 3,0; 5,0; 10,0 mg dm^{-3}.   Solution
            pH adjusted to 4,6 subsequent to the addition of Al.

            Note that gradual acidification of the nutrient solution was
            associated with most treatments (Section 2.2.3).
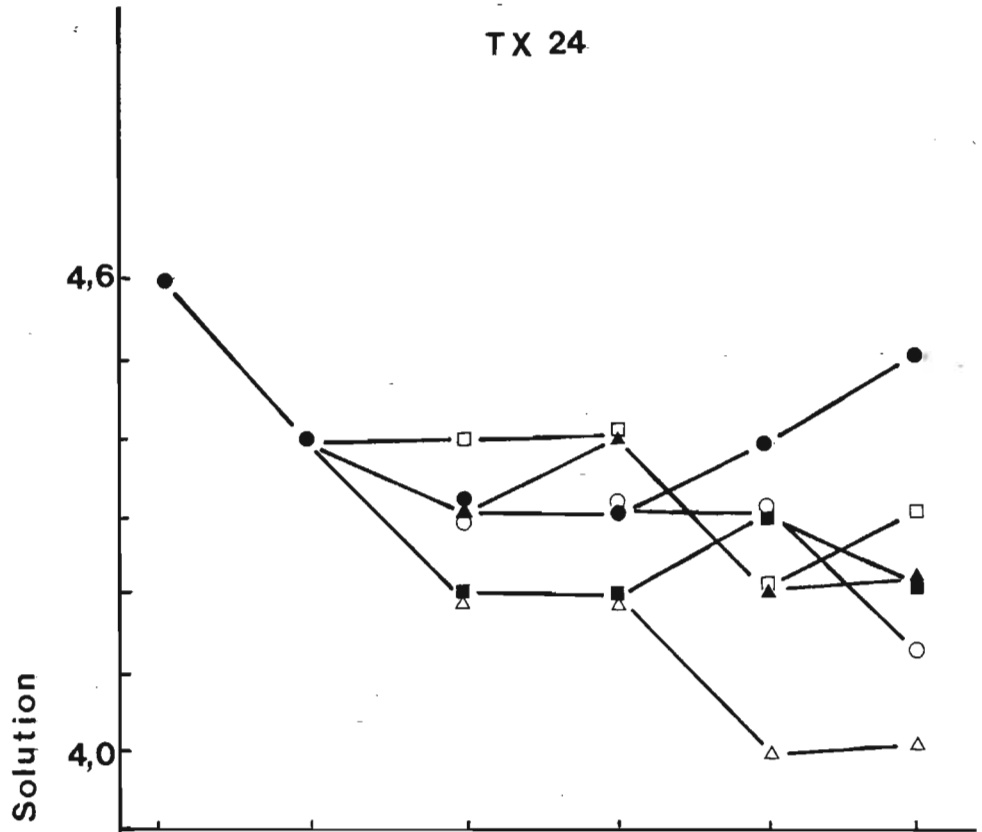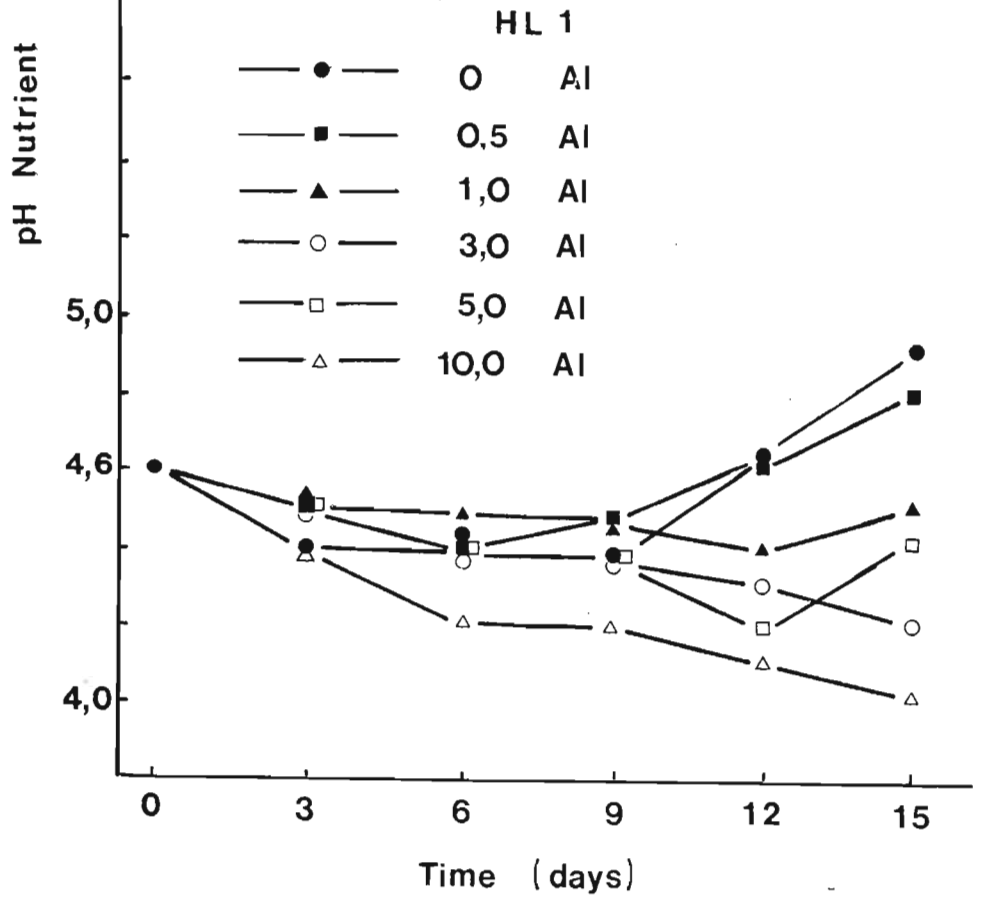
Figure 4   The response of Zea mays (cvs. TX 24 and HL 1) to different
            P sources.   In these experiments, P was supplied as either
            KH_2PO_4 (control) or amorphous aluminium phosphate.   The
            aluminium phosphate treatments included Al additions as
            $Al_2(SO_4)_3$ to give 0; 5,0; 10,0 mg dm^{-3} Al.   Dry matter
            (DM) yield and tissue P levels for the various treatments
            were expressed on a relative basis (% control; KH_2PO_4).   Dry
            matter yields were unaffected or increased when aluminium
            phosphate was the P source without added Al.   Aluminium
            added to the nutrient solutions generally depressed DM yield
            and tissue P levels were, with the exception of TX 24 (roots),
            also lower.   A rapid increase in nutrient solution pH
            (both cultivars) occurred when P was supplied as aluminium
            phosphate without added Al (Section 2.2.5).
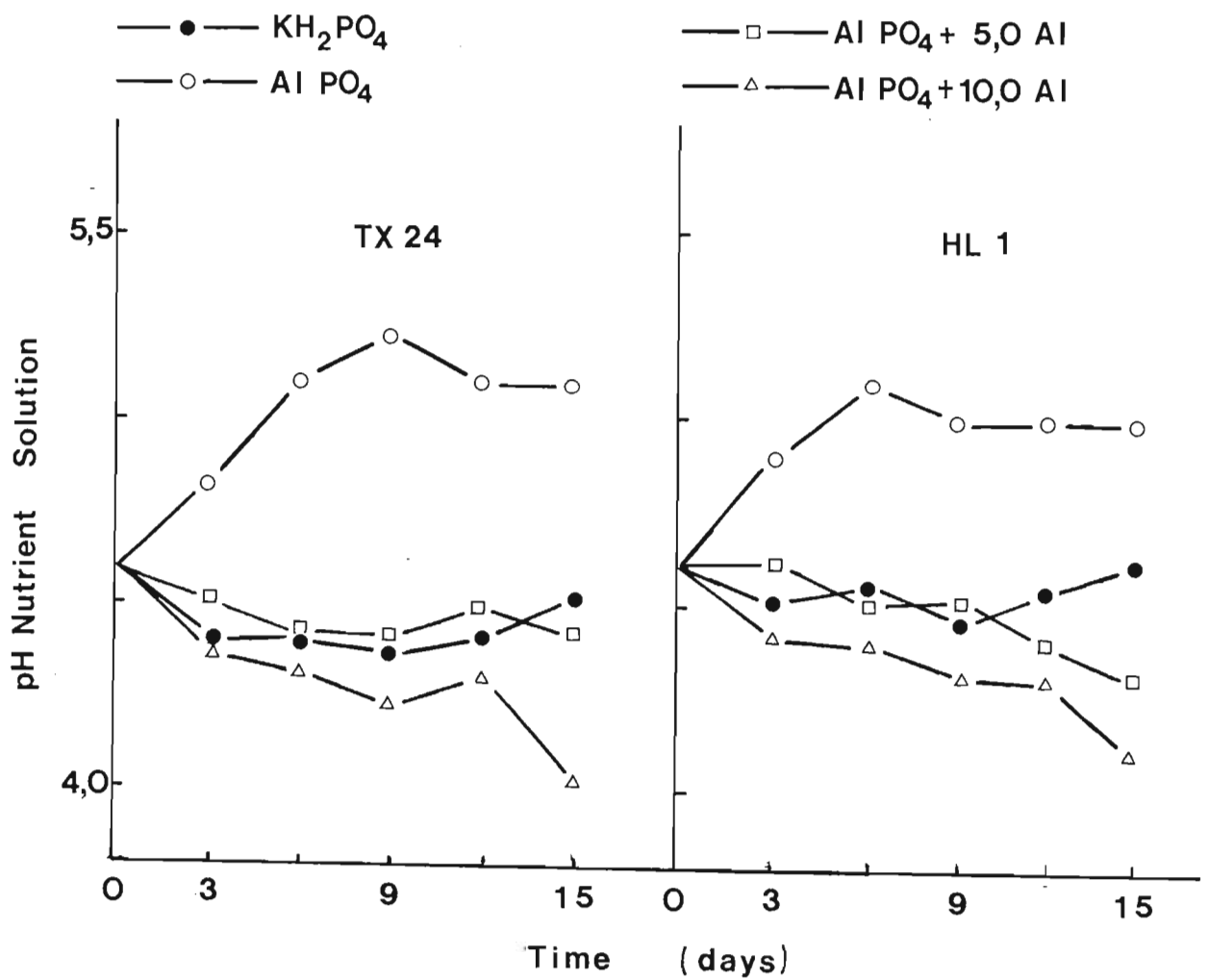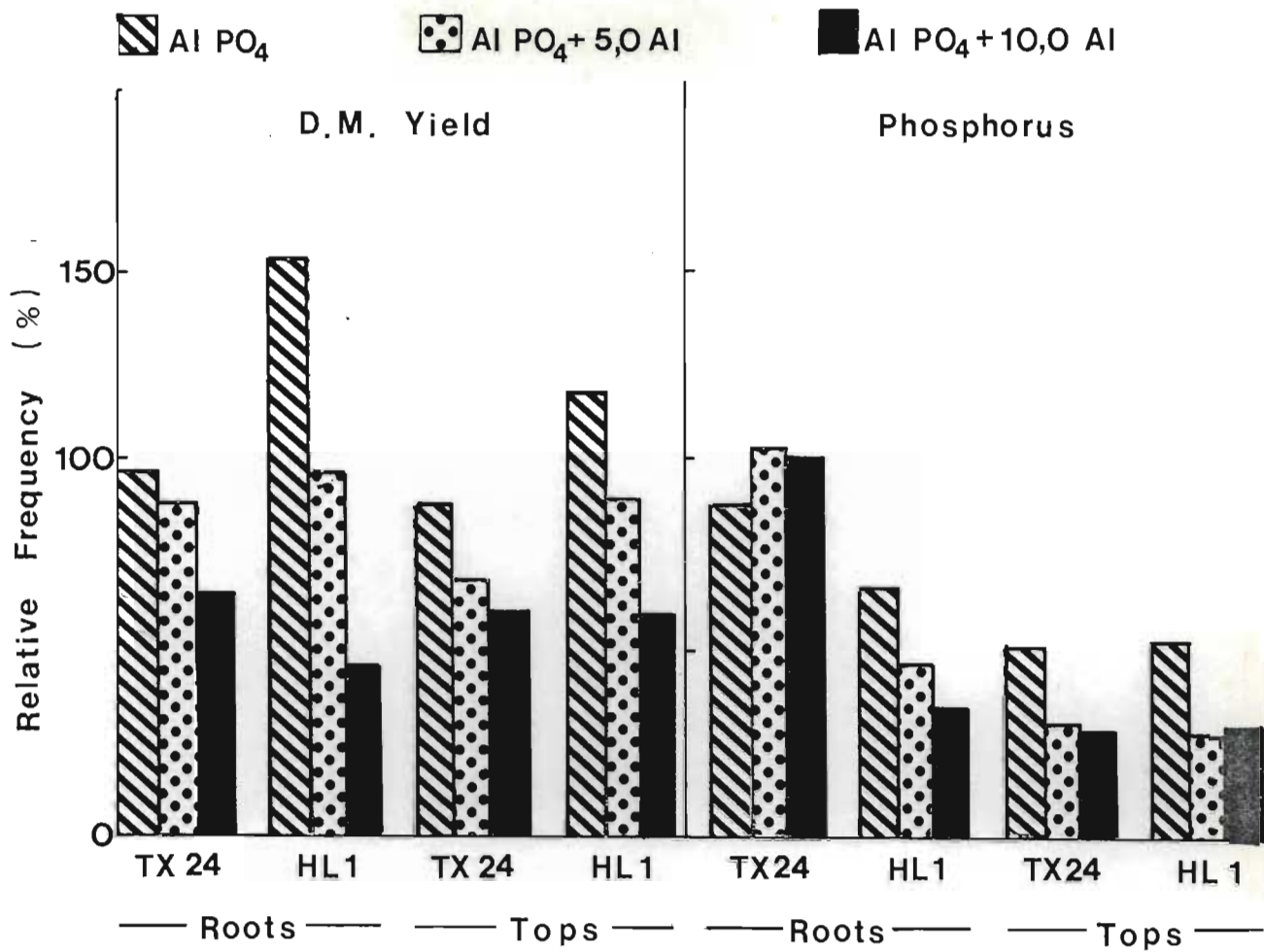
Figure 5   The effect of plant growth and nutrient uptake
            (Zea mays cv. TX 24) on nutrient solution concentrations of
            Ca, K, Mg and P.   Plants were grown for 15 days
            (Section 2.2.8).   Each point represents the mean value
            recorded from 27 pots (Section 2.2.6).   Variations between
            pots were small.
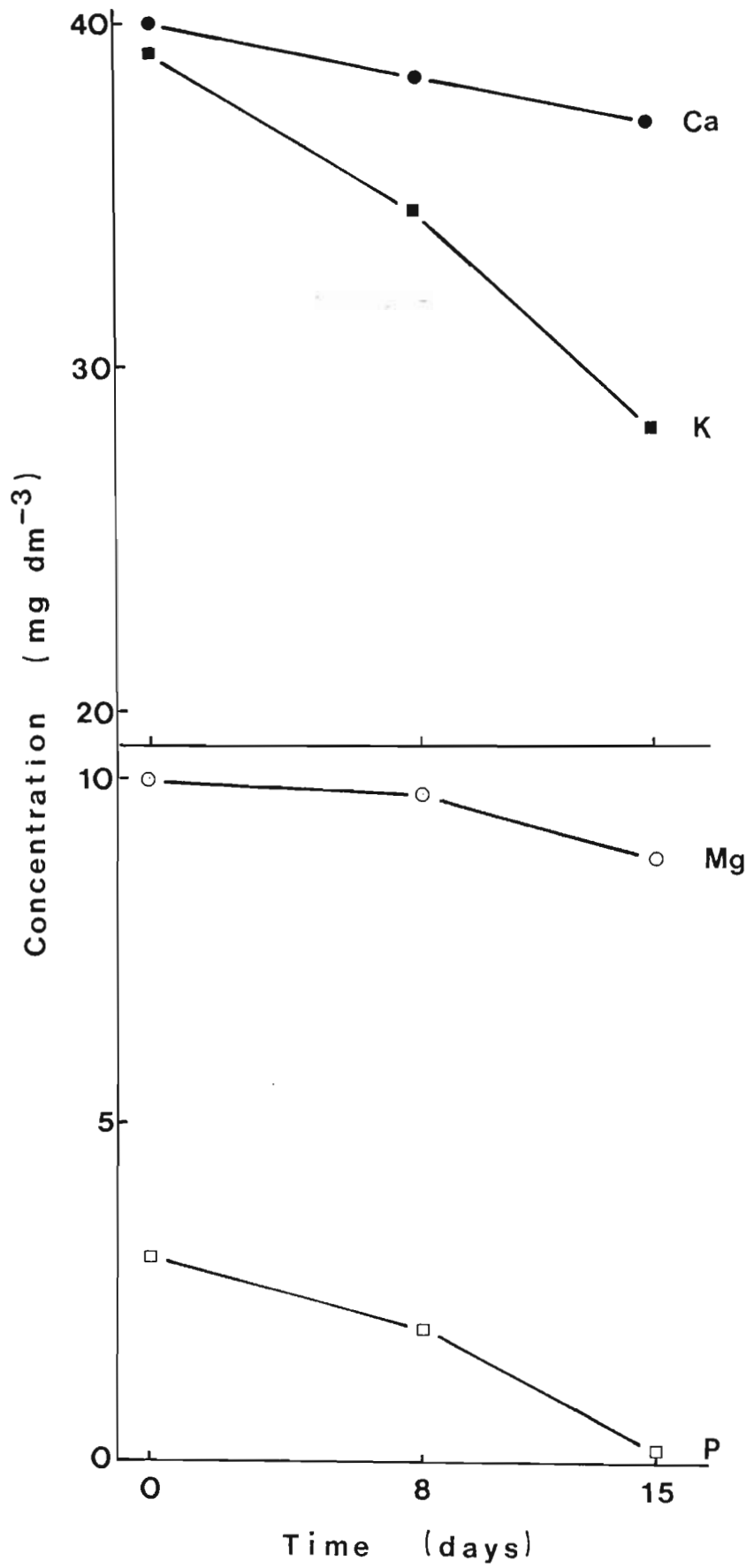
Figure 6   Unsectioned roots of <u>Zea mays</u> (cv. TX 24) treated for different times with $8,0$ mg dm^{-3} Al and stained with hematoxylin (Section 3.3.1.1) to illustrate the pattern of Al uptake by the root. The development of a dark blue/purple colour is indicative of the presence of Al (Section 2.3.1.3).

    A - control, no Al added. Roots were characterized by the complete absence of stained tissue.

    B - 2 h treatment with Al. Aluminium uptake was clearly evident and localized in the root apex.

    C - 5 h Al treatment. An increase in the intensity of stain was considered evidence for continued Al uptake. Uptake sites were however, still localized in the root apex except where there was evidence of damage to the root axial structure.

    D - 24 h treatment time. A loss of apical dominance was indicated by the initiation of lateral roots along the main axis of some roots.
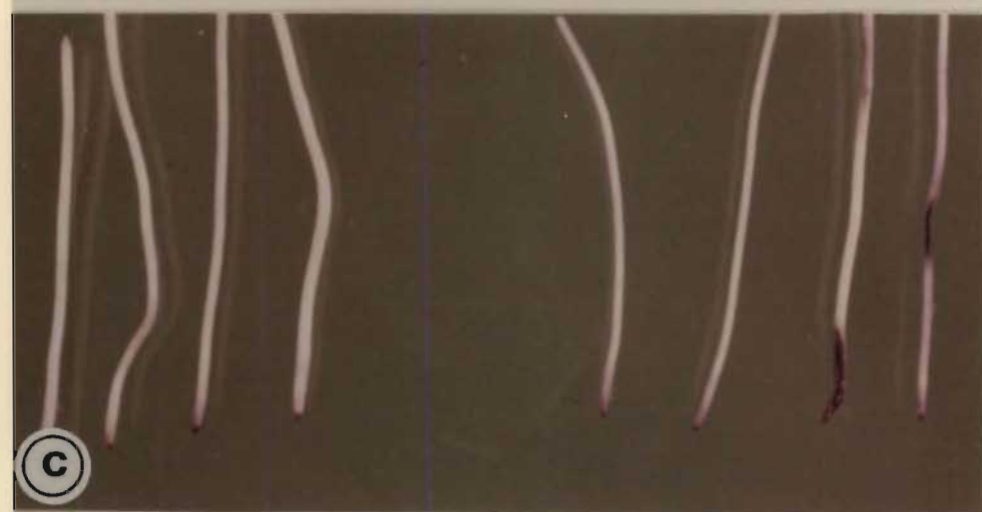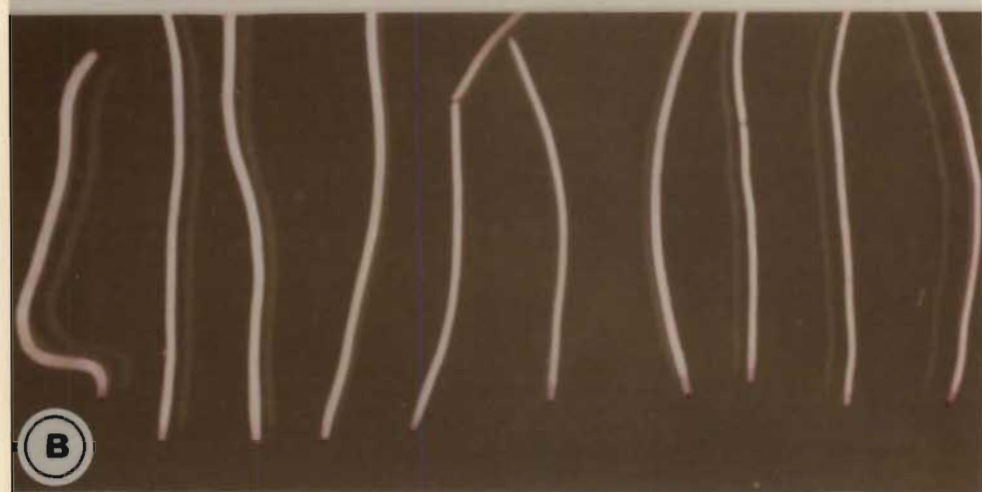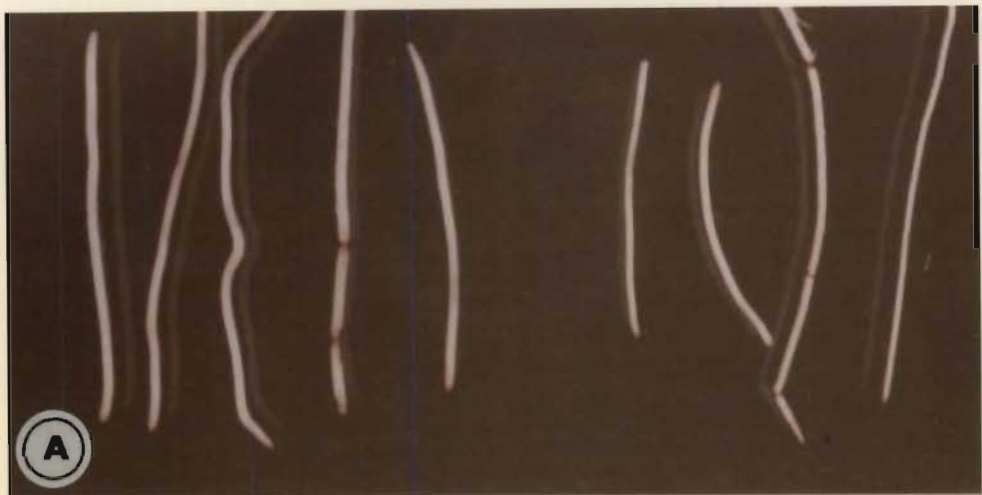
Figures 7A and 7B   Unsectioned roots of Zea mays (cv. TX 24) treated for
                    different times with 8,0 mg dm^{-3} Al and stained in hematoxylin
                    (Section 3.3.1.1) to illustrate secondary Al uptake associated
                    with lateral root development.

        A - 96 h treatment with Al.   Lateral root development occurred
                    to within a few mm of the root apex.   Emerging lateral root
                    tips stained with an intensity reminiscent of that observed
                    in the primary root apex.   Lateral root development may
                    therefore be associated with secondary Al uptake sites.

        B - 168 h treatment with Al.   Although many lateral roots were
                    initiated, few showed significant development subsequent to
                    emerging through the epidermis.

Figure 7C   Unsectioned roots of Zea mays (cv. TX 24) stained in methylene
            blue (Section 2.3.3.2) to demonstrate the presence of muco-
            polysaccharide materials (Section 3.3.3.1).   Similarities
            emerged between the regions of the root associated with Al
            uptake (Figures 6B and 6C) and the occurrence of muco-
            polysaccharide materials.
            Control roots (0 Al) are illustrated on the left of the picture,
            while the two examples on the right have been treated with
            8,0 mg dm^{-3} Al for 20 h prior to staining with methylene blue
            (Section 3.3.3.1).
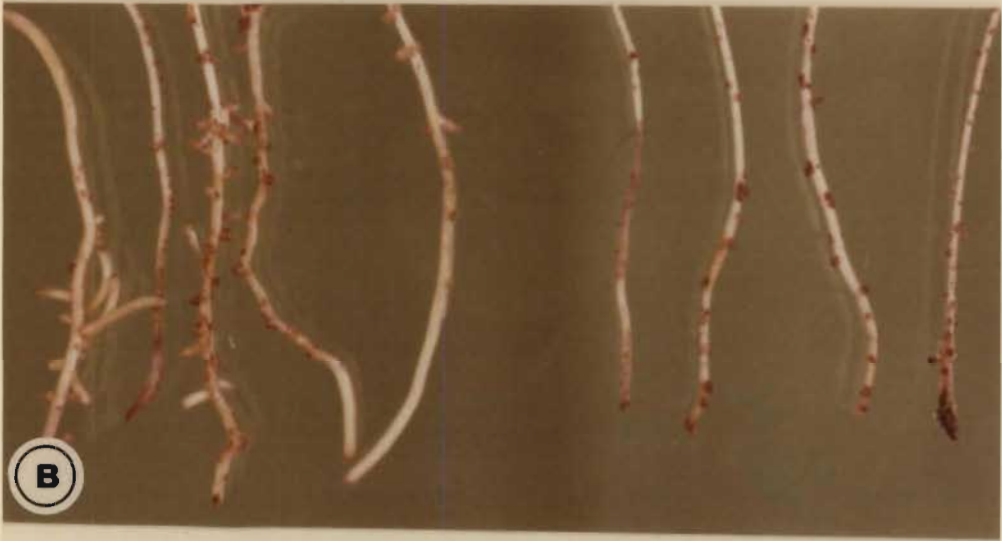
Figure 8   Micrographs of the root apices of Zea mays (cv. TX 24)
treated for different times with 8,0 mg dm^{-3} Al, and stained
with hematoxylin (Section 2.3.1.3) to illustrate the chrono-
logical pattern of Al uptake by the cell populations of the
root apex (Section 3.3.1.2).   Darkly stained cells (blue/
purple) are indicative of the presence of Al.

A - Control (0 Al).   Note the absence of stain from all
    tissue; cap periphery (PC); cap columella (CC);
    quiescent centre (Q)

B - 0,5 h Al treatment.   Staining was initially limited to
    the peripheral cap and mucilagenous secretions surrounding
    the root.

C - 2 h Al treatment resulted in staining of the cell
    populations of the root cap and the epidermal and outer
    cortical regions of the root apex.   Aluminium was
    however, essentially absent from the cap and proximal
    meristems and quiescent centre.

D - 20 h Al treatment produced extensive staining of the root
    cap and epidermal and outer cortical regions of the root.
    Morphological disturbances resulted in the fracture of the
    epidermis but, despite this, the absence of stained tissue
    in the inner cortex (unlabelled arrows) and stele
    did not indicate penetration of Al to these tissues.

Magnification A - C X 100;   D X 60.
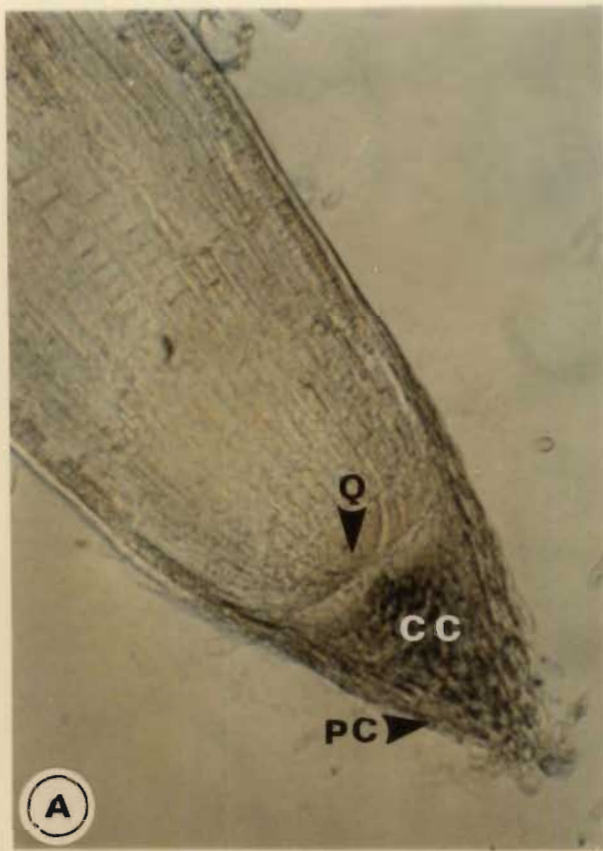
Figure 9 Outline drawing of the root tip of Zea mays indicating the
          location of the cell populations comprising the cortex (C),
          epidermis (E), quiescent centre (Q), stele (S), cap columella
          (CC) cap initials forming the cap meristem (CI) and
          peripheral cap cells (CP).   The boundary (CB) between the cap
          and root, which is characteristic of the "closed" construction
          of the root apex as found in Zea, is also illustrated
          (Section 3.3.1.2).

C

E

S

Q

CB

CI

CC

CP
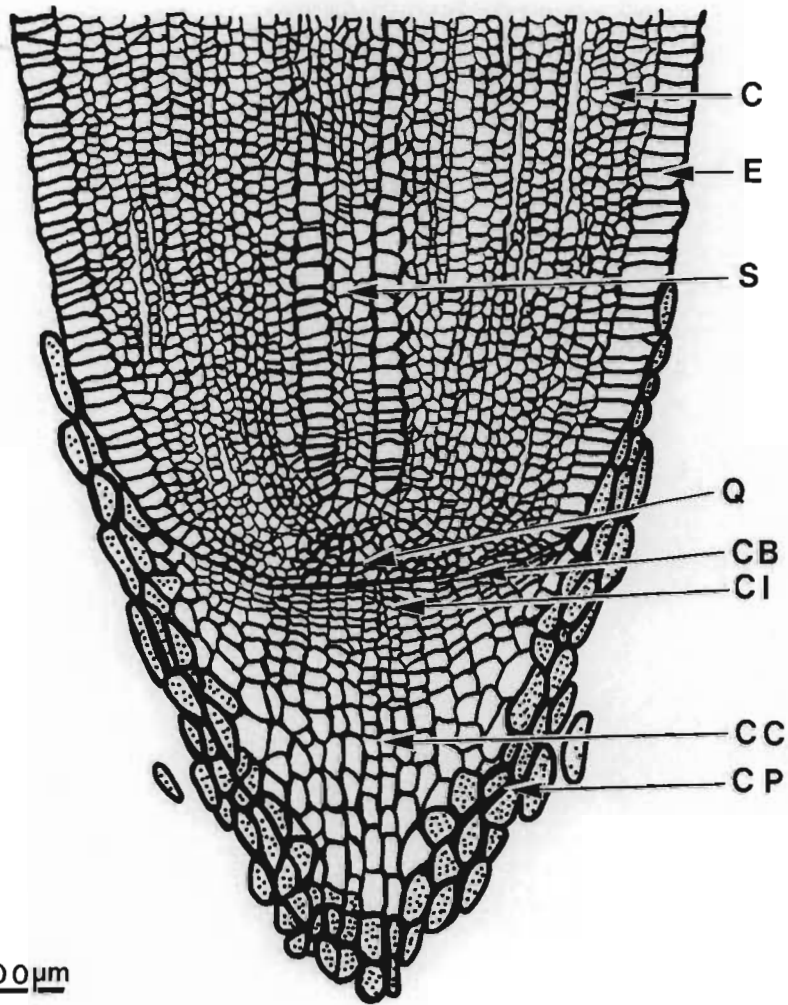
100μm

Figures 10A and 10B Micrographs of the root apices of <u>Zea mays</u>
(cv. TX 24) treated with 8,0 mg dm^{-3} Al and <u>stained</u> with
hematoxylin (Section 2.3.1.3) to illustrate the effects of cap
removal on the pattern of Al uptake (Section 3.3.2.1).

A - Control (with cap) after 20 h treatment with Al.

Note extensive staining of the cell populations comprising
the peripheral cap (PC), epidermis and outer cortex, and
the absence of stain from the cap and
proximal meristems, cap columella (CC) and quiescent
centre (Q).   The epidermis remained intact and yet the
outer cortex and epidermal cells are heavily stained
thereby confirming entry of Al into these tissues.

B - Decapped root after 20 h treatment with Al.
Cap removal has permitted Al to enter the quiescent centre
and the distal regions of the proximal meristem.
Aluminium uptake was limited to these tissues and movement
did not extend to the adjacent cells of the inner cortex
and stele.

Figures 10C and 10D Micrographs of the root apices of <u>Zea mays</u>
(cv. TX 24) stained with methylene blue (Section 2.3.3.2)
to illustrate the occurrence of mucopolysaccharide materials
as well as the effect of Al treatment (20 h; 8,0 mg dm^{-3})
on the distribution of mucopolysaccharides (Section 3.3.3.1).

C - Control (0 Al) characterized by the high intensity of
stained tissue in the root cap, outer cortex and
proximal meristem adjacent to the cap junction.   In
contrast, the inner cortex and stele showed only low
levels of stain.

D - After 20 h Al treatment, methylene blue staining showed
that the distribution of mucopolysaccharide materials
had been reduced and was principally restricted to the
root cap and proximal meristem immediately adjacent to
the cap junction.

Magnification A - D X 100
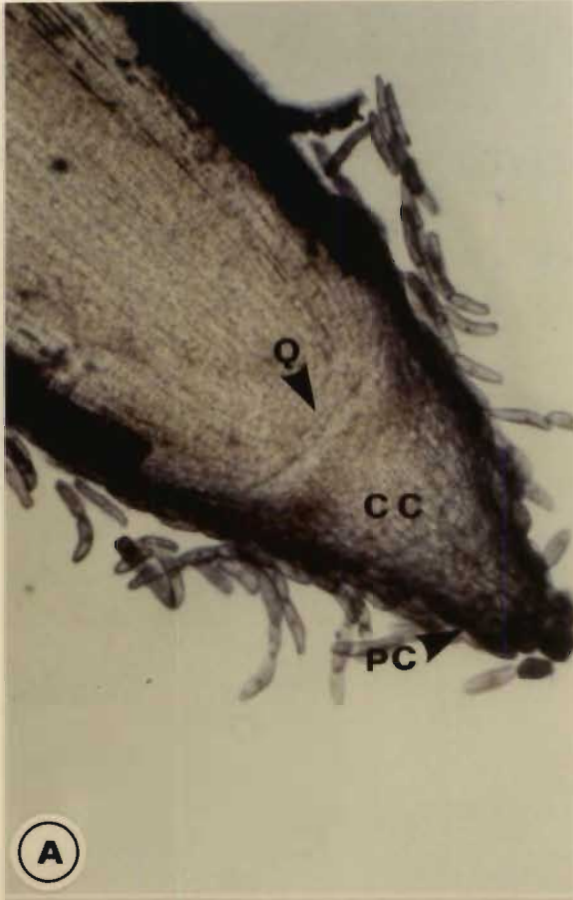
Figure 11   Micrographs of the root apices of <u>Zea mays</u> (cv. TX 24) treated with TTC to show the effect of various treatment times with 8,0 mg dm^{-3} Al on root respiratory activity (Section 4.3.1). The development of the highly pigmented, red, formazan in the root tissue as seen in longitudinal section was indicative of the occurrence and intensity of enzyme-catalysed oxidations (Sections 2.3.2.1; 2.3.2.2; 2.3.2.3).

A - Control (0 Al).

Note the high level of respiratory activity which occurred in the peripheral cap cells (PC) and in the cells of the quiescent centre (Q).

B - 2 h Al treatment has significantly reduced the level of activity in the peripheral cap cells, epidermis and quiescent centre. There was, however, evidence of a general increase in respiration in the cells of the central cap and cap and proximal meristems.

C - 12 h Al treatment has inhibited respiration throughout the root, and only isolated activity was evident.

D - 48 h Al treatment has caused further reductions in respiration, and only isolated activity of low intensity was evident in the cells of the root meristem proximal to the quiescent centre and including the pole of the stele.

Magnification A - D X 100
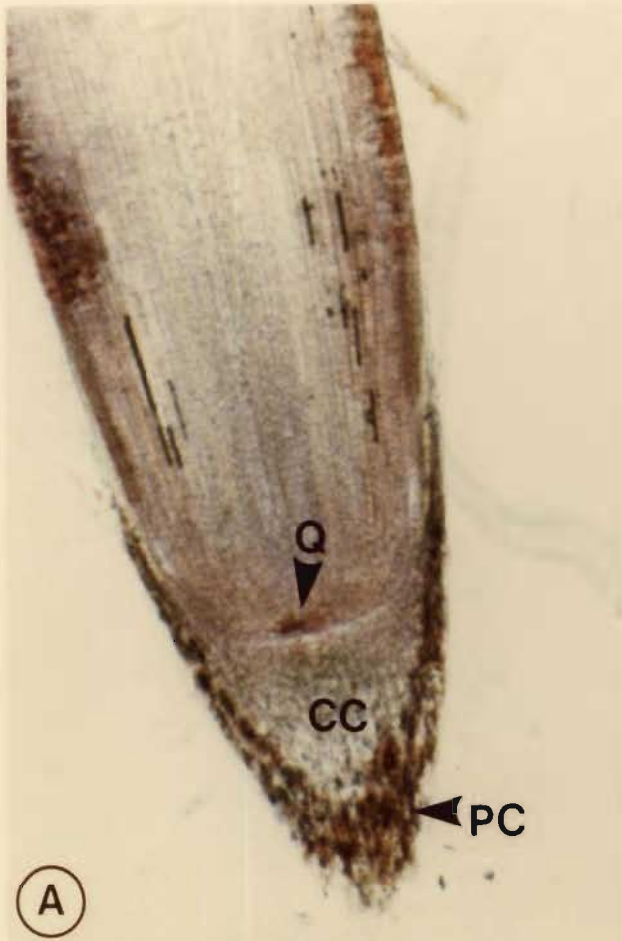
Figure 12 Low magnification electron micrographs illustrating the
          effect of treatment with 8,0 mg dm^{-3} Al for different times
          on the ultrastructure of cap cells (Section 4.3.2).

          A - Cap meristem, control (0 Al).

          B - Cap meristem, 20 h Al treatment.

              Note the resistance of the nuclear/nucleolar ultra-
              structure to Al-induced changes. Aluminium treatment
              was however, associated with an increase in vacuolation
              of these cells and, although plastids were still
              numerous in Al-treated roots, there was, in contrast to
              the control (A), little evidence of the occurrence of
              starch deposition.

          C - Cap columella, control. Cells were characterized by
              an absence of vacuoles. Amyloplasts were present with
              defined starch grains (unlabelled arrow).

          D - Cap columella, 6,5 h Al treatment produced
              an increase in the number of vacuoles, and the starch
              grains present in amyloplasts, showed an increased
              intensity of stain.

          E - Cap periphery, control.

          F - Cap periphery 20 h Al treatment. Comparisons between
              Figures E and F indicated that Al treatment was notable for
              the extensive development of vacuoles in these cells,
              accompanied by a decreased frequency with which cytoplasmic
              organelles were encountered. Cell walls were reduced in
              thickness and the appearance of wall material was altered.
              Golgi apparatus material was seen to accumulate exterior to
              the plasmalemma. Amyloplasts were characterized by a
              marked increase in the intensity of stain developed in
              starch bodies (unlabelled arrows).
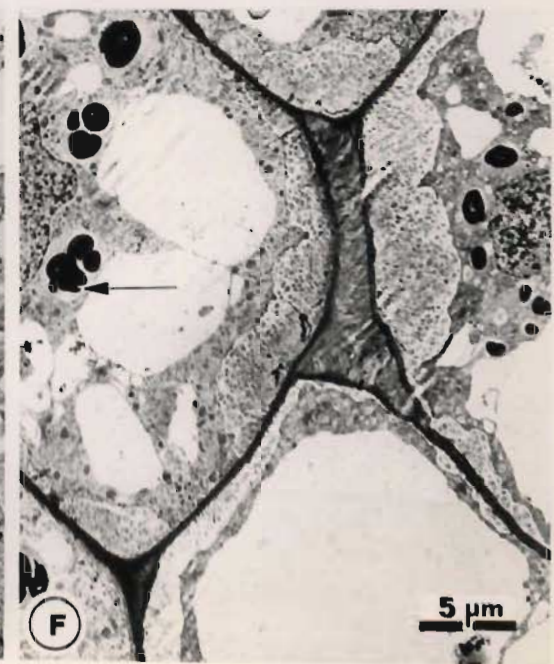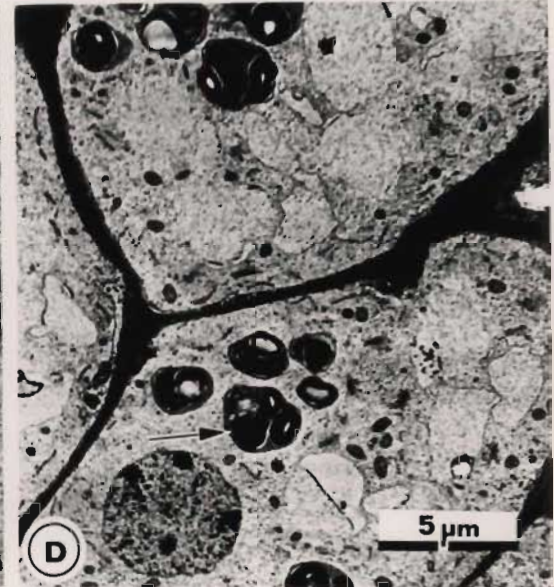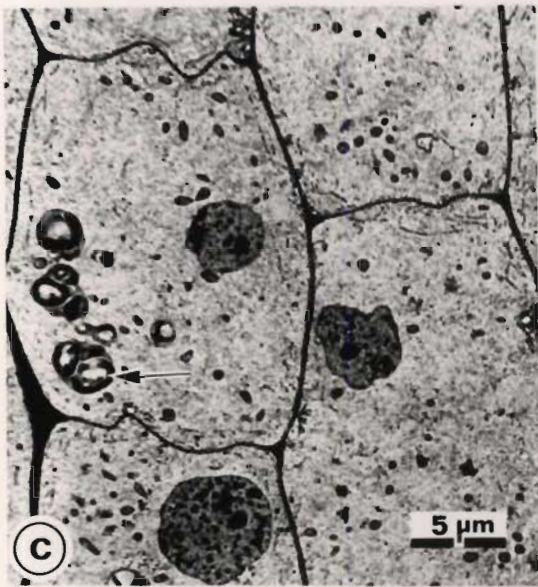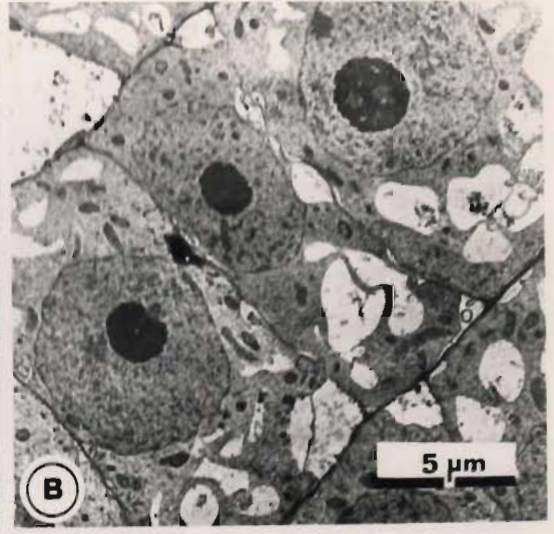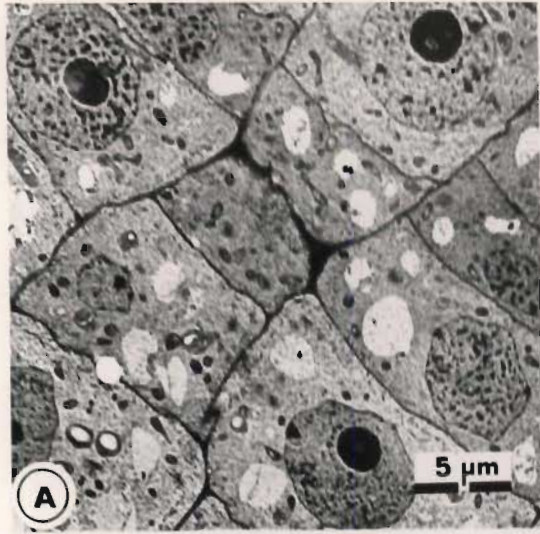
Figure 13 Electron micrographs illustrating morphological features
associated with Golgi apparatus function in peripheral root
cap cells of the control (0 Al) (Sections 4.3.2; 4.3.3).

A - Dictyosomes were characterized by their size, and many
showed a distinct polarity. Secretory vesicles (SV)
were large, and an alteration in shape from elongated to
round, occurred with maturation. Secretory vesicles
invariably contained a dense granular material.

B - Dictyosomes were frequently associated with ribosomes
on the forming face (unlabelled arrows) and ER.
Cisternal membranes were well-defined and cisternae were
regularly spaced in the dictyosomal stack.

C - The secretory activities associated with peripheral cap
cells were illustrated by the number of secretory
vesicles (SV), several of which appear near the plas-
malemma, and the abundance of Golgi apparatus-derived
material (GM) accumulated exterior to the plasmalemma.
The Golgi apparatus material retained the granular
appearance of material contained in the secretory vesicles.
Dictyosome (D); endoplasmic reticulum (ER);
mitochondria (M).
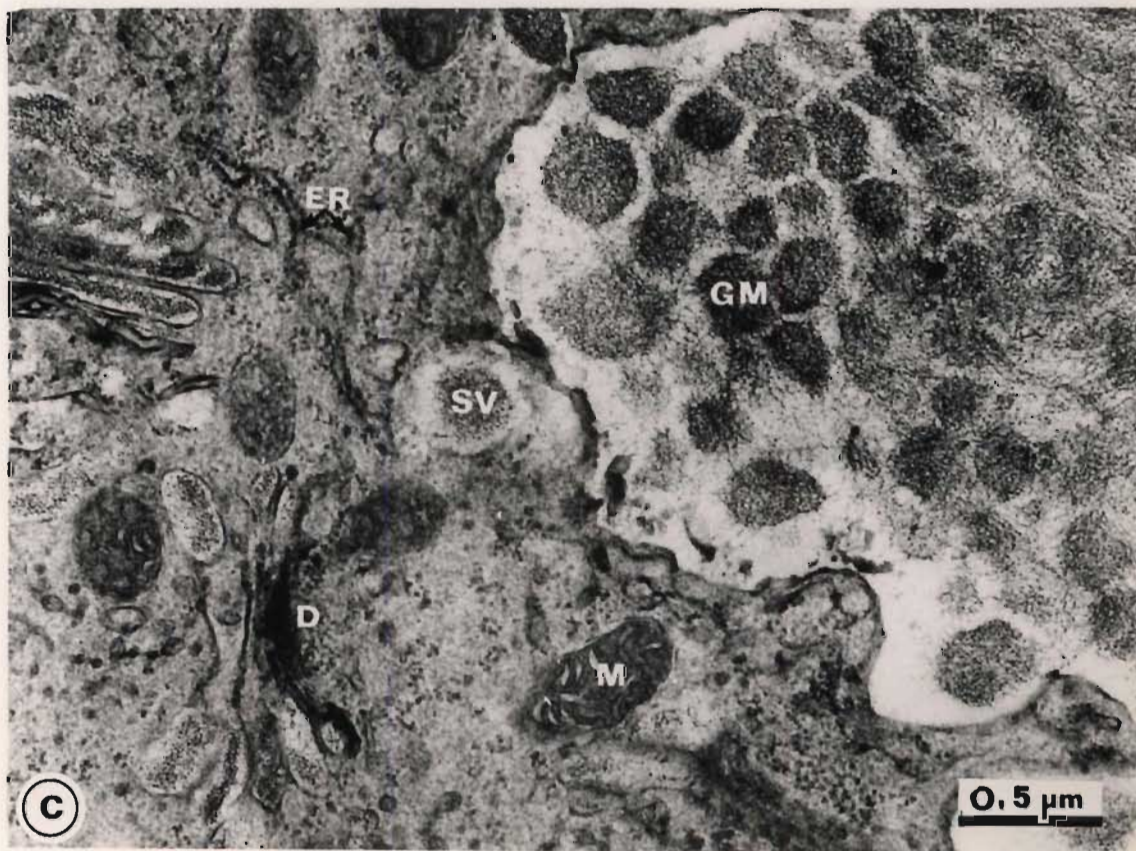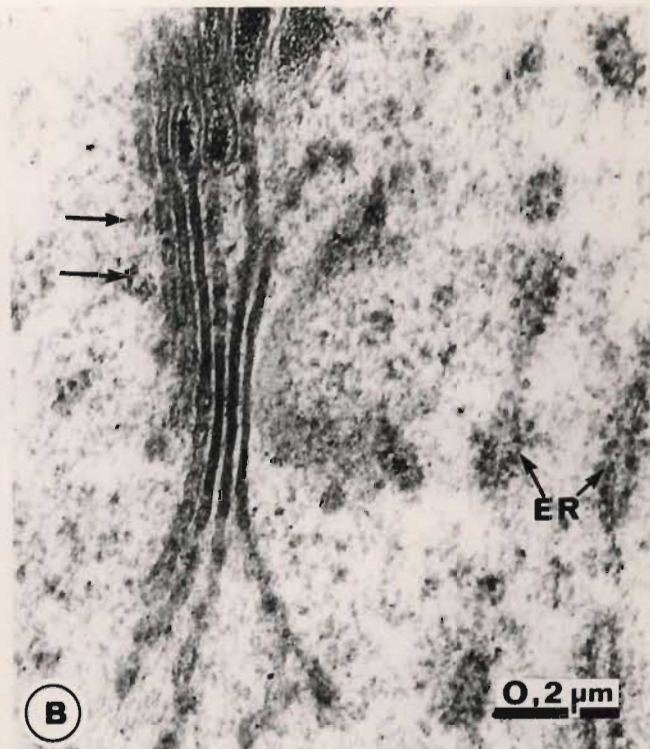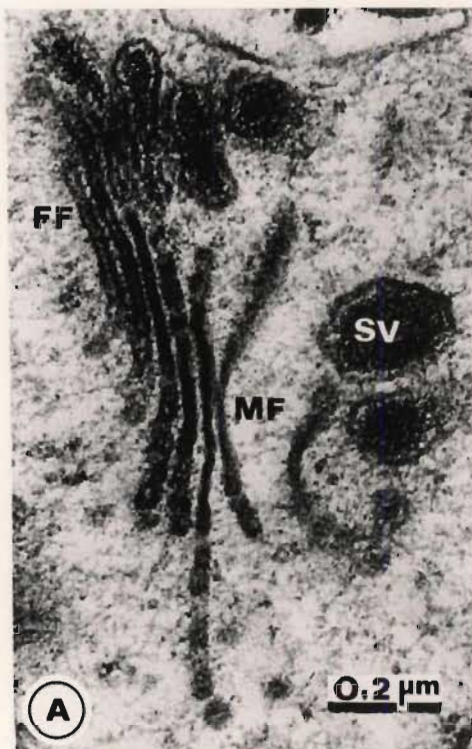
Figure 14 Electron micrographs illustrating the effect of 2 h treatment
with 8,0 mg dm^{-3} Al on the Golgi apparatus function in
peripheral root cap cells (Sections 4.3.2; 4.3.3).

   A - The effect of Al treatment was expressed through
       disintegration of many dictyosome vesicle membranes
       (unlabelled arrows) and the contents of secretory vesicles
       were found to become less granular in appearance.

   B - Severe curling of the cisternae of dictyosomes was also
       observed (unlabelled arrows).   Secretory vesicles (SV)
       tended to accumulate in the vicinity of dictyosomes and
       some of the smaller secretory vesicles appeared to be empty.

   C - Inhibition of vesicle transfer by Al was indicated by the
       accumulation of secretory vesicles (SV) in the cytoplasm
       where many vesicles could be observed in the vicinity of
       dictyosomes.   Differences in the appearance of the contents
       of secretory vesicles were also evident, and secretory
       vesicles containing either dense granular material or more
       diffuse fibrillar material, could be distinguished at this
       stage.   The Golgi apparatus-derived material (GM) exterior
       to the plasmalemma was less abundant and more fibrillar in
       appearance.

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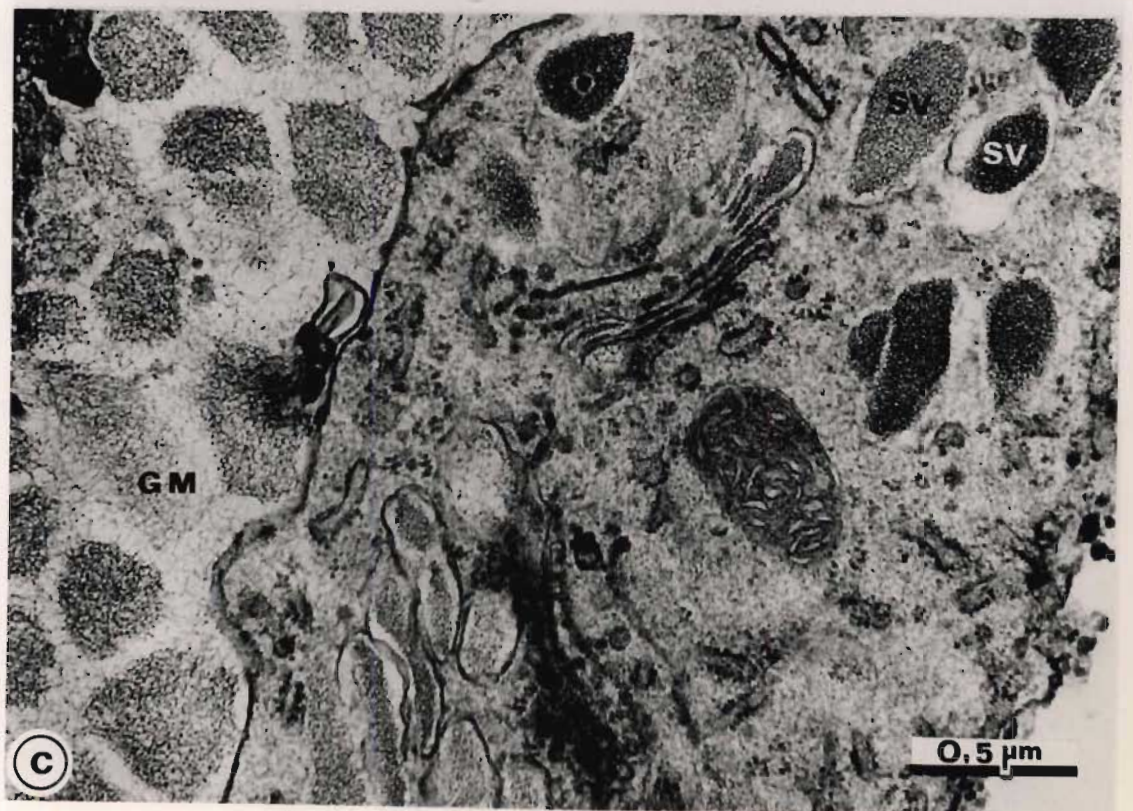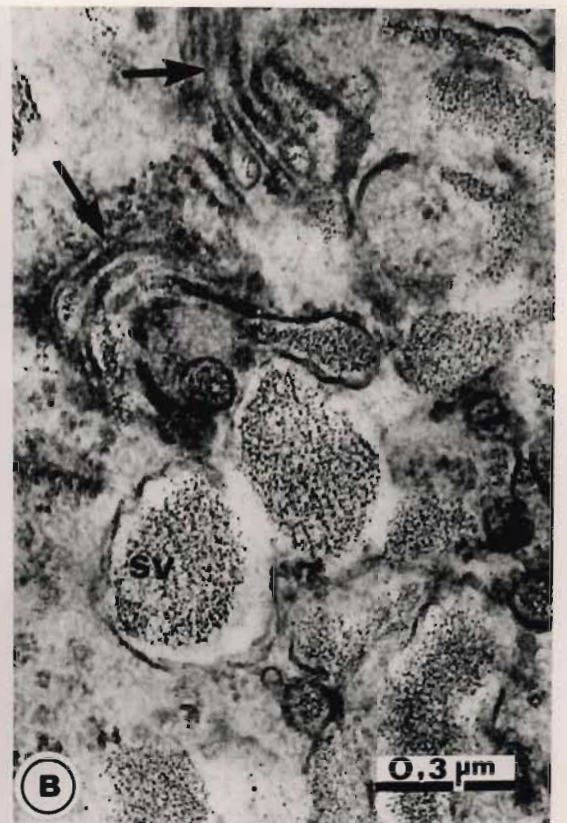
Figure 15 Electron micrographs illustrating the effect of 6,5 h treatment with 8,0 mg dm^{-3} Al on the Golgi apparatus function in peripheral root cap cells (Sections 4.3.2; 4.3.3).

A - Further disintegration of vesicle membranes (unlabelled arrows) was evident with the longer Al treatment times. The nuclear membranes (NM) of the nucleus (N) were however, still intact.

B - Complete disorganization of the cisternal structure of some dictyosomes was also evident at this treatment.

Note the accumulation of secretory material evident in the vicinity of the disintegrating dictyosome. The secretory vesicles lacked definition, and the shape was variable. The membranes of the mitochondrium (M) were still well-defined.

C - The dictyosome (D) showed no evidence of activity, many membranes of secretory vesicles (SV) have disintegrated, and the secretory material was generally diffuse and fibrillar. Endoplasmic reticulum (ER), identified by the presence of ribosomes (unlabelled arrows), was swollen and a progression was suggested from swollen ER to the formation of vacuoles (V).
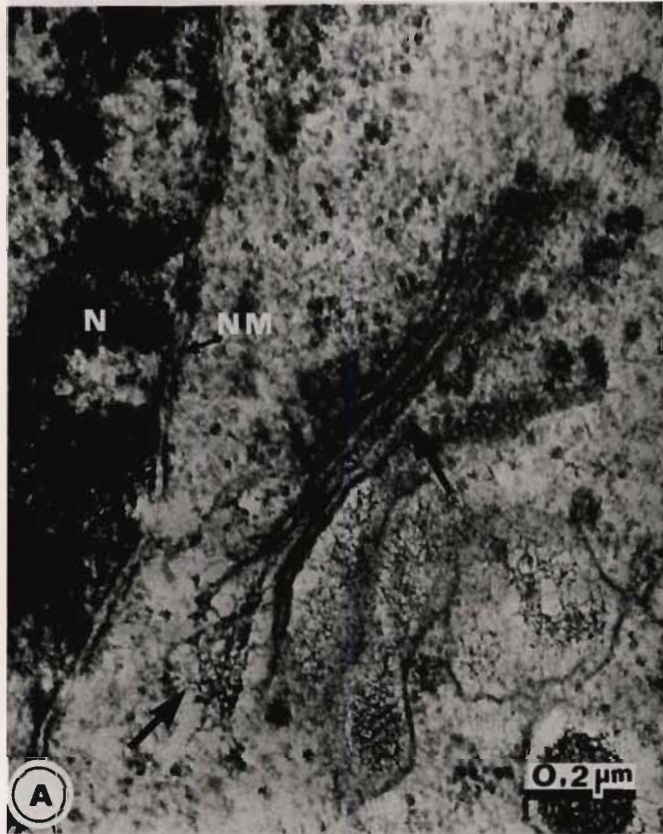
Figure 16   Micrographs of longitudinal sections of the primary root (Zea mays, cv. TX 24) treated with 8,0 mg dm^{-3} Al for various times to illustrate the progressive cellular disorganization of the root apex arising from Al treatment (Section 4.3.4).

A - Control, no Al added.

      Note the absence of cellular distortion (S - stele).

B - 6,5 h Al treatment time.   Cell enlargement (unlabelled arrow) was first evident in the cortex (S - stele).

C - 20 h Al treatment time.   Further cell enlargement, notably in the mid-cortical cells (unlabelled arrow), has resulted in the disintegration of the outer root shape.

D - 48 h Al treatment time.   Complete disruption of the cells of the root apex was clearly evident (S - stele).

Magnification A - D   X   60
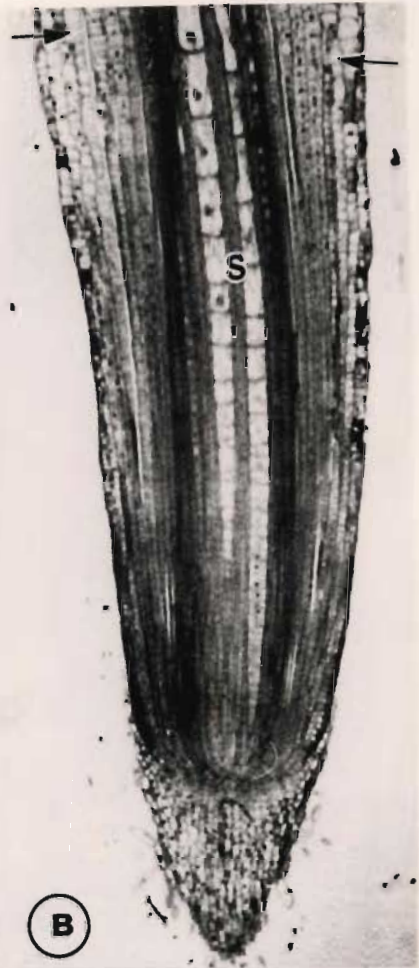
Figure 17   Micrographs of transverse sections of the primary root (<u>Zea mays</u>
            cv. TX 24) cut 1,5 mm from the root tip after treatment <u>with</u>
            8,0 mg dm^{-3} Al for different times to demonstrate the effects of
            Al-induced cell enlargement of cortical cells on the structure
            of the stele and the integrity of the outer root shape
            (Section 4.3.4).

   A - Control (0 Al), many cortical cells contained nuclei,
       and the majority were non-vacuolate.

   B - 6,5 h Al treatment.   Cells of the cortex have increased
       in size, and the majority were found to contain large
       vacuoles.   Distortion of the walls of the immature
       metaxylem (MX) vessels, arising from enlargement of
       cortical cells, was noted at this stage.

   C - 20 h Al treatment.   The anisotropic growth response of
       mid-cortical cells (unlabelled arrows) was clearly evident.
       The metaxylem (MX) vessels have now collapsed and
       disintegration of epidermal cells was also evident.

   D - 48 h Al treatment.   Disruption of the stele (S) and
       disintegration of the outer root cells through continued
       expansion of mid-cortical cells (unlabelled arrows) was
       well advanced at this stage.

   Magnification A - D X 100

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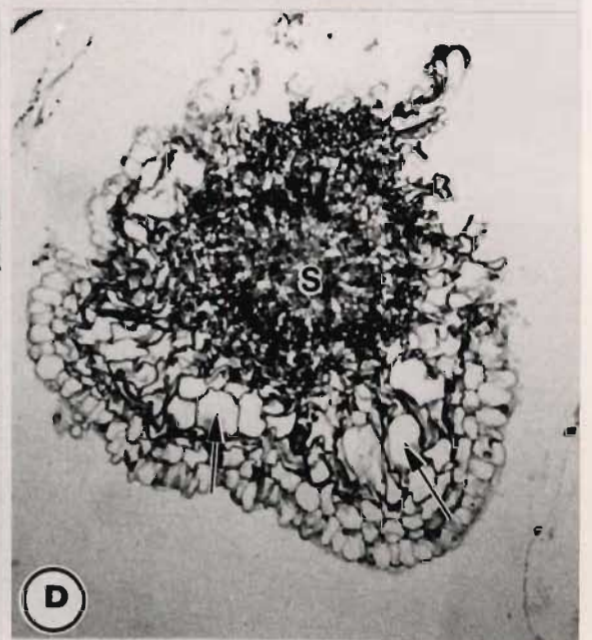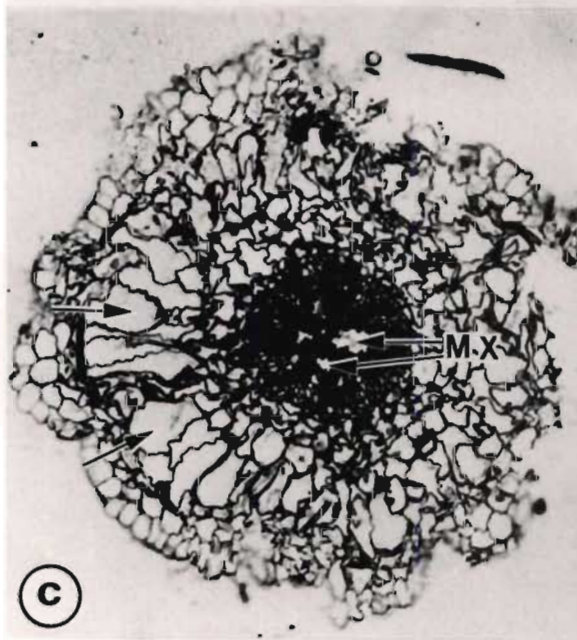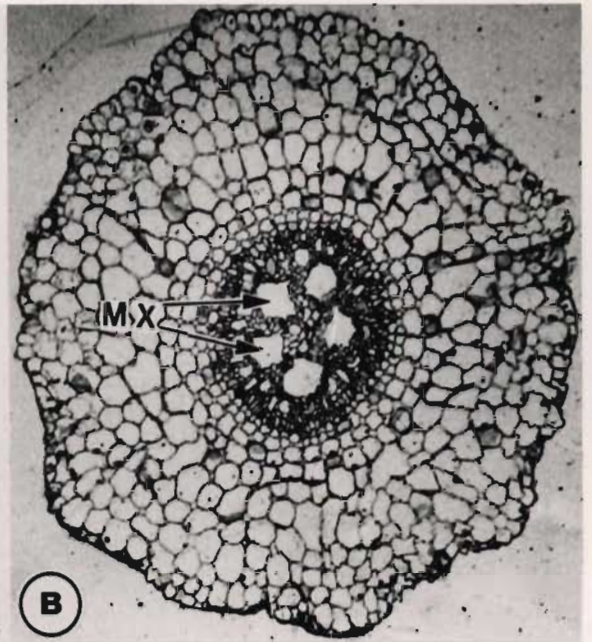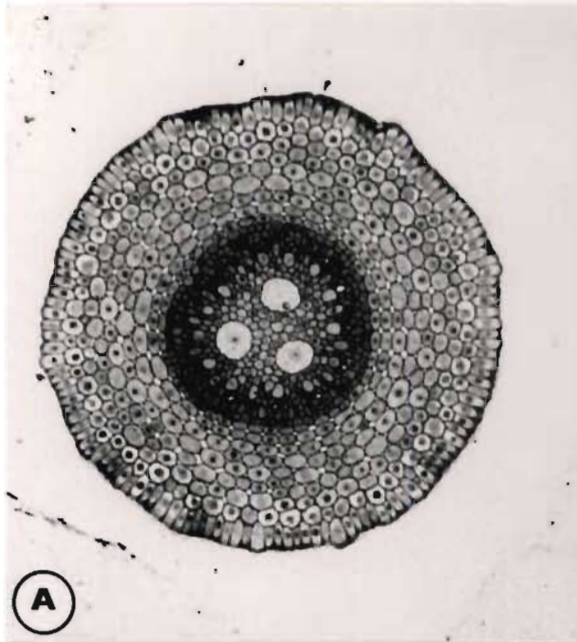
Figure 18   Relative (% of control, 0 Al) changes in mean cell volume
            brought about by treating the primary root (Zea mays cv. TX 24)
            with 8,0 mg dm⁻³ Al for different times.   Cell volume
            measurement covered the cell populations comprising the quies-
            cent centre, epidermis, outer cortex (cell file 2) and mid-
            cortex (cell file 5) analysed in 0,5 mm segments for the
            apical 2,0 mm of the root (Section 4.3.4).

            Note that preferential cell enlargement was initially recorded
            in the quiescent centre and cortex 1,0 - 2,0 mm from the root
            tip.   With longer Al treatment times, increases in mean cell
            volume were essentially a feature of mid-cortical cells
            1,0 - 1,5 mm from the root tip.   No measurement of quiescent
            centre cells was possible with 20 h treatment time.
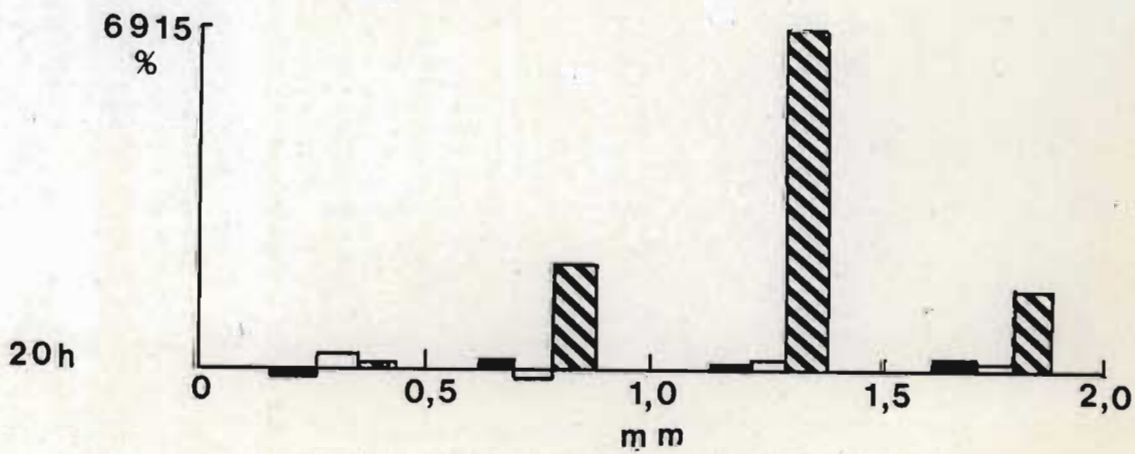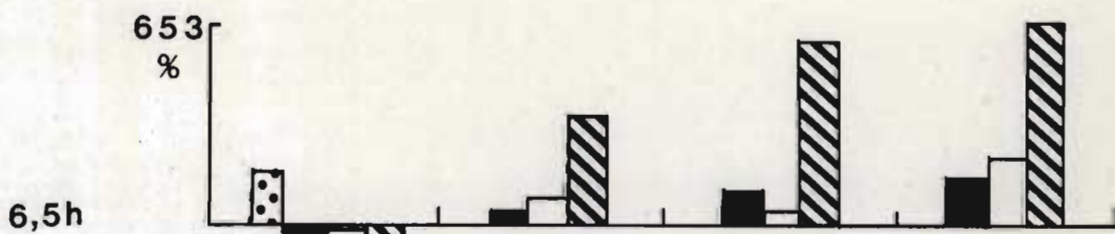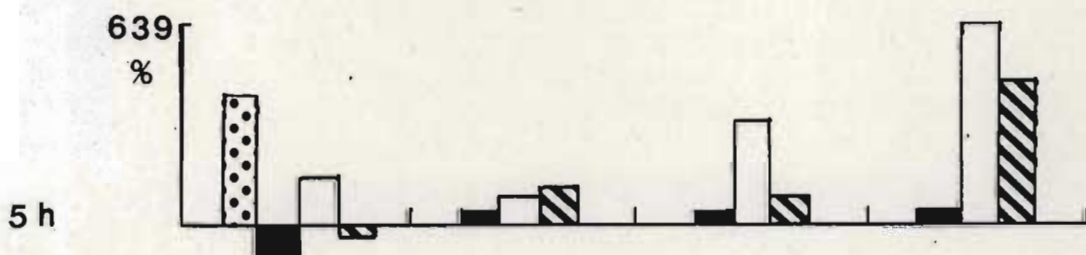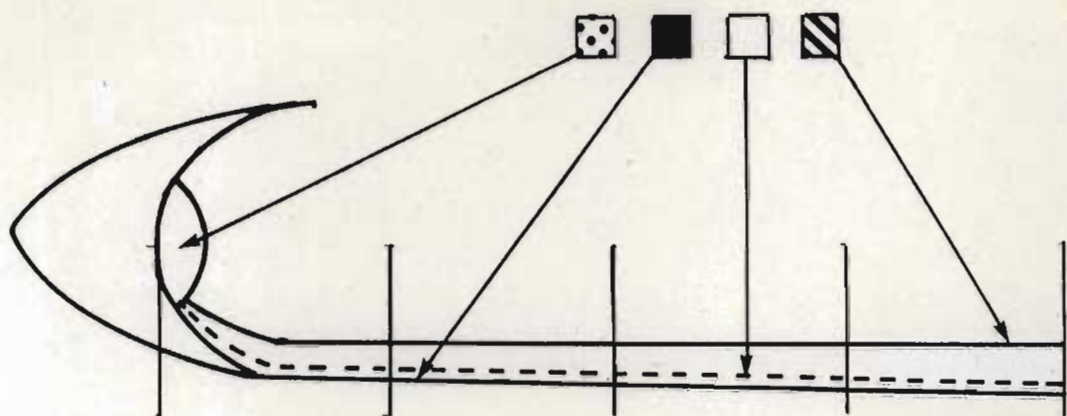
Figure 19   The effect on cell growth direction expressed as cell
            breadth (b)/cell length (l), brought about in the outer cortex
            (cell file 2) and mid-cortex (cell file 5) by treating the
            primary root (Zea mays cv. TX 24) with 8,0 mg dm^{-3} Al for
            different times (Section 4.3.4).

            Note that Al treatment caused a preferential increase in
            cell breadth in mid-cortical cells.   A change in cell
            growth direction may have arisen from changes in wall
            structure, required to accommodate cell enlargement
            against a decrease in pressure potential.

Growth Direction (b/l)

Root Exposure Time (hours)

Root Segments (mm)

Outer Cortex

Mid Cortex

39
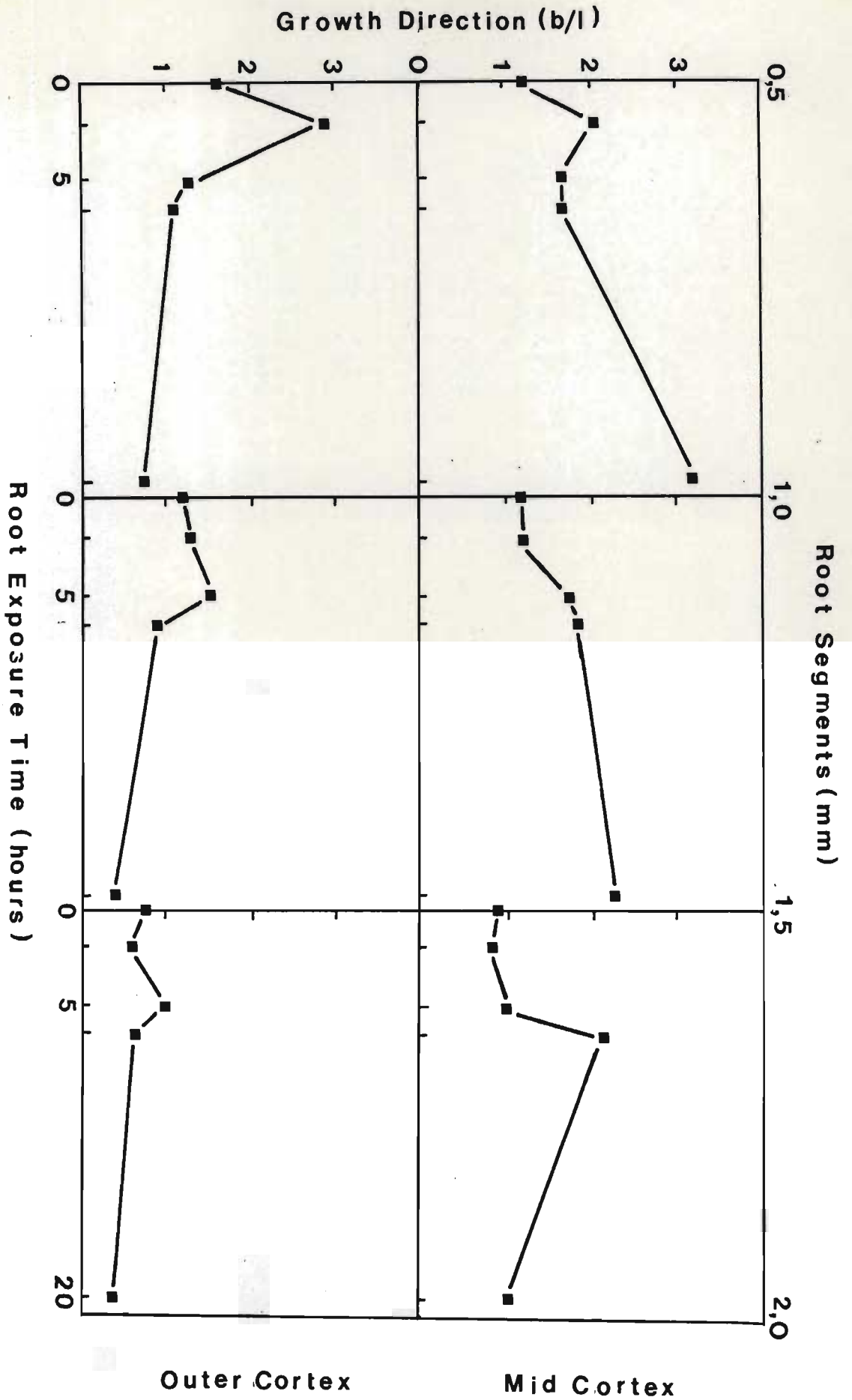
Figure 20   Electron micrographs of the cells of the proximal meristem
            adjacent to the cap junction showing the effects of treating
            the primary root (Zea mays cv. TX 24) with 8,0 mg dm^{-3} Al
            for different times (Section 4.3.5).

       A - Cortical cells showing the uncharacteristic vacuolated
           nature of these cells following 6,5 h Al treatment.

           Note that the formation of cytoplasmic vacuoles (V) pre-
           ceded changes in the structure of the nucleus/nucleolus
           which were not yet evident in these cells.

       B - The structure of the nucleus/nucleolus (NU) of the control
           (O Al).   The distribution of dense (DC) and diffuse (DIC)
           chromatin, the appearance of the nucleolus and the size
           and frequency of nucleolar vacuoles was characteristic.

       C - 20 h Al treatment increased the size of well-defined
           nucleolar vacuoles (NV) and caused the appearance of
           intensely granular areas in the nucleolus (unlabelled
           arrows).   Cell location similar to B.

       D - The distribution of dense/diffuse chromatin and the
           appearance of the nucleolus was characteristic of outer
           cortical cells of the proximal meristem of the control
           (O Al).

       E - 20 h treatment with Al caused the chromatin to become
           dispersed, while the fibrillar and granular components of the
           nucleolus segregated from each other.   These changes
           were considered indicative of altered nuclear/nucleolar
           function in these cells (Section 4.4.6).
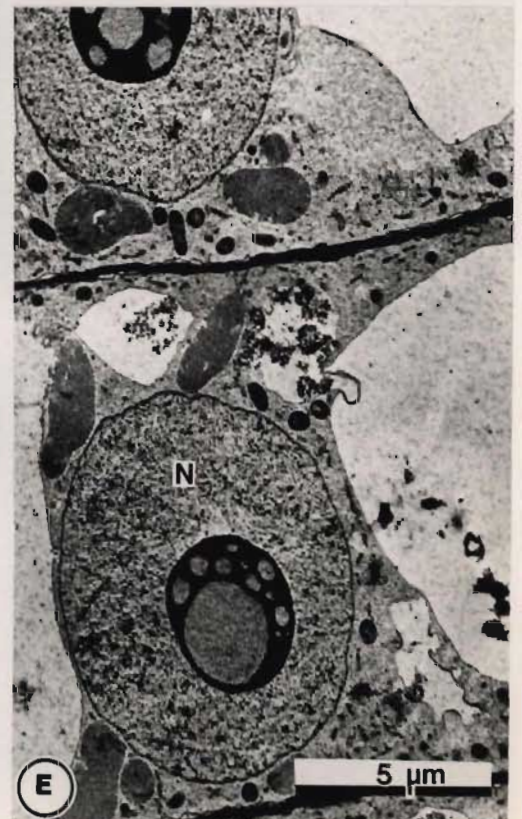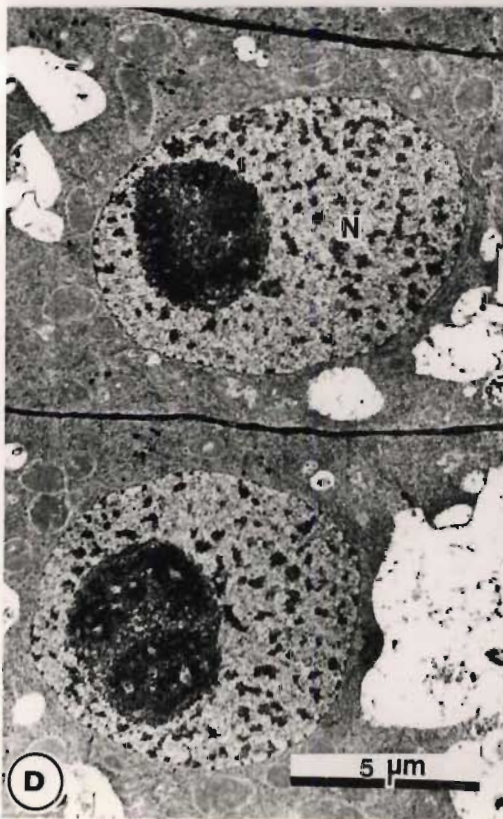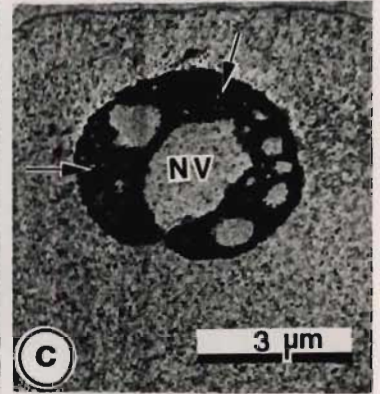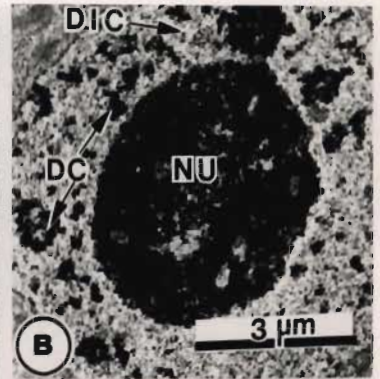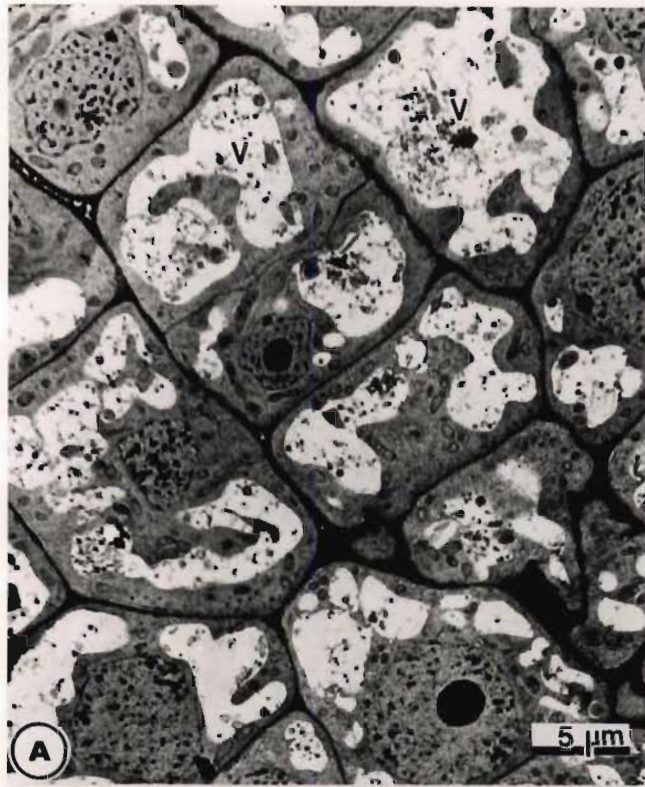
Figure 21   Changes in osmotic potential with time of cell sap expressed
            from the distal 20 mm of the primary root (Zea mays cv. TX 24)
            following treatment with 8,0 mg dm^{-3} Al (Section 4.3.6).
            Aluminium treatment initially (1 h) caused an increase in
            osmotic potential (increasingly negative value), while longer
            treatment times were connected to continued decreases in
            osmotic potential.   Each treatment comprised 69 plants.
            The experiment was repeated and the vertical scale lines
            represent the experimental range recorded over the two
            experiments (Section 4.2.2).

Osmotic Potential (– k Pa x 10²)

Root Exposure Time (hours)
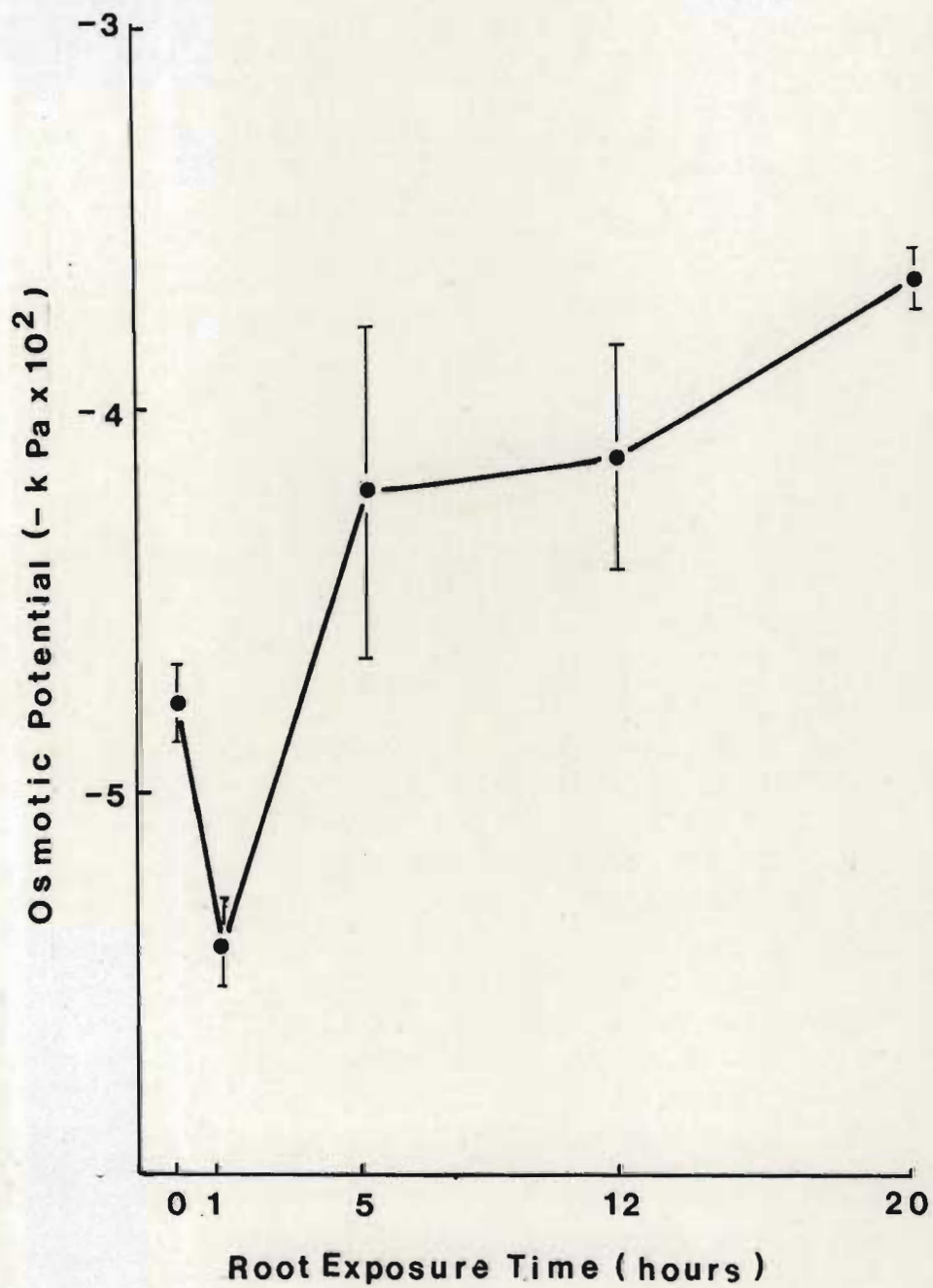
Figure 22 Longitudinal sections taken through previously decapped roots
(Zea mays cv. TX 24) to illustrate the effects of treatment
with Al (8,0 mg dm^{-3}) on the stages involved in the
regeneration of the root cap (Section 5.3).

A - Control (0 Al) 24 h after cap removal. Changes in the
pattern of cell division in the distal regions of the
root which preceded the formation of recognizable cap
tissue, were evident.

B - Decapped root treated with Al 24 h after cap removal.
The cap boundary remained intact and no evidence of cap
regeneration existed.

C - Control (0 Al) 48 h after cap removal. Recognizable
cap tissue was present, although the distinctive boundary
between the cap and proximal meristem had not yet formed.

D - Decapped root treated with Al, 48 h after cap removal.
No evidence of cap regeneration could be found.
Severe cellular disorganization of root apical cells
was, however, apparent which distorted the linear
pattern of cell files in the root apical tissues.

Magnification A - D X 90
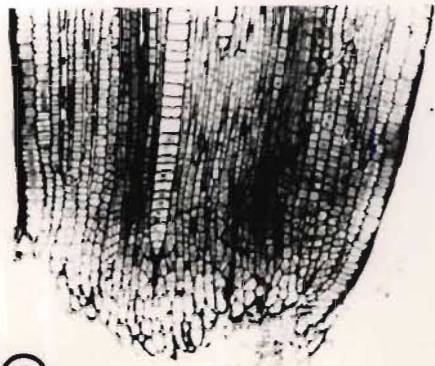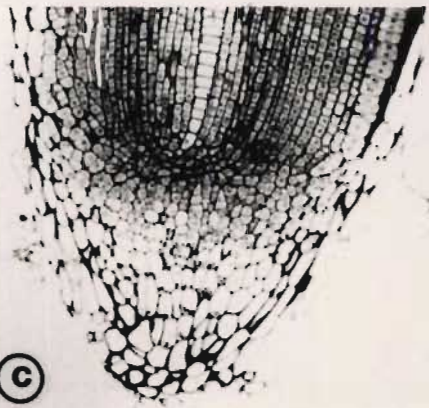
Figure 23 Electron micrographs of plastids and dictyosomes located in the cells of the quiescent centre of the control (0 Al) at different times after cap removal (Sections 5.3.1; 5.3.3).

A - Plastid in roots fixed immediately after decapping (0 h).

Note the absence of starch and presence of lipid drops (plastoglobuli)(L).

B - Dictyosome found in cells fixed immediately after decapping (0 h), characterized by the absence of secretory vesicles.

C - 6 h after decapping plastids showed the development of starch grains (S) and improved definition of membrane structure.   Lipid drops were still evident.

D - 6 h after decapping, dictyosomes were present with well-defined secretory vesicles (SV) which contained a granular material.   The shape of the vesicles contrasted with that found in the cap periphery of intact roots (Figure 13A).

E - The development of amyloplasts with well developed starch grains (S) was confirmed in cells of cortical lineage 24 h after cap removal.

Note the presence of a dictyosome with well-defined secretory vesicles near the amyloplasts.

F - Dictyosome in cell of cortical lineage 24 h after decapping showed the well-defined secretory vesicles which contained a granular material.

G - 24 h after cap removal, plastids in cells of stelar lineage showed many features consistent with repressed development.

Note the presence of electron-dense regions (DNA fibres) (unlabelled arrows), the simple structure and absence of starch grains.

H - Dictyosome in quiescent centre cell of stelar lineage characterized 24 h after cap removal, by the small size of secretory vesicles (SV) and paucity of a granular secretory product.

A

B

C

D

E

F

G

H
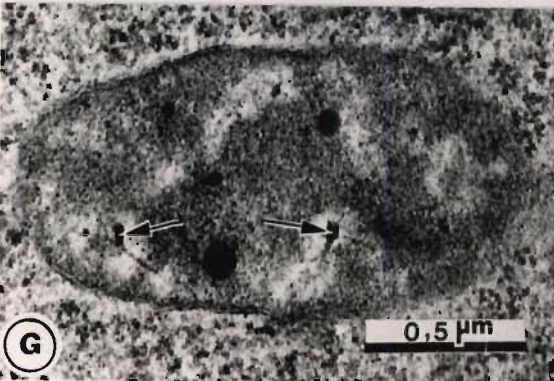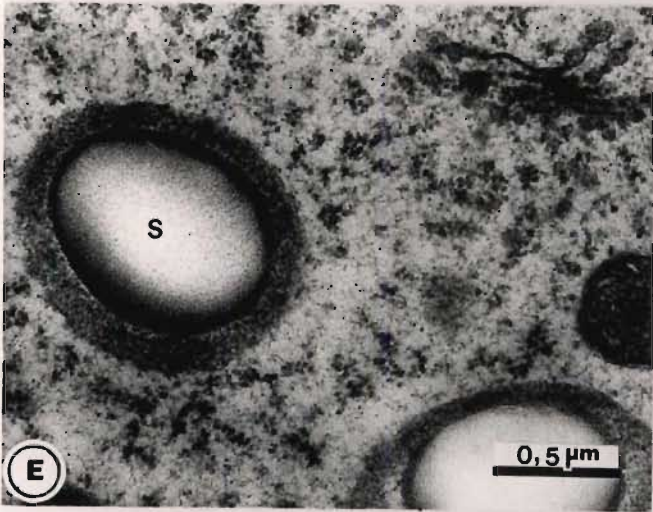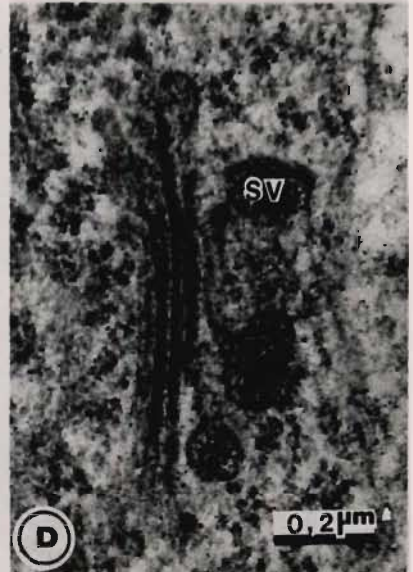
Figure 24 The effect of time after cap removal and of treatment of
          decapped roots with Al on Golgi apparatus activity in the cells
          of the quiescent centre (Section 5.3.1).

          In the absence of Al, Golgi apparatus activity increased in
          response to cap removal, as indicated by the relative fre-
          quencies (% of control) with which A dictyosomes,
          B cisternae per dictyosomal stack and C secretory vesicles
          were encountered with time after cap removal.   In contrast,
          Al treatment (8,0 mg dm^{-3}) depressed all these activities.

A. Dictyosomes

Control (−Al)

Treatment (8mgdm^{-3}Al)

B. Cisternae / Dictyosome

C. Secretory Vesicles

Relative Frequency (%)

Relative Frequency (%)(×10^2)

Time (hours) After Decapping
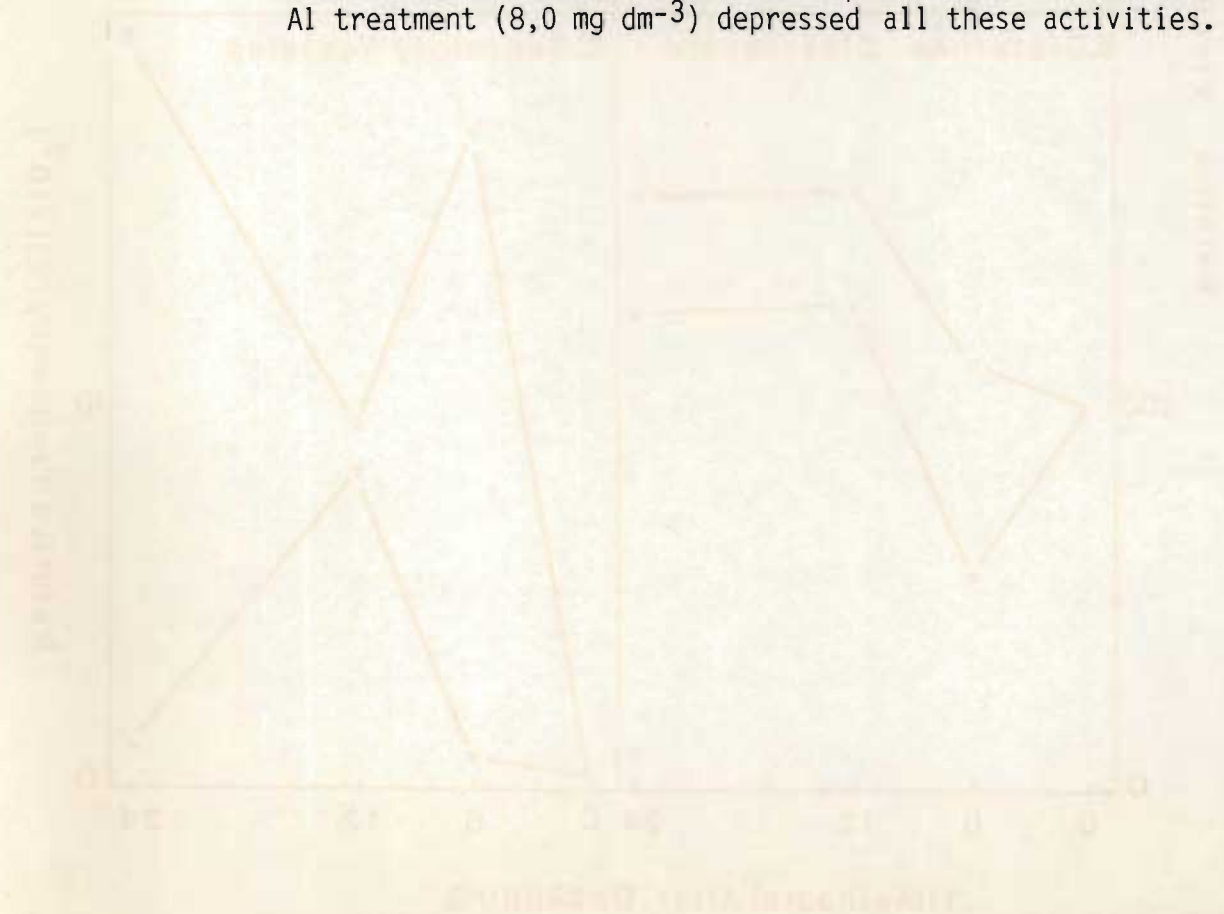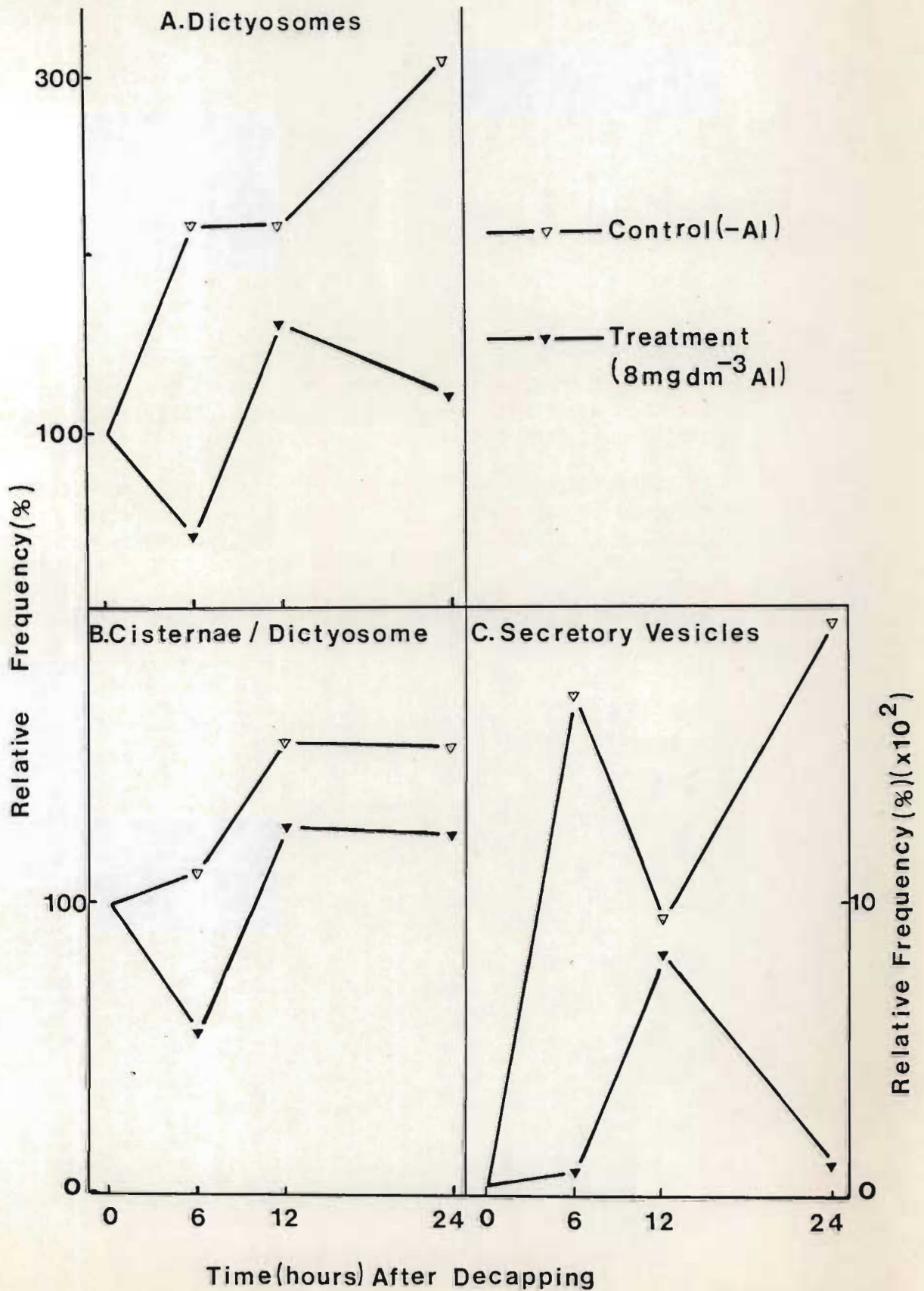
Figure 25 Electron micrographs of plastids and dictyosomes located
in the cells of the quiescent centre of decapped roots
treated with 8,0 mg dm^{-3} Al at different times after cap
removal (Sections 5.3.2; 5.3.4).

A - 6 h after cap removal, many plastids remained notable
for the absence of defined starch grains.

B - Plastid 12 h after decapping, characterized by an absence
of starch grains and poorly defined internal organization.
Membrane profiles were indistinct.

C - Dictyosomes 6 h after decapping were notable for the
absence of secretory vesicles and cisternal membrane pro-
files were often poorly defined.

D and F - Dictyosomes 24 h after decapping showed evidence of
increased secretory activity, but secretory vesicles
(unlabelled arrows) frequently accumulated near the
dictyosomes, and vesicle membranes were often poorly
defined, although there was some evidence of a granular
secretory product within the vesicles.

E - Plastids with defined starch grains 24 h after decapping.
Evidence of starch accumulation in plastids coincided with
the presence of dictyosomes.

Note the intensity of stain shown in the starch grains in
contrast to situation in the control (Figure 23E).

A   0,5 μm

B   0,2 μm

C   0,2 μm

D   0,2 μm

E   0,5 μm

F   0,2 μm
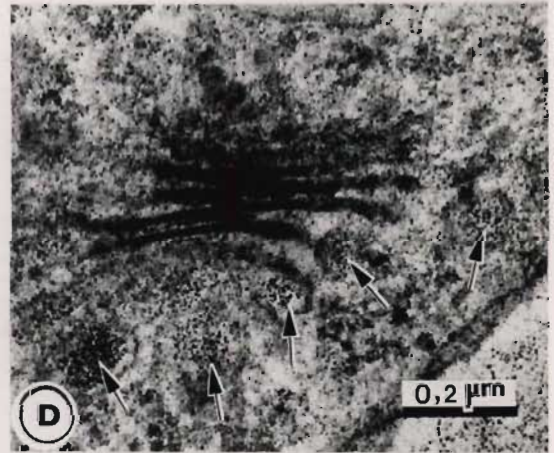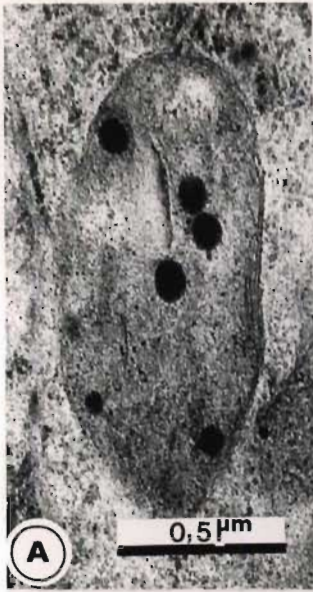
Figure 26   The effect of time after cap removal and of treatment of
            decapped roots with Al (8,0 mg dm^{-3}) on the differentiation of
            plastids located in quiescent centre cells (Sections 5.3.3;
            5.3.4).   This was indicated by the relative frequency (% of
            control) with which A plastids,   B electron-dense regions
            in plastids (DNA fibres), C starch grains and D lipid
            drops(plastoglobuli) were encountered for each treatment.

            In the absence of Al, differentiation of plastids was
            associated with morphological distinctions evident between
            plastids found in cells of cortical and stelar lineages.
            These differences which were not initially evident (12 h),
            affected the numerical frequency with which plastids, DNA
            fibres, starch grains and lipid drops were encountered in
            cortical (C) or stelar (S) tissues.   These differences are
            indicated by the dotted lines, while the solid lines
            represent the mean values.

            Aluminium-treated roots did not develop these differences, and
            treatment effects were represented by single values.

Control (−Al)
Treatment (8mgdm⁻³Al)

A. Plastids

B. DNA Fibres

C. Starch Grains

D. Lipid Drops

Relative Frequency (%)

Relative Frequency (%)

(×10²)

Time (hours) After Decapping

53
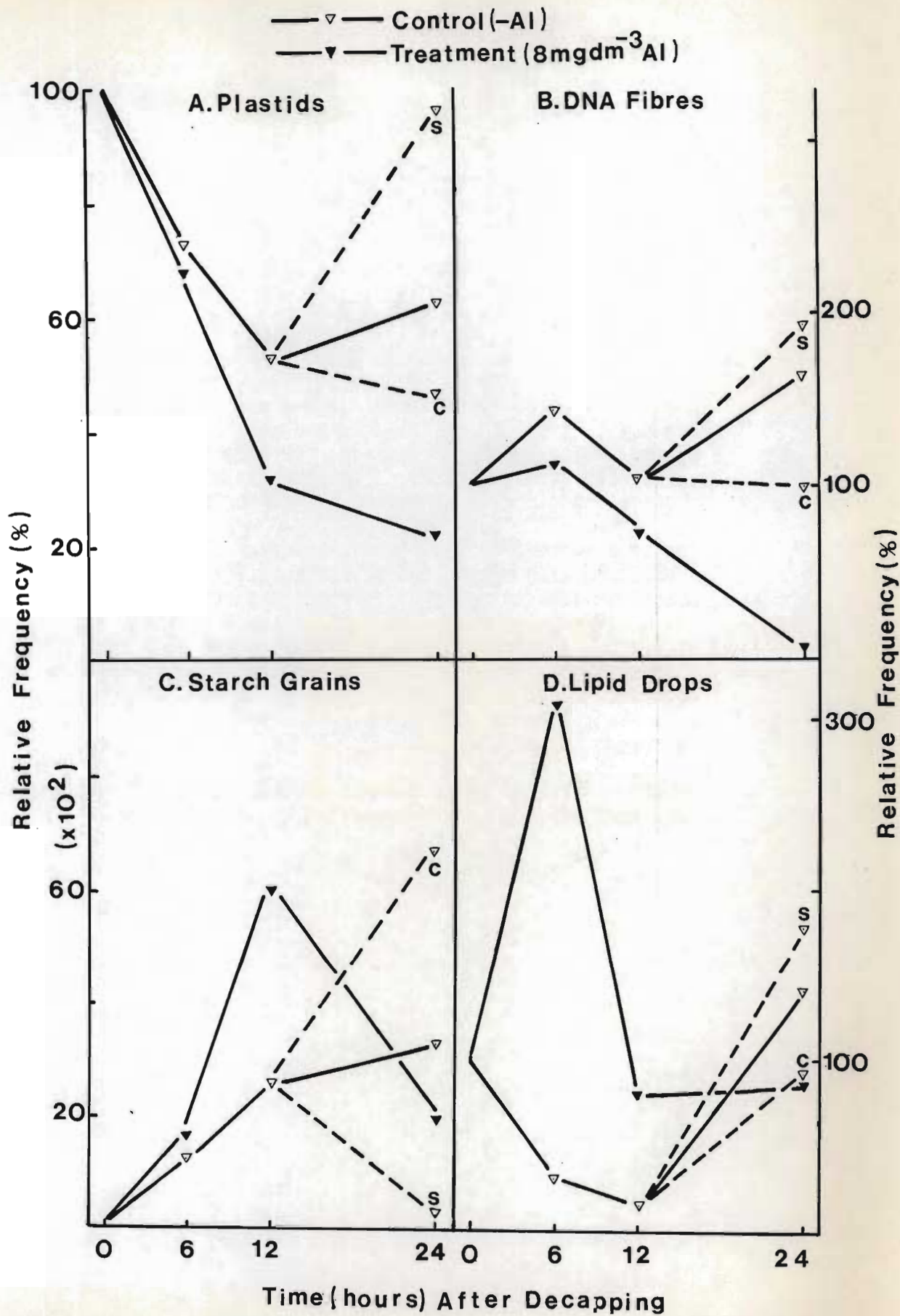
Figure 27 Electron micrographs of plastids and dictyosomes
of the Golgi apparatus in quiescent centre cells of cortical
lineage at various times after cap removal (control treatment
0 ABA) (Section 6.3).

A - Plastids immediately following cap removal were
characterized by their simple structure, absence of
starch, and many were observed to be dividing.
Lipid drops (plastoglobuli)(L) were present.

B - Dictyosome 6 h after cap removal.

Note the well-defined secretory vesicles (SV) containing
granular material.

C - Dictyosome 24 h after cap removal showing further
evidence of increased secretory activity involving the
elaboration of a granular secretory product.

D - Amyloplasts in quiescent centre cell 24 h after cap
removal showing large well-defined starch grains (S) and
dictyosome (D).
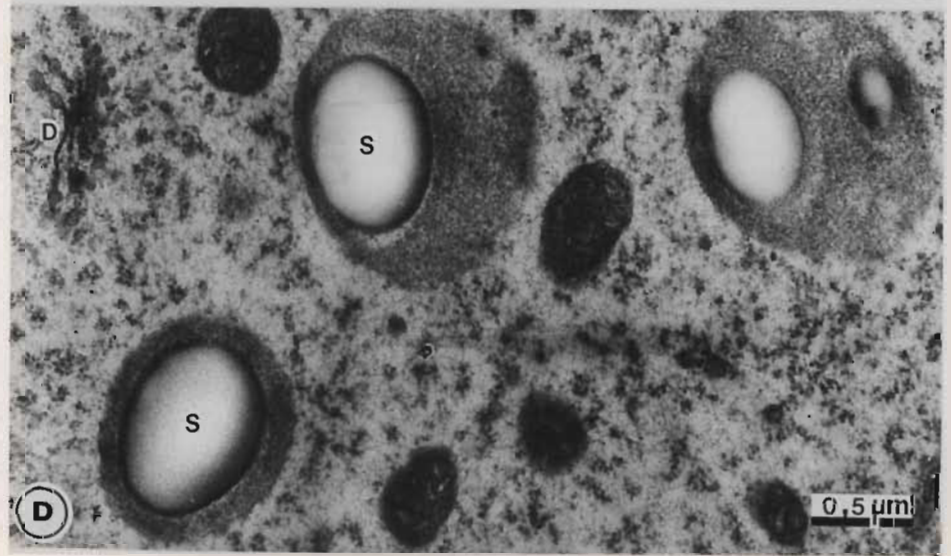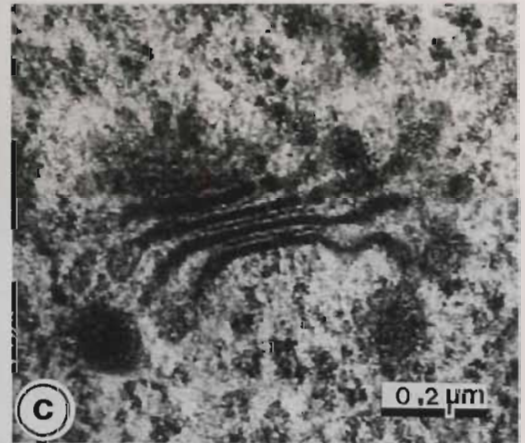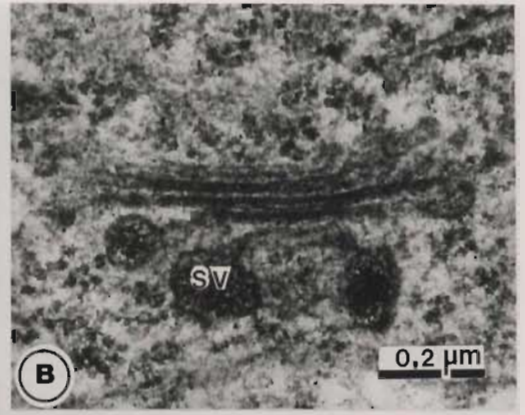
Figure 28 Electron micrographs illustrating the range of plastid structure
observed in quiescent centre cells of cortical lineage 24 h
after cap removal and treatment of the root (Zea mays cv. TX 24)
with ABA (Section 6.3).

Abscisic acid concentration Figures A, B, C 5 X 10^{-5} M:
Figures D, E 2 X 10^{-4} M.

Note the absence of differentiation. Plastids at both ABA
concentrations showed little internal organization.
Lipid drops (plastoglobuli)(L), small starch grains (S) and
occasional electron-dense regions (DNA fibres) (unlabelled
arrows) were present. In contrast to the situation in the
control (0 ABA) immediately following decapping (Figure 27),
many plastids in ABA-treated roots (24 h) were appreciably
smaller in size.

A   0,5 µm

B   S   0,5 µm

C   0,5 µm

D   S   0,5 µm

E   0,5 µm
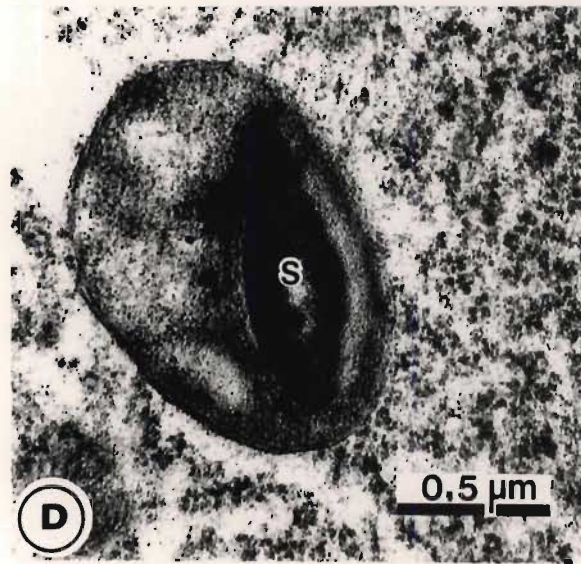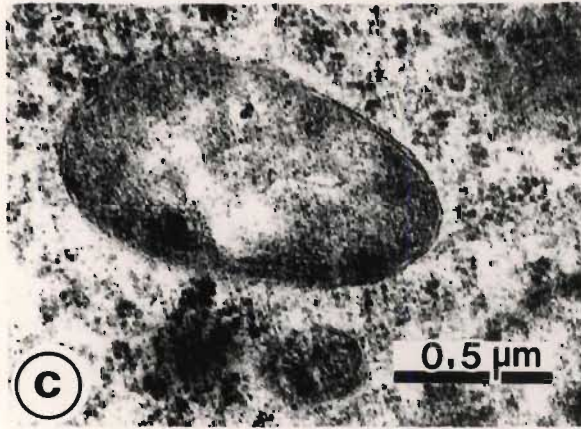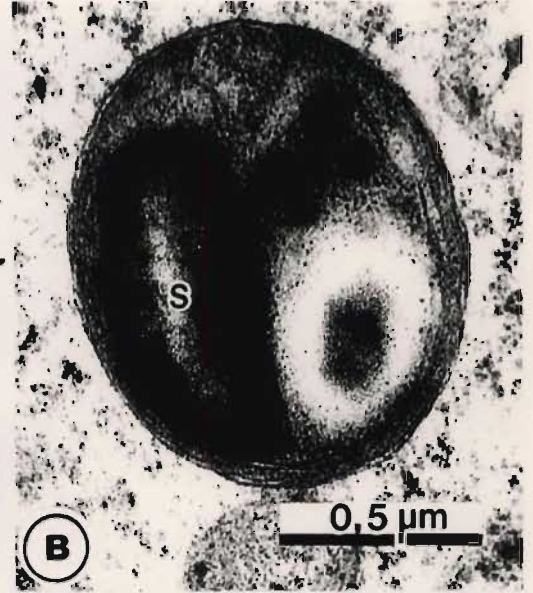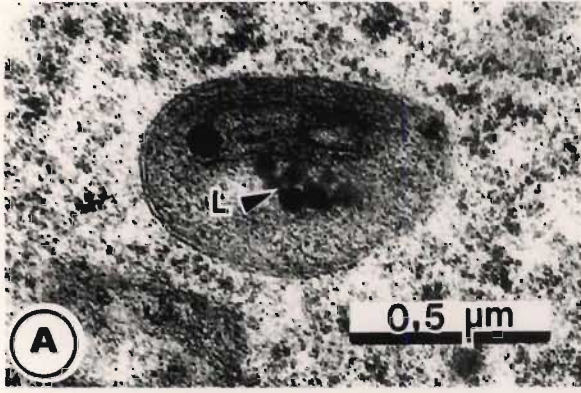
Figure 29   Electron micrographs illustrating the range of dictyosome
             structures encountered in quiescent centre cells of cortical
             lineage 24 h after cap removal and treatment of the root
             (<u>Zea</u> <u>mays</u> cv. TX 24) with ABA (Section 6.3).

             Abscisic acid concentrations FiguresA and B, 5 X 10^{-5}M
             Figures C and D 2 X 10^{-4} M.

             Note the repressed state of dictyosome development at both
             ABA concentrations, illustrated by the absence of large,
             well-defined secretory vesicles.   Dictyosomes also tended to
             be smaller at the higher ABA concentration and membrane
             profiles were usually also less distinct.
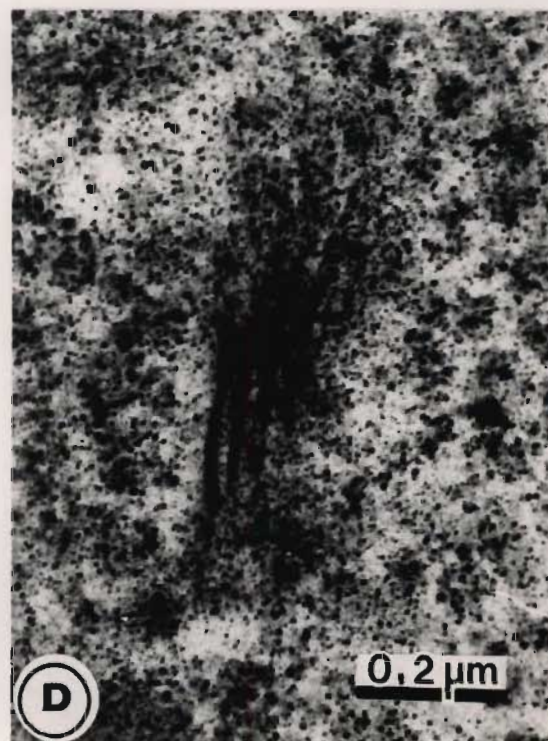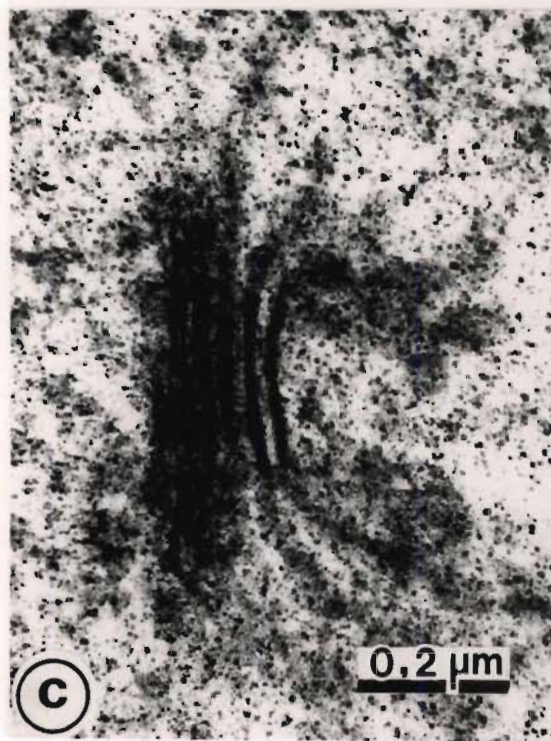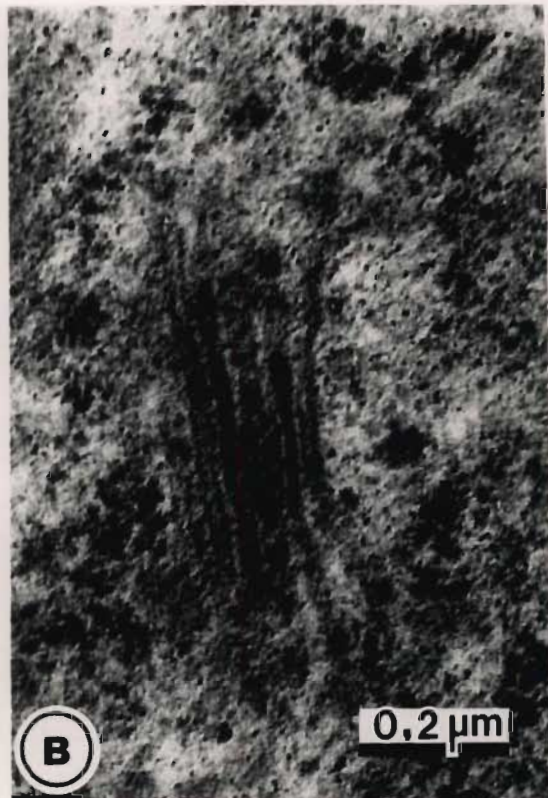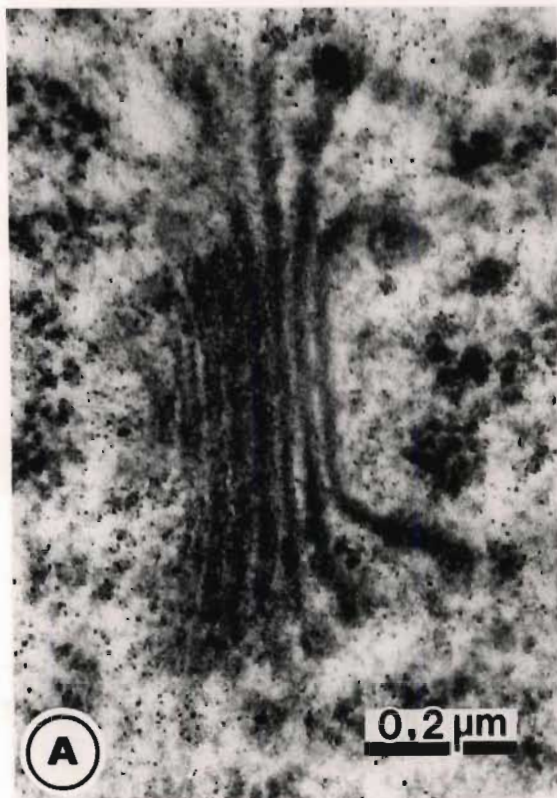
Figure 30 Diagrammatic representation of the root apex (<u>Zea mays</u>), defining parameters for the measurement of cells and cell populations referred to (Section 7.2.1).

      CC central cap, D root diameter,
      DM cap meristem, H cap height,
      PC peripheral cap, QC quiescent centre,
      W   width cap.

The central cap was defined as W/3.

61
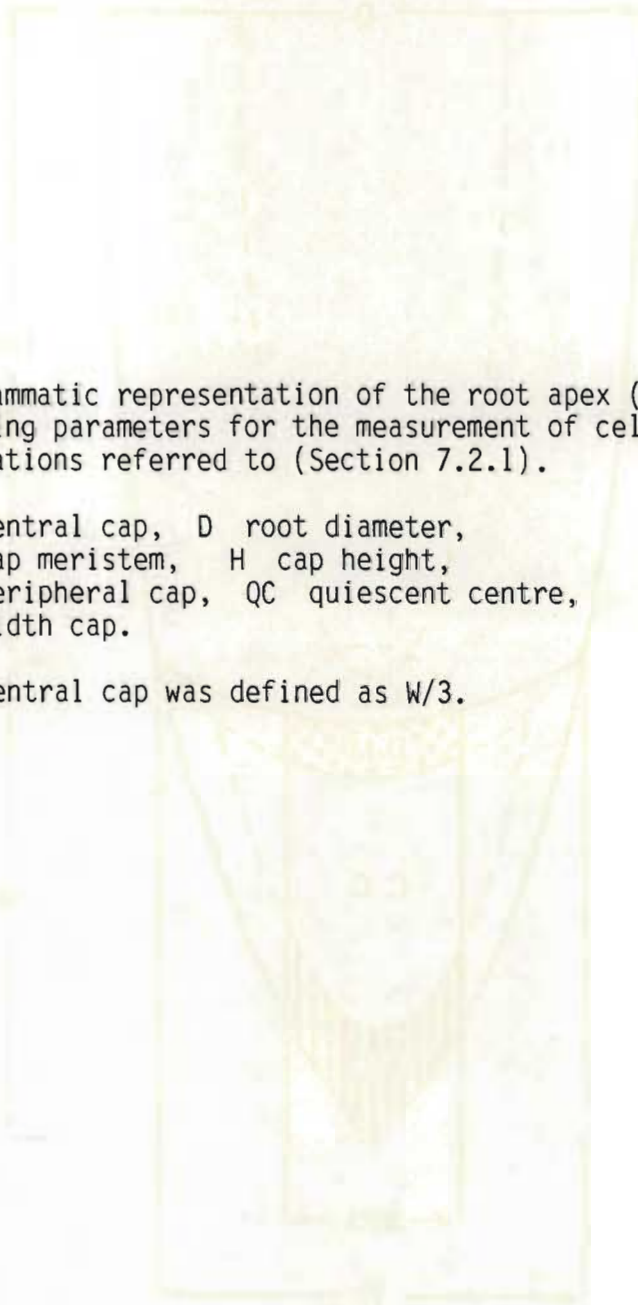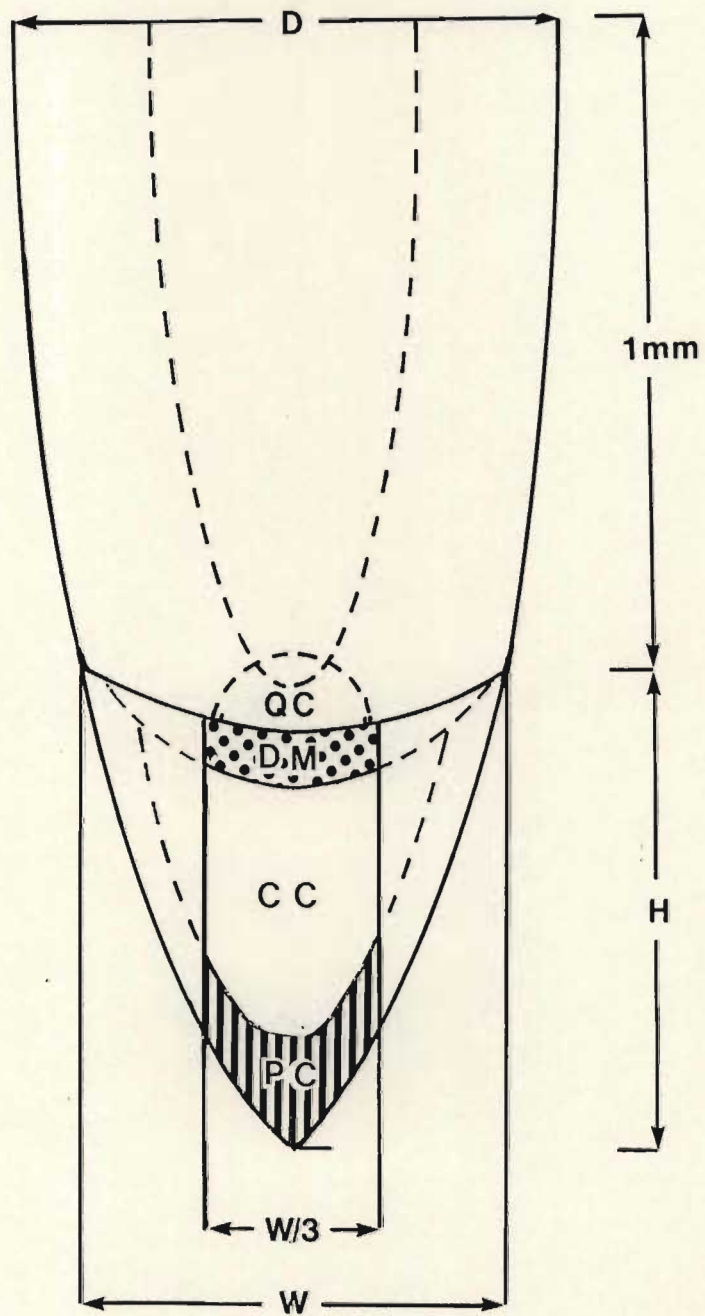
Figure 31   The effect of different Al concentrations (mg dm^{-3}) over a
29 h period on the mean volume (μm^3 X 10^6) of the root cap of
primary roots of <u>Zea</u> <u>mays</u> (cv. TX 24).   Error bars indicate
the S.E. of the mean (minimum of 5 roots included in deter-
mining each point)(Section 7.3.1).

63
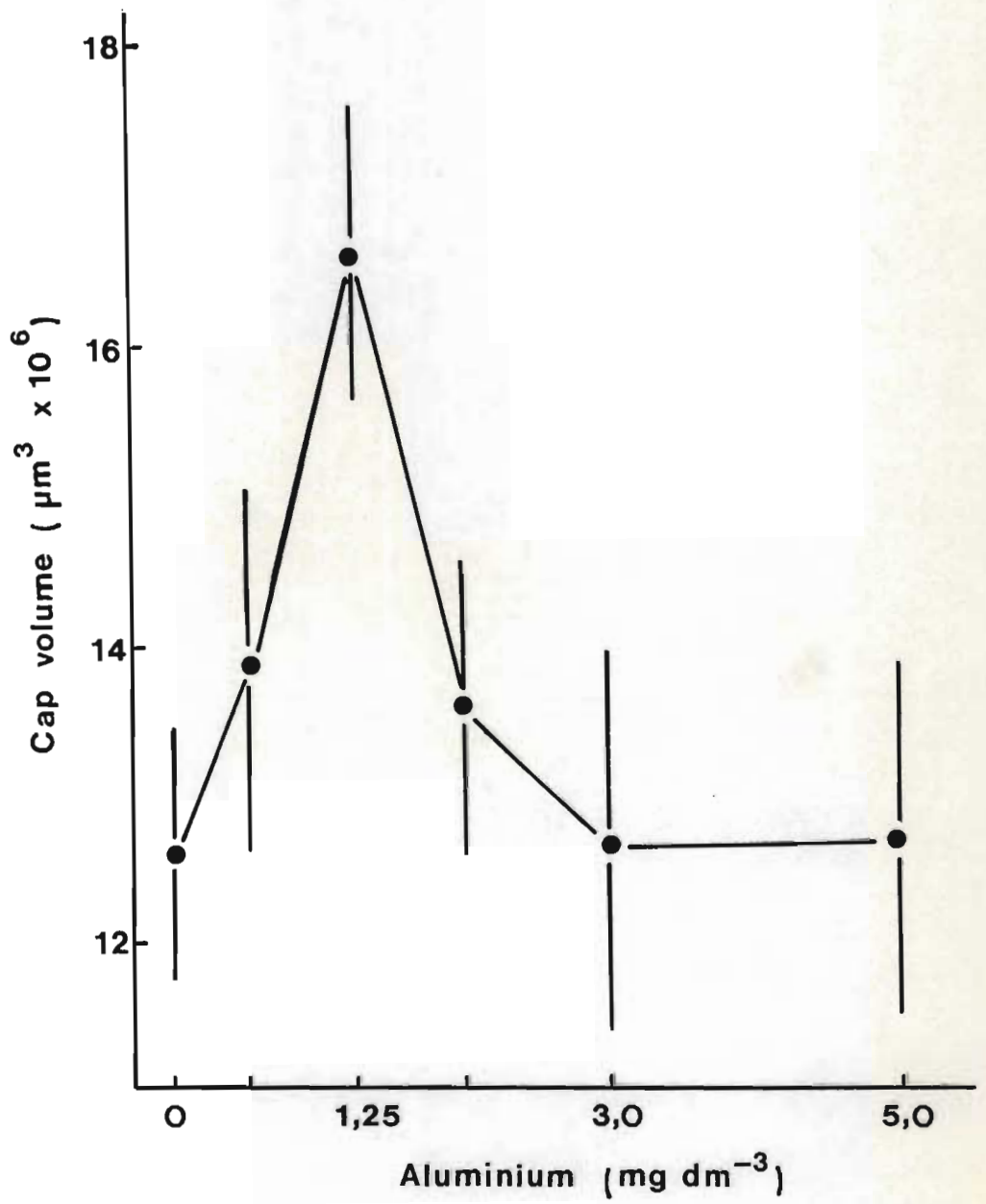
Figure 32 The effect of different Al concentrations (mg dm^{-3}) over a 29 h
period on the mean volume (μm^3 X 10^2) of peripheral cap cells
located at the distal end of the cap.   Error bars indicate
the S.E. of the mean (minimum 5 roots included in determining
each point)(Section 7.3.1).

65
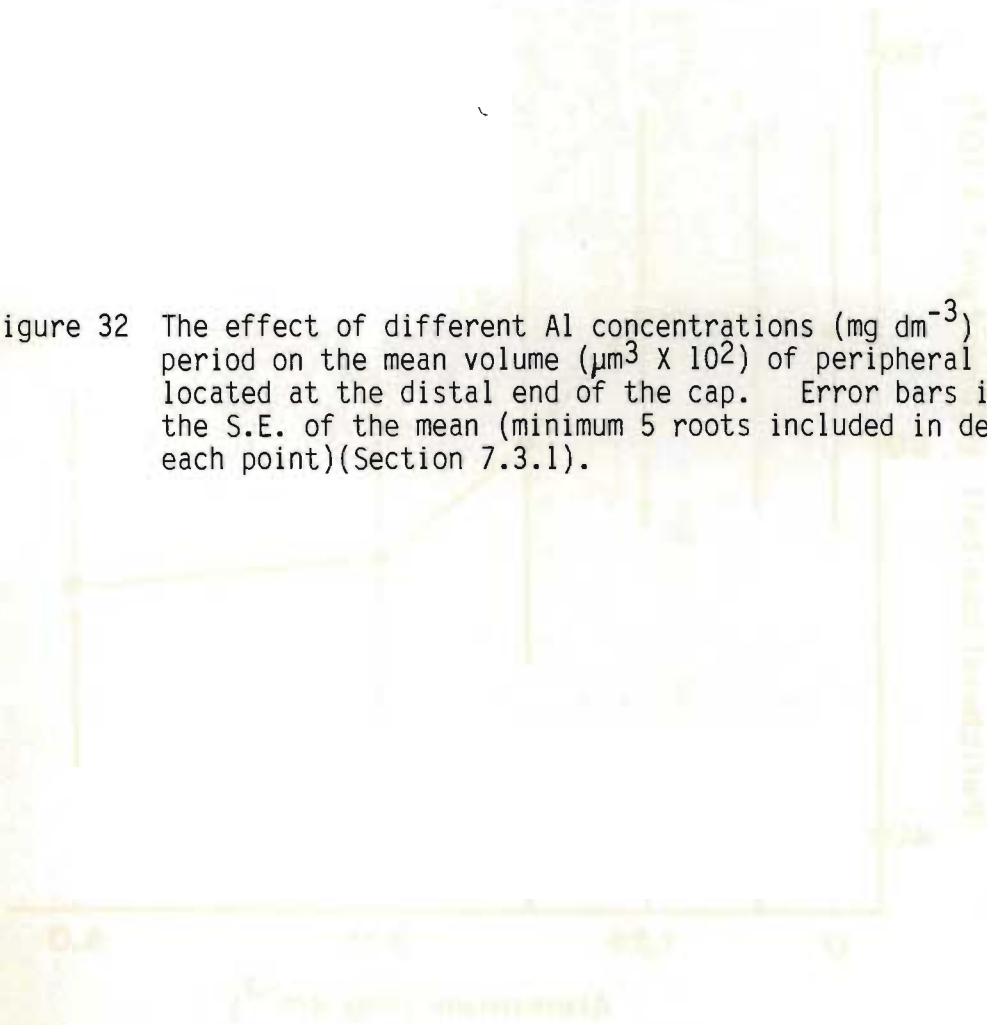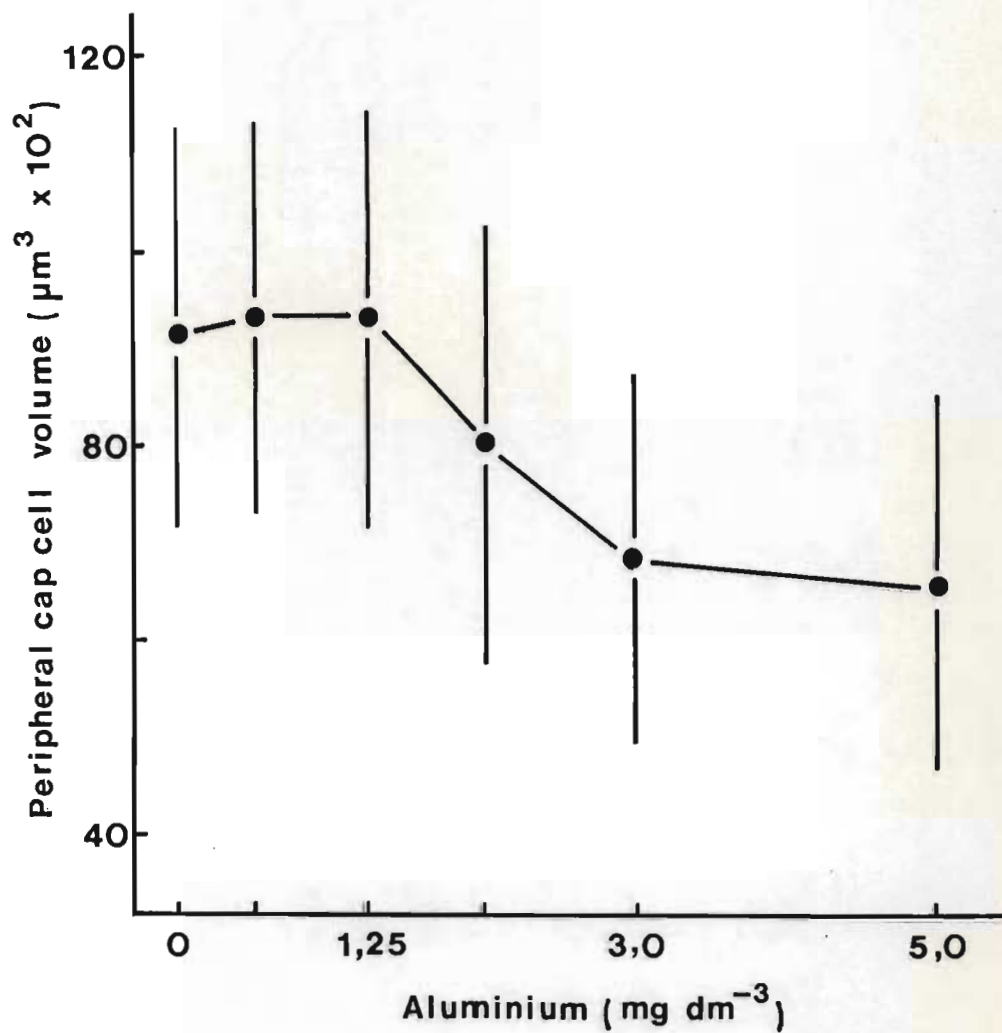
Figure 33   Micrographs of root cap cells showing the effects of different
            Al concentrations (mg dm^{-3}) on amyloplast numbers and
            distribution and cap cell geometry (Sections 7.3.1; 7.3.3).

        A - Control (0 Al).   Central cap cells, including the distal
            meristem, were characterized by the linear arrangement of
            cell files.   Amyloplasts were numerous and densely packed.
            Nuclei of cap columella cells were located towards the
            proximal pole of the cell.

        B - 2,0 mg dm^{-3} Al.   After 29 h, the linear arrangement of
            central cap cells was still evident, although amyloplasts
            were fewer and there was less evidence of sedimentation.
            Nuclei of some columella cells also appeared to be
            displaced from the proximal pole of the cell.

        C - 5,0 mg dm^{-3} Al.   After 29 h, the linear arrangement of
            central cap cells was no longer apparent.   Amyloplasts
            appeared not to sediment and cells were increasingly
            vacuolate.

        D - Control (0 Al).   Peripheral cap cells at the distal end of
            the cap.

        E - 5,0 mg dm^{-3} Al.   After 29 h, peripheral cap cells at the
            distal end of the cap showed (in comparison to D) a
            marked decrease in size.

        Magnification A - E   X 450
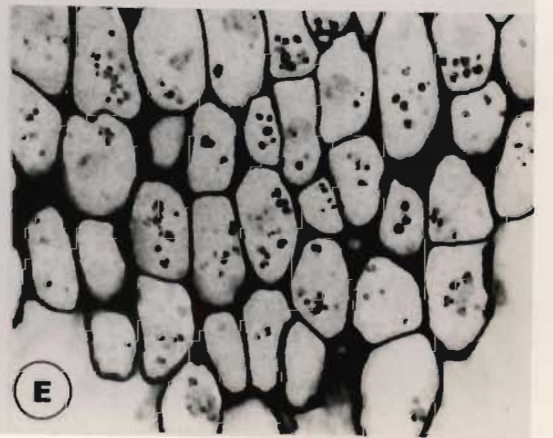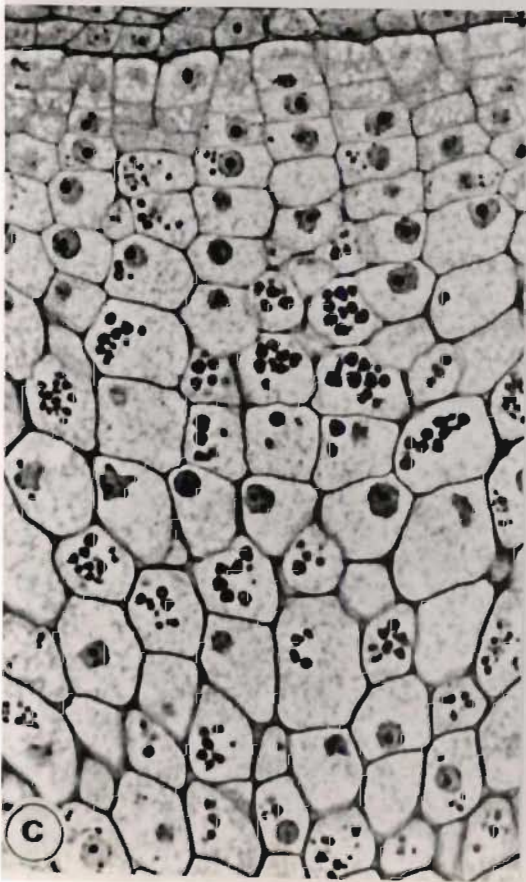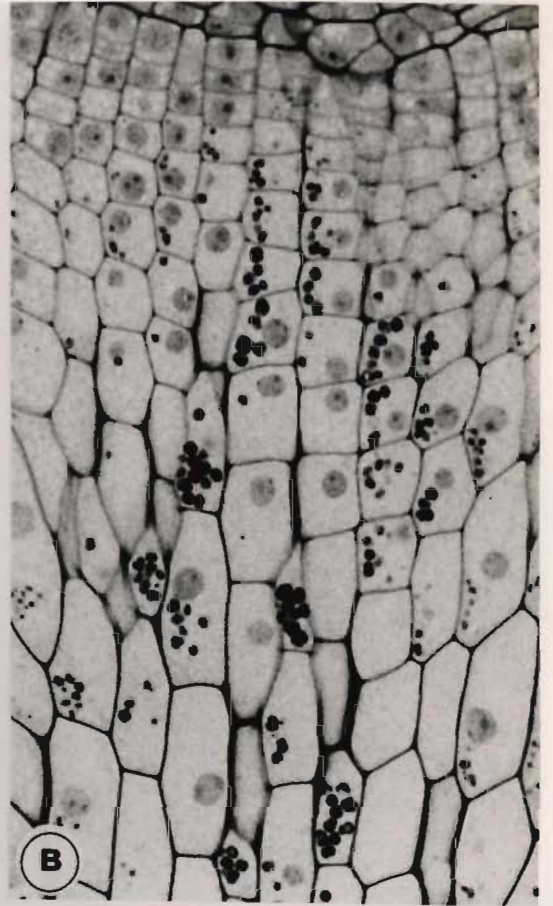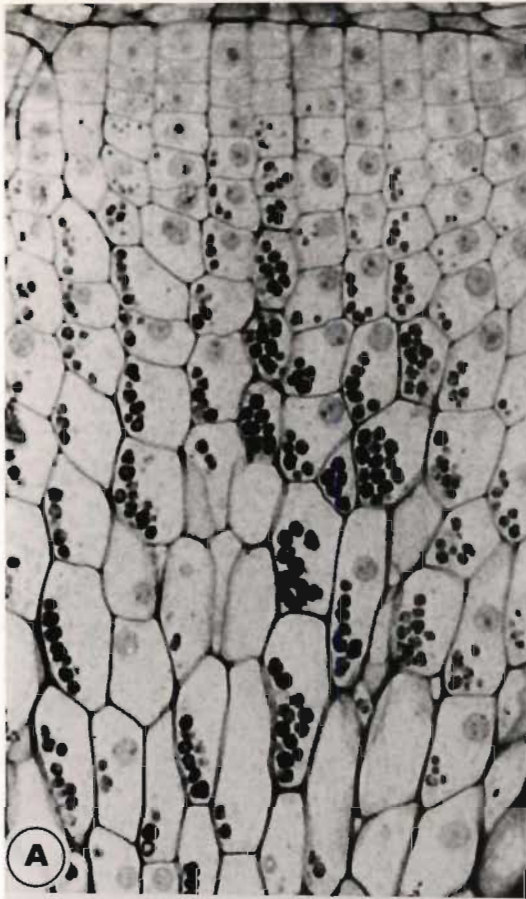
Figure 34   The effect of different Al concentrations (mg dm^{-3}) over a 14
            day growth period on the length (mm) of primary roots of Zea
            mays (cv. TX 24)(Section 7.3.2).   Each point is the mean
            value obtained from 46 plants derived from two experiments.
            Error bars represent the range for two experiments.

69
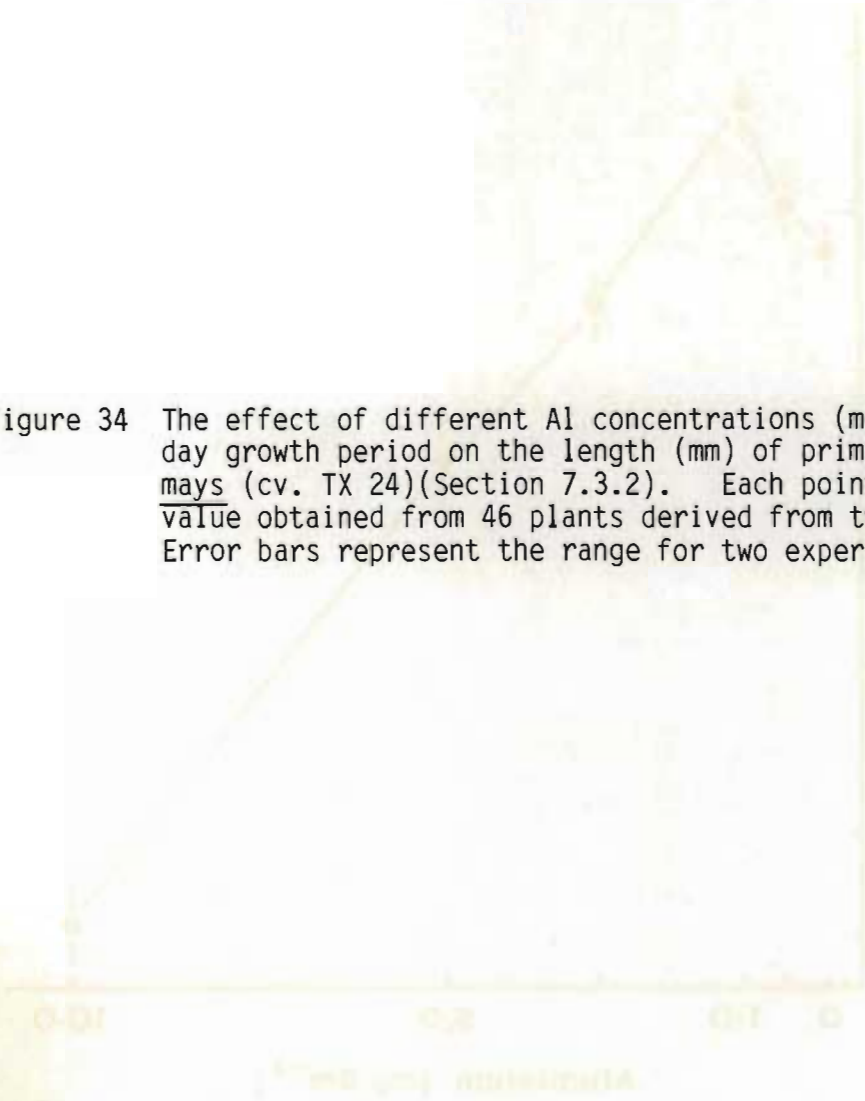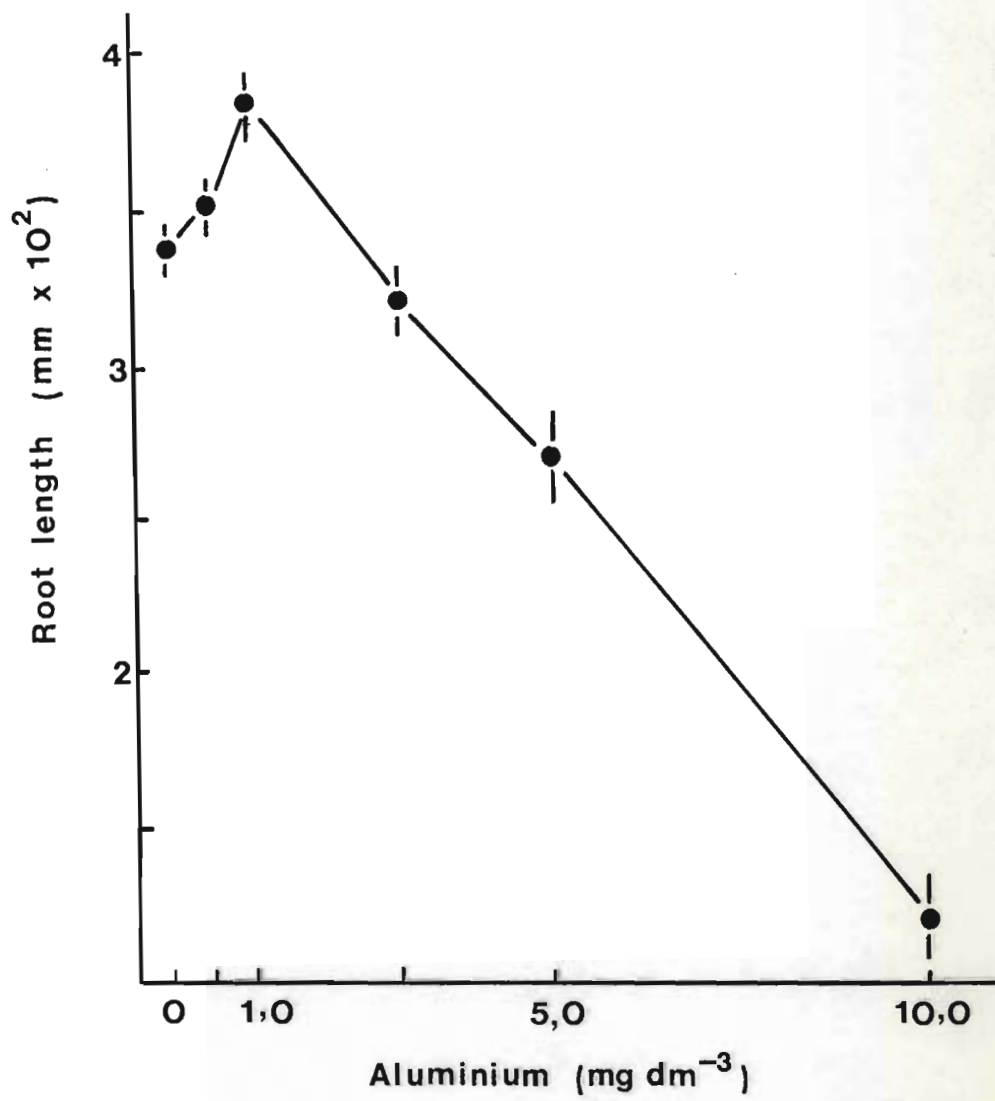
Figure 35   The effect of different Al concentrations (mg dm^{-3}) on the
number and location of amyloplasts present in the root cap
of <u>Zea mays</u> (cv. TX 24)(Section 7.3.3).
Increasing Al concentration caused a decrease in amyloplast
numbers.   Error bars indicate the S.E. of the mean, where
amyloplast counts on a minimum of 5 roots were made in
determining each point.   The frequency distribution indicated
the proportion (%) of amyloplasts observed to be present in the
proximal half of central cap column cells.
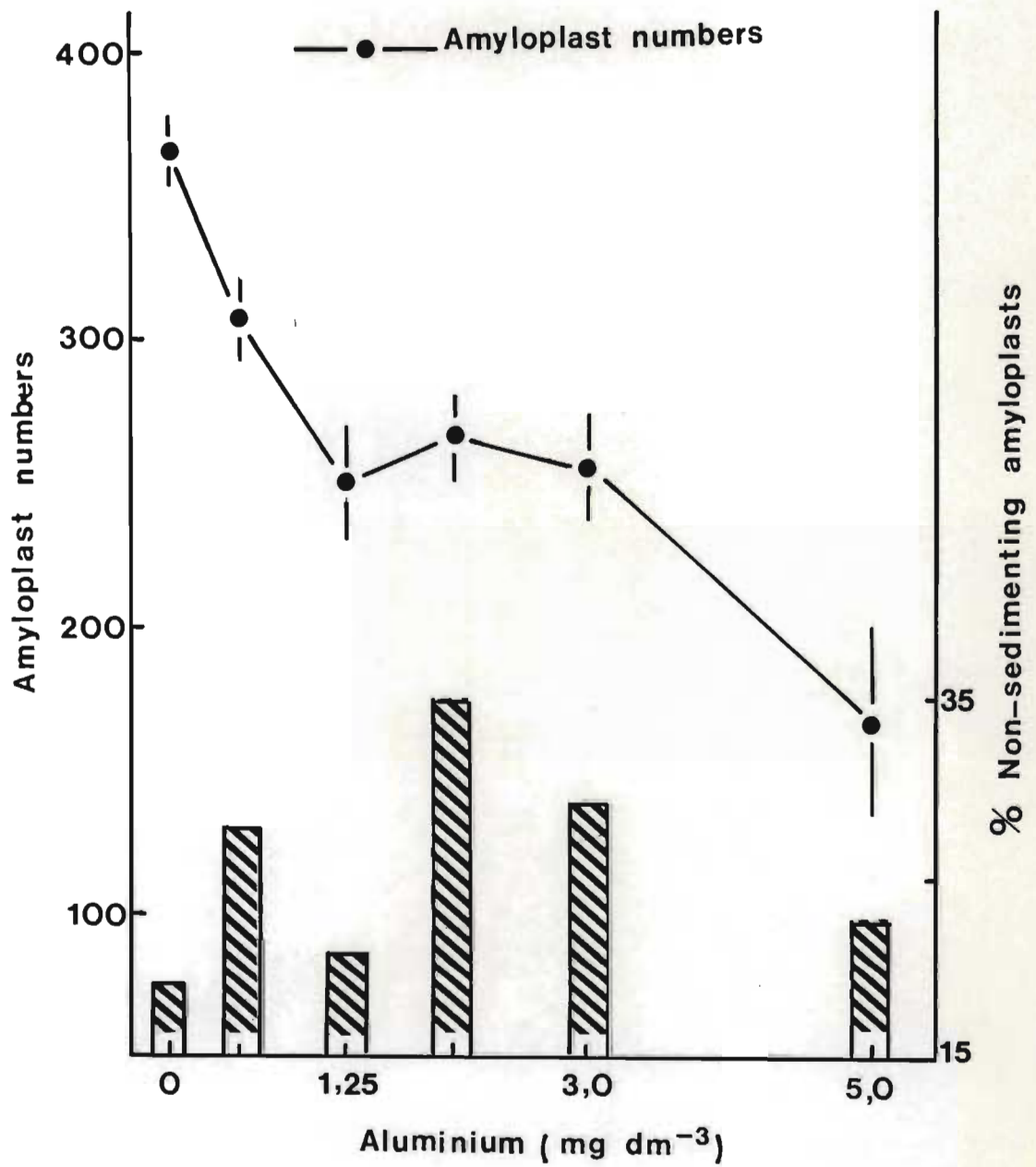
Figure 36   Electron micrograph illustrating the effects of various Al
             treatments on Golgi apparatus activity (Section 7.3.4.1;
             7.3.4.2).

        A - Control (0 Al), dictyosome from peripheral cap showed a
            well-ordered cisternal architecture, and a characteristic
            granular secretory product was present in the secretory
            vesicles.   Membrane structure of the secretory vesicles
            (SV) was intact.

        B - Control (0 Al).   Golgi apparatus activity in the peri-
            pheral cap was indicated by the accumulation of Golgi
            apparatus-derived material (GM) exterior to the
            plasmalemma.   Dictyosome (D).

        C - The effect of 0,5 mg dm^{-3} Al (29 h) on Golgi apparatus
            secretory activity was indicated by the disruption of the
            dictyosome (D) and notable changes in the appearance of
            vesicular material and Golgi apparatus-derived material
            (GM).

        D - 2,0 mg dm^{-3} Al.   Severe disruption of cisternal structure,
            vesicular membranes (unlabelled arrows) and the appearance
            of vesicular contents was evident in peripheral cap
            dictyosomes after 29 h treatment.

        E - 2,0 mg dm^{-3} Al.   In contrast to D, cisternal morphology of
            dictyosome located in the central cap was little affected
            by 29 h treatment.

            Note smaller size of secretory vesicles and the absence of
            a granular secretory material.

0,2 μm

SV

A

0,5 μm

GM

D

B

GM

D

0,5 μm

C

SV

0,2 μm

D

0,2 μm

E

73
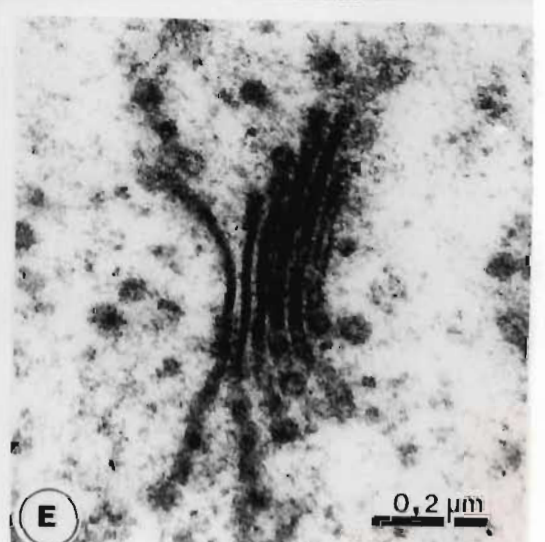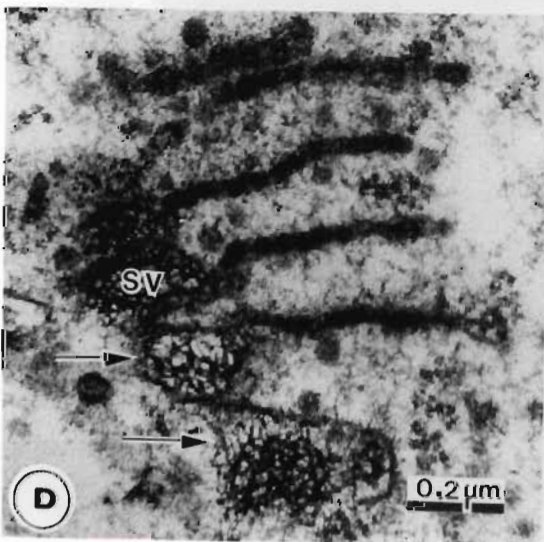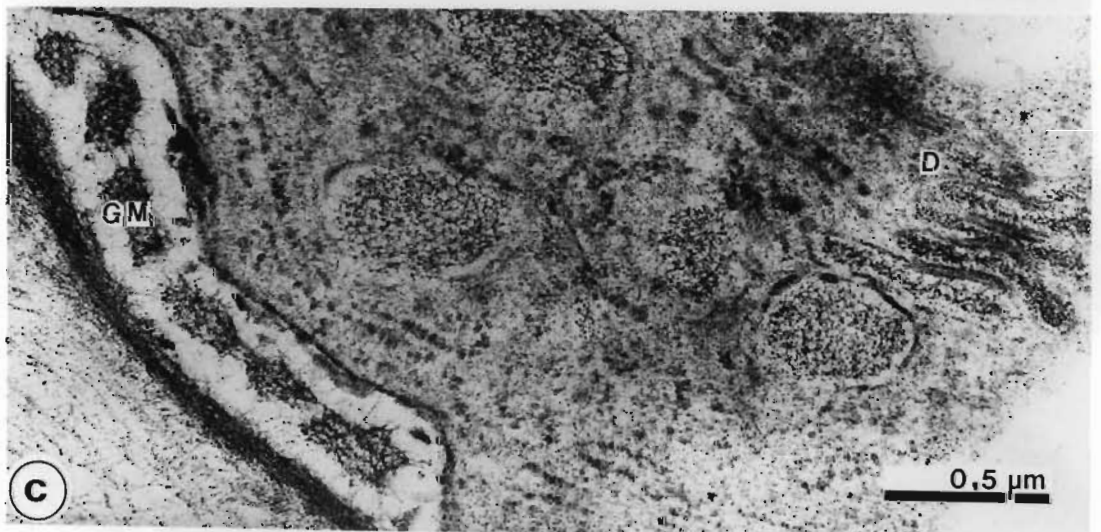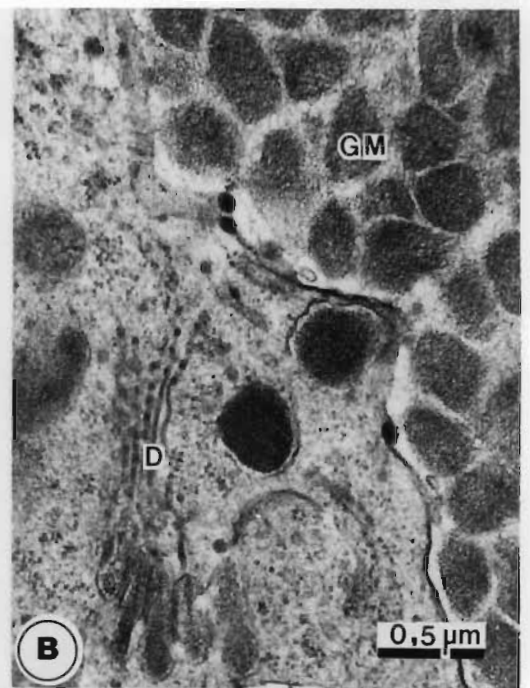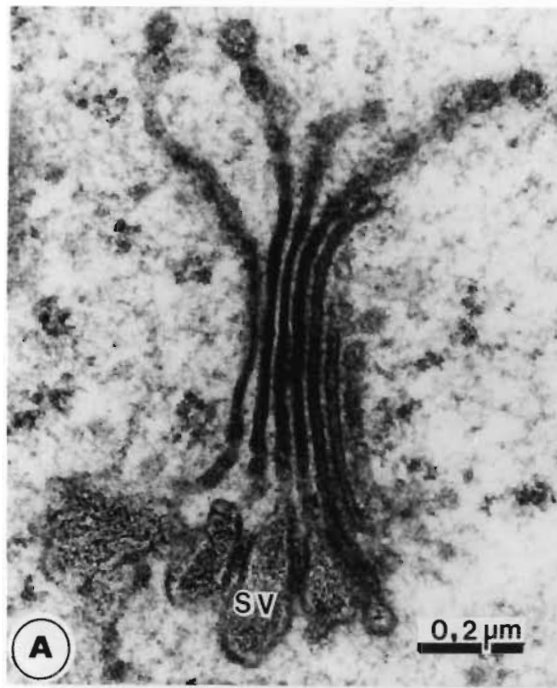
Figure 37   Electron micrograph illustrating the response of cells of the distal meristem and cap periphery to Al (Sections 7.3.4.1; 7.3.4.3).

A - Distal meristem (control, 0 Al).

B - Distal meristem 5,0 mg dm^{-3} (29 h). The nuclear structure showed little response to Al.   Cells were however, increasingly vacuolate and, while many proplastids were present, there was no evidence of starch accumulation being initiated.

C - Peripheral cap cells 5,0 mg dm^{-3} Al (29 h).   Many cells were highly vacuolate and disorganization of the cytoplasm was clearly evident.
Accumulation of Golgi material (unlabelled arrow) was limited to the inner cell.

5 µm

5 µm

5 µm

A

B

C
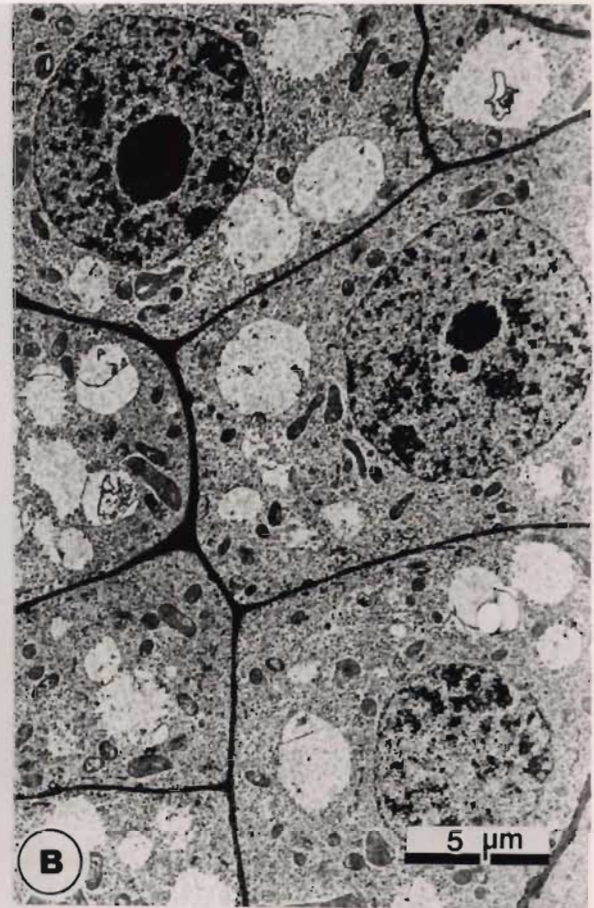
Figure 38   The effect of different Al concentrations in initiating H^+ efflux and cell enlargement (Sections 7.3.5; 7.3.6).

H^+ efflux ($\mu g\ H^+g^{-1}$ root dry mass) was determined over a 7 h treatment period.   The experiment was performed in duplicate and points represent mean values.   Error bars give the experimental range for two trials.   Cell enlargement was expressed as the relative (% of control) change in root diameter measured 1 mm from the root apex.   Each point was determined from the measurements taken from 5 roots.
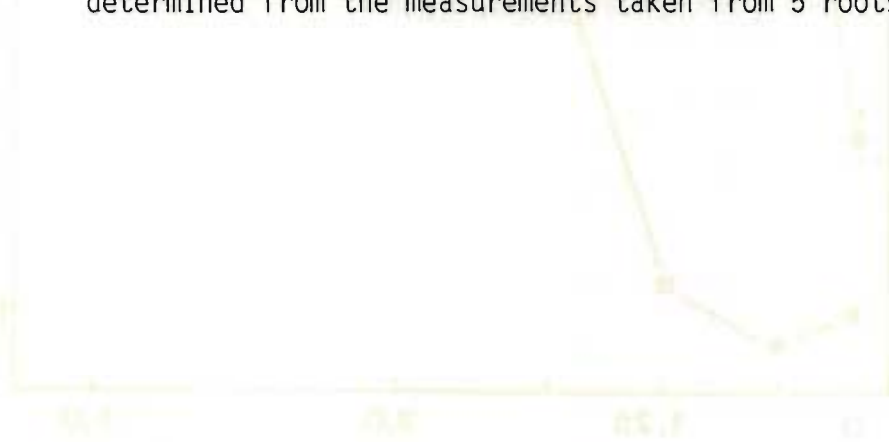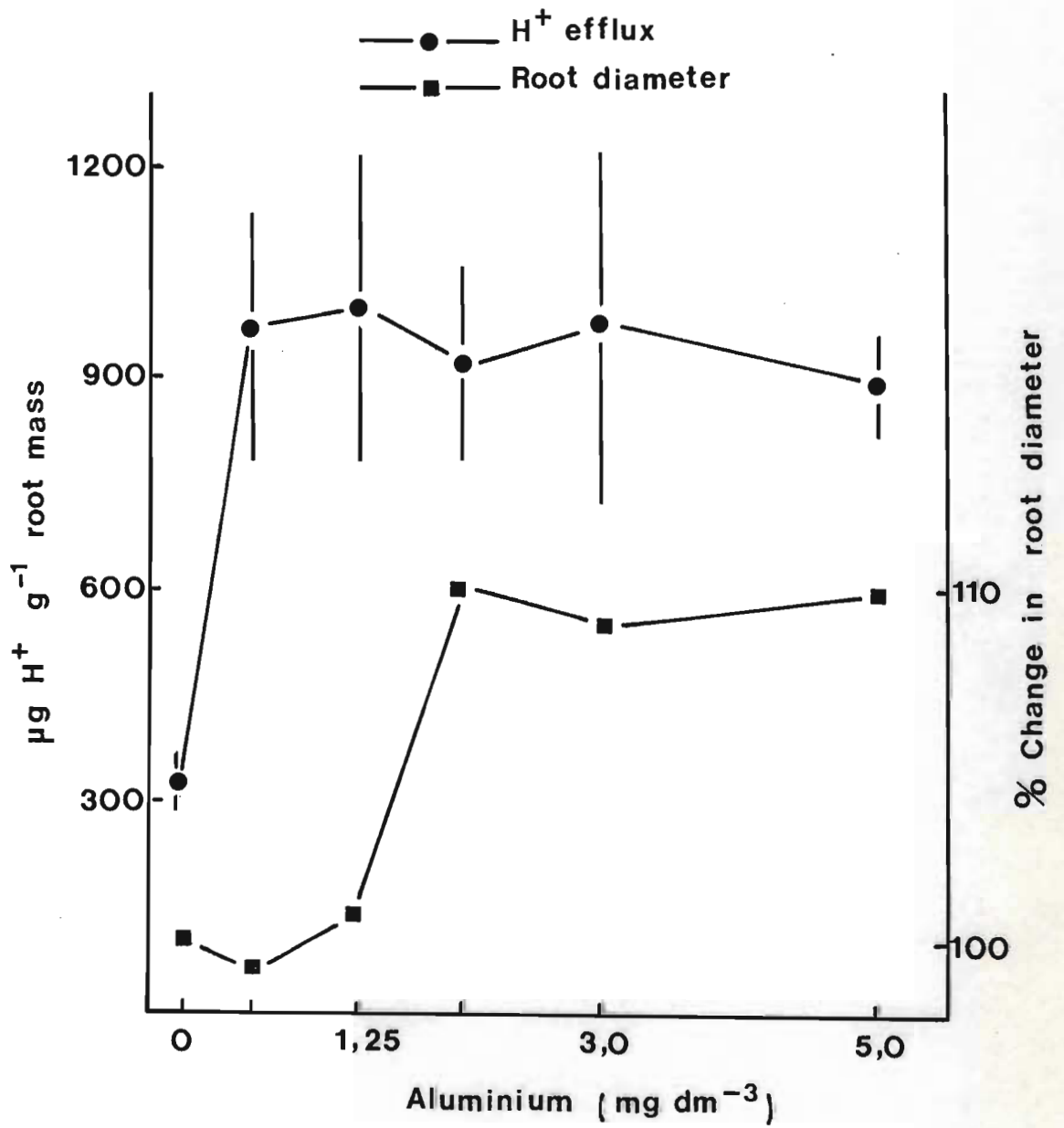
Figure 39   The effects of different Al concentrations (mg dm^{-3}) on Al
             uptake   (Zea mays cvs. TX 24 and HL 1) measured as root Al
             concentration (mg kg^{-1}), and root length (mm) (mean of both
             cvs.) over a 14 day growing period.   Vertical scale lines
             represent the 5% and 1% confidence levels for root length (L)
             and root Al concentration (Section 8.3.1.1).

             Note that in cv. HL 1, the highest root Al concentration
             coincided with the Al treatment level at which the longest
             roots were recorded.   A statistically significant reduction
             in root length, with increased Al treatment levels,
             coincided with a sharp decline in root Al concentration.
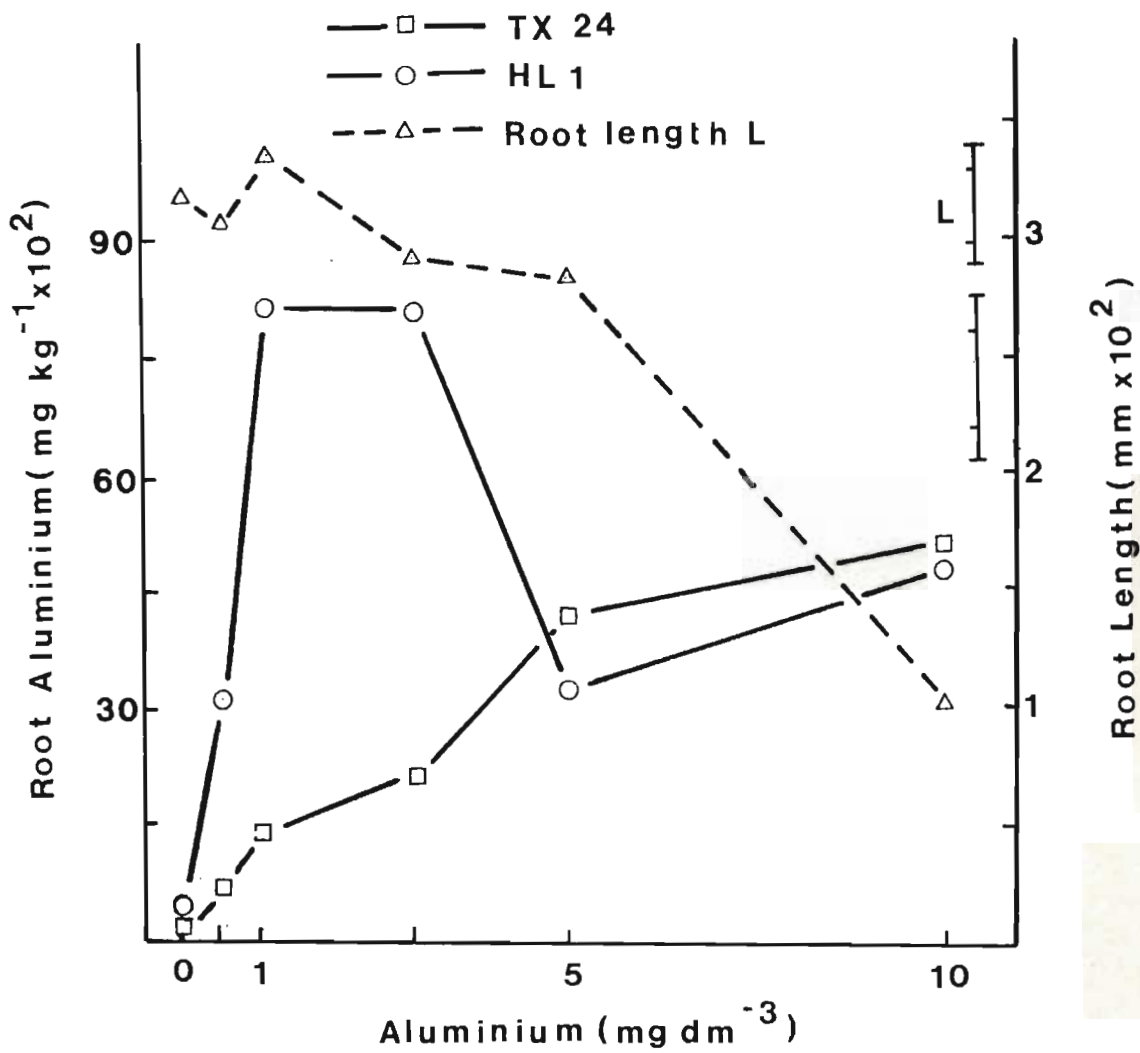
Figure 40   The effects of different Al concentrations (mg dm^{-3}) on the
levels (%) of P found in the plant tops and roots and on the
distribution (D) of P between plant fractions (Zea mays
cvs. TX 24 and HL 1).   Vertical scale lines represent the
5% and 1% confidence levels for P in the plant tops (T) and
roots (R) (Section 8.3.1.1).

Note that root P level increased rapidly in cv. HL L with
increasing Al treatment.   In contrast, statistically
significant changes in root P concentration were not
recorded for cv. TX 24.   Leaf concentrations of P were
reduced in both cultivars by Al treatment.
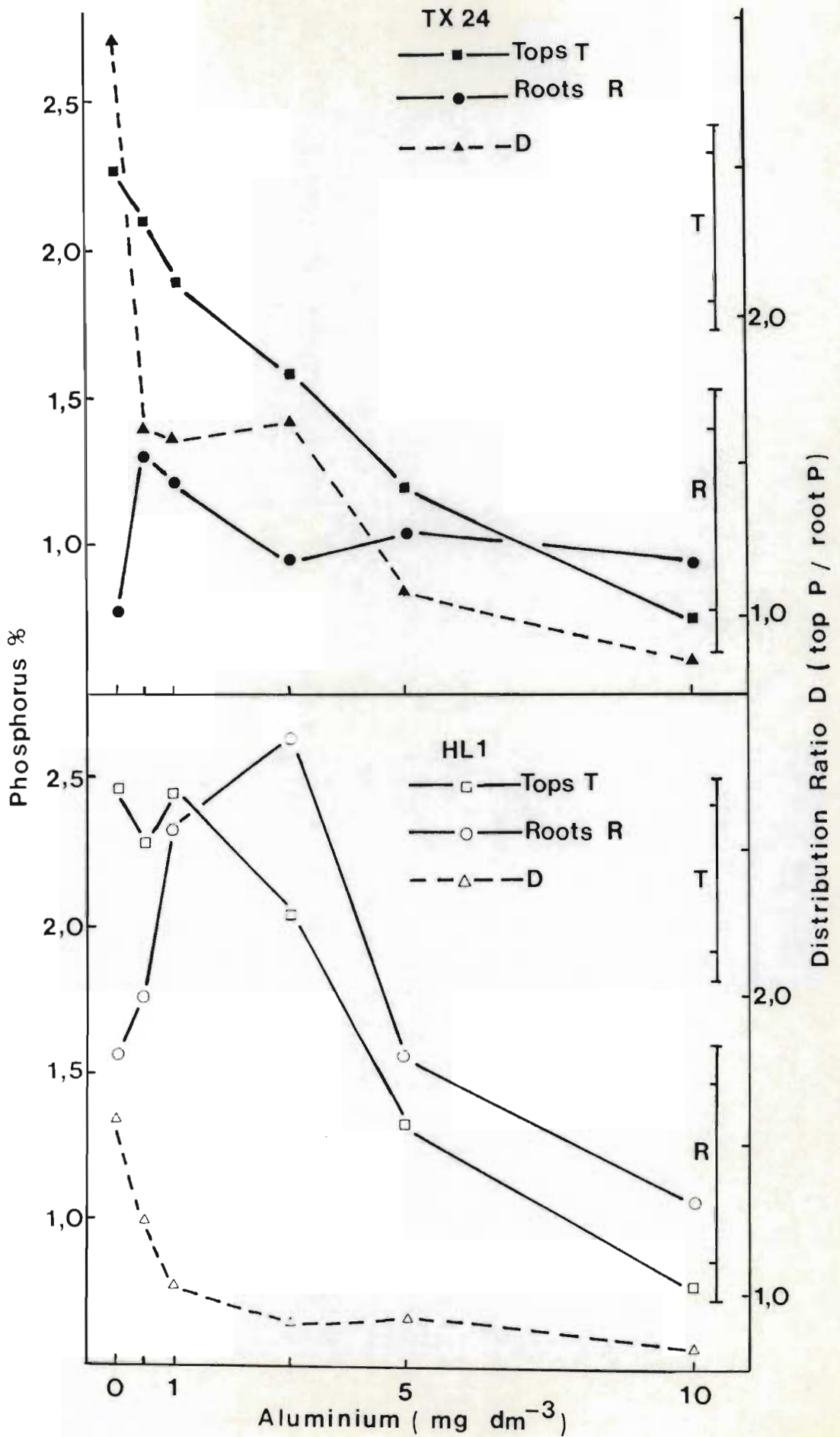
Figure 41 The effects of different Al concentrations (mg dm^{-3}) on the levels of Mn (mg kg^{-1}) and K (%) found in the plant tops and roots and on the distribution (D) of Mn and K between plant fractions (mean for both cultivars). Vertical scale lines represent the 5% and 1% confidence levels (single level 5% only) for concentrations of these elements in the plant tops (T) and roots (R)(Section 8.3.1.1).

Note that statistically significant cultivar differences did not emerge for these elements. Furthermore, Mn levels were reduced in both the plant tops and roots by increasing Al treatment levels. This may indicate that susceptibility to Al and Mn toxicities do not coincide in Zea.
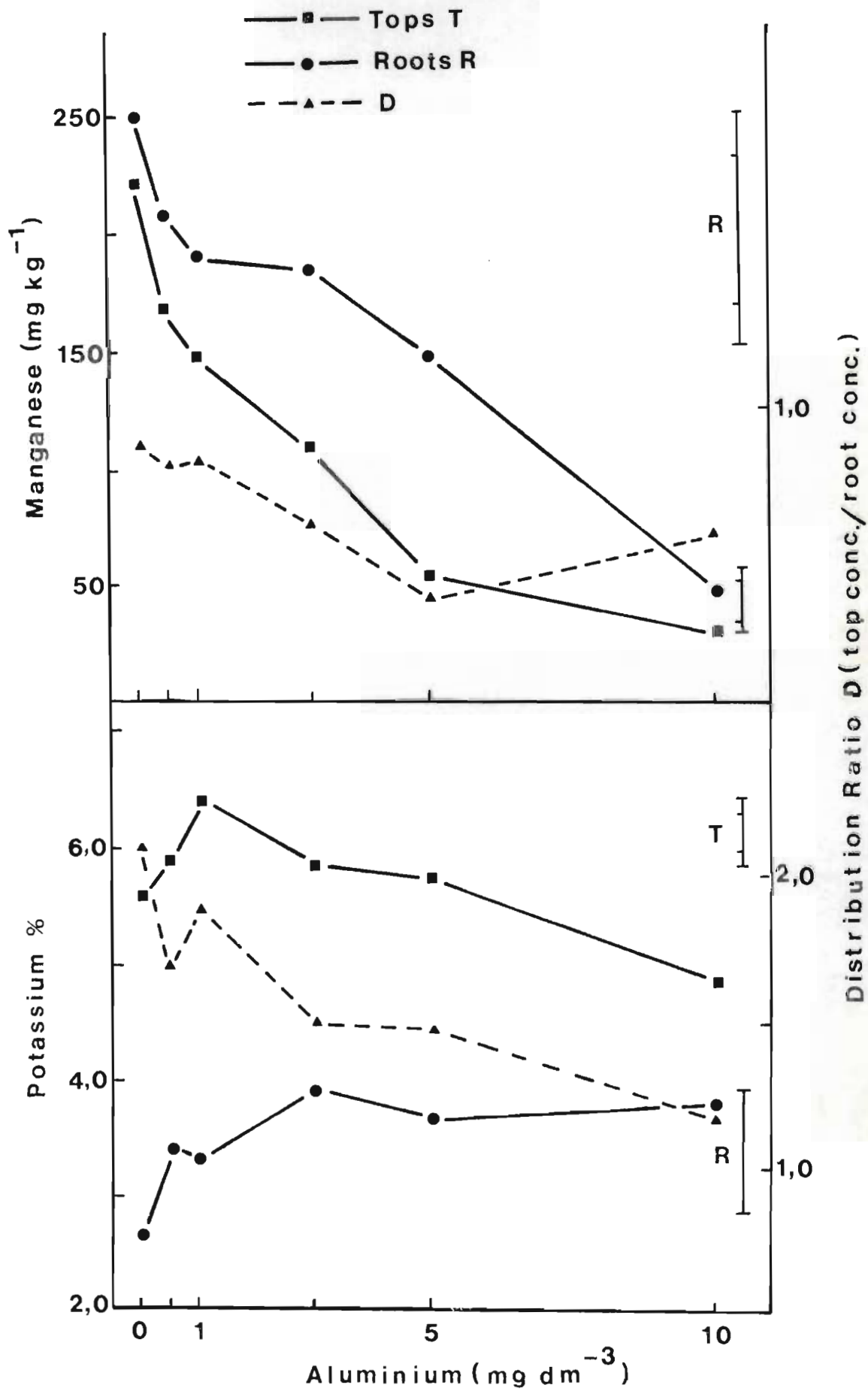
Figure 42   The effects of different Al concentrations (mg dm^{-3}) on the
levels (%) of Ca and Mg found in the plant tops and roots
and on the distribution (D) of Ca and Mg between plant
fractions (Zea mays)(mean values for both cultivars).
Vertical scale lines represent the 5% and 1% confidence
intervals (single level 1% only) for the concentrations of
these elements in the plant tops (T) and roots (R)
(Section 8.3.1.1).

Note that statistically significant differences did not emerge
between cultivars with respect to Ca and Mg concentrations.
Levels of Ca and Mg were decreased in plant tops and roots by
Al treatment.   In both cases, the distribution ratio (D)
increased with heightened Al treatment levels.   This
indicated that low root concentrations of Ca and Mg were
met by an increasing proportion of the element being
transported to the plant top.
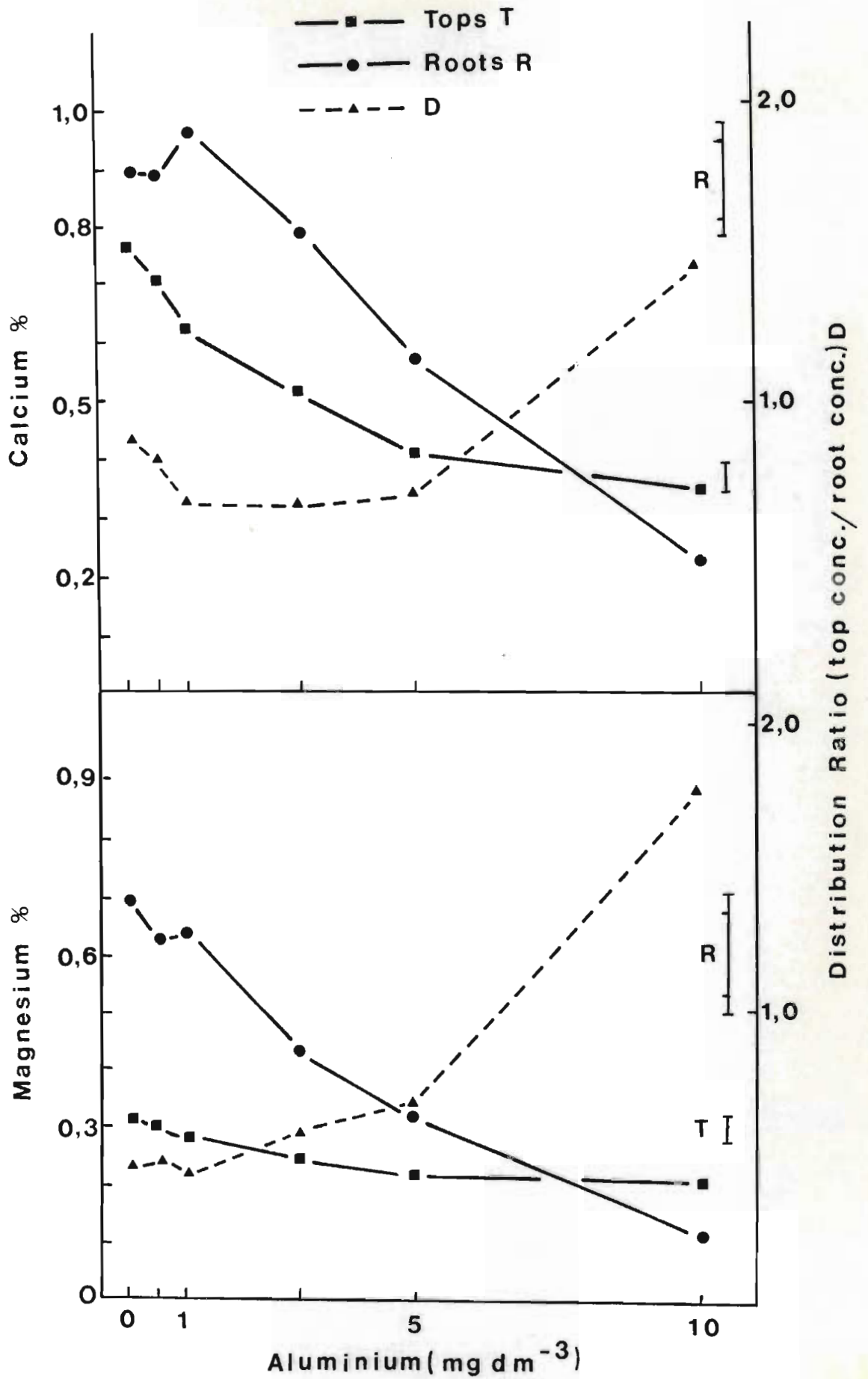
Figure 43   The effects of different solution Mg levels (mg dm^{-3})
            on the concentrations of Mg in the plant tops (%);   root
            concentrations of Mg, K, Ca (%) and the distribution of Mg
            between plant fractions (D) and total root cationic charge
            (m mol kg^{-1}).   All values are the mean values for both
            cultivars.   Vertical scale lines represent the 5% and 1%
            confidence intervals (single level 1% only) for the
            concentrations of Mg in the plant tops (T), roots (R) and
            distribution ratio (D) and the root concentrations of K and
            Ca (Section 8.3.2.1).

            Note that increased Mg supply (and uptake) was associated
            with reduced root concentrations of K and Ca.   Root cationic
            charge remained effectively constant, and a degree of
            substitution between these elements was therefore indicated.
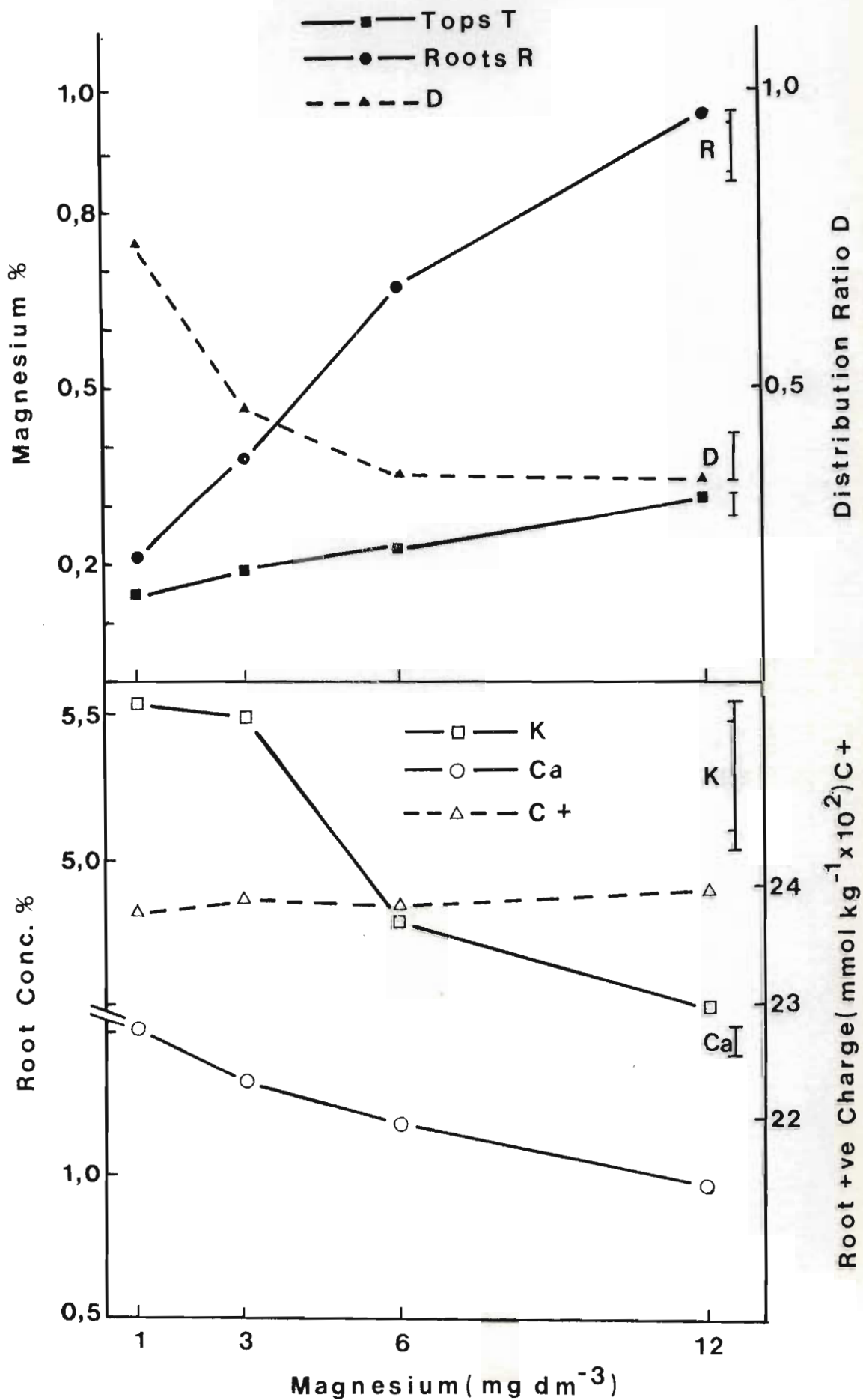
Figure 44   Unsectioned roots of <u>Zea mays</u> (cvs. TX 24 and HL 1) illustrating the effects of different Al concentrations and treatment times on the respiratory activity (TTC measured, Sections 2.3.2.1; 2.3.2.2; 2.3.2.3) of the root apex. Aluminium treatment times were indicated by 0 - Control (0 Al : 1 - 3 h : 2 - 6 h : 3 - 10 h : 4 - 20 h.

A - 2,0 mg dm^{-3} Al, cv. TX 24.

    Note that the intensity of red (formazan) colour development was undiminished over the course of the experiment.

B - 4,0 mg dm^{-3} Al, cv. TX 24.   Diminished activity was evident in some roots at the longest treatment time.

C - 8,0 mg dm^{-3} Al, cv. TX 24.   The intensity of red colour development was further diminished, although colour development of low intensity was still evident in isolated roots.

D - 2,0 mg dm^{-3} Al, cv. HL 1.   Uniform colour development was evident over the course of the experiment.

E - 4,0 mg dm^{-3} Al, cv. HL 1.   Reduced colour development was apparent in some roots at 10 h.   Severe inhibition of root activity was evident at 20 h.

F - 8,0 mg dm^{-3} Al, cv. HL 1.   Roots were characterized by an absence of red colour at 20 h.   This should be interpreted as a marked decline in activity rather than inhibition of respiratory activity in the root apex. Factors affecting the sensitivity of this technique were discussed in Section 2.3.2.2.

A
0   1   2   3   4

B
0   1   2   3   4

C
0   1   2   3   4

D
0   1   2   3   4

E
0   1   2   3   4

F
0   1   2   3   4
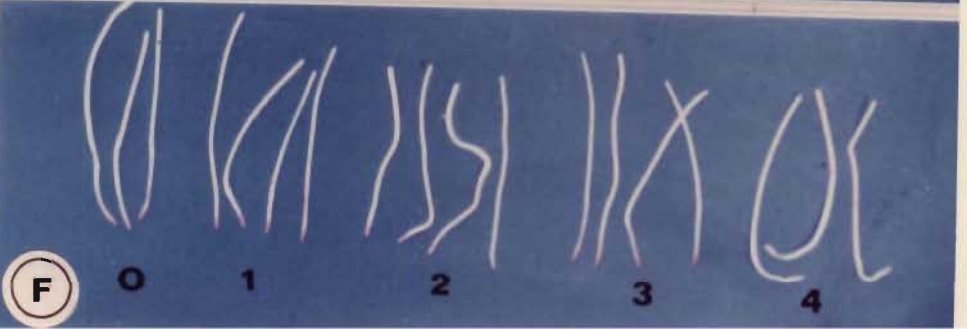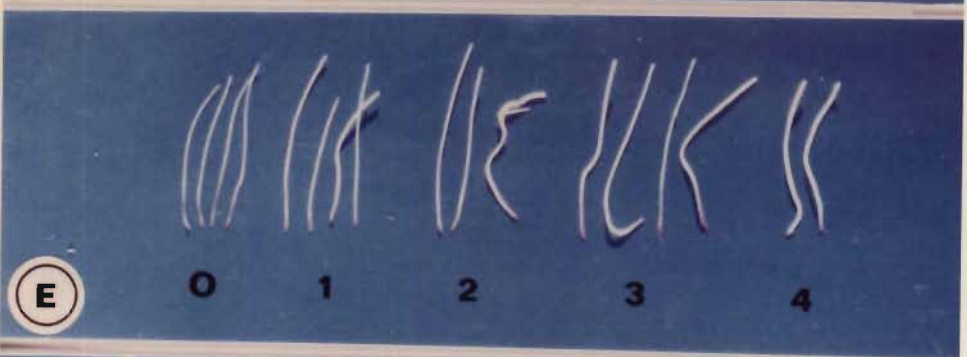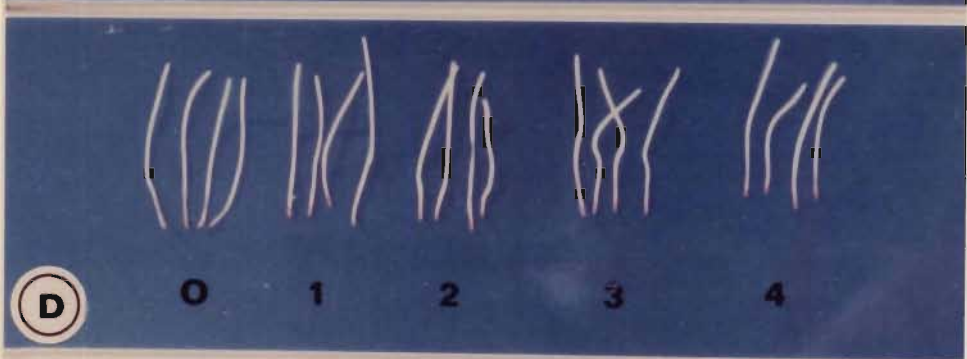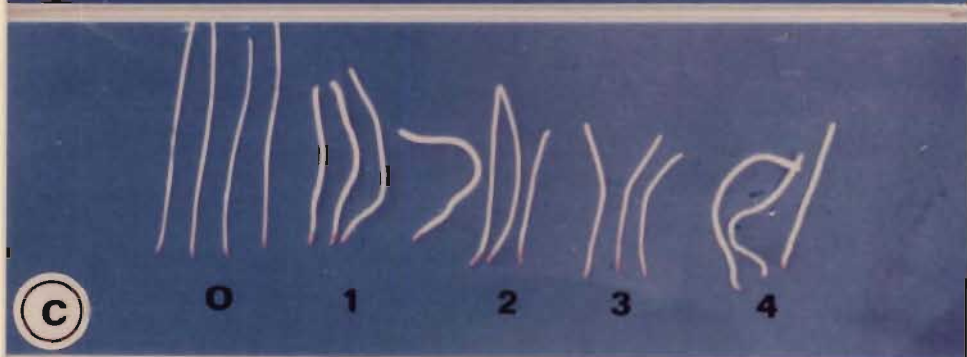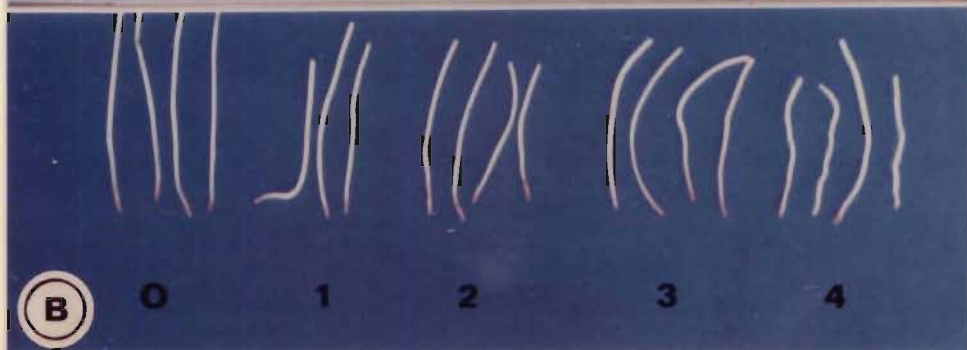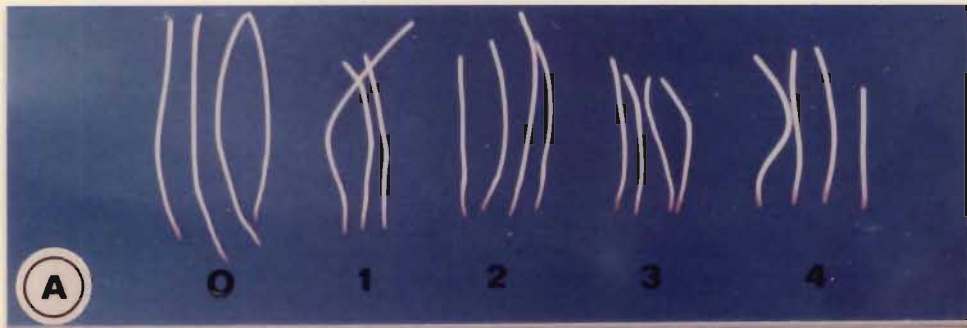
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Env. and Exp. Botany (In Press)

ALUMINIUM TOXICITY AND REGENERATION
OF THE CAP OF PRIMARY ROOTS OF Zea mays

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Previous studies[1] have attached importance to the Golgi apparatus in the peripheral cap cells in the physiology of Aluminium (Al) toxicity. Behaviour of the Golgi apparatus is correlated with cellular development[2] and the cells of the quiescent centre respond to removal of the root cap by entering mitosis and regenerating a new cap[3]. Treatment of decapped roots with Al may be expected to provide a basis for studying the effect of Al on the developmental stages of the Golgi apparatus during cap regeneration.

Ultrastructural changes in the Golgi apparatus and associated cytoplasmic organelles were followed in decapped roots grown in liquid culture containing either no Al (control) or 8 mg dm^{-3} Al at 27°C for 0h, 6h, 12h, 24h and 48h after decapping.

Development of Golgi apparatus function in the control (Fig. 1) co-incided with differentiation of proplastids, accumulation of starch (Fig.3) and development of amyloplasts. A recognisable cap was formed within 48h and resumption of biochemical activity was confirmed by detection of Golgi apparatus derived material between the plasmalemma and cell wall of the outer cells.

Al was found to inhibit development of the Golgi apparatus so that dictyosomes with defined membrane structure were infrequently encountered 24h after decapping (Fig.2). Vesicle membranes were not clearly defined and secretory vesicles accumulated in the vicinity of the dictyosome. Many plastids in Al-treated cells failed to develop internal organisation (Fig.4) and membrane profiles were indistinct. Starch accumulation was only apparent with extended exposure in isolated cells and was linked to the presence of dictyosomes. These observations may involve Golgi apparatus function in the control of amyloplast development. In Al-treated cells, lipid drops (plastoglobuli) could still be found in considerable numbers, sometimes in conjunction with starch with 24h exposure to Al. This could implicate Al in preventing the assembly of membrane material. This is consistent with the slower development of Golgi apparatus noted in Al-treated cells. Starch grains in Al-treated cells showed an increased intensity of stain which may indicate fundamental but undefined biochemical differences. Accumulation of starch and resumption of Golgi function was delayed (24h), and preceded severe cellular disorganisation in Al-treated roots which failed to regenerate a new cap with 48h exposure to Al.

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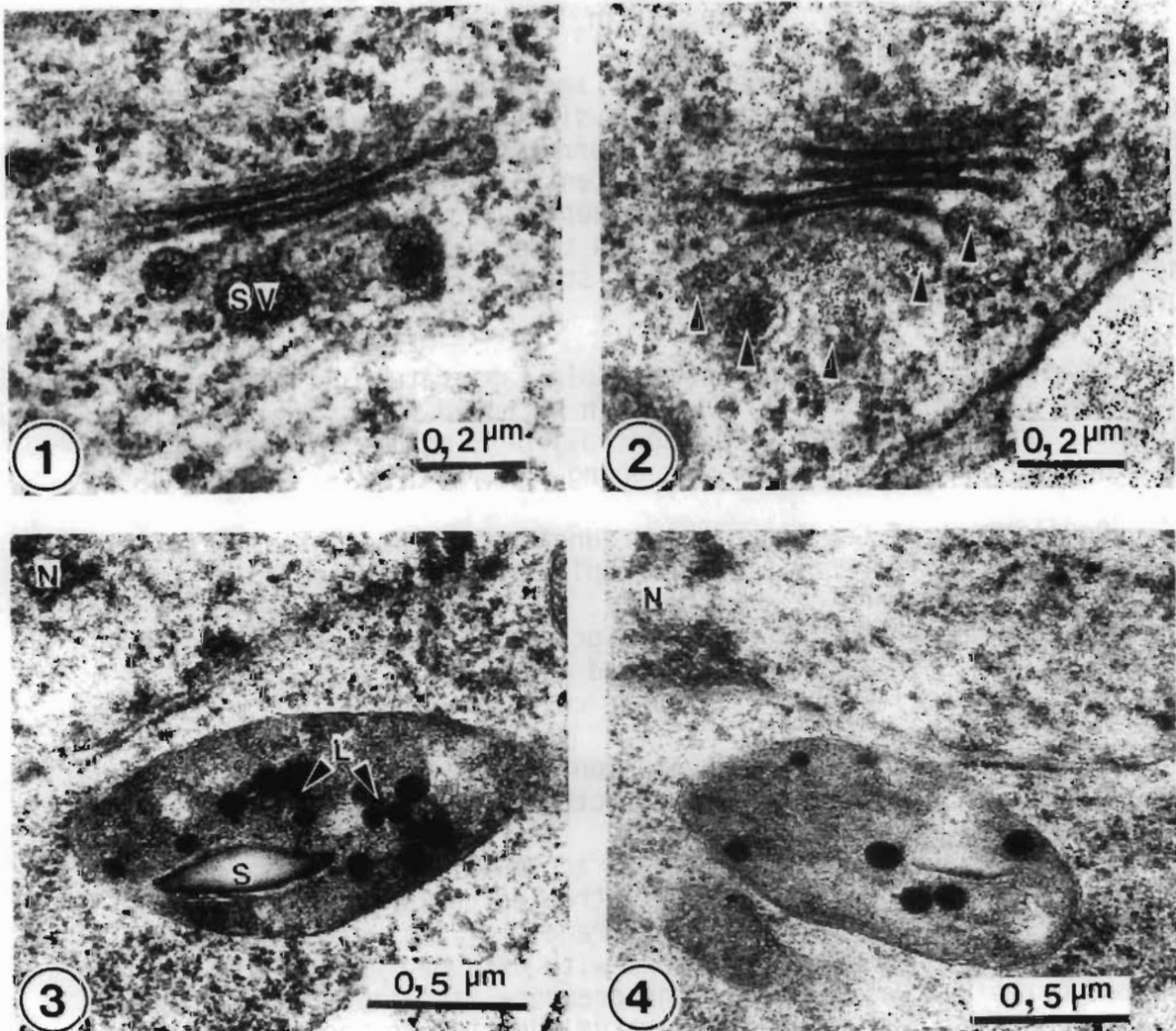
Fig.1. Dictyosome, (control) 6h after decapping.   Secretory vesicles
       (SV) are well defined.
Fig.2. Dictyosome, Al-treated cell 24h after decapping.   Secretory
       vesicles are poorly defined and accummulate near the
       dictyosome (arrowed).
Fig.3. Plastid (control) 6h after decapping showing starch grain (S),
       defined membrane structure and presence of lipid drops (L).
       Nucleus (N).
Fig.4. Plastid, Al-treated cell 6h after decapping.   Note absence
       of starch grains and poorly defined internal organisation.

Aluminium uptake sites in the primary root of *Zea mays* L.

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Histochemical studies of the chronological sequence involved in the uptake of Al by the primary root of *Zea mays* L., cv. TX 24, showed the primary sites of Al uptake to be the peripheral cells of the root cap and the mucilagenous secretions surrounding the root. Al spread rapidly through the cells of the root cap but the cap initials were the last cells to be reached. Entry of Al into the rest of the root was considerably less rapid, and was found to be limited to the outer cortical cells of the root apex. Little evidence could be found of Al reaching actively dividing cell populations of the primary root meristem during the first 20 h of this experiment. The concept of Al acting directly on cell division is consequently questioned. Decapped root experiments implied a protective function for the root cap over the quiescent centre and mitotically active cells of the root. Epidermal cells of the root apex were not an effective barrier to Al. It is postulated that Al uptake is a function of the biochemical properties particularly the presence of acid mucopolysaccharides in the cells involved.
S. Afr. J. Plant Soil 1985, 2: 1 – 7

Histochemiese studies van die primêre wortel van *Zea mays* L., cv. TX 24, het getoon dat aluminium hoofsaaklik deur die periferale wortelmusselle opgeneem is terwyl vergelykbare hoeveelhede van die element ook in die slymsekreet om die wortel waargeneem is. Aluminium het vinnig deur die selle van die wortelmus beweeg en die wortelmusinisiaalselle was die laaste selle wat bereik is. Opname van aluminium deur die res van die wortel was heelwat stadiger en selfs na 'n lang blootstelling was die element steeds tot die buitenste korteksselle van die wortelpunt beperk. Na 20 h kon min aanduidings van die beweging van aluminium tot by die delende selle van die primêre wortel se meristeem gevind word. Die siening dat aluminium 'n direkte invloed op seldeling uitoefen word dus bevraagteken. Uit eksperimente waar die wortelmus verwyder is wil dit voorkom of die wortelmus 'n beskermende invloed op die stiltegebied en mitoties-aktiewe selle van die wortelpunt uitoefen. Epidermisselle van die wortelpunt het nie as 'n effektiewe skerm teen aluminium gedien nie. Daar word veronderstel dat aluminiumopname deur selle 'n funksie van hul biochemiese eienskappe, veral die teenwoordigheid van muko-polisakkariede, is.
S.-Afr. Tydskr. Plant Grond 1985, 2: 1 – 7

Keywords: Aluminium, cap, root, toxicity, uptake

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Introduction

The toxic consequences of aluminium (Al) on plant growth have been connected to a bewildering variety of plant symptoms, comprehensively reviewed by Foy, Chaney & White (1978). There remains however, considerable speculation on the precise mechanism of Al toxicity and the physiological role of Al is poorly defined with many anomalies.

Depression of root elongation as a result of reduced mitotic activity is possibly the most obvious effect of Al uptake (Clarkson, 1965). Al does not however, enjoy a high degree of mobility in many plants (Jackson, 1967). Particular significance must therefore be attached to the root apex, for it may be reasonably argued that the rapidity with which the effect of Al is manifest (Clarkson, 1965) cannot be reconciled with low mobility, unless the sites of uptake and action are nearly co-incidental.

Published research on the sites of Al uptake is infrequent (McLean & Gilbert, 1927; Wright & Donahue, 1953; Rasmussen, 1968; Kalovoulos & Misopolinos, 1983), and the function of the root epidermis in controlling entry of Al into the root is controversial (Wright & Donahue, 1953; Rasmussen, 1968). Nevertheless, the identification of the sites of Al uptake and accumulation in the root is fundamental to the elucidation of the mechanism of Al toxicity. It is the purpose of this work to trace the chronological sequence of Al uptake in the primary root of *Zea mays* L., cv. TX 24.

Material and Methods

Experiment I

Plant growth conditions

Pre-germinated seed of *Zea mays* L., cv. TX 24, was planted in 25 ℓ plastic buckets (1,08 ℓ/plant) containing the following nutrient solution (mg dm^{-3}) at pH 4,6: N 36; P 3; K 40; Ca 40; Mg 10; S 14; Na 1,5; Mn 0,11; Zn 0,05; Cu 0,02; Fe 5,5; Mo 0,03; B 1,9.

Solution pH was not adjusted during the course of the experiment. Nitrogen was provided as NH_4^+ and NO_3^- in a molar ratio of 1:1. Sulphur was supplied as SO_4^{2-}. Fe was present as Fe CDTA.

Plants were grown with continuous aeration for a total of seven days. $Al_2(SO_4)_3$ solution was added in advance of harvest to give 0 h, 0,5 h, 2 h, 12 h, 20 h and 48 h root exposure to 8 mg dm^{-3} of Al in solution. This concentration of Al corresponds to a calculated Al^{3+} activity of $8,4 \times 10^{-5}$ mol dm^{-3}, with the balance of the Al being distributed between $AlSO_4^+$ ($2,8 \times 10^{-5}$), $AlOH^+$ ($1,5 \times 10^{-5}$) and $Al(OH)_2^+$

reference purposes because of the importance of Al activity and of ion pair formation rather than total solution concentration in determining Al toxicity (Pavan & Bingham, 1982).

Artificial light was supplied to give a 14 h light, 10 h dark regime with alternating temperature of 28 : 23 °C. The light source was Gro-lux W.S.* (731,3 Wm^{-2}) and incandescent (113,4 Wm^{-2}) to give a light intensity of 300 μE m^{-2}s^{-1} at plant height. Roots were stained with haematoxylin.

Experiments II and III

Plant growth conditions

Pre-germinated seeds of Zea mays L., cv. TX 24 were planted along the upper edge of 66 × 22 cm filter paper. Seeds were arranged with the radicles pointing downwards. The paper was loosely rolled and placed vertically in a glass container, with 5-cm depth of half-strength nutrient solution. The 'Rag-Doll' technique has been more fully described by Konzak, Polle & Kittrick (1976).

Plants were grown in the dark for three days at 25 °C to give a radicle length of 6–10 cm.

Experiment II

Experimental procedures

Plants were selected for a straight main root axis, the main root was decapped following the procedure of Feldman (1975). Plants with (control) and without root caps were transferred to half-strength nutrient solution containing either no Al (control) or 8 mg dm^{-3} Al as Al$_2$(SO$_4$)$_3$ and grown for 20 h at 22 °C with continuous aeration, but without supplementary light. Roots were stained with haematoxylin.

Experiment III

Experimental procedure

Plants were selected for a straight main root axis and transferred to half-strength nutrient solution containing either no Al (control) or 8 mg dm^{-3} Al as Al$_2$(SO$_4$)$_3$ and grown for 20 h at 27 °C with continuous aeration but without supplementary light. Roots were stained with methylene blue.

Histochemical procedures

(i) *Haematoxylin staining technique.* Plants were harvested, roots were thoroughly washed in de-ionized water and the roots of intact plants stained (Polle, Konzak & Kittrick, 1978) in haematoxylin solution for the visual detection of the distribution of Al. Haematoxylin stain was prepared by dissolving Merck 4305* haematoxylin (2 g dm^{-3}) and NaIO$_3$ (0,2 g dm^{-3}) in de-ionized water (Gill, Frost & Miller, 1974; Polle *et al.,* 1978).

(ii) *Methylene blue method.* A 1% aqueous solution of methylene blue was diluted by adding 0,64 ml to 100 ml de-ionized water (Gurr, 1958) and correcting the pH to 3,98. The roots of intact plants were washed and stained for 45 min at 27 °C. The capacity of tissue to bind methylene blue at this pH is considered indicative of the presence of acid muco-polysaccharides (Gurr, 1958).

Micro-techniques

Root tips of prestained material were sectioned frozen on a Reichert, Thermo-Electric Cold Stage Microtome.* Sections were temporarily mounted in a 50% glycerine:water mixture, viewed on an Olympus BH-2* microscope and photographed initially on Kodachrome 25* film.

*Trade and company names are included for the benefit of the reader.

Results

The cell populations of the root cap and the primary growth region of the main root axis in Zea as referred to in these experiments have been defined respectively by Barlow (1975) and Luxová (1981) and are illustrated in Figure 1.

100μm
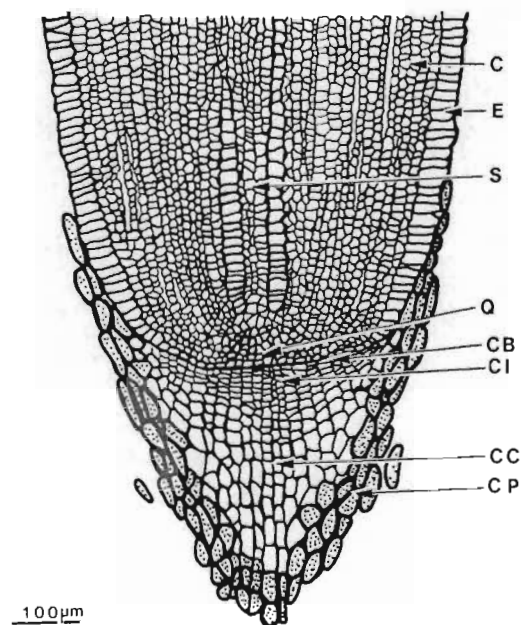
Figure 1 Cell outline drawing of the root tip of Zea mays L. (C = cortex; E = epidermis; Q = quiescent centre; S = stele of the root. CB = cap boundary; CC = cap columella; CI = cap initials; CP = peripheral cap cells).

Experiment I

This experiment represents a time-course study of Al uptake by the primary root of Zea (Figures 2A–2D). Figure 2A, the control treatment with no Al added, confirms the complete absence of stain from all tissue. The distribution of stained tissue (Figures 2B–2D) shows that the initial sites of Al uptake are the root cap and the mucilagenous secretions covering the epidermal cells of the root. The presence of Al in the peripheral cap cells could be demonstrated within 30 min exposure to 0,84 mmol dm^{-3} Al activity and Al was also rapidly adsorbed onto the outer surface of the root epidermis (Figures 2B & 2C).

Al spread rapidly through the cells of the root cap (Figures 2C & 2D), although the cap initials were the last group of cells within the root cap to be reached, with 12 h exposure (not illustrated**).

Prolonged exposure led to the accumulation of appreciable quantities of Al in the outer cortical cells (Figure 2D). These changes were apparent within 12 h, and were accompanied by gross morphological disorganization of the root apex, leading ultimately to disintegration of the outer cells of the root in the apical 1,5 to 2,0 mm (Figure 2D). The severe cellular disorganization and failure of the epidermis did not, however, result in appreciable quantities of Al being found in the inner cortical regions or the stele (Figure 2D).

**The cost of printing colour illustrations has necessitated the omission of some treatments from the plates. Limited numbers of colour prints of these treatments may be ...
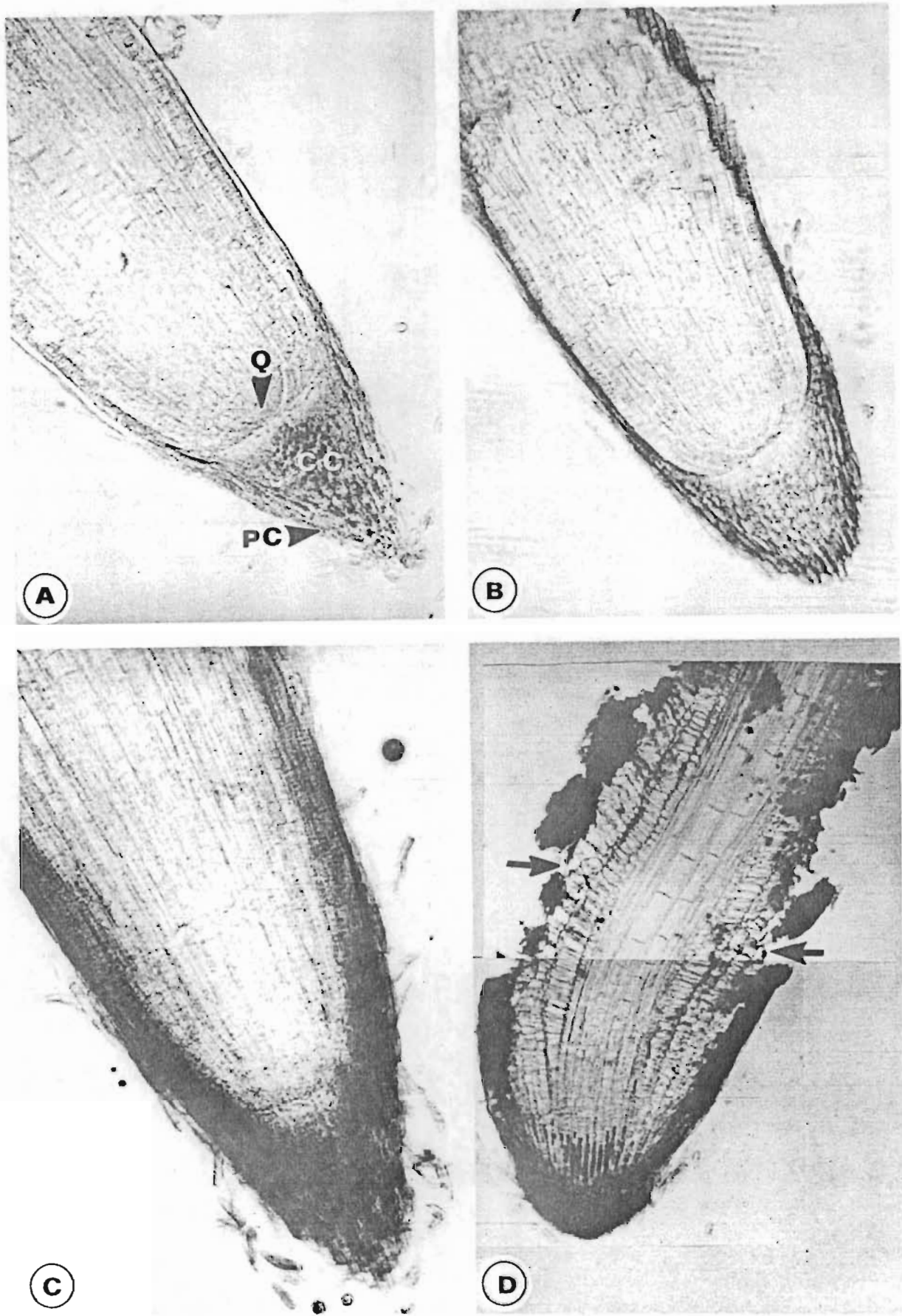
Figure 2 Micrographs of the root apices of *Zea mays* L., cultivar TX 24, treated with 0,84 mmol dm^{-3} Al activity and stained with haematoxylin. Darkly stained cells (blue/purple) are indicative of the presence of Al. **A.** Control, no Al added. Note complete absence of stain from all tissues. PC = cap periphery; CC = cap columella; Q = quiescent centre ($\times$ 100); **B.** Thirty minutes exposure to Al. Staining of peripheral cap cells and the mucilagenous secretions surrounding the root is indicative of Al uptake ($\times$ 100); **C** Two hours exposure to Al. Extensive staining of the cells of the root cap, excluding the cap initials, the outer surface of the root and the epidermal and outer cortical layers of the root apex is evident ($\times$ 100); **D.** Twenty hours exposure to Al. Heavy staining of the root cap and epidermal and outer cortical cells is evident. Considerable morphological disturbance of the cells of the root apex is apparent. Despite fracture of
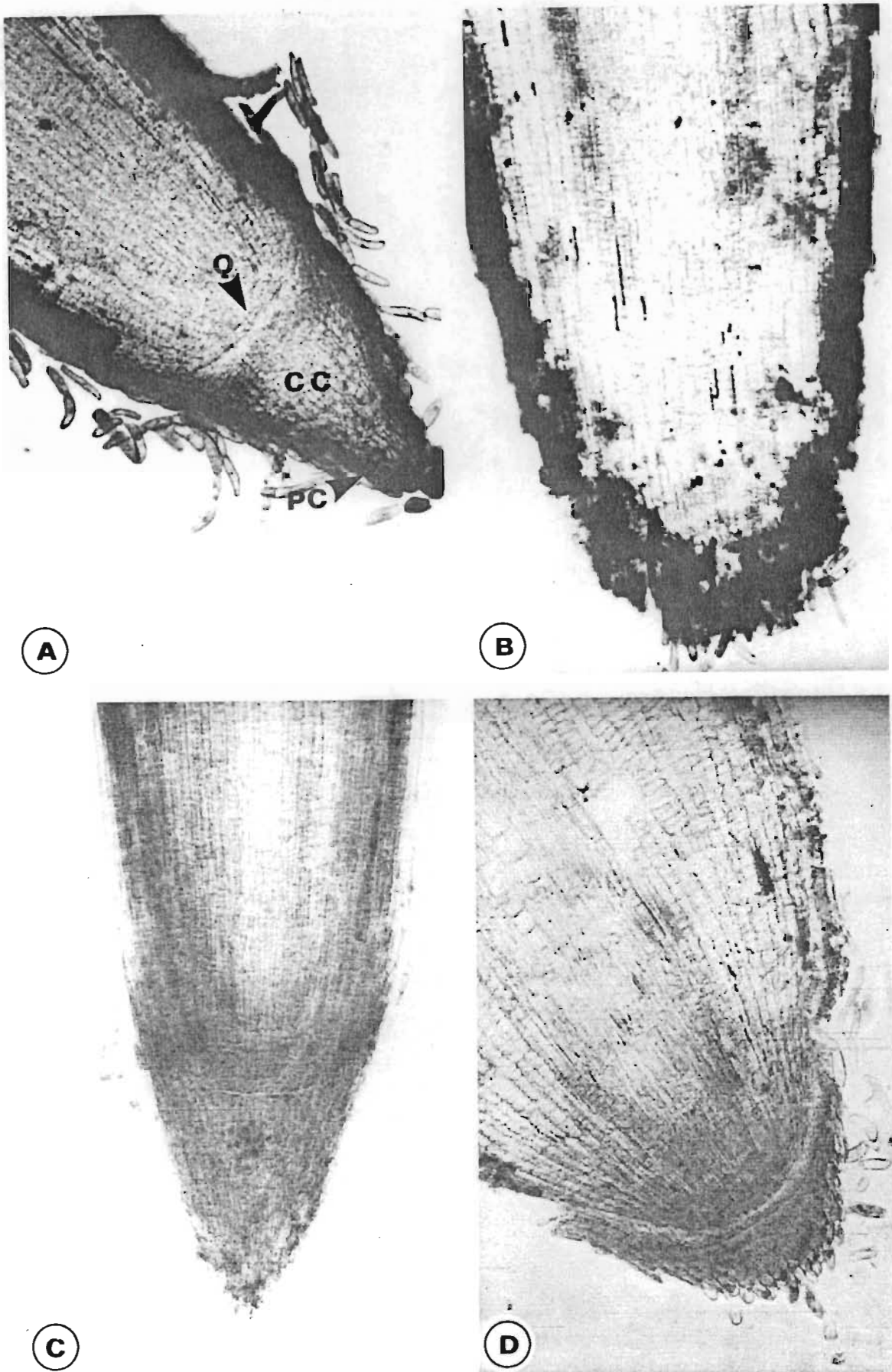
Figure 3 Micrographs of the root apices of *Zea mays* L. **A.** Twenty hours exposure to 0,84 mmol dm $^{-3}$ Al activity stained with haematoxylin. Note extensive staining of peripheral root cap cells (PC), epidermis and outer cortical cells and the absence of stain from mitotically active cells of the cap and root meristem. CC = cap columella; Q = quiescent centre ($\times$ 100); **B.** Decapped root exposed to 0,84 mmol dm $^{-3}$ Al activity for 20 h stained with haematoxylin. The epidermis is intact and yet the outer cortical and epidermal cells are heavily stained. Al has also entered the primary meristem in consequence of cap removal, penetration is limited and does not extend into the inner cortical regions or the stele ($\times$ 100); **C.** Control — no Al added, stained with methylene blue. Note that the intensity of stain is greatest in the cap, outer cortical regions and the meristem proximal to the cap/root junction. Inner cortex and central vascular tissue is characterized by a low intensity of stain ($\times$ 100); **D.** Twenty hours exposure to 0.84 mmol dm $^{-3}$ Al activity stained in methylene blue

Experiment II

The pattern of Al distribution as shown by haematoxylin staining in decapped and whole roots is illustrated in Figures 3A and 3B. The pattern of Al uptake was considerably altered in decapped roots with the accumulation of substantial amounts of Al in the cells of the quiescent centre and the primary root meristem with 20 h exposure to 0,84 mmol dm^{-3} Al activity. Significantly, penetration of Al in the decapped root did not extend to the inner cortical regions or the central vascular tissue (Figure 3B). The absence of Al from the quiescent centre and substantial areas of the root meristem of the control (Figure 3A) may implicate the root cap in protection of these tissues from Al.

Furthermore, neither the control nor the decapped root showed the level of cellular disorganization found in the previous experiment, and the epidermis remained intact for the period of exposure. Al is nevertheless found in considerable quantities in the epidermal and outer cortical cells of the root (Figures 3A & 3B), thereby confirming that failure of the epidermis is not a pre-requisite for entry of Al into the root.

Movement of Al into the cap columella and the cap initials of the control (Figure 3A) was less rapid than in the previous experiment.

Experiment III

The apical 2 – 3 mm of intact roots showed a strongly preferential capacity to bind methylene blue, while the remainder of the root axis had only a limited ability to take the stain (not illustrated**). In longitudinal section there were parallels between the cell populations stained with methylene blue (Figure 3C) and the regions of the root associated with Al uptake and accumulation (Figures 2B – 2D & Figure 3A) *viz.* the cells of the root cap and epidermal and outer cortical layers. Cells of the quiescent centre and root meristem were found to be stained with methylene blue (Figure 3C) but not haematoxylin in intact roots (Figures 2B – 2D & Figure 3A). Substantial amounts of Al were, however, found in these cells in decapped roots (Figure 3B). Significantly the inner cortex and stele had only a limited capacity to bind methylene blue. Reference to Figures 3A and 3B shows that these tissues do not accumulate Al in decapped or intact roots. Al was also shown to reduce the roots' capacity to bind the stain. With 20 h exposure to 0,84 mmol dm^{-3} Al activity, intensive methylene blue staining was limited to the root cap and the root meristem proximal to the cap/root junction (Figure 3D). In contrast to the control (Figure 3C), only isolated stain of low intensity was evident in the outer cortical-epidermal regions of the Al-treated root (Figure 3D).

Discussion

Al uptake

The results of this investigation differ principally from previous research on *Zea* (Rasmussen, 1968; Kalovoulos & Misopolinos, 1983) and *Triticum* (Wright & Donahue, 1953), with respect to the identification of the chronological pattern of Al uptake by the cell populations of the primary root. This information is a useful basis for speculating on the probable location of the site of Al injury in the root and, indeed, on the mechanism of Al toxicity.

The outer cap cells are the first to receive Al and, since the consequences of Al injury are extremely rapid (Clymo, 1962), the peripheral cap cells must be considered prime targets for Al injury. Al is also rapidly adsorbed onto the outer surface of the root where it may be complexed by mucilage

Earlier researchers (Wright & Donahue, 1953; Rasmussen, 1968) have differed with respect to the effectiveness of the epidermis in preventing entry of Al into the root meristem. While Al concentration may be relevant, this investigation has clearly shown that with prolonged exposure, Al can enter the root through the epidermis and, in this respect, results differ from the findings of Rasmussen (1968). Furthermore, morphological disturbances of the root apex leading to fracture of the epidermis as noted in Experiment I and also in *Triticum* (Hecht-Buchholz, 1983) are not a pre-requisite for the entry of Al into the cells of the root apex. However, Al does enter these cells less rapidly than those of the cap. Mobility of Al in the root cells is also low and penetration does not extend beyond the outer cortical layers. These observations may explain the association between Al accumulation sites and the outer surface of the root (Rasmussen, 1968; McCormick & Borden, 1974). These factors also suggest that the presence of Al in these tissues does not represent a primary site of Al toxicity.

Differences in Al uptake were noted between Experiments I and II. These experiments differed with respect to temperature and the size of the plants under investigation. Penetration of Al into the inner cap cells proceeded more slowly in the second experiment which featured a lower temperature and shorter growth periods. Clarkson (1967) has indicated that Al uptake is not influenced by temperature. The corollary to this observation would be that Al uptake may be affected by the physiological state of development of the plant, although the precise mechanism remains to be demonstrated.

The root cap and Al toxicity

Valuable circumstantial evidence in support of the fundamental role of the root cap in the physiology of Al toxicity arises from experiments with decapped roots. Removal of the root cap permitted entry of Al directly into the quiescent centre and root meristem. Whether the root cap actually protects cells directly from Al injury or whether the effect is entirely passive, remains to be demonstrated.

Barlow (1974) has, however, shown that removal of the cap stimulates the cells of the quiescent centre to enter mitosis and, since repeated division of these cells ultimately leads to regeneration of a new cap, the role of the root cap in delaying or restricting entry of Al into the quiescent centre may be fundamental to explaining the recovery of some root meristems from primary Al shock (Horst, Wagner & Marschner, 1983).

It is also of interest that Al-treated, decapped roots were slower than the controls to demonstrate the morphological disturbance of the apical 1,5 – 2,0 mm. This supports the hypothesis that the response to Al is initiated at a site remote from the cellular disorganization and is in accord with the absence of Al from the inner cortical regions of the root where cellular changes are more apparent.

Root biochemistry and Al uptake

These experiments have shown that Al accumulates preferentially in well-defined areas of the root. These include the root cap, epidermis and outer cortex of the root apex. Furthermore, movement to neighbouring cell populations may be restricted, or even non-existant. The immobility of Al in plant tissue has been connected to the internal precipitation of insoluble compounds, arising from interactions between Al and phosphorus (P) (Wright, 1943). This concept of chemical immobilization of Al is difficult to reconcile with the rapidity with which the toxic consequences of Al may be observed

plain the specific nature of Al uptake sites in the root.

It is therefore attractive to consider that Al uptake and distribution in the root may be determined by the biochemistry of cell populations involved. Gurr (1958) has noted that acidic substances are stained by methylene blue with increasing intensity at pH values above their isoelectric point, while below this point, the capacity to bind the stain diminishes sharply. With the exception of the root meristem proximal to the cap junction, there are clear parallels between control roots stained with methylene blue at pH 3,98 and the regions of the root associated with Al uptake. Significantly, removal of the root cap permitted entry of Al into those cells of the root meristem proximal to the cap which were stained with methylene blue, thus confirming the potential of these cells for Al uptake and accumulation.

Appearance of Al in these cells of intact roots may therefore be time dependent and this may explain the apparent anomalies between this work and the detection of Al in the nuclei of mitotically active cells of the root (Morimura, Takohashi & Matsumota, 1978; Naidoo, Stewart & Lewis, 1978).

The limited capacity of the cells of the inner cortex and stele to bind methylene blue, and the exclusion of Al from these tissues, even in decapped roots, supports the hypothesis that Al uptake is a function of cell biochemistry.

The ability of plant tissue to bind methylene blue at pH values below 4,0 is indicative of the presence of acid polysaccharides (Gurr, 1958). Obroucheva (1975) has connected the rapid synthesis of uronic acid in the root meristem with the region of cell division. Polyuronic acids are also present in the mucigel (Wright & Northcote, 1974). The biosynthetic function of cells of the root apex may therefore be a factor in the expression of Al toxicity. The precise mechanism is uncertain, but it is reasonable to expect that it may involve avoidance of the formation of insoluble Al compounds.

The noticeable effect of Al in reducing the capacity of the root to bind methylene blue stain may result from Al acting directly on the site of synthesis of the muco-polysaccharides, or from a general decline in the metabolic activity of the root. Since the symptoms of Al toxicity become apparent before the cells of the root cap and the meristem proximal to the cap/root junction receive Al, it is tempting to postulate that toxicity is effected through action at the site of synthesis of the muco-polysaccharides.

Al uptake and root growth

The widely reported effect of Al on cell division has frequently been associated with DNA synthesis (Clarkson, 1967; Morimura et al., 1978), which concept is in accord with the location of Al in the nuclei of meristematic cells (Morimura et al., 1978; Naidoo et al., 1978).

There is also evidence concerning the rapidity with which mitotic activity declines following Al treatment. Reduced mitotic activity can be observed within 2 h, followed by complete inhibition within 5–6 h (Clarkson, 1965; Horst et al., 1983).

This experiment has, however, indicated that it may take considerably longer for Al to reach mitotically active cell populations since some 12 h were required before Al was detected in the cap intitials. Furthermore, Al may take considerably longer to reach the quiescent centre and mitotically active cells of the root meristem. This suggests that Al may act indirectly on mitosis through the integrated control function of the root meristem.

Clowes (1972) has unequivocally demonstrated that the root

the root meristem and, if Al acts directly on the root cap as evidenced by this study, it is reasonable to suggest a fundamental role for the root cap in Al toxicity. The precise mechanism of regulation remains to be demonstrated, but Juniper (1972) has proposed the presence, in the root cap, of two integrated perception systems. Organization of these systems is dependent on starch metabolism within the differentiating cells of the root cap. Our experiments have implied a connection between Al toxicity and the biochemistry of the muco-polysaccharides, and it is conceivable that the interference by Al could be directed along this pathway.

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The primary site of aluminium injury in the root of *Zea mays* L.

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Histochemical and ultrastructural studies of aluminium-induced changes in root metabolism and the structure of the cytoplasmic organelles of the root cap indicated that the first observable effect of aluminium involved the migration of secretory vesicles of the Golgi apparatus which was considered indicative of interference by Al in membrane transport. Al also resulted in a reduction in the number of cisternae per dictyosome, disruption of the membranes of the cisternae and secretory vesicles as well as alteration to the appearance of the product elaborated in the secretory vesicles. Changes in the frequency and appearance of the endoplasmic reticulum were also noted. Progressive vacuolation of the cells of Al-treated roots was observed. No alteration to the nuclear structure of the cap initials involving either the appearance of the nuclear membranes, or degree of chromatin condensation could be connected to Al injury during the first 20 h of exposure. Al was shown to affect the pattern and intensity of respiratory activity in the root apex. High levels of respiratory activity were detected in control treatments in cells proximal to the cap/root junction and approximating the location of the quiescent centre. Al treatment was found to have a rapid inhibitory effect on the metabolic activity of these cells.
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Studies van aluminiumgeïnduseerde veranderings in wortelmetabolisme en organelstruktuur m.b.v. histochemiese en elektronmikroskopiese tegnieke het getoon dat die eerste waarneembare invloed van aluminium die beweging van sekreetvesikels van die Golgi-apparaat is wat as 'n aanduiding van die invloed van aluminium op membraanvervoer beskou word. Aluminium het ook 'n afname in die aantal sisternums per diktiosoom, ontwriging van membrane van die sisternums en sekreetvesikels en veranderings in voorkoms van die sekreetvesikels se produk veroorsaak. Veranderings in die hoeveelheid en voorkoms van die endoplasmiese retikulum is ook opgemerk. Progressiewe vakuolering van die selle van aluminiumbehandelde wortels is waargeneem. Geen wysiging in die nukleêre struktuur van die wortelmusinisiaalselle wat voorkoms van die membrane of graad van chromatienverdigting betref, wat met aluminiumbeskadiging verband hou, is gedurende die eerste 20 h van blootstelling waargeneem nie. Aluminium het die patroon en omvang van respiratoriese aktiwiteit in die wortelpunt beïnvloed. In kontrolebehandelings is hoë vlakke van respiratoriese aktiwiteit in selle proksimaal tot die wortelmus/wortelaansluiting en naastenby by die posisie van die stiltegebied gevind. Aluminiumtoediening het 'n vinnig inhiberende invloed op die metaboliese aktiwiteit van hierdie selle uitgeoefen.
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Introduction

Aluminium (Al) toxicity has frequently been connected to inhibition of root growth (Rorison, 1960), acting through reduced mitotic activity (Clarkson, 1965; Morimura, Takohashi & Matsumoto, 1978; Horst, Wagner & Marschner, 1983). Time-course experiments on Al uptake by the primary root of *Zea mays* L. have, however, indicated that cessation of cell division may not be a direct consequence of the presence of Al in mitotically active cells (Bennet, Breen & Fey, 1985).

While the accumulation of Al in the root has many of the characteristics indicative of non-metabolic ion uptake (Clarkson, 1967; Rhue, 1976), Al uptake sites in the root apex are located in areas of intense physiological activity. Not only does the root apex contain mitotically active cell populations (Luxová, 1981), it is also an area of high respiratory activity (Yemm, 1965), has been connected to uronic acid production (Obroucheva, 1975), and accumulation of plant growth substances (Torrey, 1976). The root cap also has many unique features involving polysaccharide metabolism (Barlow, 1975), as well as perception of geotropic stimuli (Juniper, 1972).

The possible failure of cell division resulting secondarily from impairment of other metabolic functions by Al has, however, received little consideration. Furthermore, Clarkson (1969) was unable to relate reduced mitosis to lower respiration rates in Al-treated roots of barley (*Hordeum vulgare*) and onion (*Allium cepa*).

Al uptake studies (Bennet *et al.*, 1985) have, nevertheless, attached a fundamental significance to the root cap, since the peripheral cap cells are the first to take up Al. Clowes (1972a) has also demonstrated that the root cap has an important function in controlling the cycling rate of cells within the root meristem. The precise mechanism of control and how Al may influence this function remains to be defined.

This investigation is concerned with Al-induced changes in the metabolic activity of the cell populations of the root apex, and associated ultra-structural changes which might lead to identification of the site of Al injury.

Material and Method
Plant growth conditions

The experiment was conducted under identical conditions to those in Experiment 1 (Bennet *et al.*, 1985), with the exception that the root exposure times were 0 h; 2 h; 5 h; 6,5 h; 12 h; 20 h and 48 h.

Histochemical procedures

The histochemical demonstration of the sites of dehydrogenase/diaphorase enzyme activity with tetrazolium salts is

widely practised (Barka & Anderson, 1963; Pearse, 1972).

The following procedure was found to give reproducible results:

- 4,6 mM Tetrazolium solution was prepared from 2 : 3 : 5 triphenyl-tetrazolium chloride (TTC) (BDH 34062;* M W = 334,81) in phosphate buffer at pH 7,2.
- Phosphate buffer was prepared from M/15 Na_2HPO_4 and M/15 KH_2PO_4 to give pH 7,2 (Vogel, 1955).
- Roots of intact plants were treated in TTC solution for 1,5 h at 25 °C.

Microtechniques

Pre-treated roots of selected plants from each treatment were sectioned frozen on a Reichert, Thermo-Electric Cold Stage Microtome.* Sections were temporarily mounted in a 50% glycerine water mixture, viewed on an Olympus BH-2* microscope and photographed on Kodachrome 25* film.

Root tips from each treatment were also fixed in 6% glutaraldehyde in 0,05 M sodium cacodylate buffer at pH 7,2, with post fixation (2 h) in 2% OsO_4 in 0,05 M sodium cacodylate buffer (Hayat, 1981a).

Fixed material was dehydrated in graded alcohol, treated with propylene oxide and embedded in Epon/Araldite resin.

Ultra-thin (gold) sections for transmission electron microscope viewing were stained in uranyl acetate and lead citrate (Hayat, 1981b). Material was viewed on Jeol 100 TEM.*

Results

The cell populations of the root cap and the primary growth region of the main root axis in *Zea mays*, as referred to in this study, have been defined respectively by Barlow (1975), Luxová (1981) and outlined by Bennet *et al.* (1985). It is pertinent to note that the Al activity of 0,84 mmol dm^{-3} used in these experiments corresponds to the highest level of toxicity reported by Pavan and Bingham (1982) in coffee (*Coffea arabica*).

Al and the metabolism of the root meristem

In the absence of Al, the highest rate of respiratory activity was associated with the cap periphery and epidermis (Figure 1A). Figure 1A also shows an area of intense respiratory activity in the cells proximal to the cap/root junction, corresponding to the region of the root forming the quiescent centre (Clowes, 1956).

Changes in the overall metabolic pattern were evident within 2 h exposure to 0,84 mmol dm^{-3} Al activity. Reduced respiration was first observed in the peripheral cap cells, the epidermal cells of the root apex and the quiescent centre (Figure 1B). Limited exposure to Al concurrently stimulated the inner cap cells and the cells of the root meristem to increased metabolic activity (Figure 1B). Increased activity was, however, short-lived (Figure 1C and Figure 1D) and within 12 h exposure, only isolated respiratory activity remained.

Results with prolonged exposure (48 h) were variable. Some roots continued to show some respiratory activity, chiefly in the root meristem proximal to the quiescent centre and including the pole of the stele (Figure 1D), while in others, no activity could be detected.

Ultrastructural changes in the root cap

Al-induced changes in cell structure are presented in Figures 2A – 2F.

In the control treatments, the outer peripheral cap cells were found to be rich in dictyosomes, secretory vesicles, mitochondria and endoplasmic reticulum (E.R.). Nuclei and amyloplasts also occurred (Figure 2E). Cells of the cap columella were essentially non-vacuolate, contained amyloplasts and E.R., while dictyosomes were less well developed than those in the outer cap (Figure 2C). The cap initials were poor in all organelles (Figure 2A).

Progressive vacuolation of the cells of the root cap was one of the most easily identifiable consequences of Al toxicity. Vacuolation was initiated in the outer cap cells, where it was evident within 2 h exposure to 0,84 mmol dm^{-3} Al activity and was followed by severe disorganization of the cytoplasmic contents of the outer cap cells within 20 h (Figure 2F).

Vacuolation of the cap columella and cap initials proceeded more slowly. There was, however, evidence of vacuolation of the cells of the columella within 6,5 h (Figure 2D) and of the cap initials within 20 h exposure (Figure 2B).

The frequency of cytoplasmic organelles was also reduced in Al-treated cells, this included an almost total disappearance of E.R. with prolonged exposure to Al. Comparison between Figures 3B – 5C also suggests that disappearance of the E.R. was preceded by swelling of the E.R. These changes were accompanied by an alteration in the appearance of the amyloplasts which showed an increased intensity of stain in the starch bodies of Al-treated cells of the cap periphery and cap columella (Figures 2D – 2F).

No change in the nuclear structure of the cap initials could be detected during the first 20 h of this experiment. Cells contained well-defined nuclei, the nuclear membrane remained intact, and no apparent change in the degree of chromatin condensation as a consequence of Al treatment was observed (Figures 2A, 2B, 5A). No evidence was found to connect Al treatment with the rapid disruption of the plasmalemma (Figures 3C, 4C and 5C).

Al-induced changes in the structure of the Golgi apparatus

Dictyosomes of the peripheral cap cells of the control treatment reflected structural features characteristically associated with the secretory function of the outer cap cells (Figures 3A – 3B). These included an easily recognizable polarity of the dictyosome based on the appearance of the cisternal contents and the size, shape and frequency of the secretory vesicles. Dictyosomes were also frequently associated with ribosomes on the forming face and E.R.

Al treatment was connected to a rapid alteration to the dictyosomes of the Golgi apparatus. This was observed with 2 h exposure to Al as a disruption in vesicle production and change in membrane structure with many vesicles showing disintegrated outer membranes (Figures 4A – 5A). Severe curling of the cisternae (Figure 4B) and a reduction in the number of cisternae per dictyosome from a mean of 5,5 (control), to a mean of 3,7 (6,5 h exposure) were also found.

Change in the secretory function of the Golgi apparatus

The relative absence of secretory vesicles from the cytoplasm of the control treatment (Figure 3C) was considered indicative of the rapidity of transfer of vesicular contents across the plasmalemma. Limited exposure to Al (2 h) was characterized by the accumulation of secretory vesicles around the dictyosomes (Figures 4B – 4C). Furthermore, the contents of the secretory vesicles were progressively altered from a dense, granular material (Figures 3A and 3B), to a diffuse, fibrillar
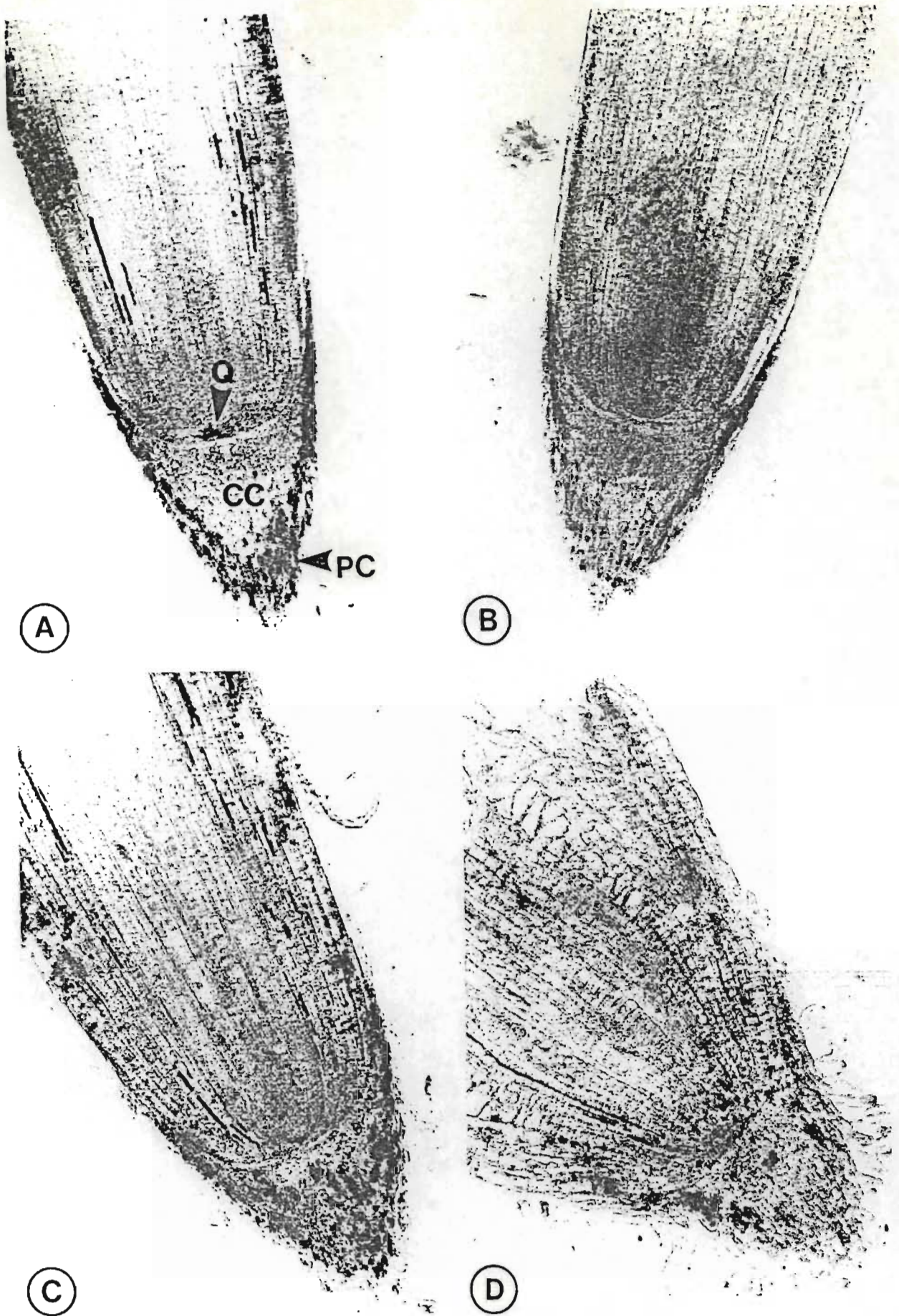
Figure 1 Micrographs of the root apices of *Zea* treated with TTC to show the effect of 8 mg dm^{-3} Al on root metabolism. The development of the highly pigmented, red, formazan in the root tissue as seen in longitudinal section is indicative of the occurrence and intensity of enzyme-catalysed oxidations. A. Control — note the high level of respiratory activity in the peripheral cap cells (PC) and in the cells of the quiescent centre (Q) (× 100); B. Two hours' exposure to Al has significantly reduced the level of respiration in the peripheral cap cells, epidermis and quiescent centre. There is evidence of a general increase in respiration in the remaining cell populations (× 100); C. Twelve hours' exposure to Al has inhibited respiration throughout the root and only isolated activity is now evident (× 100); D. Forty-eight hours' exposure to Al and only isolated activity of low intensity is evident in the cells of the root meristem proximal to the quiescent centre and including the pole of the stele (× 100).
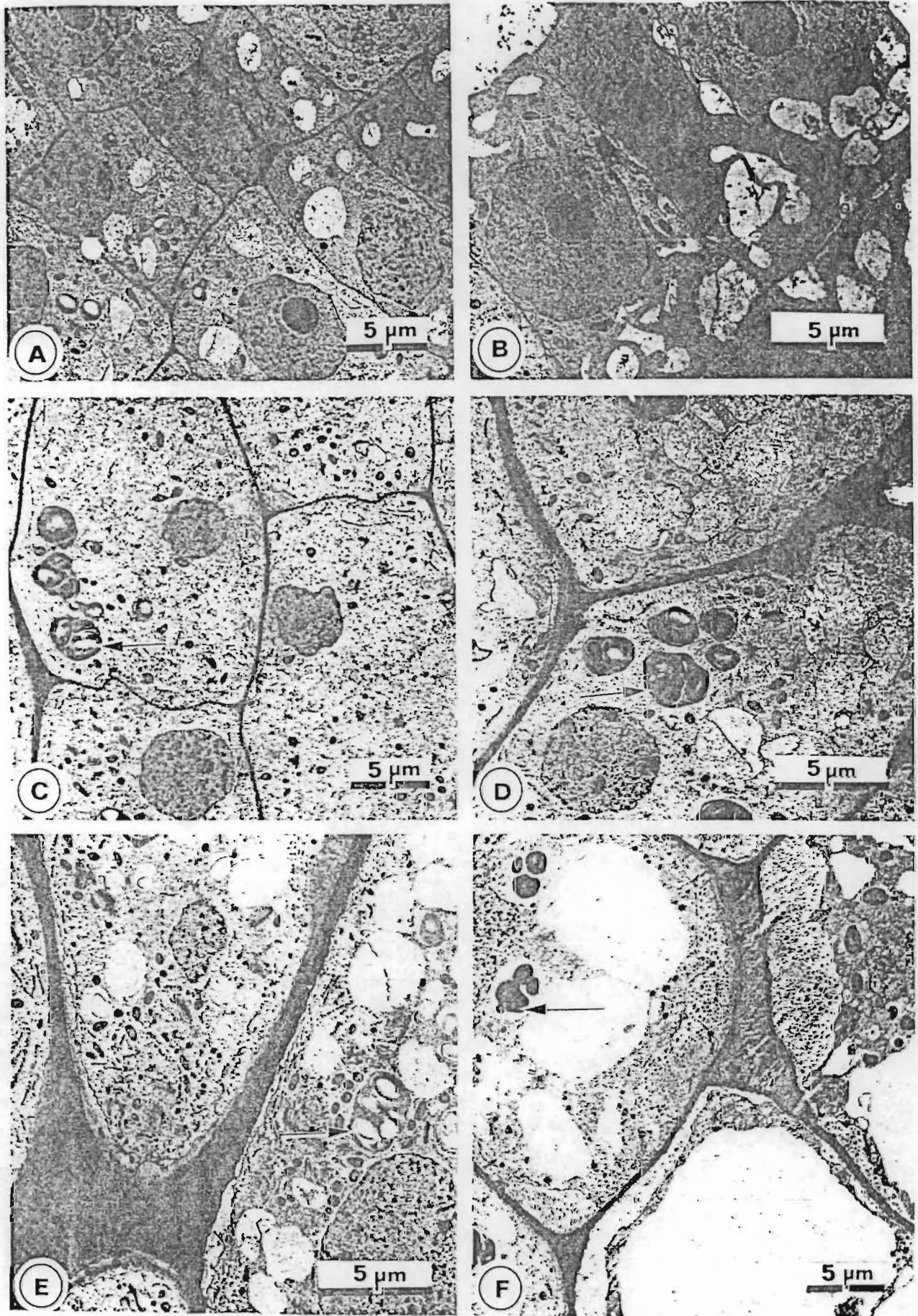
Figure 2 Electron micrographs of the cells of the root cap of *Zea* illustrating Al-induced changes in ultrastructure. Material glutaraldehyde — OsO_4 fixation, stained in uranyl acetate and lead citrate. A. Cap initials, control; B. Cap initials, 20 h exposure 8 mg dm^{-3} Al. Note the apparent stability in nuclear structure. Changes in cell structure are essentially limited to increased vacuolation; C. Cap columella, control, cells are non-vacuolated and contain amyloplasts (arrowed); D. Cap columella, 6,5 h exposure 8 mg dm^{-3} Al. Note initiation of vacuoles and increased intensity of stain in starch grains within amyloplasts (arrowed); E. Cap periphery, control, note extensive development of cytoplasmic organelles, and appearance of amyloplasts (arrowed); F. Cap periphery 20 h exposure to 8 mg dm^{-3} Al. Note the extensive development of vacuoles, the accumulation of Golgi apparatus-derived material between the plasmalemma and the cell wall, and the

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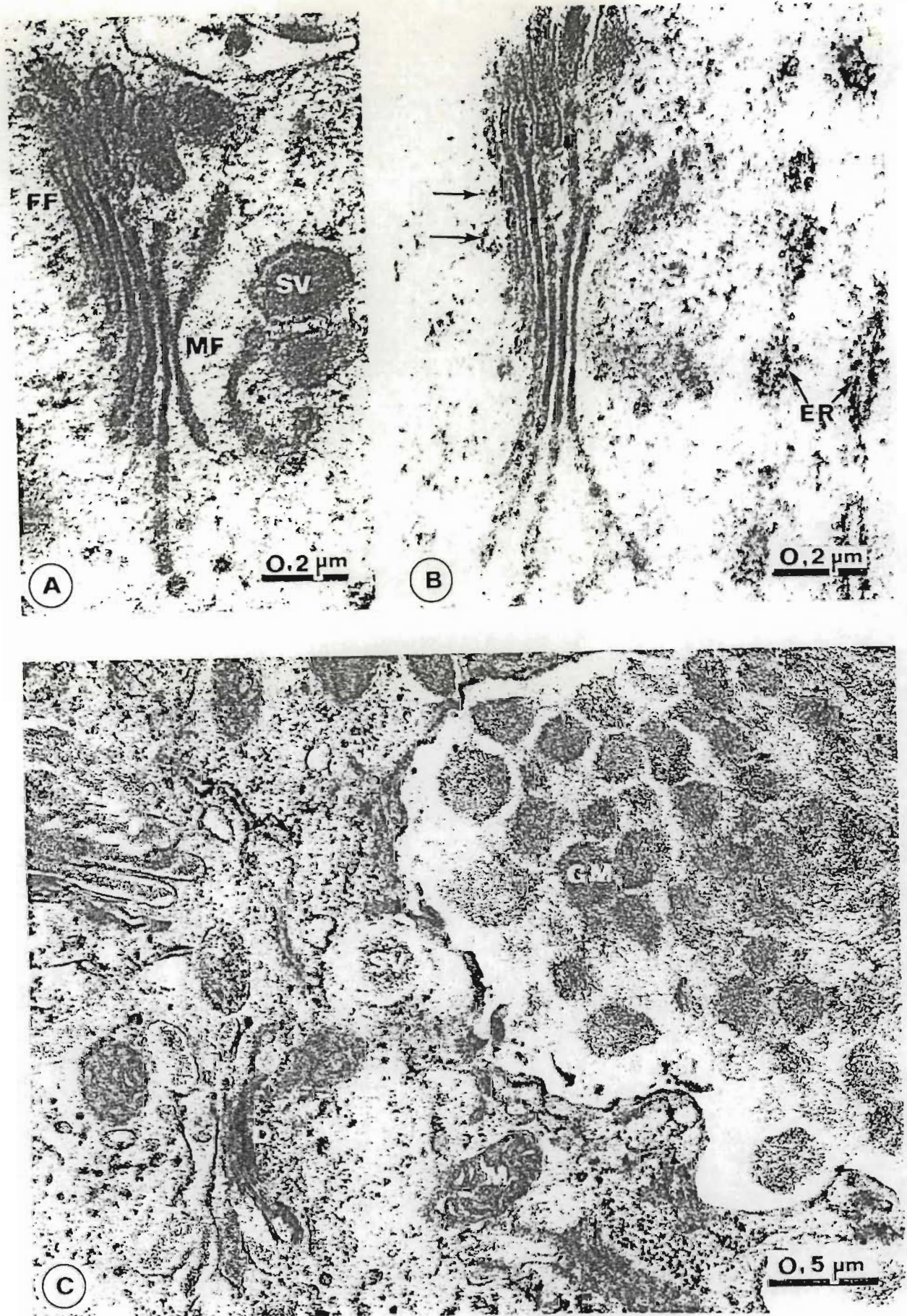
Figure 3 Electron micrographs of peripheral root cap cells in *Zea* illustrating the secretory function of the dictyosomes of the Golgi apparatus in the control treatment. Material glutaraldehyde — OsO₄ fixation, stained in uranyl acetate and lead citrate. **A.** Most dictyosomes show a distinct polarity with the cisternae developing sequentially across the stack from the forming face (FF) to the maturing face (MF). Note the fine granular appearance of the contents of the secretory vesicle (SV) and the alteration in shape from elongated to round of the secretory vesicles with maturation; **B.** Dictyosomes are frequently associated with ribosomes on the forming face (arrowed) and E.R., **C.** The secretory pattern in the peripheral cap cells is characterized by the activity of dictyosomes (D), the frequency of secretory vesicles (SV) (several of which appear near the plasmalemma), the occurrence of mitochondria (M) and a well-developed E.R., the granular appear-
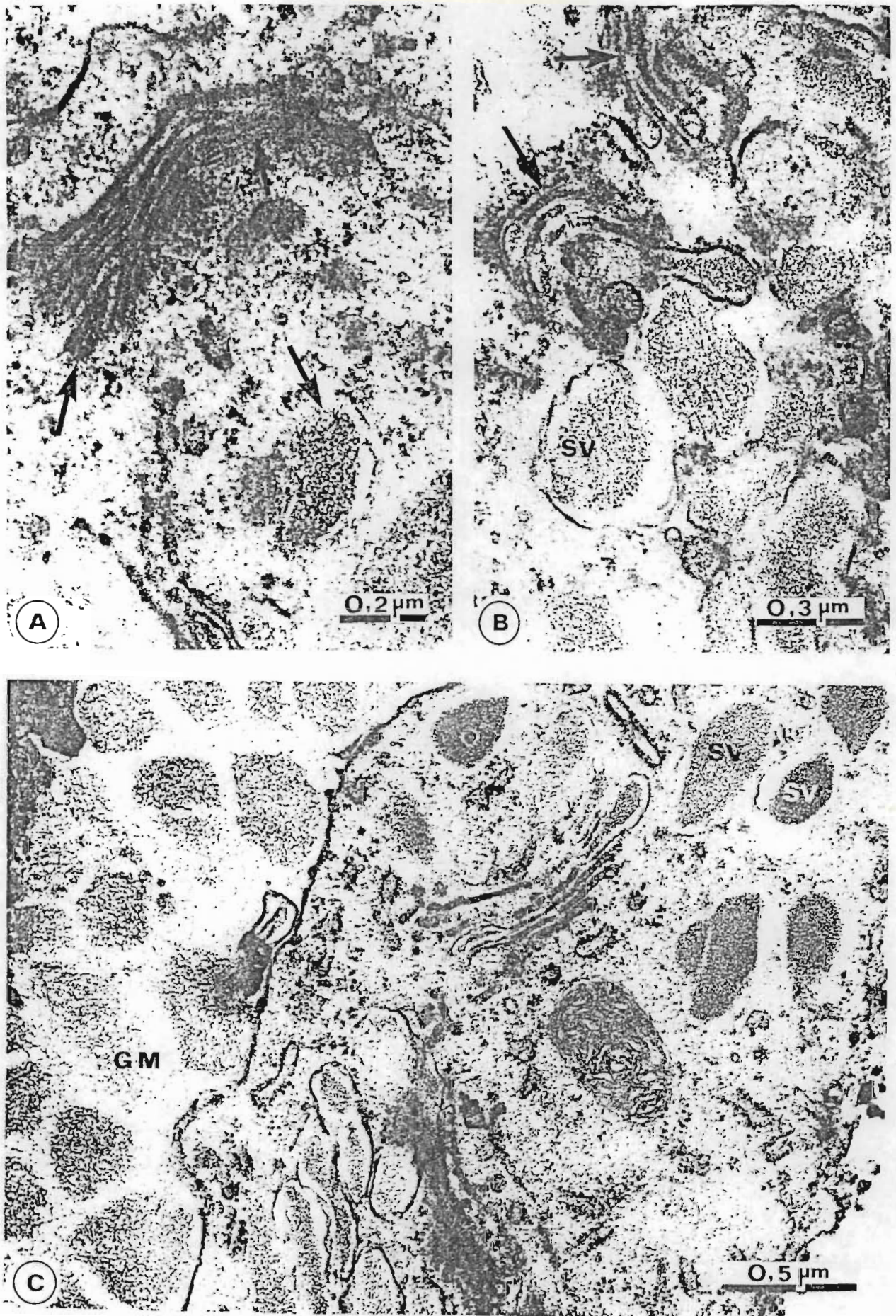
Figure 4 Electron micrographs of peripheral root cap cells in *Zea* illustrating the effect of 2 h exposure to 8 mg dm^{-3} Al on the secretory function of the dictyosomes of the Golgi apparatus. Material glutaraldehyde — OsO$_4$ fixation, stained in uranyl acetate and lead citrate. **A.** Many of the membranes of the secretory vesicles and cisternae have begun to disintegrate (arrowed). Vesicular contents may still retain much of the granular appearance of the control; **B.** Severe curling of the cisternae is evident (arrowed). The contents of the secretory vesicles (SV) is also altered and some vesicles even appear empty. Many vesicles are accumulating in the vicinity of the dictyosome; **C.** The rapid accumulation of secretory vesicles (SV) in the cytoplasm is clearly evident. Differences in the appearance of vesicular contents are also

Figure 5 Electron micrographs of the peripheral root cap cells of *Zea* illustrating the effect of 6,5 h exposure to 8 mg dm^{-3} Al on the secretory function of the dictyosomes of the Golgi apparatus. Material glutaraldehyde — OsO$_4$ fixation, stained in uranyl acetate and lead citrate. A. Disintegration of the membranes of the cisternae and secretory vesicles (arrowed) is clearly evident. The nuclear membrane (NM) of the nucleus (N) is still intact; B. Complete disorganization of the dictyosome (arrowed) is evident. Note the accumulation of the secretory material in the vicinity of the disintegrating dictyosome. The secretory vesicles lack definition and the shape is highly variable. The membranes of mitchondrium (M) are still well defined; C. The dictyosome (D) is no longer active. Membranes of many secretory vesicles (SV) have disintegrated and the secretory material is diffuse and fibrillar. E.R. identified by the presence of ribosomes (arrowed) is swollen and there is a progression evident from swollen E.R. to vacuole (V).

secretory vesicles could still be observed with 2 h exposure to Al (Figure 4C). However, longer exposure resulted in the complete disappearance of vesicles with finely granular contents (Figure 5C). Similar changes involving the appearance of Golgi apparatus-derived material, accumulated between the plasmalemma and cell wall, were also noted (Figures 3C, 4C and 5C).

Discussion

Al-induced changes in root metabolism have confirmed a fundamental importance for the peripheral cap cells in the physiology of Al toxicity. This observation is in accord with investigations into the site(s) of Al uptake (Bennet et al., 1985), since the outer cap cells are also the first cell population of the root apex to receive Al. The most conspicuous activity of these cells involves the secretion of slimes and mucilages of polysaccharide or polysaccharide-protein complexes (Mollenhauer & Morré, 1980). Earlier investigations (Bennet et al., 1985) have also implied that the biochemistry of the mucopolysaccharide substances may be essential to the expression of Al toxicity.

Al toxicity and the physiology and structure of the Golgi apparatus

The Golgi apparatus has been connected to a remarkably diverse range of functions, including synthesis of polysaccharide materials, membrane transformation, export of materials from the cell including mucilages (Mollenhauer & Morré, 1980), while rearrangement of the dictyosomes of the Golgi apparatus following gravitropic stimulation has also been observed (Gressel & Horwitz, 1982). The secretory function of the Golgi apparatus of the peripheral cap cells has been extensively reviewed (Mollenhauer & Morré, 1980). Furthermore, Mollenhauer (1965) has drawn attention to morphological distinctions between the dictyosomes of the cap periphery and those of the cap columella and epidermis, which are considered indicative of changes in biochemical function and which may be relevant to determining the chronological sequence noted with respect to Al uptake (Bennet et al., 1985) in the cell populations of the root apex. Structural changes noted in the dictyosomes of the Golgi apparatus following Al treatment are therefore of considerable interest. Mollenhauer and Morré (1980) have indicated that three structural responses characterize stressed Golgi apparatus:

(i)   change in secretory pattern,
(ii) change in the number of cisternae per dictyosome, and
(iii) change in cisternal architecture,

while in secretory cells, the most immediate response to stress may involve the cessation of secretory activities. All these changes may be identified in the Golgi apparatus of the peripheral cap cells within 2 h exposure to 0,84 mmol dm^{-3} Al activity. Furthermore, the curling of cisternae noted in these experiments with Al is indicative of severe physiological stress, since this symptom has previously been connected to shock occurring during isolation and treatment with potassium cyanide (Mollenhauer & Morré, 1980).

Since Al elicits a number of ultrastructural responses, considerable relevance must be attached to the chronological sequence in which symptoms appear. Possibly the first consequence of Al injury concerns the transfer of secretory vesicles from the Golgi apparatus to the cell exterior. Evidence includes the rapid accumulation of secretory vesicles in the vicinity of the dictyosomes even with limited exposure to Al (2 h). This is considered indicative of interference in

brane transport function (Mollenhauer & Morré, 1980; Newcomb, 1980). Since apparently normal vesicles, based on the appearance of the vesicular contents, are intermingled with those of altered appearance, it may be argued that failure of the transfer mechanism must precede the change in vesicular contents. This implies that movement of Golgi apparatus-derived vesicles to the cell surface may represent the primary site of Al action. This observation may be supported by an increase in reactivity which has been observed across the dictyosomal stack from forming to maturing face (Morré, 1977a).

Changes in the number of cisternae per dictyosome as a consequence of Al treatment is strongly indicative of an alteration to the secretory activities of the Golgi apparatus, since during steady state secretion, the number of cisternae per dictyosomal stack may be expected to remain constant (Morré, 1977b). In addition, the appearance of the vesicular contents is altered from a dense, finely granular material to a more diffuse, fibrillar material. This observation is indicative of an effect by Al on the nature of the product being elaborated since Newcomb (1980) has indicated that the ultrastructural appearance of the cisternal contents may be characteristic of the product being synthesized. Similar changes in appearance were also noted in the Golgi apparatus-derived material exported from the cytoplasm and accumulated between the plasmalemma and cell wall.

Al toxicity within the endomembrane system

The Golgi apparatus cannot, however, be considered as an autonomous organelle and, for the more complex functions of the Golgi apparatus to proceed, the structured relationship with E.R. and the plasmalemma must also be considered (Mollenhauer & Morré, 1980). It is significant therefore that the short-term consequences of Al toxicity are essentially directed at the Golgi apparatus, and it is tempting to implicate specific functions of the Golgi apparatus directly to Al injury. Activities characteristic of the Golgi apparatus involve only the enzymes thiamine pyrophosphatase and certain glycosyl transferases (Morré & Mollenhauer, 1974), all other enzymes showing activities in the Golgi apparatus intermediate between E.R. and the plasmalemma. The glycosyl transferases have been connected to the ordered sequential addition of sugars within the Golgi apparatus (Morré, 1977a). Interference by Al in this function is supported by the altered appearance of the vesicular contents.

These experiments did not confirm the plasmalemma as a primary site of Al action (Hecht-Buchholz & Foy, 1981) since no change in appearance was evident with short-term exposure to Al. The activities of the Golgi apparatus almost certainly include the biosynthesis of new membrane material destined for incorporation in the plasmalemma (Newcomb, 1980). Failure of the Golgi apparatus may therefore be expected to precede damage to the plasmalemma.

Vacuolation

Progressive vacuolation of cap cells is one of the most easily observed consequences of Al injury. It is, however, unlikely to represent a primary site of Al toxicity since vacuolation is preceded by disturbances to the cytoplasmic organelles. Membrane differentiation within the concept of the endomembrane system must also be seen to involve the vacuolar apparatus of the cell interior (Morré & Mollenhauer, 1974). The initiation and origin of vacuoles remains to be defined with certainty and has been variously considered to involve E.R.

peels, 1980) or the Golgi/E.R./lysosome complex as possible origins of the tonoplast (Marty, Branton & Leigh, 1980).

The results of this investigation suggest that in Al-stressed cells, the vacuoles arise from swollen, rough E.R. as a direct consequence of Al injury. Furthermore, the extensive damage to the membranes of the Golgi apparatus may preclude the involvement of these structures in the formation of vacuoles.

Respiration within the root meristem

There is little direct evidence to connect Al toxicity directly to failure of the respiratory mechanism, since the appearance of the mitochondria, even with relatively long exposure to Al, indicates that these organelles are conservative to change. This observation is consistent with the findings of Hanson & Day (1980) who investigated the general response of mitochondria to stress. Furthermore, it is important to consider that the ultrastructural changes to the Golgi apparatus precede severe inhibition of respiratory activity in the peripheral cap cells. The observed changes in respiration following Al treatment may therefore reflect a decline in energy requirements rather than the direct consequence of Al action.

Although the respiratory mechanism may not represent a primary site of Al injury, the results of our experiment are useful in confirming the role of the peripheral cap cells in Al toxicity. Not only are these cells the first to take up Al (Bennet et al., 1985) but the outer cap is also amongst the first cell populations to show a physiological consequence of Al injury. It is pertinent that the mitotically active cell populations of the root apex showed increased, but short-lived, respiratory activity during the first 2 h of the experiment. It is difficult to connect this observation to a rapid decline in mitotic activity and it seems improbable therefore that Al can influence cell division through the respiratory pathway. This observation is in agreement with the results of Clarkson (1969) who was unable to connect oxygen uptake directly to reduced mitosis in the Al-treated roots of Hordeum vulgare on a time basis.

The high level of metabolic activity of the cells proximal to the cap/root junction corresponding to the quiescent centre requires comment. Relative to neighbouring cells, the cells of the quiescent centre show low rates of DNA synthesis (Clowes, 1956), amino acid incorporation (Clowes, 1958) and cell division (Clowes, 1975). The unique position of the quiescent centre to mediate in the distribution of materials exported from the root cap or transported down the stele has, however, prompted numerous suggestions with regard to an undefined role in the organization of the root apex (Torrey, 1972).

It is not easy to account for the result of our experiment, particularly as these cells are among the first to show reduced respiratory activity following Al treatment. It is, however, important that previous investigations (Bennet et al., 1985) have indicated that this change is not the direct consequence of Al, since considerable time may be required before Al can be detected in these cells. It is indeed tempting to associate the changed level of metabolism with a fundamental, but as yet, undefined function of the quiescent centre in the control of the root meristem.

Nuclear structure

These experiments have not provided evidence to directly connect Al toxicity with changes in the nuclear structure of the cap initials. To the contrary, observations with respect to the distribution of dense and diffuse chromatin show that most nuclei retained a balance between the two forms with up to 20 h exposure to Al. Jordan, Time & Trec.

attached considerable importance to the degree of chromatin condensation in the interphase nucleus, since a conversion from diffuse to dense chromatin is considered indicative of reduced RNA synthesis.

This finding is in accord with earlier work on the nuclear structure of Al-stressed cells (Clarkson, 1965), and Al uptake sites (Bennet et al., 1985) and provides further evidence for the hypothesis that Al acts indirectly on mitosis through the integrated control function of the root.

A hypothesis for Al toxicity

How Al-induced changes in the outer cap cells influence control of the root meristem remains to be demonstrated with certainty. Our experiments indicate that control must operate at two levels, which include the function of the Golgi apparatus of the peripheral cap and communication with mitotically active cell populations.

The first consequence of Al involves the regulation of Golgi apparatus function through interference in the flow of secretory vesicles. Morré (1977b) has indicated that the flow of secretory vesicles must satisfy the criteria of being directional and selective. Although the precise driving mechanism remains a mystery, Morré (1977b) has, however, hypothesized that control could be exercised through the zone of exclusion (Morré, 1977b) which surrounds all Golgi apparatus. Significantly, Giulian and Diacumakes (1976) have intimated that the zone of exclusion is derived from electrical properties which differ from those of the bulk cytoplasm. It is reasonable to anticipate that Al could be involved in the disruption of this field through the charge on the Al^{3+} ion.

Control of the root meristem through the cap was first suggested by Clowes (1972a). Clowes (1972b), however, connected hypersensitivity of the cap initials in Zea mays directly to the high cycling rate of these cells. Our experiments do not support this concept with respect to Al toxicity and have instead assigned a fundamental role to the peripheral cap cells. There is, however, as yet no evidence to indicate how the response of the Golgi apparatus is transmitted to the mitotically active cells.

Raven & Rubery (1982) have indicated that communication between cells can involve chemical or electrical impulses. The rapid effect of Al on the metabolism of the cells of the quiescent centre may represent valuable evidence in support of a stress response mechanism and furthermore, the metabolic changes may seem to favour a chemical pathway for transmission of the stimulus. The high level of functional interdependence between components of the endomembrane system (Morré & Mollenhauer, 1974) may indicate a role for E.R. in the transmission of intracellular messages between Al-stressed cells. Support for this view may be found in the widely defined function of E.R. as a hormone-binding site (Raven & Rubery, 1982), and it is conceivable that hormone gradients within the root meristem are altered as a consequence of damage to the E.R. noted in these experiments. An alternative path might involve the as yet undefined role of hydrolysing enzymes contained in the Golgi apparatus (Morré, 1977b) which may be released on disruption of the membrane structures.

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Aluminium induced changes in the morphology of the quiescent centre, proximal meristem and growth region of the root of *Zea mays*

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Treatment of the primary root with 8 mg dm^{-3} Al altered the pattern of cell growth so that with 48-h treatment, the root apex was no longer an organized, cytologically heterogeneous complex. An increase in mean cell volume was first observed with 2-h treatment, primarily in the cells of the quiescent centre where cell enlargement was associated with the removal of the constraint to quiescence by Al and in the mid-cortex 1 – 2 mm from the root tip where growth stimulation was associated with a departure from osmotic equilibrium. An initial increase in the osmotic potential of the root cell sap arising from Al treatment (1 h) was not maintained and subsequent decreases in the osmotic potential coincided with changes in the cell growth direction. Stress patterns arising from the anisotropic growth response of cortical cells with 20-h root exposure to Al were associated with the collapse of the conducting tissue of the stele and disintegration of the outer cells of the root. Advanced vacuolation of the cells of the root apex, first observed with 5-h treatment, was a feature of Al-stressed roots. Alteration to the nuclear structure in cells of the proximal meristem involving the degree of chromatin condensation and structure of the nucleolus was considered indicative of senescence.

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Behandeling van die primêre wortel met 8 mg dm^{-3} Al het die patroon van selgroei verander, sodat die wortelpunt na blootstelling van 48 h nie meer 'n sitologies-georganiseerde geheel was nie. 'n Toename in die gemiddelde selvolume is na blootstelling van 2 h waargeneem, hoofsaaklik in die selle van die rustende gebied, waar die vergroting van selle geassosieer is met die opheffing van die rustoestand, en in die sentrale korteks, 1 – 2 mm vanaf die wortelpunt, waar die aanvangsgroei gestimuleer is deur 'n afwyking vanaf osmotiese ewewig. Die aanvanklike verhoging in die osmotiese potensiaal van die selsap, as gevolg van die Al-behandeling (1 h), is nie volgehou nie; die verlaging in osmotiese potensiaal het ooreengestem met veranderings in die rigting van selgroei. Spanningspatrone wat voortspruit uit die anisotropiese groeireaksie van die korteksselle na 20 h se blootstelling van die wortel aan Al, is geassosieer met die ineenstorting van die geleidingsweefsel van die stele en die disintegrasie van die wortels se epidermis. Gevorderde vakuolering van die wortelpuntselle wat vir die eerste keer opgemerk is met die 5 h-blootstelling is 'n kenmerk van wortels wat aan Al blootgestel is. Veranderings in die kernstruktuur van die selle van die proksimale meristeem ten opsigte van die graad van chromatienkondensering in die struktuur van die nukleolus is beskou as aanduidings van veroudering.

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Keywords: Aluminium, growth, morphology, osmotic potential, quiescence

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Introduction

Aluminium (Al) toxicity has been widely associated with a decline in mitotic activity (Clarkson 1965; Morimura *et al.* 1978; Horst *et al.* 1983) and to gross morphological disturbances in the root apex (Hecht-Buchholz 1983; Bennet *et al.* 1985a), and Fleming & Foy (1968) have intimated that varietal differences in the response of wheat cultivars to Al may directly reflect the resistance of the meristem to damage.

Very little is known of the cellular changes occurring in Al-stressed roots, and Al-uptake studies (Bennet *et al.* 1985a) have failed to demonstrate the presence of Al in many of the tissues affected until some time after the response has been detected. Communication between the point of injury and the root meristem has consequently been suggested (Bennet *et al.* 1985b).

An increasingly animated view of root function has implied that the root meristem should not be considered as a single meristem, but rather as a number of discrete cell populations, not all of which are meristematic (Ellmore 1982). Co-ordination of function of the root is nevertheless dependent on the integration of activities between these cell populations. It was, therefore, the aim of this investigation to examine the changes brought about in the cells of the quiescent centre (Clowes 1956), proximal meristem (Feldman & Torrey 1975) and primary growth region (Luxová 1981) through treatment of the root with Al.

Materials and Method

Plant growth conditions

Pre-germinated seed of *Zea mays* L., cv. TX 24 was planted in 25-l plastic buckets (1,08 l/plant) containing the following nutrient solution (mg dm^{-3}) at pH 4,6: N 36; P 3; K 40; Ca 40; Mg 10; S 14; Na 1,5; Mn 0,11; Zn 0,05; Cu 0,02; Fe 5,5; Mo 0,03; B 1,9. Solution pH was not adjusted during the course of the experiment. Nitrogen was provided as NH_4^+ and NO_3^- in a molar ratio of 1:1. Sulphur was supplied as SO_4^{2-}. Fe was present as Fe CDTA. Artificial light was supplied to give a 14 h light 10 h dark regime with alternating temperatures of 28:23°C. The light source was Grolux W.S.* (731,3 W m^{-2}) and incandescent (113,4 W m^{-2}) to give a light intensity of 300 μE m^{-2}s^{-1} at plant height.

Experiment I

Plants were grown with continuous aeration for seven days. $Al_2(SO_4)_3$ solution was added in advance of harvesting to give 0, 2, 5, 6,5, 20 and 48-h root exposure to 8 mg dm^{-3} Al in solution. Root tips from each treatment were fixed in 6%
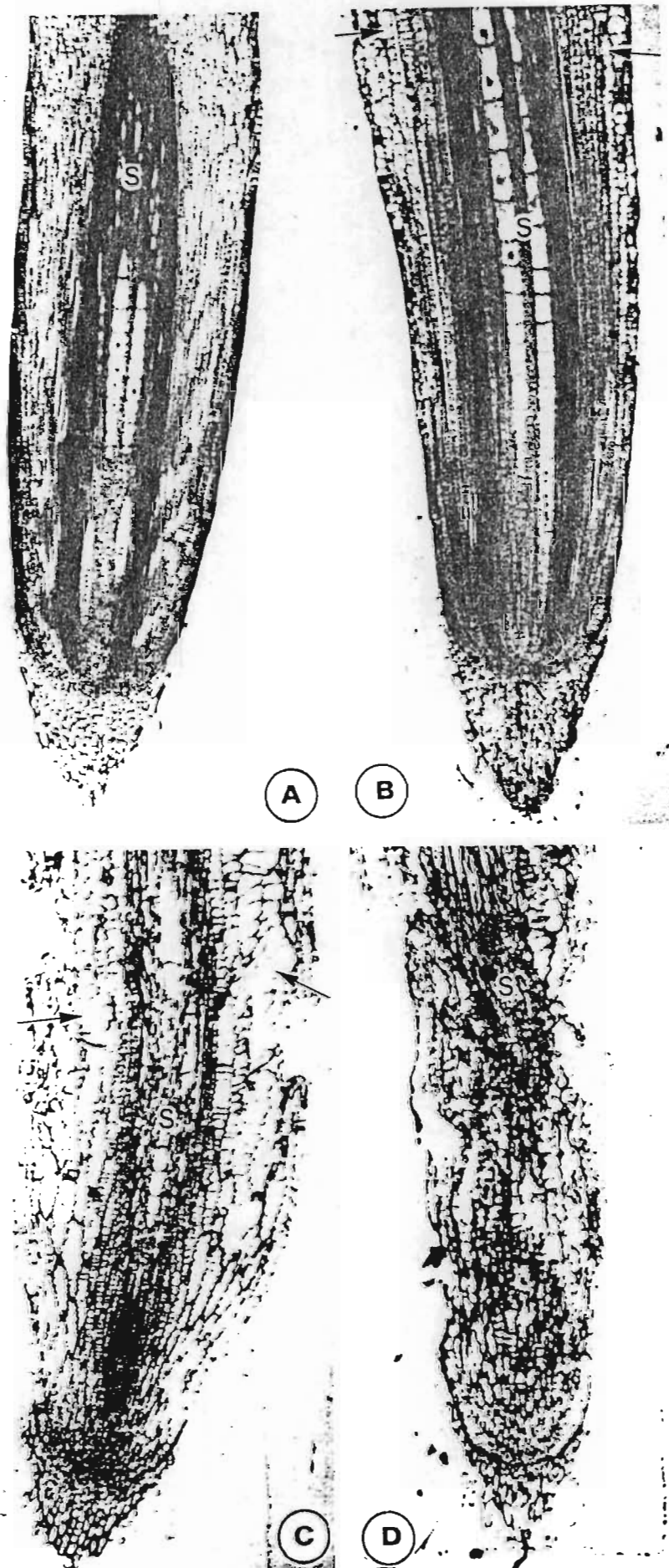
Figure 1 Micrographs of longitudinal sections of the primary root treated with 8 mg dm^{-3} Al to illustrate the progressive cellular disorganization of the root apex associated with Al treatment. **A.** Control, no Al added. Note absence of cellular distortion (S — stele). **B.** 6,5-h treatment time. Cell enlargement (arrowed) is first evident in the cortex (S — stele). **C.** 20-h treatment time. Cell enlargement, notably in the mid-cortical cells (arrowed) has resulted in the disintegration of the outer root shape. **D.** 48-h treatment time. Complete disruption of the cells of the root apex is now clearly evident (S — stele). Magnification × 60.

with post fixation (2 h) in 2% OsO_4 in 0,05 M sodium cacodylate buffer (Hayat 1981a). Fixed material was dehydrated in graded alcohol, treated with propylene oxide and embedded in Epon/Araldite resin. Sections for light microscope viewing were stained in methylene blue. Ultra-thin (gold) sections for transmission electron microscopy were stained in uranyl acetate and lead citrate (Hayat 1981b).

Micrographs of longitudinal sections taken to include the central cylinder were prepared for the apical 2 mm of the primary root, projected for additional magnification and analysed in 0,5-mm segments to cover the following cell populations:
(i)   Epidermis,
(ii) outer cortex, cell file 2,
(iii) mid-cortex, cell file 5, and
(iv) quiescent centre.
Procedures for the measurement of cell dimensions and the calculation of cell volumes have been described by Barlow (1974).

Micrographs of transverse sections 1,5 mm from the root tip were also prepared to assess the effect of cell enlargement on the structure of the stele.

Experiment II

Plants were grown for a total of six days, with $Al_2(SO_4)_3$ solution added in advance of harvesting to give 0, 1, 5, 12 and 20-h root exposure to 8 mg dm^{-3} Al. Sixty-nine plants were sampled from each treatment. Roots of plants were rinsed in de-ionised water. The sap was expressed from the distal 20 mm of the primary root and immediately frozen in solid carbon dioxide (dry ice). Sap was thawed, centrifuged and the osmolality determined with the minimum delay on a Westcor Vapor Pressure Osmometer.*

Statistical treatment of results

The availability of plant material was a limitation to the number of measurements made during these experiments. Because of the importance attached to the interpretation of changes in osmotic potential (Experiment II), this experiment was repeated to provide a second set of results. Ranges are given for these measurements.

Changes in cell volume were considered to be so massive (Experiment I) that the frequency of measurements was limited to the number of cells found within the defined cell populations of single roots sampled from each treatment.

Results
Experiment I
Morphological changes in the root apex
Severe cellular disorganization of the root apex was associated with treatment of the primary root with Al (Figures 1 & 2) so that with prolonged treatment (48 h), complete disruption of the organized, cytologically heterogeneous root apex occurred (Figure 1D). Aluminium treatment caused a rapid alteration in the size of the cells of the root apex. Analysis of the relative extent of these changes with time for the different cell populations (Figure 3) indicated that increased cell volume was most notable in the cells of the mid-cortex 1 mm – 2 mm from the root tip. Cell volume changes were associated with alterations in the growth directions of these cells (Figure 4) and resulted in:
Increased root thickness evident with 6,5-h exposure to Al (Figures 1 & 2),
inward pressure on the stele which is indicated by distortion

(Figure 2B) and
progressive collapse of metaxylem vessels of the stele with longer exposure times (Figures 2C & 2D).

Disintegration of the epidermis (20 h) was associated with pressure directed towards the root exterior (Figures 1C, 1D & 2C, 2D) through cellular expansion of the mid-cortex.

A conspicuous effect of Al involved the cells of the quiescent centre, where increased mean cell volume was evident with 2-h treatment. It was considered pertinent that during the first 5 h of the experiment, the rate of cell enlargement recorded in the quiescent centre substantially exceeded that shown by neighbouring cell populations. This pattern was not maintained and a decline in growth of quiescent centre cells (6,5 h) coincided with progressive disorganization of the root apex. No measurement of quiescent centre cells was possible with 20-h treatment. Little attention was given to the remaining cell populations since the initial growth of the outer cortical cells associated with short-term Al exposure was not maintained during the course of the experiment, and changes in the size of epidermal cells were comparatively modest.

Ultrastructure of the proximal meristem
Ultrastructural features of roots treated with Al (Figure 5) included, progressive vacuolation of the cells of the proximal meristem observed with 5-h treatment (Figure 5A), an almost total disappearance of dense chromatin from interphase nuclei (20 h) (Figure 5C) and improved definition of areas of the nucleolus, which was also found to become progressively granular with an increase in the frequency and size of nucleolar vacuoles. The nuclear membranes remained intact, at least during the first 20 h of the experiment.

Experiment III
Osmotic potential of the cells of the root apex
Aluminium treatment resulted in an initial (1 h) increase in the osmotic potential of expressed cell sap. This was followed (5 h) by a sharp decline, and subsequent treatments which involved longer times were connected to a continued decrease in osmotic potential (Figure 6).

Discussion
Within the context of the root meristem, development should be seen to include cell division, cell growth and differentiation (O'Brien 1982). It is, however, clear from these experiments that the influence of Al extends beyond the well documented inhibitory effect on cell division (Clarkson 1965; Morimura *et al.* 1978; Horst *et al.* 1983), and that Al-induced changes in the pattern of cell growth and differentiation may include some of the most impressive plant responses to toxic levels of Al.

Many of the cellular changes which have been identified in the root apex may not coincide with the presence of Al in the tissues involved (Bennet *et al.* 1985a & b), which confirms the complexity of plant reaction to Al and indicates that the physiology of Al toxicity may involve several responses occurring at different rates and at different levels of scale.

The quiescent centre
The cells of the quiescent centre have become characterized by low levels of activity, judged largely by criteria associated with cell division (Clowes 1972) and the incorporation of radioactive materials (Barlow 1974). The autonomy of the quiescent centre has nevertheless been explicitly demonstrated through the *in vitro* culture of well organized roots of normal morphology from isolated quiescent centres (Feldman &
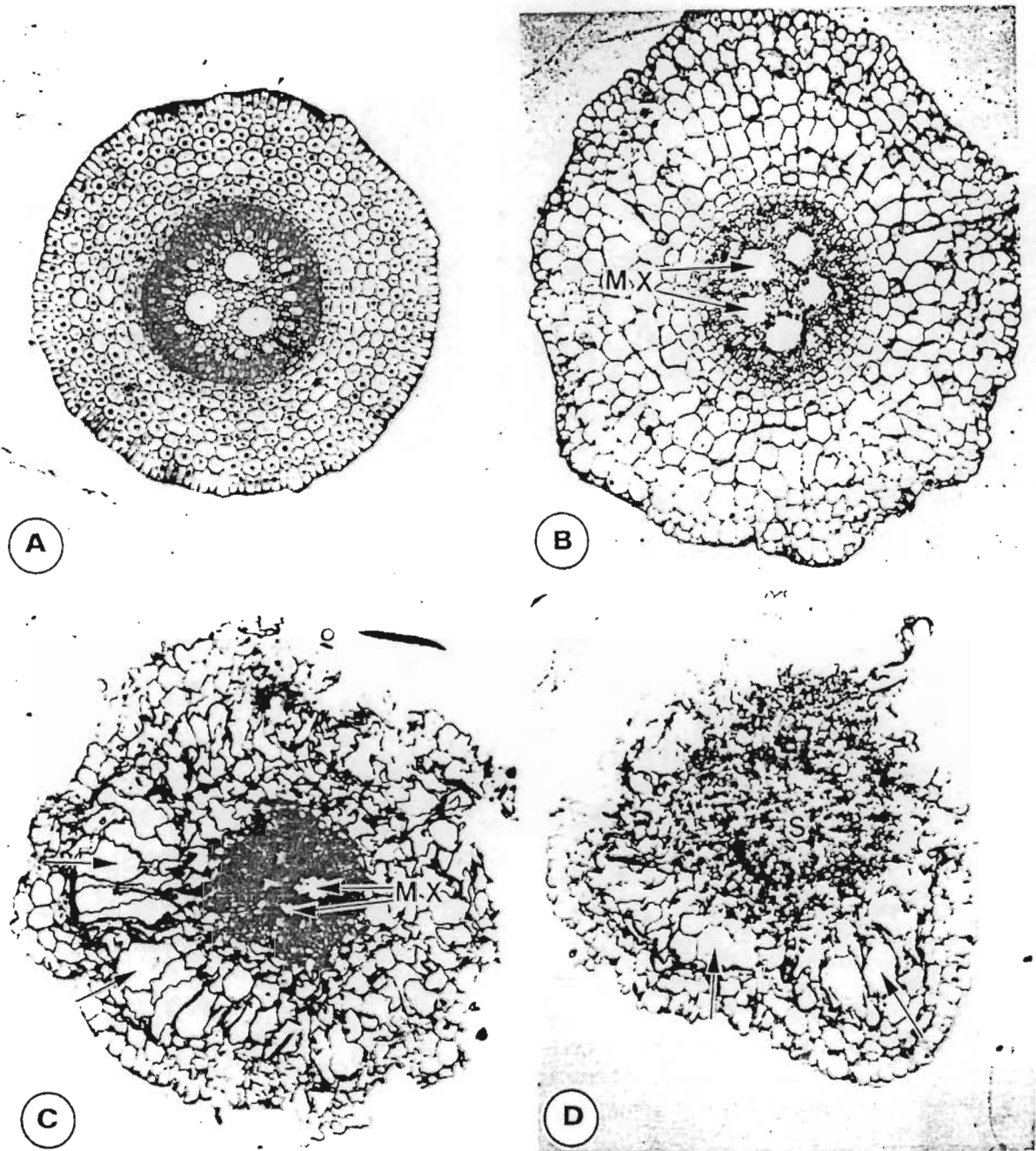
Figure 2 Micrographs of transverse sections of the primary root cut 1,5 mm from the root tip after treatment with 8 mg dm^{-3} Al to demonstrate the effect of Al-induced cell enlargement on the stele and outer root shape. **A.** Control, no Al added. Many cells contain nuclei and are non-vacuolate. **B.** 6,5-h treatment time. Cells of the cortex are vacuolated and distortion of the walls of the immature metaxylem (MX) vessels is evident. **C.** 20-h treatment time. Anisotropic growth response of mid-cortical cells (unlabelled arrows) and collapse of metaxylem (MX) vessels is clearly evident. **D.** 48-h treatment time. Disruption of the stele (S) and disintegration of the outer root cells through expansion of mid-cortical cells (unlabelled arrow) is clearly evident. Magnification × 100

The universal occurrence of quiescent centres in the roots of angiosperm plants (Clowes 1984) may underlie a fundamental role in root physiology which remains to be explored. Published research on the quiescent centre has indicated possible functional involvement in regulating intercellular relationships with the proximal meristem (Feldman 1976), the development of primary vascular tissue (Feldman & Torrey 1975) and, less clearly, as a site of cytokinin synthesis (Barlow 1978; Feldman 1979).

The mechanism through which quiescence is imposed on these cells is a highly controversial question (Torrey 1972; Barlow 1974; Clowes 1975, 1984). It is, however, relevant that the constraint leading to quiescence does not in itself prevent the quiescent centre from re-assuming a meristematic function (Feldman & Torrey 1976) and there are numerous studies

involving surgical manipulation of the cell populations of the root apex which attempt to define the forces creating the quiescent state (Barlow 1974; Feldman 1976; Feldman & Torrey 1976). These procedures inevitably affect many features of the integrated system of the root apex and questions concerning cellular interactions remain largely unresolved.

The rapid increase in the size of the cells of the quiescent centre of Al-stressed roots is therefore of considerable interest, since this change coincides with an alteration in the function of the quiescent centre suggested by a decline in metabolic activity following Al treatment which we reported in a previous paper (Bennet *et al.* 1985b). Similar changes are not shown by either the distal or proximal meristem with short-term Al exposure and it may therefore be argued that Al
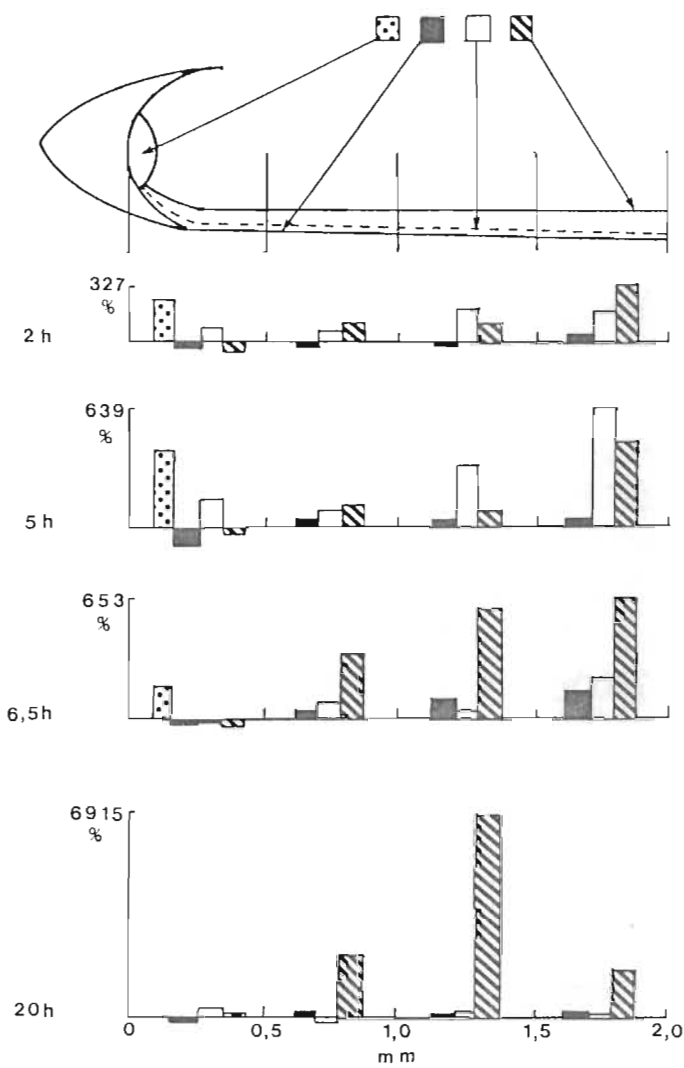
Figure 3 Relative (% of control) changes in mean cell volume with different Al exposure times recorded for the apical 2,0 mm of the primary root covering cell populations of the quiescent centre, epidermis, outer cortex (file 2) and mid-cortex (file 5) following Al treatment. Note the preferential cell growth initially recorded in the quiescent centre and cortex 1,0–2,0 mm from the root tip. With longer treatment times enlargement is essentially a feature of the mid-cortex.

imposing quiescence. According to Barlow (1974), excision of the root cap produces a similar result. In our experiments, the root cap remained intact and metabolically active for some hours after Al treatment (Bennet et al. 1985b). These observations suggest, therefore, that the root cap may represent the source of chemical control of quiescence imposed on the cells of the quiescent centre. The nature of such control is presently unknown, but it is clearly influenced by toxic levels of Al and removal of the root cap. Previous studies on Al toxicity (Bennet et al. 1985b) have attached considerable emphasis to Golgi apparatus function in the peripheral cap cells as a primary site of Al action. It is therefore conceivable that the Golgi apparatus function is involved in imposing quiescence on the cells of the quiescent centre. Re-establishment of a quiescent state during cap regeneration in decapped roots may therefore be allied to the resumption of this activity rather than to the presence of physical constraints associated with cap regeneration (Barlow 1974).

Aluminium initiated growth response of cortical cells

These experiments have demonstrated a strong element of growth stimulation arising from Al treatment and occurring

This coincides with the region of the root associated with cellular changes initiated by gravistimulation (Shen-Miller et al. 1978).

An increase in cell volume may be expected to depend heavily on the uptake of water (Steward 1969; O'Brien 1982). In Al-stressed roots, the short-term increase in osmotic potential of the expressed sap may indicate that the inflow of water is initiated by a rapid departure from osmotic equilibrium. It is, however, pertinent that continued growth of mid-cortical cells which occurred over the entire course of our experiment could only be maintained against the subsequent decline in osmotic potential by a change in pressure potential. Initially, decreased pressure potential may involve alterations in cell wall structure (Ray & Green 1972; Green 1980), which is supported by the change in growth direction shown with time in these cells. Furthermore, it is considered significant to this argument that the timing of the change in growth direction coincided with a decline in osmotic potential.

Growth stimulation of the mid-cortical cells is of such great magnitude that stresses directed towards the stele result in the collapse of the primary vascular tissue, while the continued requirement for reductions in pressure potential to accommodate further growth may ultimately involve the disintegration of the outer root cells.
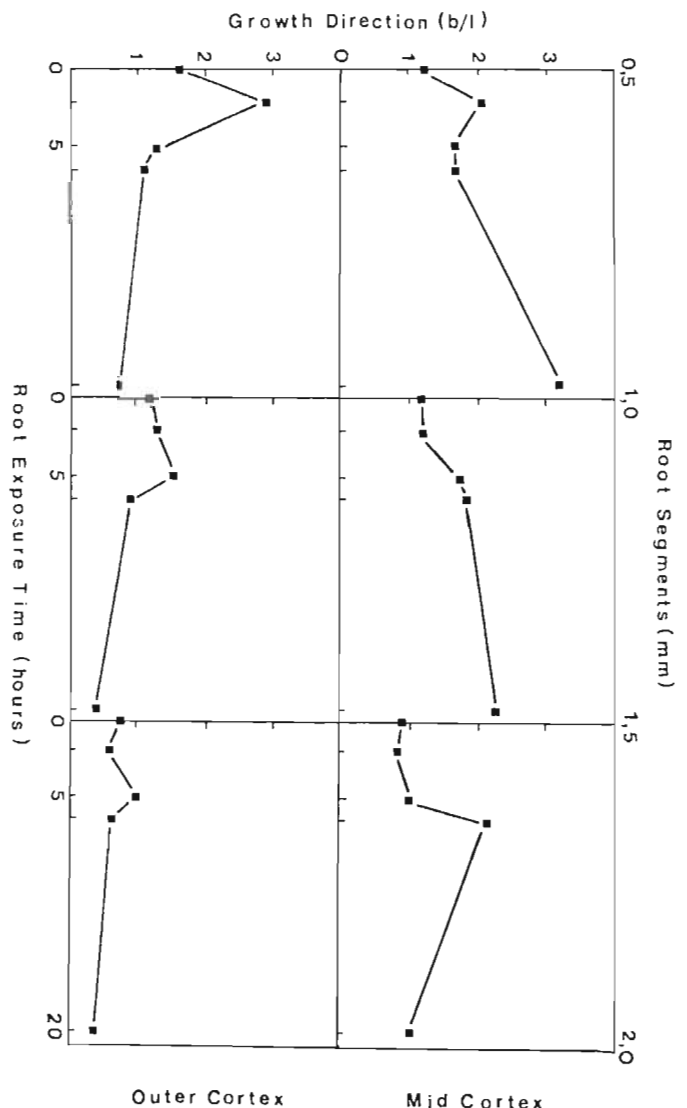
Figure 4 Alteration in the growth direction (cell breadth/cell length) of the outer (file 2) and mid-cortical (file 5) cells of the primary root associated with Al induced cell enlargement. Preferential increase in
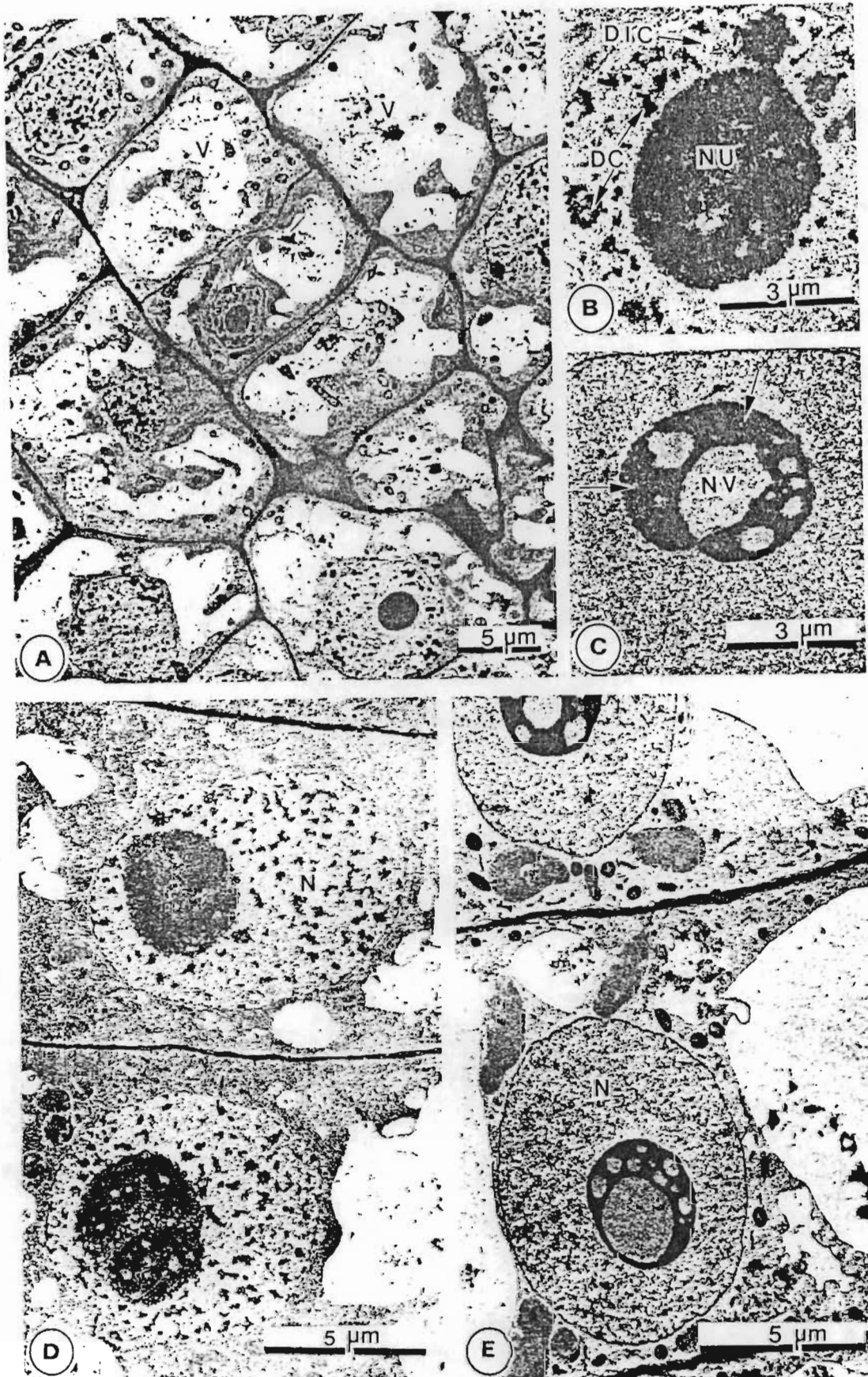
Figure 5 Electron micrographs of cells of the proximal meristem adjacent to the cap junction showing the effects of Al treatment. **A.** Cortical cells showing the highly vacuolated (V) nature of these cells following 6,5-h Al treatment. Formation of cytoplasmic vacuoles clearly precedes changes in the structure of the nucleus/nucleolus not yet evident in these cells. **B.** Nucleus/nucleolus (NU) of the control. Dense (DC), diffuse (DIC) chromatin. Cell located in outer cortex behind cap. **C.** Nucleus/nucleolus, 20-h treatment time, note the presence of large well defined nucleolar vacuoles (NV) and the appearance of intensely granular areas (unlabelled arrows) in the nucleolus. Cell location similar to 5B. **D.** Nucleus (N) of the control. The distribution of dense/diffuse chromatin and the appearance of the nucleolus is characteristic of outer cortical cells of the proximal meristem. **E.** Nucleus (N) following 20-h exposure to Al. The disappearance of dense chromatin and ......
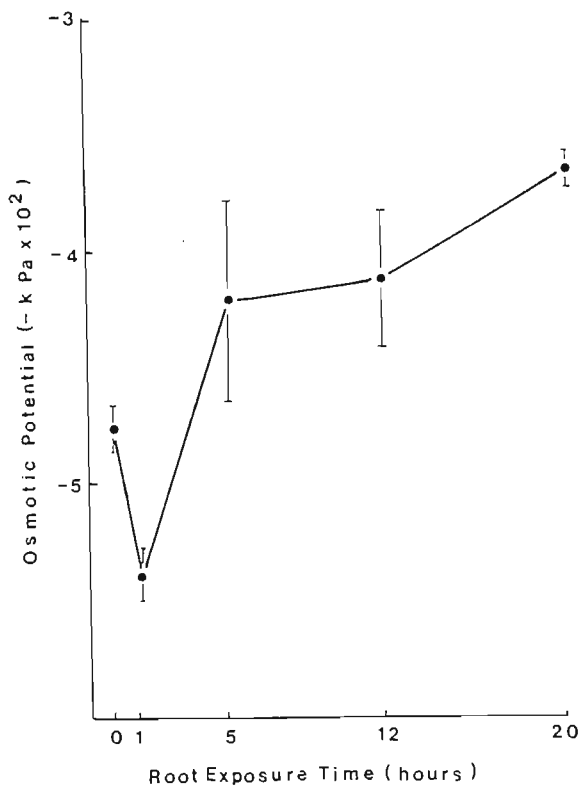
Figure 6 Al-induced changes in the osmotic potential of cell sap expressed from the distal 20 mm of the primary root. Vertical scale lines represent the ranges for two experiments.

The mechanism of cell growth stimulation in Al-stressed roots is presently unknown. It is, however, relevant to this concept that progressive vacuolation is a feature of the cells of Al-stressed roots, as one of the fundamental functions which has been assigned to the vacuole is storing water taken up during growth (Green 1969; O'Brien 1982).

Aluminium toxicity and nuclear structure in the proximal meristem

These experiments do not indicate rapid structural or functional changes in the nuclei of meristematic cells which could be associated with the reported decline in mitotic activity (Clarkson 1965) following Al treatment.

Nuclear changes were seen to involve a decrease in chromatin condensation of the nucleus and an increase in size and frequency of vacuoles in the nucleoli. Both these ultrastructural features have been considered as possible indicators of increased nuclear activity involving RNA synthesis (Barlow 1970; Jordan et al. 1980). However, this conclusion is not compatible with the established decline in metabolic activity of the proximal meristem (Bennet et al. 1985b) and it is suggested that ultra-structural features of Al-stressed nuclei observed with extended exposure are indicative of senescence. In support of this hypothesis, Hyde (1967) has intimated that the large nucleoli of active cells may have their basic synthetic machinery obscured by the products of synthesis. Differences in the capacity of fixatives to define ultrastructural features of nucleoli may therefore relate to the extraction of protein during fixation, which sharpens and clarifies the structure. It is, therefore, conceivable that the increasingly vacuolated and granular appearance of the nucleoli of Al-stressed cells of the proximal meristem may reflect an inhibition of function, which produces a similar ultrastructural response through progressively reducing the products of synthesis present in the

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Aluminium toxicity and regeneration of the root cap: Preliminary evidence for a Golgi apparatus derived morphogen in the primary root of *Zea mays*

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Ultrastructural changes in the Golgi apparatus and associated cytoplasmic organelles were followed in decapped roots grown in liquid culture containing either no aluminium (Al) (control) or 8 mg dm^{-3} Al. Development of Golgi apparatus function in the control coincided with differentiation of proplastids and the formation of amyloplasts. A recognizable cap was formed 48 h after decapitation. Aluminium inhibited development of the Golgi apparatus function. Vesicle membranes were not clearly defined and secretory vesicles accumulated in the vicinity of the dictyosome. Many plastids in Al-treated cells failed to develop internal organization. Starch accumulation was only apparent in isolated plastids with extended exposure to Al and was linked to the presence of dictyosomes. Lipid drops (plastoglobuli) were found in considerable numbers in plastids, sometimes in conjunction with starch, even with 24-h exposure to Al. This could implicate Al in preventing the assembly of membrane material. A partial resumption of Golgi function 24 h after decapping preceded severe cellular disorganization in Al-treated roots which failed to regenerate a new cap. An hypothesis concerning the control of Golgi apparatus function and plastid differentiation was developed.

S. Afr. J. Bot. 1985, 51: 363 – 370

Ultrastrukturele veranderinge in die Golgi-apparaat en verwante sitoplasmiese organelle is bestudeer na die verwydering van die kaliptra van wortels wat gekweek is in 'n vloeibare medium wat of geen aluminium (Al) (kontrole) of 8 mg dm^{-3} Al bevat het. Die ontwikkeling van Golgi-apparaatfunksie in die kontrole het ooreengekom met die differensiasie van proplastiede, en ontwikkeling van amiloplaste. 'n Herkenbare wortelmussie is binne 48 h na verwydering gevorm. Daar is gevind dat Al die ontwikkeling van die Golgi-apparaatfunksie geïnhibeer het. Vesikelmembrane was nie duidelik gedefinieer nie en sekresievesikels het in die nabyheid van die diktiosome versamel. Baie plastiede in Al-behandelde selle het geen interne organisasie ontwikkel nie. Stysel-versameling was slegs in geïsoleerde plastiede duidelik na verlengde Al-behandeling, en is gekoppel aan die teenwoordigheid van diktiosome. Lipiedliggaampies (plastoglobuli) was steeds in aansienlike getalle in plastiede teenwoordig, soms tesame met stysel in 24-h Al-blootgestelde materiaal. Dit mag impliseer dat Al die akkumulasie van membraanmateriaal verhoed. 'n Gedeeltelike hervatting van Golgi-funksie is waargeneem 24 h na wortelmusverwydering en het die ernstige sellulère wanorganisasie vooruitgegaan in die Al-behandelde wortels wat nie daarin kon slaag om 'n nuwe wortelmus binne 48 h na behandeling te regenereer nie. 'n Hipotese in verband met die kontrole van Golgi-apparaatfunksie en plastieddifferensiasie is ontwikkel.

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Keywords: Aluminium, differentiation, Golgi apparatus, morphogen, plastid

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Introduction

The cells of the quiescent centre (Clowes 1956) are characterized by an inherent capacity to express a developmental pathway not normally seen in rapidly elongating roots (Feldman & Torrey 1976). These cells respond to the removal of the root cap by entering mitosis and regenerating a new cap (Barlow 1974), which has many properties shown by normal caps (Barlow 1974; Barlow & Grundwag 1974).

Studies on Al toxicity (Bennet *et al.* 1985b) have attached particular relevance to the Golgi apparatus function in the peripheral cap cells in the physiology of Al toxicity. Golgi apparatus function is correlated with cellular development (Mollenhauer 1965) and the dictyosomes of the quiescent centre are characterized by low levels of activity indicated by their small size and absence of secretory vesicles (Clowes & Juniper 1964). The response of the Golgi apparatus to cap removal is presently unknown. Decapping has however been shown to permit Al to enter the quiescent centre (Bennet *et al.* 1985a). Consideration of the role of Al in influencing developmental stages involved in cap regeneration may therefore be expected to promote understanding of the mechanism of Al toxicity.

Materials and Methods

Plant growth conditions

Pre-germinated seeds of *Zea mays* L. cv TX 24 were planted along the upper edge of 660 × 220 mm filter paper. Seeds were arranged with the radicles pointing downwards. The paper was loosely rolled and placed vertically in a glass container, with 50 mm deep nutrient solution. This technique has been more fully described by Konzak *et al.* (1976).

Experimental procedure

Plants were selected for a straight main root axis, the main root was decapped, following the procedure of Feldman (1975). Plants with decapped roots were transferred to the following nutrient solution; (mg dm^{-3}) N 18; P 1,5; K 20; Ca 20; Mg 5; S 7; Na 0,75; Mn 0,05; Zn 0,025; Cu 0,01; Fe 3; Mo 0,015; B1 containing either no Al (control) or 8 mg dm^{-3} Al as Al$_2$(SO$_4$)$_3$ and grown at 27 °C with continuous aeration, but without supplementary light. At 0 h, 6 h, 12 h, 24 h and 48 h after decapping, root tips from each treatment were processed for light and electron microscopy by fixation in 6% glutaraldehyde in 0,05 M sodium cacodylate buffer at pH 7,2 with post fixation (2 h) in 2% OsO$_4$ in 0,05 M sodium cacodylate buffer (Hayat 1981a). Fixed material was dehydrated in graded alcohol, treated in propylene oxide and

microscope viewing were stained in methylene blue. Ultra-thin (gold) serial sections for transmission electron microscope viewing were stained in uranyl acetate and lead citrate (Hayat 1981b).

To follow the effect of cap removal on the fine structure of the cells of the quiescent centre on a quantitative basis, photographs of these cells were built into mosaics and counts were made of the number of plastids, starch grains, lipid drops, dictyosomes, cisternae per dictyosomal stack and secretory vesicles for single roots sampled from each treatment.

Results

In the control treatments (0 Al) changes were observed in the plane of cell division (24 h) preceding formation of recognizable cap tissue which was evident 48 h after cap removal (Figures 1A & 1C). Al-treated roots failed to regenerate a cap (Figure 1D) and Al treatment was associated with severe cellular disorganization and progressive vacuolation of the cells of the root apex first apparent 24 h after cap removal (Figure 1B).

Golgi apparatus function in decapped roots in the absence of Aluminium

Changes in the cells of the quiescent centre arising from cap removal involved the activity and secretory pattern of the Golgi apparatus. In root tips fixed immediately after decapping, the dictyosomes of the quiescent centre were characterized by an absence of secretory vesicles (Figure 2B) and contained the least number of cisternae per dictyosomal stack (Figure 3). Increases in the number of dictyosomes, secretory vesicles, and cisternae per dictyosomal stack were observed within 6 h of cap removal (Figure 3), suggesting enhanced secretory activity. Steady state secretion (Morré 1977a) was, however, indicated 12 h after decapping since further increases in the number of cisternae per dictyosomal stack were not observed.

Changes in the appearance of vesicular contents were also detected and many vesicles containing a dense granular material were observed in the outer cells of decapped roots within 6 h of cap removal (Figure 2D). With longer times (24 h) after cap removal, differences in Golgi apparatus morphology were evident between cells of cortical and stelar lineages, and the secretory vesicles in the latter were often poorly developed and rarely contained granular material (Figure 2H).

Re-establishment of full Golgi apparatus function, usually associated with the outer cap and based on the presence of elongated secretory vesicles and the accumulation of Golgi apparatus-derived material (Morré et al. 1967) between the plasmalemma and cell wall, could only be identified 48 h after decapping.

Golgi apparatus in decapped roots treated with Aluminium

Golgi apparatus development was rapidly inhibited by Al. Within 6 h the number of dictyosomes and cisternae per dictyosomal stack were reduced (Figure 3). Some temporary recovery of Golgi apparatus activity was evident with longer times after cap removal (Figure 3), but it was notable that
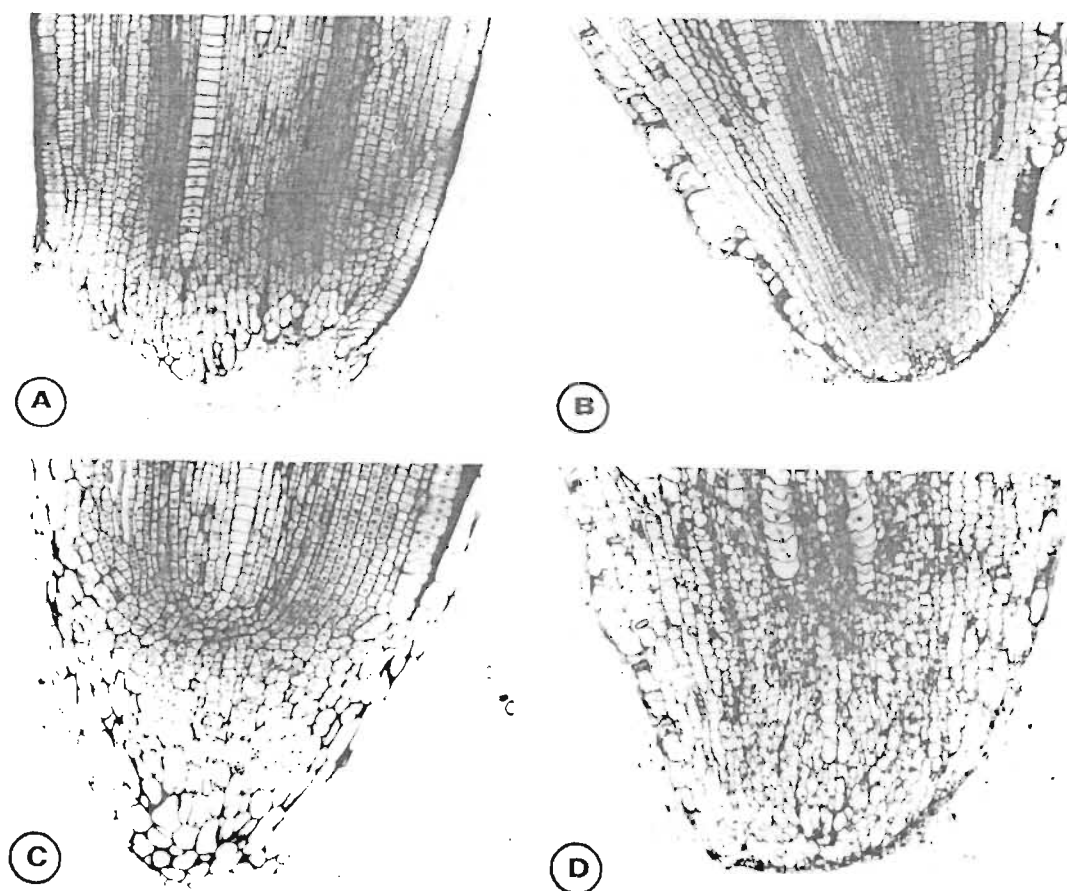
Figure 1 Longitudinal sections through decapped roots of Zea mays illustrating the effect of Al on regeneration of the root cap. A. Control (0 Al) 24 h after cap removal. Initiation of a new cap is evident. B. Decapped root treated with Al 24 h after cap removal. Cap boundary remains intact and no evidence of cap regeneration exists. C. Control (0 Al) 48 h after cap removal. Recognizable cap tissue is apparent. D. Decapped root treated with Al 48 h after cap removal with no evidence of cap regeneration. Cellular distortion of root cells is h...
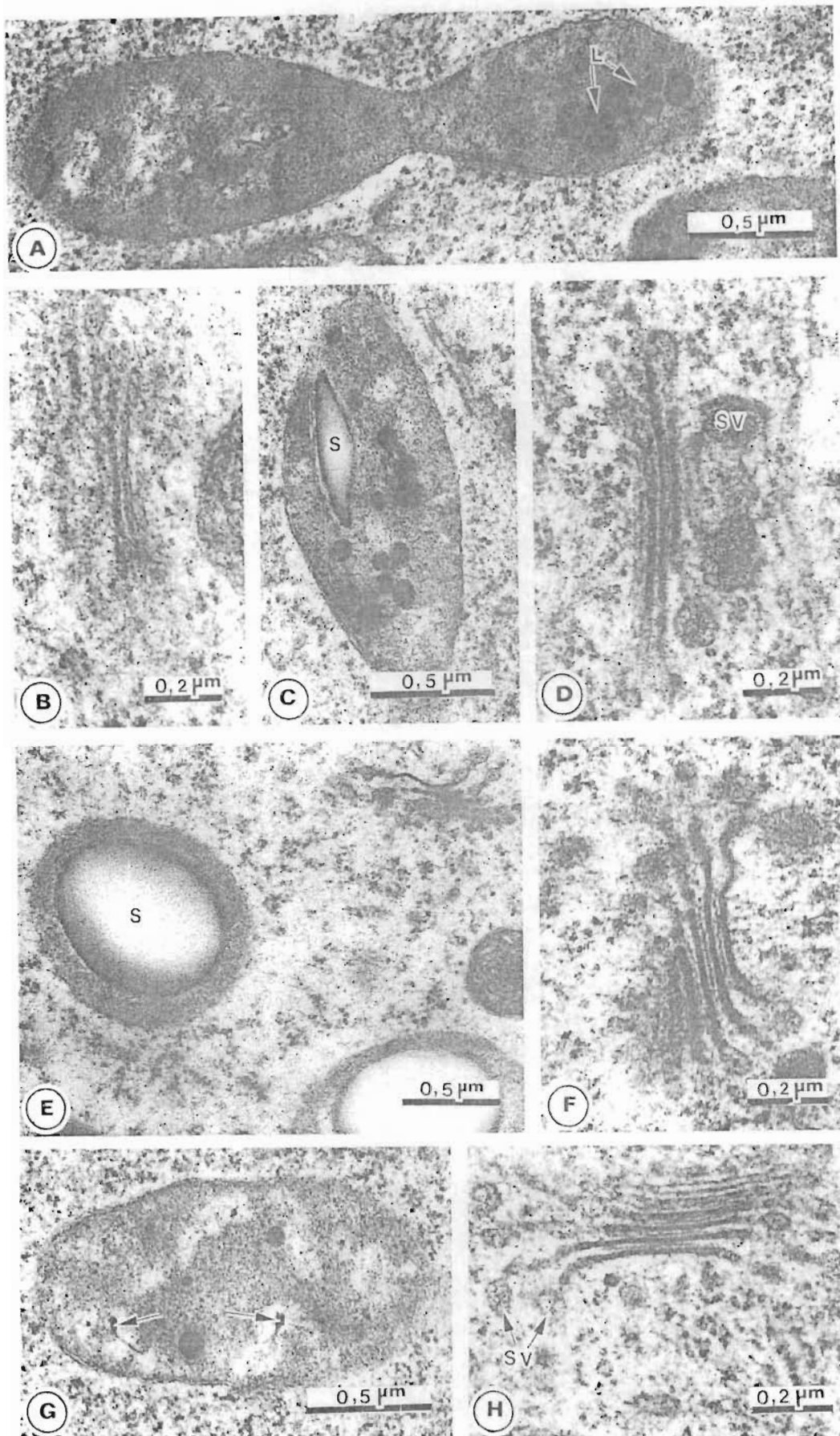
Figure 2 Electron micrographs of plastids and dictyosomes in the cells of the quiescent centre of the control (0 Al) at different times after cap removal. **A.** Plastid fixed immediately after decapping (0 h). Note absence of starch and presence of lipid drops (L). **B.** Dictyosome fixed immediately after decapping (0 h) characterized by the absence of secretory vesicles. **C.** Plastid 6 h after decapping showing developing starch grain (S) and defined membrane structure. Lipid drops are also still evident. **D.** Dictyosome 6 h after decapping. Secretory vesicles (SV) are well defined and contain granular material. **E.** Developing amyloplasts in cell of cortical lineage containing defined starch grains (S) 24 h after decapping. Note the presence of dictyosome. **F.** Dictyosome in cell of cortical lineage 24 h after decapping with defined secretory vesicles containing granular material. **G.** Plastid in cell of stelar lineage; note presence of DNA fibres (arrowed) and absence of starch. **H.** Dictyosome in cell of
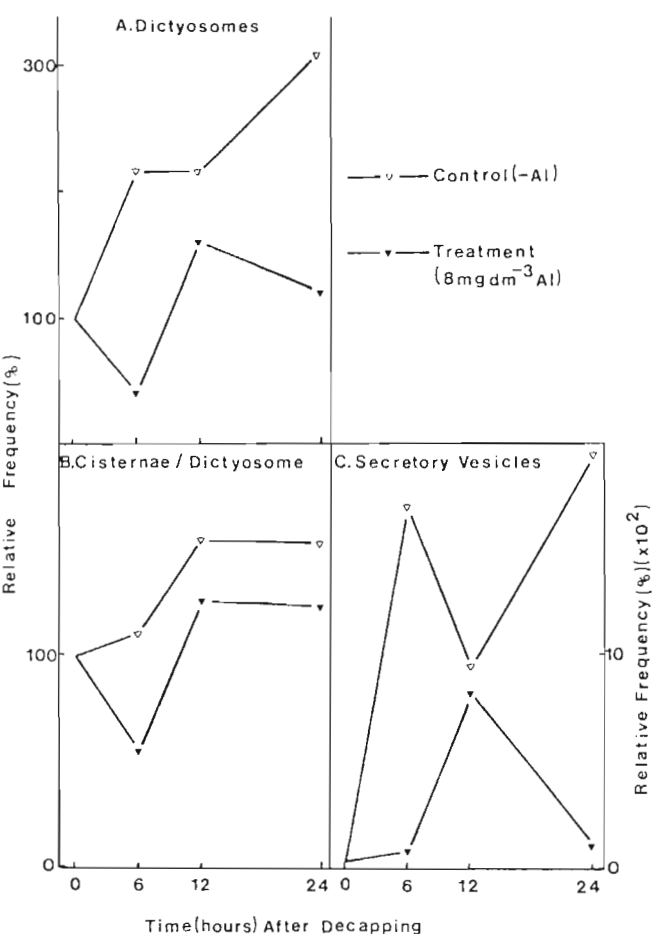
Figure 3 The effect of time, after cap removal and treatment of decapped roots with Al, on Golgi apparatus activity in the cells of the quiescent centre, indicated by the relative frequency of **A.** dictyosomes, **B.** cisternae per dictyosomal stack, **C.** secretory vesicles.

at no stage did the numbers of dictyosomes, cisternae per dictyosomal stack, and secretory vesicles approach the levels found in the control treatment (Figure 3).

Secretory vesicles containing the granular material were infrequently encountered and only with extended periods after cap removal (24 h). Vesicle membranes were not clearly defined and secretory vesicles were found to accumulate in the vicinity of dictyosomes (Figure 4D). Dictyosomes with defined membrane structure were infrequent 24 h after decapping and occurrence coincided with isolated plastids containing defined starch grains. No structural distinction was evident between the Golgi apparatus located in cells of cortical and stelar lineages. Curling of dictyosomal cisternae (Bennet *et al.* 1985b) was not observed.

Plastid differentiation in decapped roots in the absence of Aluminium

Plastids showed a high frequency (Figure 5) in the cells of the quiescent centre immediately following cap removal, and there was evidence of multiplication (Figure 2A). Plastids showed little internal organization, membrane profiles were indistinct, lipid drops and irregular patches of electron-dense material were numerous (Figure 5). Starch grains were infrequent and always small.

Plastids were observed to follow a defined developmental sequence with increasing intervals after cap removal. These changes involved increases in the frequency with which starch

number of lipid drops (Figure 5). Initially plastids found in cells of cortical and stelar origin were not morphologically distinct, but 24 h after cap removal, plastids in cells of stelar lineage were clearly distinguishable and characterized by many of the features observed in plastids immediately after cap removal (Figures 2 & 5). Many of these plastids were particularly rich in electron-dense areas (Figure 2G). Plastids in cells of cortical origin were distinguished by the presence of well defined starch grains (Figure 2E), while lipid drops were rarely present in plastids which contained large starch grains.

Plastids in decapped Aluminium-treated roots

Unlike the control, many plastids in Al-treated cells failed to develop internal organization during the course of the experiment, and membrane profiles usually remained indistinct (Figures 4A, 4B). A conspicuous consequence of Al treatment involved the rapid increase (6 h) in the number of lipid drops (Figure 5) which could still be observed in considerable numbers, sometimes in conjunction with starch, 24 h after cap removal.

Aluminium treatment also prevented starch accumulation and, although there was initially little difference in the frequency of occurrence of starch deposition sites between the control and Al-treated roots, starch grains in Al-treated cells were usually smaller and large grains were infrequently found with extended time after cap removal (24 h) (Figure 4E).
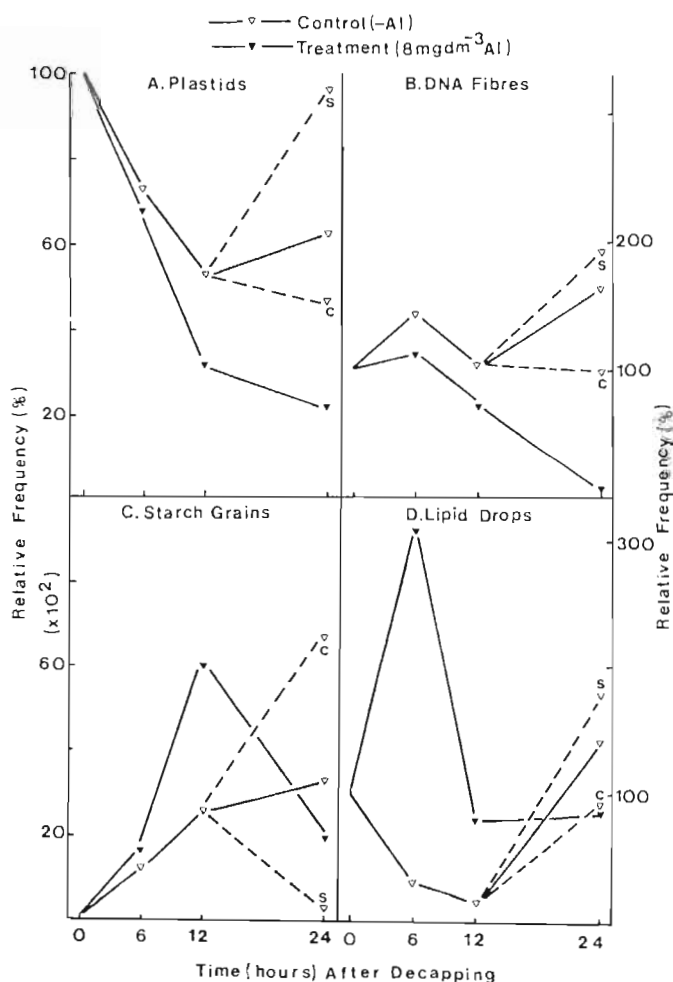
Figure 5 The effect of cap removal and treatment of decapped roots with Al, on differentiation of plastids located in the quiescent centre, indicated by the frequency of **A.** plastids, **B.** electron-dense regions
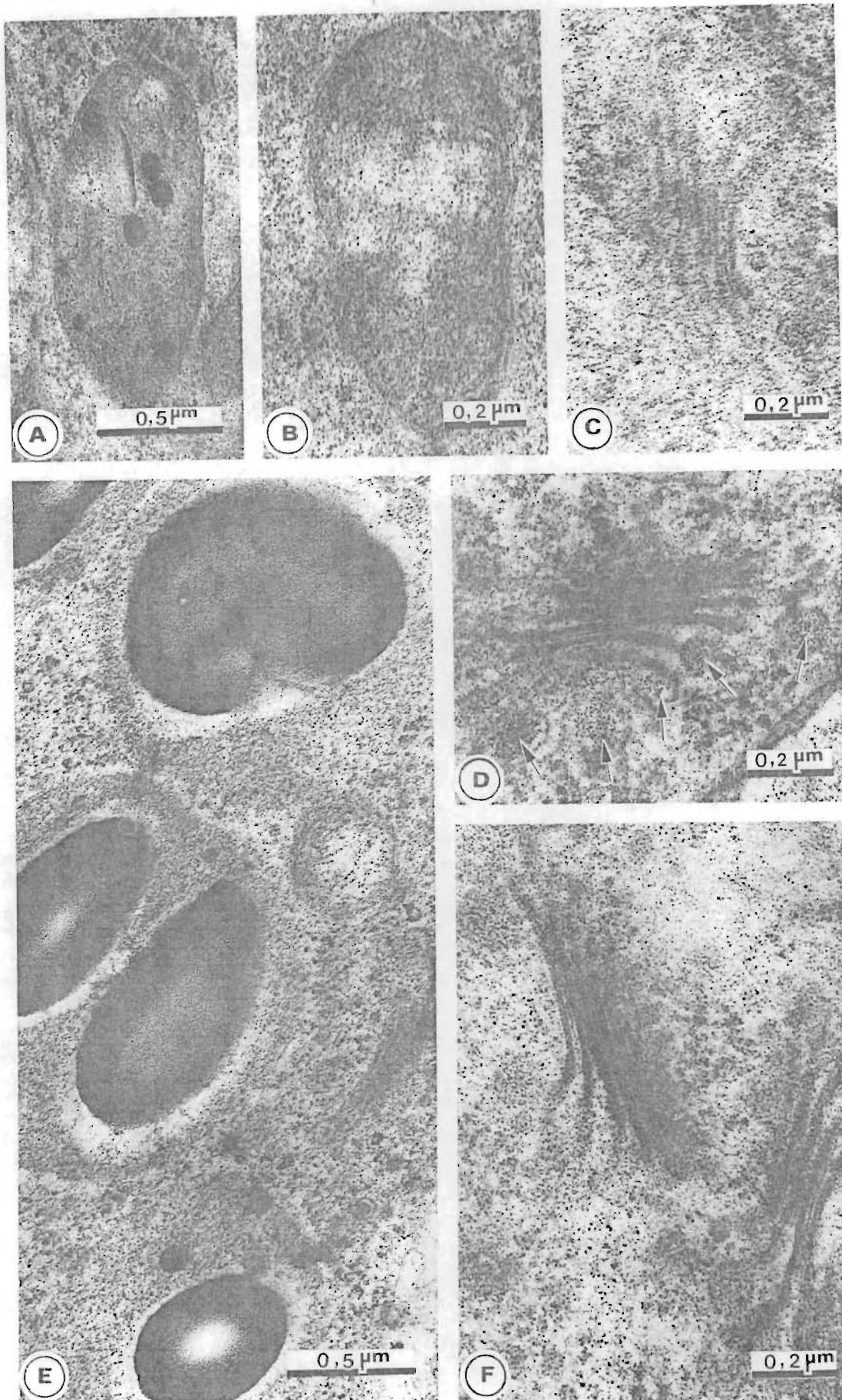
Figure 4 Electron micrographs of plastids and dictyosomes present in the cells of the quiescent centre of roots treated with 8 mg dm^{-3} Al at different times after cap removal. **A.** Plastid 6 h after decapping. Note absence of starch and scarcity of lipid drops. **B.** Plastid 12 h after decapping, characterized by an absence of starch grains and poorly defined internal organization. **C.** Dictyosomes 6 h after decapping. Secretory vesicles are absent and membrane profiles poorly defined. **D.** and **F.** Dictyosomes 24 h after decapping. Secretory vesicles (arrowed) contain some granular material and frequently accumulate near the dictyosome. **E.** Plastids containing defined starch grains in the vicinity of dictyo

Aluminium treatment caused the disappearance of electron-dense patches observed principally in undifferentiated plastids and, with 24 h exposure, these could no longer be detected (Figure 5). No morphological distinction could be drawn between plastids located in cells of cortical and stelar origin.

Discussion

Barlow (1981) has indicated that differentiation of cells within the root apex may occur in response to cap removal, in the absence of cell division, so that cells showing the correct spatial arrangement may develop properties more usually associated with the cellular function of the root cap. He suggests that differentiation arises in response to cap-determining gradients of morphogens induced within the remaining cells of the root apex. Very little is known of the origin of these gradients and the ultra-structural changes in cytoplasmic organelles following decapping, and in the presence of Al, are pertinent to elucidating the processes involved in the re-establishment of biochemical gradients within the root apex.

The control of Golgi apparatus function in response to cap removal

The behaviour of the Golgi apparatus is correlated with cellular development (Mollenhauer 1965; Mollenhauer & Mollenhauer 1978) and the dictyosomes of the quiescent centre are notable for the low level of activity, suggested by their small size and absence of secretory vesicles (Clowes & Juniper 1964; Mollenhauer & Morré 1966). Abrupt increases in the secretory activity of the control treatments in response to decapping, implied by an almost linear increase in the frequency of occurrence of dictyosomes with time, an initial increase in the number of cisternae per dictyosomal stack, and distinctive changes in the appearance of vesicular contents, may be considered evidence that the loss of the cap coincides with the temporary removal of a constraint on the function of the Golgi apparatus.

Characteristic differences in the secretory patterns of the Golgi apparatus, found in the cell populations of the root apex, have been widely described (Mollenhauer 1965; Mollenhauer & Morré 1966; Mollenhauer & Mollenhauer 1978; Barlow 1975) and have generally been associated with cellular differentiation (Barlow 1975). The Golgi apparatus of the peripheral cap cells are distinguished by the granular appearance of the vesicular contents (Mollenhauer & Morré 1980). This is not characteristic of neighbouring cell populations of the cap or epidermal regions of the root apex where vesicles containing electron-dense material are usually encountered (Mollenhauer & Morré 1980). These observations suggest that Golgi apparatus activity associated with the presence of granular secretory vesicles coincides with the spatial arrangement of cells with cap properties, as described by Barlow (1981), in decapped roots and is of fundamental importance in root physiology.

The re-establishment of biochemical gradients in decapped roots, as suggested by morphological differences between Golgi apparatus located in cells of the quiescent centre of cortical and stelar lineages and based largely on the occurrence of secretory vesicles containing granular material, infer that the Golgi apparatus function may be involved in re-imposing constraints on Golgi apparatus function. Furthermore, the differentiation of plastids with large, well defined starch grains which Barlow (1981) has considered a marker of resumed cap function, coincided with this distinctive Golgi apparatus secretory pattern. Elaboration of a granular secretory product

of the outer cap cells, since the oval-shaped secretory vesicles and accumulation of Golgi apparatus-derived material typically associated with slime production in the peripheral cap region (Mollenhauer & Morré 1980), was only evident with regeneration of recognizable cap tissue.

Inhibition of Golgi apparatus function by Aluminium

Aluminium toxicity has been connected with inhibition of vesicle transfer and alteration to the secretory product elaborated in the dictyosomes of the peripheral cap cells (Bennet et al. 1985b). A principal function of the Golgi apparatus involves the production of secretory vesicles (Morré 1977a) and it is therefore of consequence that in decapped roots, Al action was directed at the elaboration of granular secretory product, since vesicles containing this material were infrequently observed and only with extended times after cap removal. Inhibition of vesicle transfer, suggested by an accumulation of secretory vesicles in the vicinity of dictyosomes was indicated only in the case of vesicles which contained granular material. Aluminium-treated roots also failed to re-establish the biochemical gradients observed in the control, and differences in Golgi apparatus morphology between quiescent centre cells of cortical and stelar lineage were not observed.

The bulk of the Golgi apparatus membranes are thought to be contributed by the endoplasmic reticulum (Morré 1977b). Our experiments have indicated that the initial reduction in the number of dictyosomes coincided with a sharp increase in the number of lipid drops present in the plastids. Lipids are important constituents of plant membranes (Vickery & Vickery 1981) and these observations may imply a role for Al in preventing the assembly of membrane material, which accords with the slower development of the Golgi apparatus noted in Al-treated cells.

These experiments also suggest that growth stimulation of the cells of the root apex in response to Al treatment (Bennet et al. 1985c) may be delayed in decapped roots, and that the appearance of these symptoms may correlate with the development of the secretory activity in the Golgi apparatus.

Plastid differentiation in response to cap removal in the absence of Aluminium

Barlow & Grundwag (1974) have demonstrated that cap removal initiates a developmental sequence in the plastids of the cells of the quiescent centre, and have intimated that structural features of the majority of plastids, immediately following cap removal, are indicative of repression of plastid development. The elaboration of the membrane system and accumulation of starch in plastids found in cells of cortical lineage essentially followed the pattern described (Barlow & Grundwag 1974).

Factors which govern plastid development are as yet unknown (Barlow & Grundwag 1974). Re-establishment of axial gradients in plastid differentiation coincided with changes in Golgi apparatus function and, as the plastids in quiescent centre cells of stelar lineage show many of the features previously connected to repression (Barlow & Grundwag 1974), it is inferred that gradients of morphogens are re-established before recognizable cap tissue is formed.

Aluminium toxicity and plastid differentiation

Many plastids in Al-treated cells failed to develop in response to decapping and membrane profiles were frequently indistinct. The action of Al is, however, not considered to

many of the changes in plastids involving starch accumulation and associated with cap removal showed little quantitative difference between Al-treated roots and the control. Furthermore, the role of the electron-dense regions observed in many plastids is anomalous. Barlow & Grundwag (1974) have connected the presence of these regions, thought to represent DNA fibres (Salema & Badenhuizen 1969), to sites of starch synthesis and accumulation. Aluminium has a clear inhibitory effect on the occurrence of these fibres which could be interpreted as inhibition of DNA synthesis by Al, which accords with the work of Wallace & Anderson (1984). Barlow (1981) has, however, shown that differentiation of apical cells in which amyloplast development was used as a marker of cap function, proceeded in the absence of mitosis, which was suppressed by the use of DNA inhibitors. Amyloplast development is inhibited by Al and a primary role of Al, preventing DNA synthesis, therefore seems improbable. As a corollary, the arrested condition of plastids in Al-treated cells may arise as a consequence of failure of substrate induction mechanisms.

Observations with respect to the effect of Al on plastid differentiation support the idea of functional interdependence between Golgi apparatus and plastid differentiation. The presence of large starch grains was not a feature of Al-treated roots, even with extended periods after cap removal, and the occurrence of isolated plastids containing defined starch grains invariably coincided with the evidence of Golgi activity.

An hypothesis for a morphogen originating in the outer cap

There are numerous suggestions of growth inhibiting substances originating in the root cap (Gibbons & Wilkins 1970; Pilet 1975). Experiments with decapped roots in the presence and absence of Al have inferred that the Golgi apparatus secretory activity involving a granular secretory product may be connected to the occurrence of a morphogen mediating Golgi apparatus activity and plastid differentiation. Furthermore, as the re-establishment of biochemical gradients in decapped roots in the absence of Al, interpreted in terms of Golgi apparatus and plastid morphology, occurs prior to the formation of recognizable cap tissue and precedes a return to the quiescent state, it is conceivable that the morphogen may also be involved in imposing quiescence on the cells of the quiescent centre. This idea accords with the previously expressed hypothesis that disruption of Golgi apparatus function by Al in the peripheral cap cells of intact roots (Bennet et al. 1985b) precedes removal of the constraint on the growth of the cells of the quiescent centre (Bennet et al. 1985c).

Aluminium decreases the incidence of the morphogen by the disruption of Golgi apparatus function and a considerably wider involvement for this morphogenic substance in root physiology may be indicated, since Al toxicity has been connected to a decline in mitotic activity (Clarkson 1965) and DNA synthesis (Wallace & Anderson 1984). The endomembrane system, including the Golgi apparatus, is, however, characterized by the absence of DNA (Morré & Mollenhauer 1974) and, furthermore, the presence of the morphogen is not in itself dependent on either DNA synthesis or cell division (Barlow 1981). Since direct action between Al and nucleic acids appears improbable, it may be appropriate to consider that reduced mitotic activity and DNA levels following Al treatment are indicative of a functional involvement of the morphogen in controlling these activities. The precise biochemical pathway and the identity of the morphogen

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CONTROL OF PLASTID DIFFERENTIATION AND GOLGI APPARATUS ACTIVITY IN DECAPPED ROOTS BY ABSCISIC ACID

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The pattern of differentiation induced in quiescent centre cells by cap removal[1,2,3] is altered considerably by the presence of aluminium[3], and these experiments have provided evidence that the formation of amyloplasts may be controlled by a morphogen associated with alterations in Golgi apparatus activity. The identity of the morphogen is unknown, and although it has been suggested[1] that amyloplast development in response to decapping may reflect alterations in the distribution of growth substances, ions or starch precursor levels, roles for gibberellic acid, indole acetic acid or sucrose have not been demonstrated[1].

The presence of abscisic acid (ABA) in the roots of a number of plants[4,5] has prompted an investigation into the effect on plastid differentiation and Golgi apparatus activity of growing plants (Zea mays L) with decapped roots in water culture containing either no ABA (control) or 5 X 10^{-5}M and 2 X 10^{-4}M ABA for 24h after cap removal.

The control (no ABA) followed the previously reported[1,2] developmental sequence involving increases in Golgi activity as evidenced by the appearance of secretory vesicles and elaboration of a granular secretory product (Fig.1). Amyloplasts with well developed starch grains (Fig.2) were clearly evident in the quiescent centre 24h after cap removal.

ABA at both concentrations was effective in duplicating many of the functions associated with the presence of the root cap. Plastids located in the cells of the quiescent centre and neighbouring meristem failed to differentiate (Fig.3) and amyloplasts were still absent from these cells 24h after cap removal. Comparison with the control (Fig.1) showed that ABA also inhibited Golgi apparatus activity and dictyosomes present in the quiescent centre were characterised by their small size and low level of activity implied by the paucity of secretory vesicles (Fig.4). Vesicles containing a granular secretory product, rarely observed at the lower ABA concentration, were absent at the higher ABA level.

It was therefore proposed that ABA may be the morphogenic substance occurring in the root meristem associated with Golgi apparatus activity and involved in the control of plastid differentiation.

Aluminium toxicity and induced nutrient disorders involving the uptake and transport of P, K, Ca and Mg in *Zea mays* L.

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Chemical analysis of plant fractions of maize cultivars TX 24 and HL 1 grown in liquid culture containing Al showed that the action of Al was directed at the uptake of Ca and Mg and the transport of P and K. Highly significant cultivar differences in the pattern of Al uptake, notably at low solution Al concentrations were identified. P transport between roots and shoots diminished with increased root Al concentrations. An Al-induced decline in root metabolism was found to occur in intact roots (20 h) and cultivar differences in the ability to maintain root respiration in the presence of Al coincided with cultivar differences in P transport. In the absence of Al, both cultivars responded to low Mg levels by taking up correspondingly more Ca and K without significantly affecting yield. It is therefore suggested that a major requirement for these ions involves charge neutralization. Furthermore, since the root Mg concentrations recorded in these experiments were comparable to those found in plants showing a high degree of Al injury, it was also concluded that these Mg levels were adequate to meet specific functional requirements for this element in the plant. A hypothesis was developed which involved interference by Al in root metabolism which depressed active ion movement and thereby lowered the cation retention capacity of the root. This concept accorded with the linear relationships observed between leaf P and root concentrations of Ca and Mg found in Al-stressed plants.
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Chemiese analise van plantgedeeltes van mielie-kultivars TX 24 en HL 1 in 'n kultuuroplossing wat Al bevat, het getoon dat die teenwoordigheid van Al in oplossing die inname van Ca en Mg en die vervoer van P en K beïnvloed het. Hoogs betekenisvolle verskille in die patroon van Al-inname, veral by lae-oplossing Al-konsentrate, het voorgekom. P-verspreiding tussen wortels en spruite, het verminder met toename in Al-konsentrasies in wortels. Dit is gevind dat 'n Al-geïnduseerde daling in wortelmetabolisme voorgekom het by ongeskonde wortels met 'n inhoud van meer as 20 h Al. Kultivarverskille in die vermoë om wortelrespirasie vol te hou in die teenwoordigheid van Al, kom ooreen met kultivarverskille in P-distribusie tussen plantgedeeltes onder Al-spanning. In die afwesigheid van Al, kon Mg deur Ca en K vervang word vir albei kultivars sonder om opbrengs merkwaardig te beïnvloed. Daar word dus voorgestel dat 'n vername vereiste vir opname van hierdie ione, ladingneutralisasie behels. Verder, aangesien Mg-konsentrasies van wortels in hierdie eksperimente aangeteken, vergelykbaar was met dié gevind in plante met 'n hoë graad van Al-beskadiging, kon daar afgelei word dat hierdie Mg-vlakke voldoende was vir die spesifieke funksie in die plant. 'n Hipotese is ontwikkel wat behels dat die rol van Al in wortelmetabolisme die aktiewe ioonbeweging onderdruk en daardeur die katioonretensievermoë van die wortel verlaag. Hierdie konsep het ooreengestem met die lineêre verhouding gevind tussen blaar-P en wortelkonsentrasies van Ca en Mg in plante onder Al-spanning.
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Keywords: Aluminium, nutrient, toxicity, transport, uptake

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Introduction

Aluminium toxicity has been linked to a number of nutrient disorders in plants stressed with high levels of Al, notably those involving P (Wright, 1943; Foy & Brown, 1963; Andrew & Vanden Berg, 1973), Ca (Foy, Fleming & Gerloff, 1972; Foy 1974), Mg and Mn (Clark, 1977; Grimme, 1982). Attempts to equate induced nutrient deficiencies with the physiological expression of Al toxicity are frequent (Wright, 1943; Foy & Brown, 1963; Foy, Chaney & White, 1978) and the demonstration of a differential ability to acquire nutrient elements in the presence of Al has been interpreted as an expression of varietal differences in plant response to Al (Foy *et al.*, 1972; Clark, 1977; Krizek & Foy, 1982).

Several authors (Wallihan, 1948; Clarkson, 1965; Clarkson, 1969) have questioned the concept that interactions between Al and P involving the precipitation of aluminium phosphates in plant roots represent a primary response to Al, and there are also indications that the uptake sites for Al (Bennet, Breen & Fey, 1985a) and P (Clarkson & Hanson, 1980; Barber, 1982) may not coincide. Attempts to explain the effect of Al on the uptake of nutrient cations are infrequent (Clarkson & Sanderson, 1971).

Al is, however, known to elicit a range of responses in the root (Clarkson, 1965; Bennet, Breen & Fey, 1985b, c) and it is possible that alterations in chemical composition of plants arising from Al treatment are initiated through changes in root physiology. The purpose of this study was to investigate Al toxicity effects on two maize cultivars with reference to the acquisition of P, K, Ca, Mg and Mn.

Materials and Methods

Plant growth conditions

Pre-germinated seed of *Zea mays* L. was planted in 25 ℓ plastic buckets (1,08 ℓ/plant; 23 plants per bucket) containing the following nutrient solution (mg dm^{-3}) N 36; P 3; K 40; Ca 40; Mg 10; S 14; Na 1,5; Mn 0,11; Zn 0,05; Cu 0,02; Fe 5,5; Mo 0,03; B 1,9. Solution pH was adjusted to 4,6 prior to planting by the addition of KOH or HCl. No further adjustments to pH were made. During the experiment the nutrient solution was gradually acidified reaching a pH of not less than 4,1 at termination. pH Changes within this range are not expected to influence plant growth or mineral uptake (Munns, 1965; Hewitt, 1966; Clark, 1982). Nitrogen was provided as NH_4^+ and NO_3^- in a molar ratio of 1:1. Ca was added as $CaCl_2$. Sulphur was supplied as SO_4^{2-}. F

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cent cool white (269 W m^{-2}) and incandescent (180 W m^{-2}) to give an estimated light intensity of 35 W m^{-2} at plant height (Cathey & Campbell, 1980). Artificial light was supplied to give a 14 h light, 10 h dark regime with alternating temperatures of 28 : 23°C. Plants were grown with continuous aeration.

Experiment I

To test the effect of Al (added as $Al_2(SO_4)_3$) on the uptake and transport of P, K, Ca, Mg and Mn, a randomized, factorial design was employed which incorporated two maize cultivars TX 24 and HL 1, selected for previously demonstrated differences in acid tolerance (Farina, Mendes, Gevers & Channon, 1982) grown at six levels of Al (0; 0,5; 1,0; 3,0; 5,0; and 10 mg dm^{-3}) with two replications. Pots were rotated on a random basis every three days. After 14 days, the plant tops were separated from the roots, the length of the primary root was measured and roots were rinsed in de-ionised water. Plant material was dried at 70°C, weighed, ground to pass a 0,84 mm stainless steel screen, and analysed for K, Ca, Mg, Mn and Al using atomic absorption spectrophotometry (Allan, 1970; I.S.F.E.I.P., 1971 & 1972). A nitrous oxide – acetylene flame was used in the determination of Al. Sr (2500 mg dm^{-3}) was used to suppress interferences. P was determined colourimetrically using the molybdovanadate procedure (Technicon, 1972).

Experiment II

The effect on P uptake and transport of different Mg and Mn levels in the absence of Al was tested by means of a randomized factorial design in which Mg and Mn levels in the nutrient solution (Experiment I) were adjusted to give concentrations in combination of 1,0; 3,0; 6,0; 12,0 mg dm^{-3} Mg and 0,11 and 0,22 mg dm^{-3} Mn. Procedures were otherwise similar to those followed in the preceding experiment.

Experiment III

The effect of Al on the respiration of intact roots was investigated by growing plants, initially without Al, for a total of 10 days under conditions similar to those in Experiment I. $Al_2(SO_4)_3$ solution was added in advance of harvest to give 0 h, 3 h, 6 h, 10 h and 20 h root exposure to 2,0; 4,0 and 8,0 mg dm^{-3} Al.

Ten plants from each treatment were sampled and the roots of intact plants treated with tetrazolium chloride solution as previously described by Bennet et al. (1985b).

Results

Statistical treatment of results

These experiments were designed to test for differences in performance between cultivars. Where statistically significant differences did not emerge, data presentation has been simplified by grouping results which are expressed as the mean values for both cultivars.

Experiment I

Root Al concentration and root length responded to an increase in solution Al (Figure 1). Foliar concentrations of Al were appreciably lower than root levels (< 0,10 × root concentration). Highly significant cultivar differences were found in the pattern of Al uptake. Cultivar TX 24 showed a progressive increase in root Al with increasing Al in solution, whereas cv. HL 1 accumulated up to five times more Al at

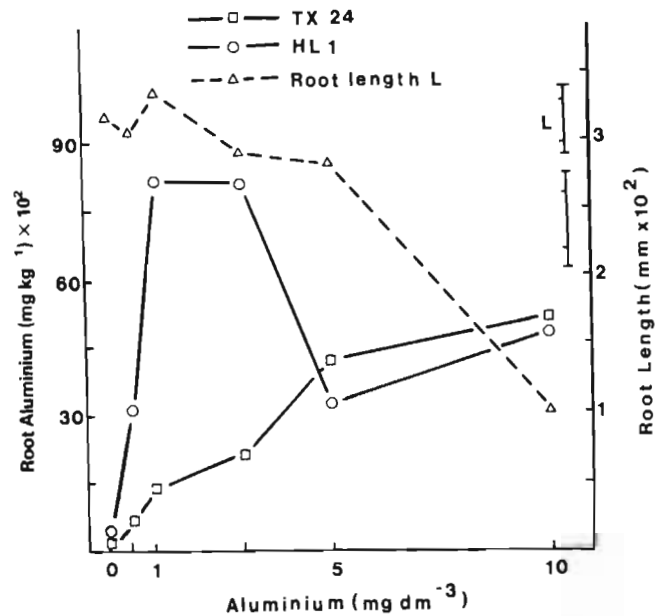**Figure 1** Cultivar response to solution Al level measured as root Al concentration (cvs. TX 24 and HL 1) and root length (mean of both cvs.) Vertical scale lines represent 5% and 1% confidence levels for root length (L) and root Al concentration.
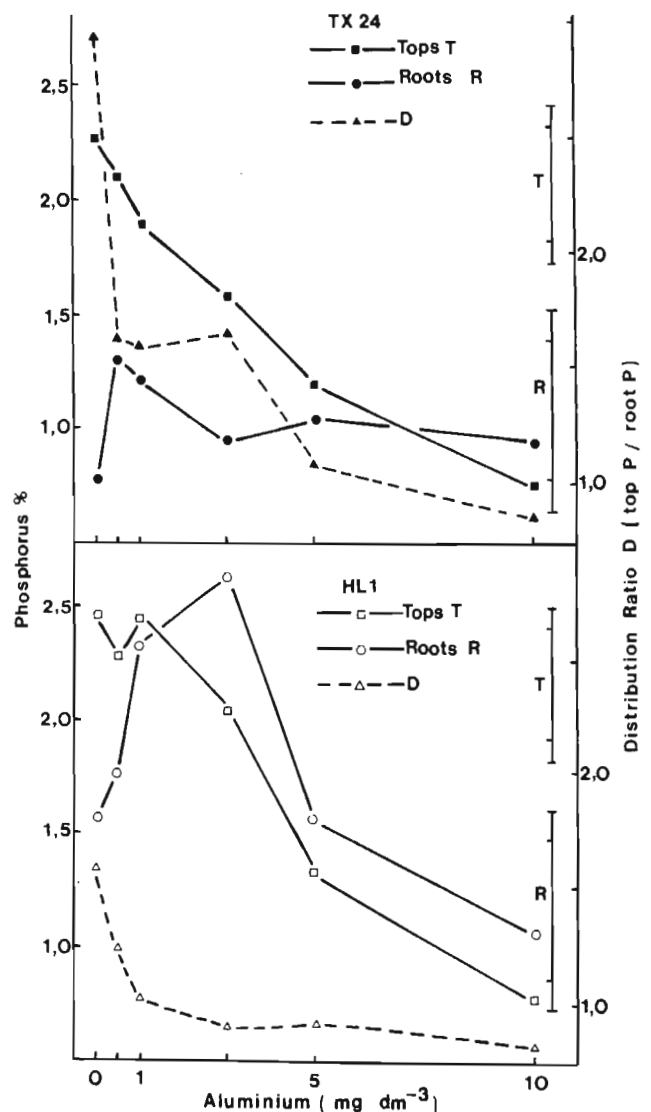

Figure 2 The effect of solution Al levels on the top and root concentrations of P and the distribution of P between plant fractions (cvs. TX 24, HL 1)....

dm^{-3}) were associated with diminished root Al levels in cv. HL 1 and at these levels cultivar differences in root Al concentrations were not significant.

Root Al was correlated (negatively) with root length only in the case of cv. TX 24 [$r = -0,81$** (*significant at 5% level; **significant at 1% level)]. Root Al was not significantly related to dry matter yield of either tops or roots. Highly significant differences in mean root yield were, however, observed between cv. TX 24 (1,18 g per pot) and cv. HL 1 (0,79 g per pot). A loss of apical dominance manifest by a proliferation of lateral roots along the main axis was apparent at the two highest solution Al levels.

Cultivar HL 1 was characterized by significantly higher concentrations of P in both roots and shoots (Figure 2). Al treatment initially increased root P levels where the observed increases were noticeably greater in cv. HL 1 (Figure 2). In the same direction, K increased slightly, while Ca, Mg and Mn concentrations in the root declined (Figures 3 and 4). The concentrations of all these elements were progressively diminished in the aerial fractions by Al (Figures 2 to 4).

Leaf concentrations of P and K were not correlated with the root levels of these elements. Highly significant linear

relationships emerged between leaf and root levels of Ca [$r = 0,81$** (TX 24); 0,75** (HL 1)] and Mg [$r = 0,84$** (TX 24); 0,72** (HL 1)]. Leaf concentrations of P were however significantly correlated with root concentrations of Ca [$r = 0,88$** (TX 24); 0,75** (HL 1)] and Mg [$r = 0,94$** (TX 24); 0,82** (HL 1)].

Distinctive differences in the effect of Al on the distribution of elements between plant fractions emerged. The decrease in distribution ratio (leaf concentration/root concentration) for P and K with increased Al stress (Figures 2 and 3) indicated that reduced foliar levels were associated with an accumulation of these elements in the roots. It was therefore concluded that transport rather than uptake was the rate-limiting step. Although Al treatment resulted in reduced Ca and Mg levels in plant roots and tops, the distribution ratio for both elements increased notably at the highest Al stress level (Figure 4). This suggested that the transport mechanisms were not affected by Al and reduced plant concentrations reflected lower uptake. Cultivar TX 24 maintained a higher distribution ratio for P in the presence and absence of Al.

A clear relationship between root concentrations of P and Al did not emerge from these experiments. With cv. TX 24

Figure 3 The effect of solution Al levels on the top and root concentrations of Mn and K and the distribution of Mn and K between plant fractions (mean of both cvs.) Vertical scale lines represent 5% and 1% confidence levels (single level 5% only) for concentrations of the
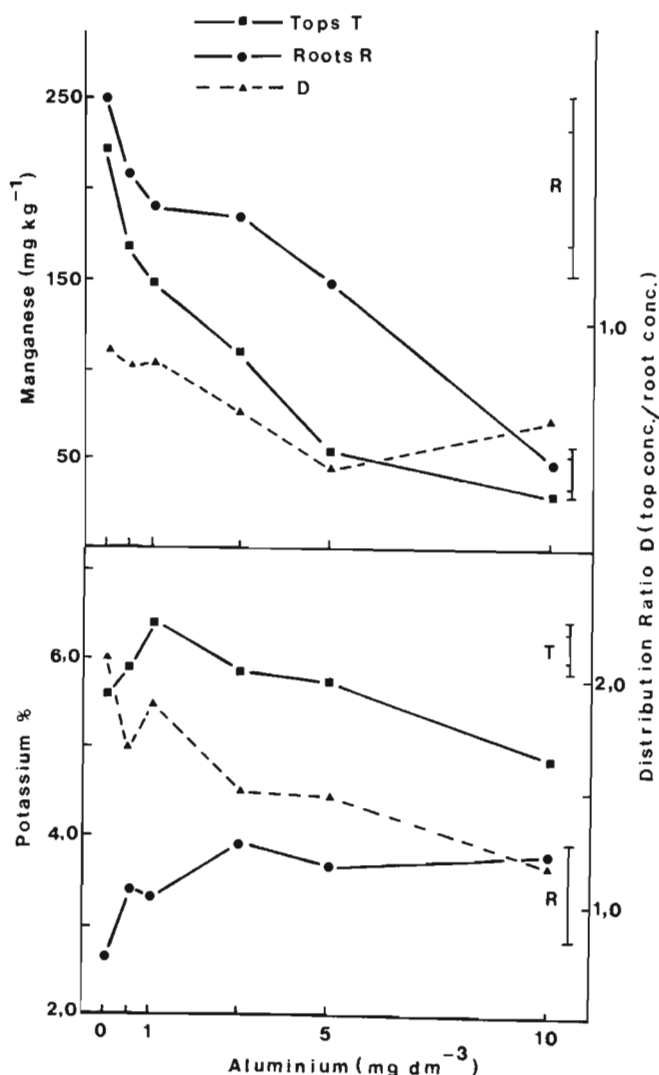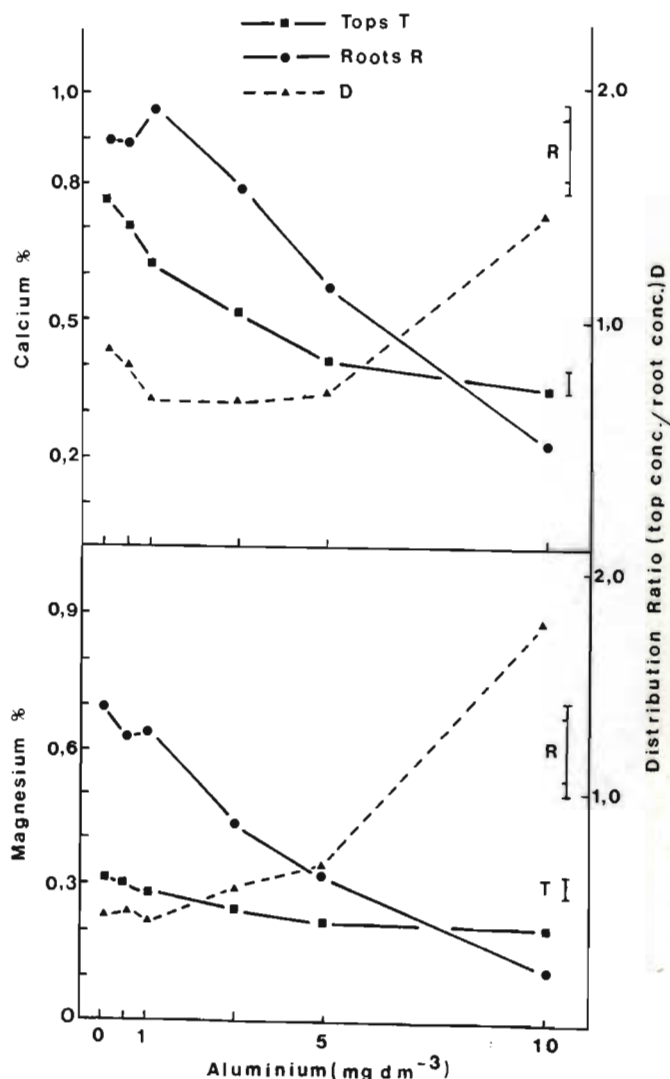
Figure 4 The effect of solution Al level on the top and root concentrations of Ca and Mg and the distribution of Ca and Mg between plant fractions (means of both cvs.) Vertical scale lines represent 5% and 1%

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no significant correlation was observed, while with cv. HL 1, a barely significant ($r = 0,59*$) correlation existed between these elements. It is therefore important that a distinctive negative relationship between the distribution ratio for P and root Al was identified, notably in cv. TX 24 [$r = -0,95**$ (TX 24); $-0,82$ (HL 1)].

Experiment II

No significant differences could be observed in the effects produced by the two levels of Mn used in this experiment. Neither P concentration in the roots and tops, nor dry matter yields (roots and tops) of either cultivar varied significantly with Mg supply (data not shown).

Root Mg concentrations were negatively correlated with K [$r = -0,71**$ (TX 24); $-0,88**$ (HL 1)] and Ca [$r = -0,70**$ (TX 24); $-0,94**$ (HL 1)] concentrations (Figure 5) and since the net positive charge on the root calculated as Σ root cations remained constant (Figure 5), a considerable degree of substitution between Mg, Ca and K was indicated. The distribution ratio for Mg (Figure 5) confirmed that low root Mg concentrations were associated with preferential movement of Mg into the plants tops in the absence of Al.
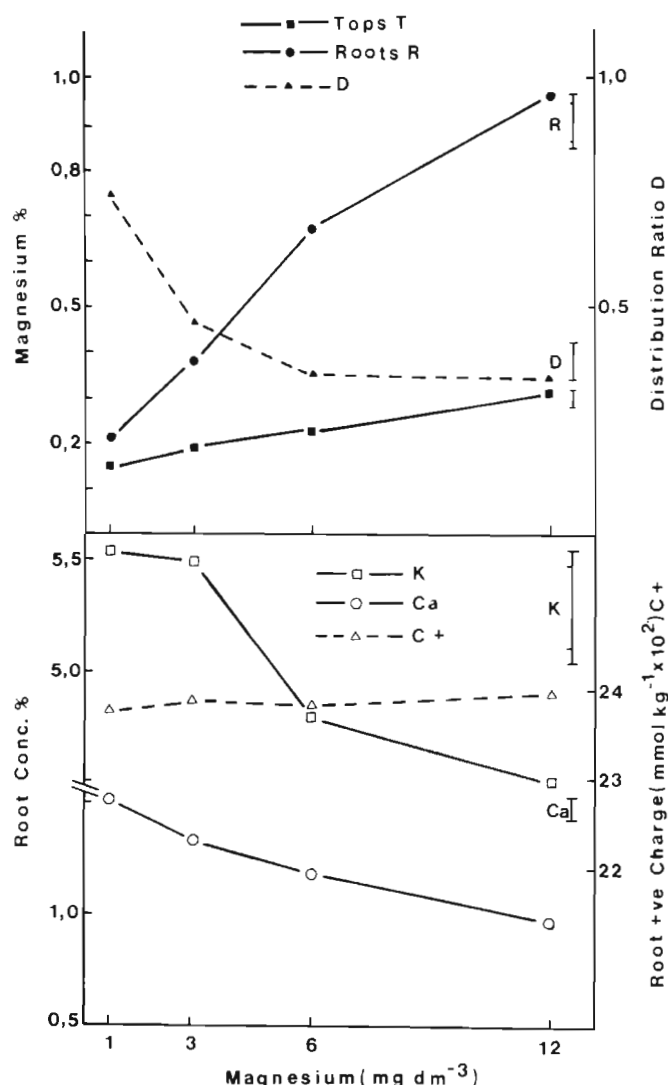

Figure 5 The effect of solution Mg levels on the top concentration of Mg; root concentrations of Mg, K, Ca; the distribution of Mg between plant fractions and total root cationic charge (means of both cultivars). Vertical scale lines represent 5% and 1% confidence interval (single level 1% only) for the concentration of magnesium in the plant tops (T) and

Experiment III

A reduction in the metabolic activity of intact roots was detected on the basis of a loss of intensity of red (formazan) colour development at Al concentrations of 4 and 8 mg dm^{-3} in the nutrient solution, with root exposure times of 20 h. Cultivar TX 24 proved superior to cv. HL 1 in maintaining root respiration in the presence of Al, and while low levels of respiratory activity were still evident in the primary root of cv. TX 24 with 20-h exposure to 8 mg dm^{-3} Al, no corresponding activity was visually apparent in the roots of cv. HL 1.

Discussion

Physiology of Al uptake

Divergences in Al uptake patterns between cvs. TX 24 and Hl 1 suggest physiological differences which determine Al uptake kinetics. Previously reported data (Bennet et al., 1985a) has connected Al uptake to biochemical properties of specific cell populations of the root apex. The appreciable uptake of Al observed in cv. HL 1 at low stress levels is clearly favoured by these differences, while the subsequent decline, with increased Al stress, may be attributed to an Al-induced failure of the biosynthetic pathways controlling Al uptake. Alterations in root physiology associated with increased Al stress are supported by the observations that statistically significant changes in root length, a loss of apical dominance and an observable decline in root metabolism all occurred at Al stress levels commensurate with those initiating reduced root Al concentrations in the roots of cv. HL 1. It may also be argued that the action of Al cannot primarily be directed at the mitotically active cell populations of the root meristem (Bennet et al., 1985b, c) since the high root Al levels, associated with low Al stress in cv. HL 1 did not produce a coincidental decline in root growth. The biochemical incorporation of Al into the root differs from the concept of a non-metabolic uptake pathway implicit in the data of Clarkson (1967) and Rhue (1976).

Mineral nutrition and Al toxicity

Disordered mineral nutrition as a consequence of Al treatment has been reviewed by Foy et al. (1978). Al-induced changes in the chemical composition of plants are subject to a variety of possible interpretations, including inactivation of the processes engaged in uptake/transport as well as competition between Al and the nutrient ions for common uptake sites.

Because of the importance attached to interactions between Al and P in plant cells (Adams, 1980) and the changes initiated through the Ca dependent regulatory protein, Calmodulin, (Haug, 1984) as a consequence of Al-induced Ca deficiency, it is relevant to consider whether the Al-induced changes in composition arise as a primary response to Al.

Evidence connecting Al-induced nutrient deficiencies with the primary expression of toxicity is, however, not supported by the observation (Horst, Wagner & Marschner, 1983) that short-term Al exposure, although depressing root elongation, is not connected to the induction of Ca or P deficiencies over the course of the experiment. Chemical immobilization of P by Al in the root does not accord with the low correlations observed in these experiments between the root concentrations of these elements. The concept is also difficult to reconcile with the rapidity of expression of

Nutrient uptake, notably of P and K is also influenced by root morphology (Schenk & Barber, 1980; Barber, 1982). The effect of Al in restricting root length and altering root structure (Bennet et al. 1985c) must be considered as a possible mechanism for restricting nutrient uptake. It is therefore important that these experiments have demonstrated that the action of Al is primarily directed at the transport of P and K rather than uptake. This observation implies that altered root morphology is unlikely to be a significant factor accounting for the changes in composition of Al-treated plants as noted in these experiments.

Functional involvement of nutrient cations in P acquisition

Relevance must, however, be attached to the accumulation of P in the roots of Al-stressed plants observed in these experiments, since similar results have been recorded for a variety of plant species (Clarkson, 1969; Andrew & Vanden Berg, 1973). The highly significant correlations observed between leaf levels of P and root concentrations of Ca and Mg may be indicative of interactions between these elements which influence P transport. Mg has well-established links in salt acquisition involving the stimulation of ATPase activity (Leonard & Hodges, 1973; Bowling, 1976; Marrè, Romani, Cocucci, Moloney & Marrè, 1982). A functional involvement for Ca is less well defined since Ca has been shown to be ineffective as a replacement for Mg in this role (Bowling, 1976).

Clarkson & Sanderson (1971) have suggested that the inhibition of Ca uptake by Al, reflects a reversal of the electrical charge by the polyvalent ion at the entry point into the root-free space. Similarities exist in the patterns of uptake between Ca and Mg (Ferguson & Clarkson, 1976) and it is tempting to consider that the altered P status of Al-stressed plants reflects an Al-induced repression of Mg uptake.

Experiments involving the effect of Mg (and Mn) supply on P uptake and transport in the absence of Al do not, however, support this hypothesis, since low Mg levels were not connected to altered P status or changes in dry matter yield of plants over the range of the experiment. Furthermore, as the lowest root Mg levels recorded in the absence of Al are comparable to those found in plants showing a high degree of Al injury (Experiment I), it was concluded that these levels are adequate to meet specific functional requirements for this element in the plant.

Negative correlations observed between Mg and Ca and K in the absence of Al are indicative that reduced Mg uptake can, to some extent, be compensated for by increased Ca and K uptake. It is therefore probable that a major requirement for these nutrient cations in the root involves a non-specific charge neutralization role. This idea accords with observations regarding the stability of root (+ve) charge and is compatible with the established non-specific stimulatory effect of some polyvalent cations on anion uptake (Viets, 1944; Franklin, 1970).

Root metabolism, ion uptake and transport

Clarkson (1967) has observed that in Al-stressed plants, much of the P retained in the root is not metabolized and is therefore readily exchangeable. Many aspects of P uptake and transfer remain uncertain, but Loughman (1981) has demonstrated that the rapid metabolic incorporation of the $H_2PO_4^-$ ion is a prerequisite for the radial transfer of P across the cortex. Furthermore, there is evidence to show

are selectively influenced by low oxygen supply, since the respiratory inhibitor 2,4 di-nitrophenol causes a cessation of P transport rather than uptake (Loughman, 1969).

In a previous paper (Bennet et al., 1985b) evidence was presented for the inhibitory effect of Al on the respiration of the cell populations of the root apex and the present experiments have confirmed that a reduction in metabolic activity can be extended to intact roots, albeit with longer root exposure times. If the observed decline in respiratory activity is seen to be selectively directed at P transport, a rapid accumulation of non-metabolized P in the roots of Al-treated plants may be anticipated. This accords with observed experimental data. The indirect action of Al on P transport through an Al-induced decline in root respiration is also consistent with the highly specific nature of Al uptake sites (Bennet et al., 1985a), the demonstrated relationship between P transport and root Al concentration and the observation that cultivar differences in the ability to maintain root respiration in the presence of Al coincided with cultivar efficiencies involving the fraction of P transported to the plant tops.

The concept that the effect of Al on P transport can be linked to an Al-induced decline in cellular metabolism clearly challenges the idea that P accumulation in Al-treated roots is independent of metabolism (Clarkson, 1969). A basis exists to reconsider Clarkson's findings since the present experiments have indicated that Al and the respiratory inhibitor 2,4 di-nitrophenol on which Clarkson's work was based may produce a similar result.

A hypothesis to connect Al toxicity and anion transport with diminished uptake of Ca and Mg

The major anion species are actively accumulated against electrochemical gradients (Higinbotham, 1973) and although the mechanism remains largely theoretical, there is considerable evidence to connect the function of membrane-bound electrogenic ion pumps involved with energy dependent ATPase activity (Fisher, Hansen & Hodges, 1970; Rao & Rains, 1976; Cheeseman, La Fayette, Gronewald & Hanson, 1980; Rasi-Caldogno, Cerana & Pugliarello, 1980). Active ion movement coincidentally generates a negative electropotential at the cell wall to drive cation uptake on a non-selective basis. The passive uptake of Ca and Mg occurs along these electrochemical gradients (Bowling, 1976; Mengel & Kirkby, 1979) so that the concentration of these elements in plant cells does not exceed the physical equilibrium levels (Higinbotham, 1973).

Experiment I indicates that Al-induced changes in plant concentrations of Ca and Mg are primarily connected to uptake as evidenced by the linear correlations observed between leaf and root concentrations of these elements. Furthermore, in contrast to P and K, the distribution ratios for these elements indicate that a reduction in root concentration may be associated with an increase in the fraction transported to the plant top, implying that the transport mechanisms remain functional.

Inhibition of Ca and Mg uptake may therefore be a secondary consequence of an Al-induced reduction in respiration which acts indirectly through active ion movement by reducing the net negative charge, with the consequent de-activation of negatively charged exchange sites. This hypothesis differs from the interpretation of Clarkson & Sanderson (1971) who considered the effect of Al on Ca uptake to be primarily a

however, also demonstrated interrelationships between plant concentrations of P, Ca and Mg, interpretation of which is consistent with the disruption of metabolically dependent transport processes. On the contrary, correlations between these elements are not adequately explained by the concept of surface charge neutralization.

Acknowledgement

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THE EFFECTS OF ALUMINIUM ON ROOT CAP FUNCTION AND ROOT DEVELOPMENT IN Zea mays L.

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Aluminium concentrations ≤ 1.25 mg dm^{-3} promoted a positive growth response in the primary root of Zea mays and this coincided with an increase in mean cap volume. Cap volume correlated with cap cell numbers. Significant alterations in mean cell volume were limited to the cap periphery where Al caused a decrease in cell volume. This implied that the action of Al was directed at differentiation of peripheral cap cells and that the observed changes in cap volume arose through interference by Al in mechanisms which control cell loss from the cap. This observation was supported by a reduction in the number of detached, mature cap cells surrounding Al-treated roots.

Ultrastructural investigation revealed that disruption of Golgi apparatus activity in the peripheral cap cells occurred at the lowest Al concentration (0.5 mg dm^{-3}). A decrease in amyloplast numbers coincided with diminished Golgi apparatus activity in the peripheral cap and these changes preceded reductions in mitotic activity, indicated by decreases in cap volume and root length which were only evident at Al concentrations in

excess of 1.25 mg dm^{-3}.

Al treatment also resulted in a redistribution of amyloplasts to the proximal halves of central cap cells as well as alterations to the linear arrangement of these cells.

The presence of Al was associated with an efflux of H$^+$. Some of the more adverse responses to Al, including inhibition of cell division, were observed to be initiated at Al concentrations where the molar ratio of charge between Ca^{2+} and Al^{3+} favoured Al. This may attach relevance to the electrical properties of the Al ion in the initiation of an electro-physiological plant response to Al.

KEY WORDS   aluminium,   Golgi apparatus (root cap),

polysaccharide metabolism,   root development.

EXPERIMENTAL AND ENVIRONMENTAL BOTANY

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INTRODUCTION

Reports dealing with plant response to aluminium (Al) have attached importance to the root cap in the expression of toxicity,[13,15] where the earliest ultrastructural effect was directed at the Golgi apparatus of the peripheral cap cells.[13] Reduction of root elongation arising from diminished mitotic activity nevertheless remains one of the most obvious consequences of toxicity.[18] The structure of nuclei in cells of the cap and proximal meristems is, however, resistant to Al-induced damage [13,14] and an understanding of the physiological response to Al is additionally complicated by the observation that many of the changes identified in the roots of Al-stressed plants do not directly coincide with the presence of Al in the tissues involved.[13,14,15]

Although Clowes[20] has unequivocally demonstrated an interaction between the root cap and cell proliferation in the root meristem, little is known to indicate how removal of mature cap cells influences the cycling rate of the remaining cap meristem and quiescent centre.[6] The cell populations comprising the root apex are principally characterized by marked differences in cell cycle times.[6,19,20,22] Co-ordination of the activities of these cells must therefore represent a finely balanced equilibrium.

Interactions between cell populations have been experimentally verified,[9,17,21,24] but definition of how this co-ordination is achieved in actively growing roots remains less than satisfactory. How Al disrupts intercellular relationships in the root meristem has not previously been considered. Aluminium uptake has, nevertheless, been connected to polysaccharide metabolism,[15] and Golgi apparatus function is thought to extend to include a morphogenic material involved in controlling plastid differentiation[12].

In this paper we report on the effects of different concentrations of Al on the function of the cap and the concomitant consequences for root development.

MATERIALS AND METHODS

Plant Growth Conditions

Pre-germinated "seed" of <u>Zea mays</u> L. cv. TX 24 with radicle lengths 7 - 10 mm were supported in 25ℓ plastic buckets (1,08ℓ/plant) containing the following nutrients (mg dm^{-3}) at pH 4.6:

N 36; P 3; K 40; Ca 40; Mg 10; S 14; Na 1.5; Mn 0.11; Zn 0.05; Cu 0.02; Fe 5.5; Mo 0.03; B 1.9. Nitrogen was provided as NH_4^+ and NO_3^- in a molar ratio of 1 : 1. Sulphur was supplied as SO_4^{2-}. Fe was present as Fe CDTA (cyclohexanediaminetetraacetic acid, Merck Art 8424*).

Artificial light was supplied to give a 14 hr light : 10 hr dark regime with corresponding temperatures of 28 : 23° C. The light source was Grolux W.S.* (731.3 $W.m^{-2}$) and incandescent (113.4 $W.m^{-2}$) lamps to give a light intensity of 300 $\mu Em.^{-2}s^{-1}$ at plant height. Plants were grown with continuous aeration.

Experiment 1

Plants were grown for 7 days. The nutrient solutions were then replaced with solutions of similar composition to which $Al_2(SO_4)_3 \cdot 18H_2O$ had been added to give root treatments of 0 (control); 0.5; 1.25; 2.0; 3.0; 5.0 mg dm^{-3} Al. pH was adjusted to 4.6 subsequent to the addition of Al. Solution pH was maintained at pH $4.6^{\pm}$ 0.1 over 29 hr by the addition of measured volumes of 0.1 M NaOH. Hydrogen ion efflux determinations were repeated in a second experiment with 7 hr Al treatment times. To assess the effect of factors not related to plant growth on nutrient solution pH, a solution containing 5.0 mg dm^{-3} Al was prepared in which no plants were placed. The pH of this solution was monitored but no change in

* Trade and company names are included for the benefit of the reader and are not endorsed by the authors.

solution pH was recorded over 29 hr.

At the conclusion of the experiment (29 hr), root tips from each treatment were fixed in 6% glutaraldehyde in 0.05 M sodium cacodylate buffer at pH 7.2 with post-fixation (2 hr) in 2% OsO_4 in sodium cacodylate buffer.[30] Fixed material was de-hydrated in graded alcohol, treated with propylene oxide and embedded in Epon/Araldite resin. Sections for light microscope viewing were stained with Ladd Multiple stain (Ladd 70955*). Ultrathin (gold) sections for transmission electron microscopy were stained in uranyl acetate and lead citrate.[31]

Measurements of cells and cell populations included determination of the volume of the root cap; mean cell volume of cells of the cap meristem; central cap columella and peripheral cap as defined in Fig. 1. A minimum of 5 roots was examined from each treatment to determine mean values. Longitudinal sections were cut sequentially through each root tip. Median sections were then selected for further measurement so as to ensure a maximum cap length (H). Measurements were made at a magnification of X 240 following the procedures and assumptions outlined by Barlow[4] and Barlow and Rathfelder.[9]

Root diameter measurements on longitudinal sections were made 1 mm from the root tip (Fig. 1)[14] by using a calibrated eyepiece on an optical microscope.

Counts were also made of the number of amyloplasts found in the root cap. Numerical estimates of the degree of amyloplast sedimentation in the central cap were based on the frequency with which amyloplasts were observed to be present in the proximal half of central cap cells. A minimum of 5 roots was examined to determine mean values from each treatment.

Experiment 2

To test the effect of Al treatment on root growth, plants (cv. TX 24) were grown for 14 days at six Al levels (0; 0.5; 1.0; 3.0; 5.0 and 10.0 mg dm^{-3}). Nutrient solution pH was adjusted to 4.6 prior to planting. No further adjustments to pH were

made. During the experiment, the nutrient solution was gradually acidified, reaching a pH of not less than 4.1 at termination. After 14 days, the plants were harvested and the length of the primary root measured. The experiment was duplicated to provide a second set of measurements. Results were expressed as the mean value for 46 plants which comprised each treatment.

Experiment 3

To identify the regions of the root where H^+ efflux was initiated by Al treatment, pre-germinated "seeds" (cv. TX 24) were planted along the upper edge of 66 X 22 cm filter paper with their radicles (7 - 10 mm) pointing downwards. The paper was loosely rolled and placed vertically in a glass container filled to a depth of 5 cm with half-strength nutrient solution as described by Konzak et al.[37] Plants were grown for 3 days at 28°C, selected for a straight root axis measuring $100^{\pm}$ 10 mm.

Ten plants were transferred to a water culture containing either 0 Al (control) or 10 mg dm^{-3} Al as $Al_2(SO_4)_3$ for 10 minutes. Roots were rinsed in de-ionised water for 5 minutes before transfer to an agar block containing bromocresol purple at pH 6.0. Agar plates (3 mm) were prepared from 0.6% agar and included plant nutrients (m gm^{-3}) N 18; P 1.5; K 20; Ca 20; Mg 5; S 7; Na 0.75; Mn 0.05; Zn 0.03; Cu 0.01; Fe 3; Mo 0.02; B 1.

H^+ efflux from the root caused the indicator to become yellow. The method for preparation of agar plates and measurement of H^+ efflux was similar to that described in detail by Mulkey et al.[41] and Moore.[40]

RESULTS

Cap Shape and Construction

Aluminium treatment was effective in altering the mean volume of the root cap over a 29 hr period. An increase in Al concentration up to 1.25 mg dm^{-3} produced increases in cap volume, while further escalation in Al

levels beyond this point resulted in a decrease in mean cap volume so that at the highest Al concentration (5.0 mg dm^{-3}), cap volume was not different from the control. Alterations in cap function arising from Al treatment were, however, indicated by changes in peripheral cap cell volume (Fig. 3) and cap cell morphology (Fig. 4). Changes in cap volume coincided with alterations to the number of cap cells viewed in median longitudinal section (r = 0.59; p <0.05). Aluminium concentrations $\leqslant$ 1.25 mg dm^{-3} produced an increase in cell numbers over the control (302). These changes preceded a drop in numbers from a root Al treatment of 1.25 mg dm^{-3} (327) with the fewest cells (270) being recorded at the highest Al level (5.0 mg dm^{-3}).

Differentiation of cells within the cap is usually accompanied by an increase in cell size.[6] Notwithstanding variation in the size of cells resulting in a substantial standard error, Al-induced changes in cell differentiation were supported by decreases in mean volume of peripheral cap cells, evident at Al levels above 1.25 mg dm^{-3} (Figs. 3, 4). In contrast to the situation in the cap periphery, Al treatment produced modest but statistically insignificant increases in mean cell volume in the cap meristem (12.3 μm^3 X 10^2; 0 Al; 14.3 μm^3 X 10^2; 5.0 mg dm^{-3} Al) and central cap (54.9 μm^3 X 10^2; 0 Al; 66.9 μm^3 X 10^2; 5.0 mg dm^{-3} Al). Notwithstanding the stability of central cap cells to changes in size, the geometry of these tissues involving the longitudinal arrangement of cell files was extensively altered at Al concentrations $\geqslant$ 3.0 mg dm^{-3} (Fig. 4).

Counts of the number of detached mature cap cells present in the mucilaginous material surrounding the root and cap showed that a decrease of 52% was associated with the presence of Al in the nutrient solution. No treatment effect connecting cell loss to Al concentration was, however, detected.

Root Growth

Over a 14-day growth period, low Al concentrations (≤ 1.0 mg dm^{-3})
were associated with an Al-stimulated growth response of the primary root,
evidenced by an increase in mean primary root length over the control.
In contrast, intensification of Al concentration beyond this point resulted
in a sharp drop in root elongation (Fig. 5).

Amyloplast Distribution

Over a 29 hr period, the number of amyloplasts present in the cap
showed a sharp decrease with increasing Al concentration (Figs. 6,4).
The distribution of amyloplasts in central cap columella cells was also
altered by Al treatment, so that the number of amyloplasts present in the
proximal half of central cap cells of Al-treated roots was higher than that
found in the control.   The highest frequency of non-sedimenting amyloplasts
was observed at an Al concentration of 2.0 mg dm^{-3}.

Ultrastructural Response to Aluminium
Peripheral Cap Cells

The present experiments showed that the action of Al was primarily
directed at the Golgi apparatus in the peripheral cap cells, and the
previously reported[13] effects of Al, which included disruption of
cisternal structure, damage to vesicular membranes and changes in the
appearance of vesicle contents, were all evident at 0.5 mg dm^{-3} Al (Fig.7).
A progressive deterioration in Golgi apparatus activity was also connected
to increases in Al concentration (Fig.7D) and at 5.0 mg dm^{-3}, characteristic
Golgi apparatus activity was no longer apparent in many peripheral cap cells
(Fig.8C).   Comparisons between Figs. 7B and 7C indicated that changes in
Golgi apparatus activity coincided with an altered appearance and decrease
in frequency with which Golgi apparatus-derived material was accumulated

between the plasmalemma and cell wall. Some accumulation of Golgi material could, however, still be detected at 5.0 mg dm^{-3} Al (Fig. 8C), although inhibition of transfer of mucilagenous material to the root exterior was indicated by the absence of slimes which were no longer evident on the root exterior of living roots.

Treatment with Al was also associated with increased vacuolation of outer cap cells (Fig. 8C).

The Central Cap Cells

Golgi apparatus secretory patterns correlate with cellular development[38] and the Golgi apparatus in central cap cells narrowly removed from the peripheral cap regions and distinguished by the size and appearance of the secretory vesicles (Fig. 7E), showed little response to low concentrations of Al ($\leqslant$ 2.0 mg dm^{-3}). Increasing Al concentrations disrupted the Golgi apparatus in these cells, although the response was notably less dramatic than that observed in the peripheral cap cells.

Changes in amyloplast structure, which involved the size and number of starch grains, were only suggested at higher Al levels ($\geqslant$ 3.0 mg dm^{-3}). In other respects, amyloplast structure was notably resistant to Al-induced changes.

Vacuolation of central cap cells was evident at an Al concentration of 5.0 mg dm^{-3}.

The Cap Meristem

Changes in nuclear structure, involving the degree of chromatin condensation, appearance of the nucleolus and the structure of the nuclear membranes, were not discernible after 29 hr treatment with 5.0 mg dm^{-3} Al (Figs. 8A, 8B). Cells of the cap meristem were, however, characterized by increased vacuolation at an Al concentration of

5.0 mg dm^{-3} (Fig. 8B) and while many proplastids were present, few showed evidence of starch deposition (Figs. 8A, 8B).

Cell Enlargement

Alterations in the polarity of cell enlargement[14] in the primary growth region of the root was indicated by an increase in mean root diameter, evident after 29 hr treatment at Al concentrations $\geq$ 1.25 mg dm^{-3} (Fig. 9).

Efflux of H$^+$ from the Root

Aluminium treatments over the full experimental range were effective in more than doubling the efflux in H$^+$ from the roots (Fig. 9), and measurable pH changes of the nutrient solution were detected within 1 hr. Quantitative estimates of H$^+$ efflux were derived from NaOH additions required to maintain the nutrient solution pH within the prescribed limits and on total root dry matter yield. Differences in H$^+$ efflux were not initially evident at different Al concentrations (7 hr) (Fig. 9) but, with longer times, diminished H$^+$ efflux was observed at the higher Al concentrations, and the difference between 0.5 mg dm^{-3} Al (4.01 mg H$^+$ g^{-1} root dry mass) and 5.0 mg dm^{-3} Al (2.10 mg H$^+$ g^{-1} root dry mass) with a 29 hr treatment duration was almost twofold.

Root Zones Associated with H$^+$ Efflux

Acid (H$^+$) efflux in vertically oriented roots treated with Al was initiated simultaneously in the root apex, cap and elongation zone of the root and was evident within 3 minutes. Control roots were appreciably slower to demonstrate H$^+$ efflux, which was only detected after 12 minutes. The lower level of colour development also implied that H$^+$ efflux was of a lower intensity in the control.

DISCUSSION

The present experiments add to the idea that a functional integration of intercellular activities in the root meristem may be directed through polysaccharide metabolism in the root cap. Furthermore, the absence of evidence to connect Al treatment with damage to the nuclear structure, and the initial exclusion of Al from tissues containing the actively dividing cells[13,14], imply the existence of a stress response link between the Golgi apparatus of the outer cap, where the earliest ultrastructural response to Al was evident, and the mitotically active cells of the root meristem.

Aluminium Toxicity and Abscission of Cap Cells

Cap cells are constantly displaced from the cap meristem at the base of the cap to the tip where they are sloughed off.[6] In actively growing roots, the total number of cap cells remains nearly constant, and it has been argued that the loss of cap cells must represent a highly organized and co-ordinated process.[6,34]

The response of the root cap to low concentrations of Al principally involved the peripheral cap cells where Al is effective in influencing mechanisms which control the loss of mature cap cells. The increase in cap volume observed at low Al concentrations is brought about by increased cap cell numbers, and these events suggest that an Al-induced reduction in cell loss is not, under these conditions, met by a coincidental decline in the activity of the cap meristem. Decreases in cap volume and cell numbers which occur at higher Al concentrations, presumably reflect reductions in mitotic activity.

Cell enlargement and abscission, which normally proceed nearly synchronously in the outer cap, must both depend on a concomitant loosening of cell wall material. Aluminium-induced decreases in volume of peripheral cap cells observed with heightened Al concentration, may therefore serve to confirm the ultimate failure of the mechanisms controlling these processes.

Growth Response of Primary Roots

A positive growth response to low concentrations of Al has been reported for a number of plant species.[27] Mechanisms whereby small amounts of Al may increase plant growth remain obscure and have been ascribed to the application of nutrient culture techniques.[1]

It is therefore pertinent that an Al-induced increase in cap volume corresponded with an increase in mean root length. These observations attach physiological relevance to the finding[9] that statistical correlations exist in a number of species between cap volume and the size of the proximal meristem. The results of these experiments also imply that the relatively short-term effects (29 hr Experiment 1) of Al on root development are translated into a permanent growth response still evident after 14 days (Experiment 2).

Interpretation of the effect of Al on root growth may depend initially on changes in the root cap being reflected through a partial release of the proximal meristem from growth inhibition. Similar responses involving growth stimulation have been reported in decapped roots.[43] These observations accord with the numerous suggestions which have attempted to link the basipetal movement of growth inhibiting substances originating in the cap with the regulation of root development.[8,16,25,26,28,43] Expression of Al toxicity may therefore be directed through the synthesis of naturally occurring endogenous root growth inhibitors.

Integration of Intercellular Activity

Juniper and French[36] have highlighted the substantial amount of polysaccharide material moved through the cap by the passage of amyloplasts contained in differentiating cells. The present experiments have identified a rapid reduction with increasing Al concentration, in the number of amyloplast present in the root cap. Evidence to connect starch degradation and

slime synthesis with the activity of the Golgi apparatus is not unequivocal.[10]
In intact roots, the secretion of mucilagenous polysaccharide slimes from
peripheral cap cells occurs while intercellular starch grains diminish
in size.[23,42]   Furthermore, inhibition of Golgi apparatus function in the
outer cap by Al proceeded synchronously with diminished slime synthesis, which
accords with the view[39] that the secretory functions of dictyosomes in the
cap-periphery include the packaging and export of mucilagenous materials
from the cap.

Haberlandt[29] originally considered that these materials facilitated
root movement through the soil.   A considerably wider involvement in root
physiology for polysaccharide metabolism is, however, indicated.   Barlow and
Hines[7] have intimated that these mucous slimes may control the separation
of cells from the root cap, a view supported by the present experiments which
additionally serve to implicate the Golgi apparatus in this function.

Amyloplasts located in central cap cells were resistant to Al-induced
changes in structure.   Evidence exists to indicate that Al was, however,
effective in preventing starch deposition in proplastids located in the
cap meristem.   These observations suggest that control of amyloplast numbers
may be effected through starch induction mechanisms functioning in the
cap meristem.   An involvement for the peripheral cap Golgi apparatus
in these activities is indicated by the finding that extends specific
secretory activities of the Golgi apparatus to include a morphogenic material
which is involved in the control of plastid differentiation.[12]

Changes in Golgi apparatus activity and a decline in amyloplast numbers
preceded a reduction in root length.   Although the quantity of polysaccharide
material moved to the cap periphery in differentiating cells must relate to

the cycling rate of the cap meristem, it is not yet known how Al-induced

changes in polysaccharide metabolism may be translated into root

growth responses. Moreover, a paradoxical situation, exists, in that small

changes in activity produce a positive growth response, while more

substantial declines, as evidenced by further reductions in amyloplast

numbers, coincided with progressive reductions in root length. An under-

standing of these events may depend on demonstrating that the initial

response to low concentrations of Al is expressed through a release of the

root from growth inhibition originating in the cap. Root growth at higher

Al concentrations may, however, depend on minimum levels of activity being

maintained in the root cap.

The Central Cap Cells

Amyloplasts present in central cap cells have been widely connected

to a gravi-perception function[2,3,5,33,47] where they are believed to respond

to gravity in predictable ways.[34]

The present experiments are therefore of interest in connecting the

frequency of non-sedimenting amyloplasts to Al treatment. Juniper[34]

has intimated that the relative absence of vacuoles from central cap cells

may influence amyloplast sedimentation. It is pertinent to this view that

the present experiments have shown that Al-induced increases in the frequency

of non-sedimenting amyloplasts preceded changes in the morphology of

the central cap, where an increase in vacuolation and altered cell geometry

were only detected at appreciably higher Al treatment levels.

The mechanism through which Al acts on amyloplast sedimentation is

unknown. The present experiments do, however, serve to implicate factors

additional to gravity and structural features of the central cap in

influencing the behaviour of these organelles. It may be relevant to

these ideas that Hillman and Wilkins[32] have observed that it is not only the size and apparent stage of amyloplast development which determines the ability of the amyloplasts to sediment.

Mechanisms of Aluminium Action

An early response to Al treatment involved the rapid efflux of H^+. Similar effects are reported in gravi-stimulated roots[40,41], which Mulkey et al.[41] attributed, in the case of gravi-stimulation, to an auxin-induced growth response. An alternative interpretation of these events may, however, depend on the observation that in roots treated with different Al concentrations, H^+ efflux preceded a detectable increase in root diameter arising from changes in the polarity of cell enlargement.[14]

Evidence exists to connect a net H^+ efflux occurring against electro-chemical gradients, to membrane hyperpolarisation[44,45] where membrane-bound electrogenic pumps are thought to function by stabilizing membrane potential through the transport of protons (H^+)[46]. The present experiments attach importance to the charge on the Al ion, since the initiation of a number of adverse plant responses to Al occurred at Al concentrations above 1.25 mg dm^{-3}. At this Al concentration (1.25 mg dm^{-3}), Al and Ca were present in the nutrient solution in equivalent moles of charge, that is, when $1/2 \log Ca^{2+} - 1/3 \log Al^{3+} = 0$.

Confidence in an electrophysiological plant response to Al is increased by the observations that the trivalent ions Sc and Ga are effective inhibitors of cell division in the root meristem[18]. Volkmann and Sievers[47] also reported a loss in the polarity of central cap cells arising from the application of an electric field, while Behrens et al.[11] associated asymmetry in gravi-electrical properties of root caps, arising from depolarisatio of membrane potential, subsequent to gravi-stimulation to changes in the sedimentation of amyloplasts.

Acknowledgements

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Figure 1   Diagrammatic representation of the root apex (Zea mays)
defining parameters for the measurement of cells and cell
populations referred to in the text.

CC central cap,   D root diameter,
DM cap meristem,   H cap height,   PC peripheral cap,
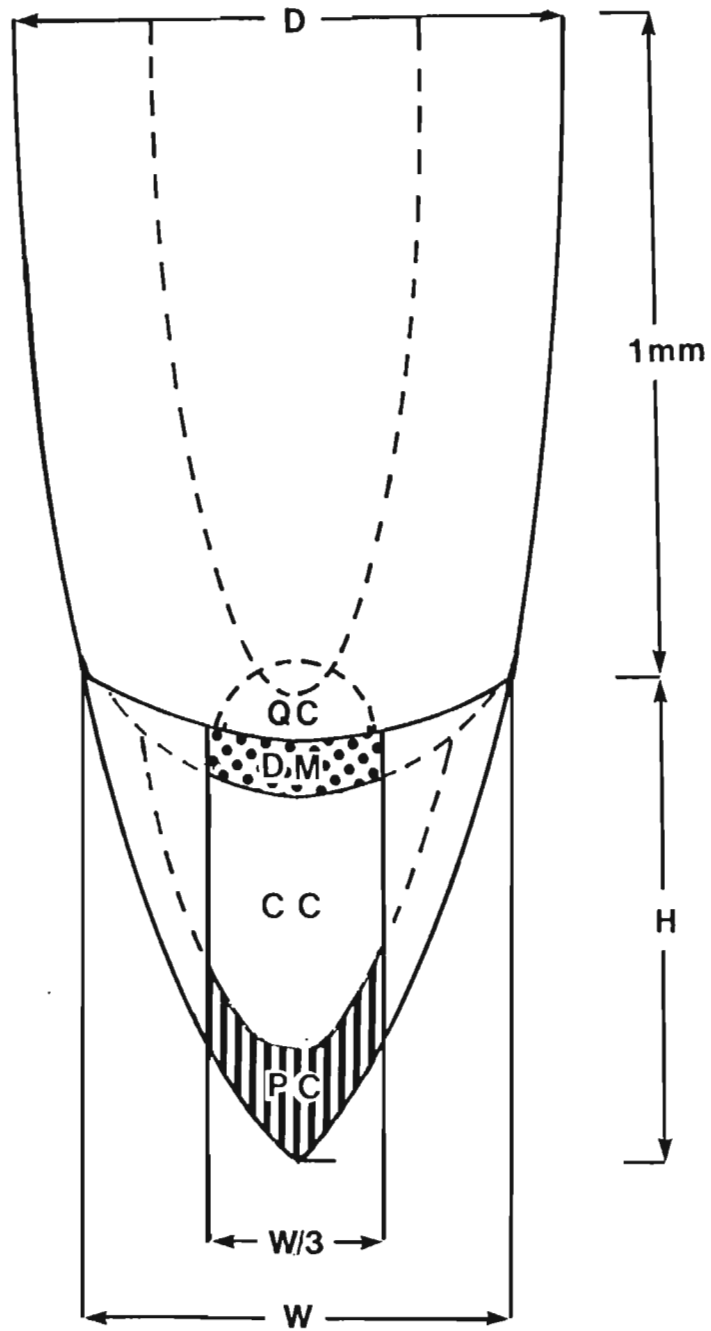QC quiescent centre,   W width cap.
The central cap is defined as W/3.

D

1mm

QC

DM

C C

H

P C

W/3

W

Figure 2   The effect of different Al concentrations ($mg \ dm^{-3}$) over a 29 hr period on the mean volume ($\mu m^3 \ X \ 10^6$) of the root cap of primary roots of Zea mays (cv. TX 24). Error bars indicate the S.E. of the mean (minimum of 5 roots included in determining each point).
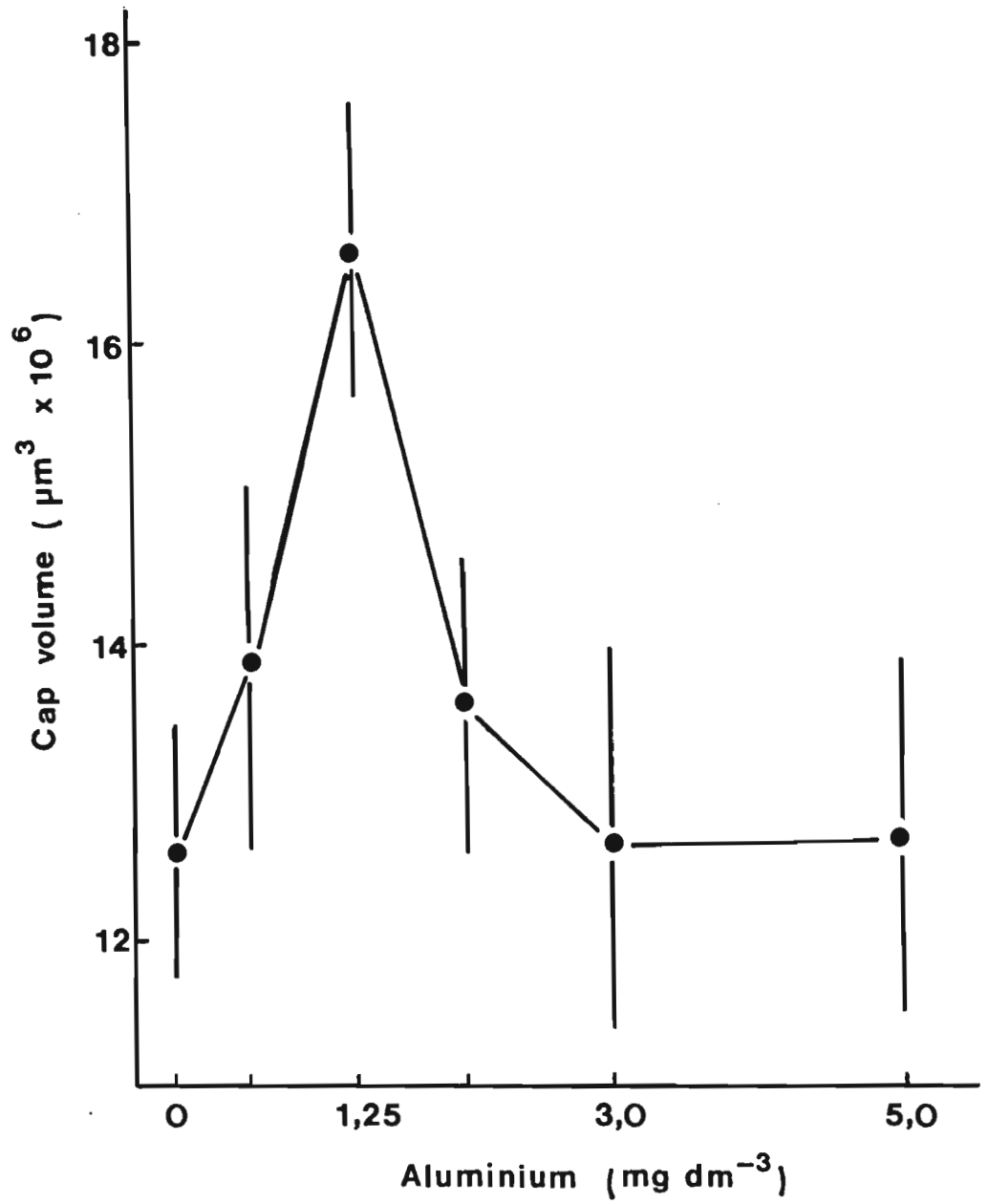
Figure 3   The effect of different Al concentrations $(mg\ dm^{-3})$
             over a 29 hr period on the mean volume $(\mu m^3\ X\ 10^2)$
             of peripheral cap cells located at the distal end of the
             cap.   Error bars indicate the S.E. of the mean
             (minimum 5 roots included in determining each point).
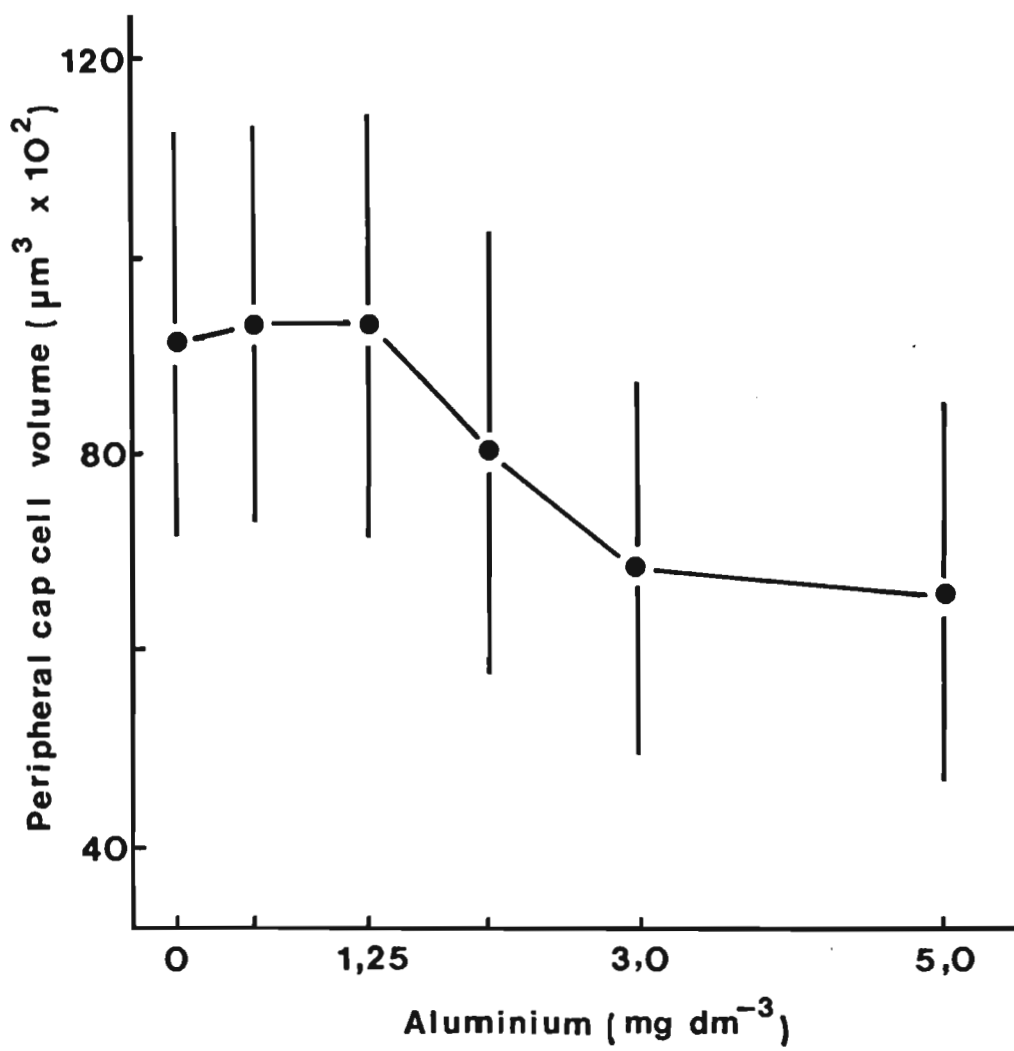
Figure 4   Micrograph of root cap cells showing the effects of
                different Al concentrations on amyloplast numbers and
                distribution and cell geometry.
                A control (0 Al), central cap cells, including
                the cap meristem, are characterized by the linear
                arrangement of cell files.   Amyloplasts are numerous
                and densely packed.   Nuclei of cap columella are located
                towards the proximal pole of the cell.
                B 2.0 mg dm^{-3} Al, linear arrangement of central cap
                cells is still evident, amyloplasts are fewer and there
                is less evidence of sedimentation.
                Nuclei of some columella cells also appear to be
                displaced from the proximal pole of the cell.
                C 5.0 mg dm^{-3} Al, the arrangement of central
                cap cells has been extensively altered, amyloplasts
                appear not to sediment and cells are increasingly
                vacuolate.
                D control (0 Al), peripheral cap cells, distal end of cap.
                E 5.0 mg dm^{-3} Al, peripheral cap cells showing decrease
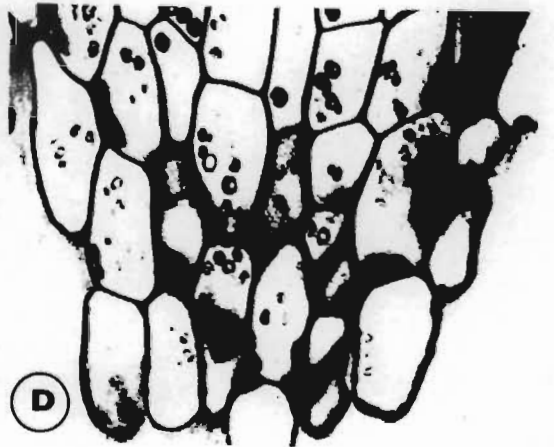   in size of these cells in comparison to D.
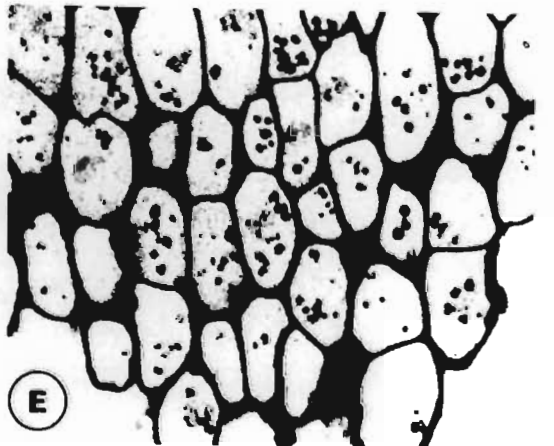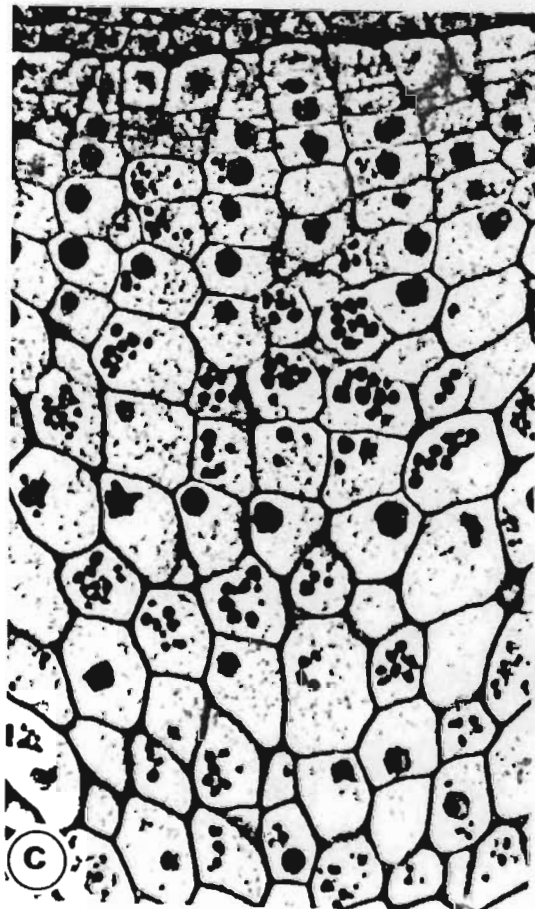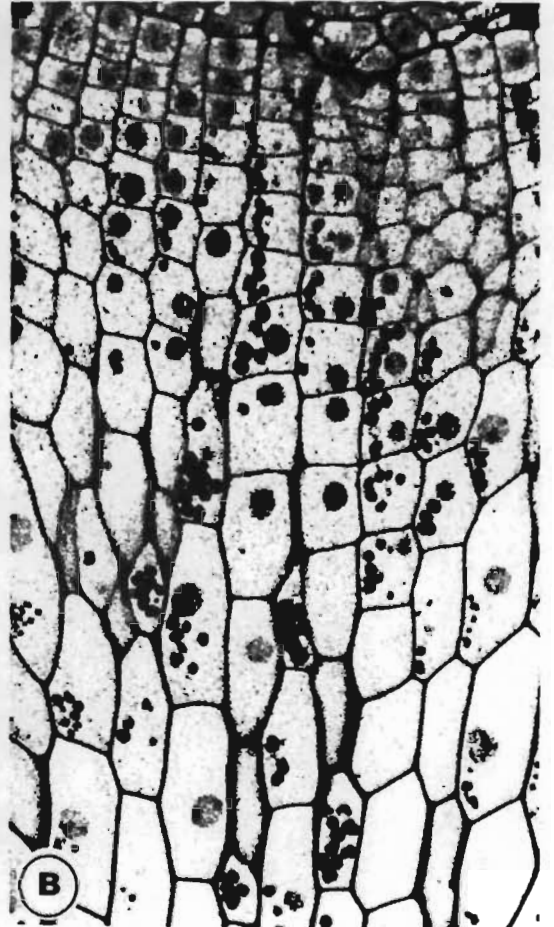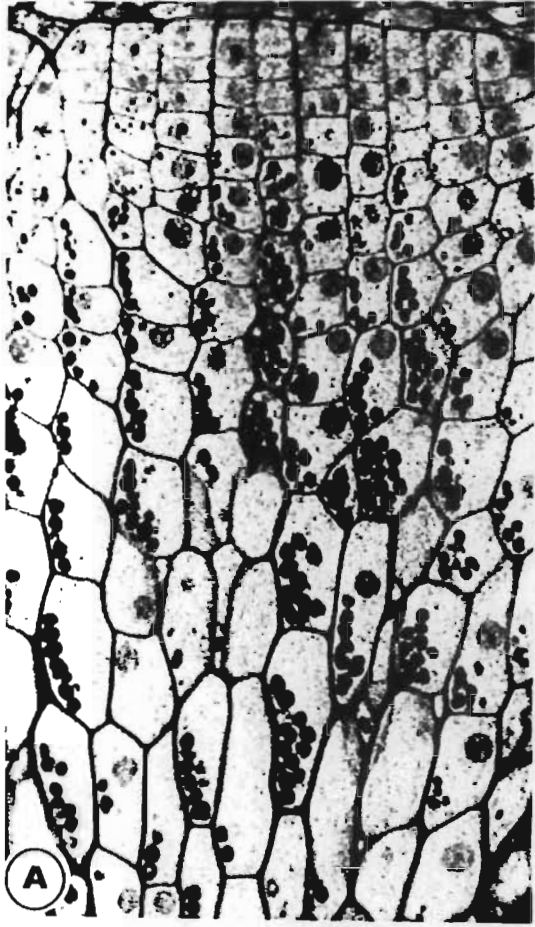
Figure 5   The effect of different Al concentrations (mg dm^{-3})
over a 14 day growth period on the length (mm) of primary
roots of Zea mays.   Each point is the mean value obtained
from 46 plants derived from two experiments.
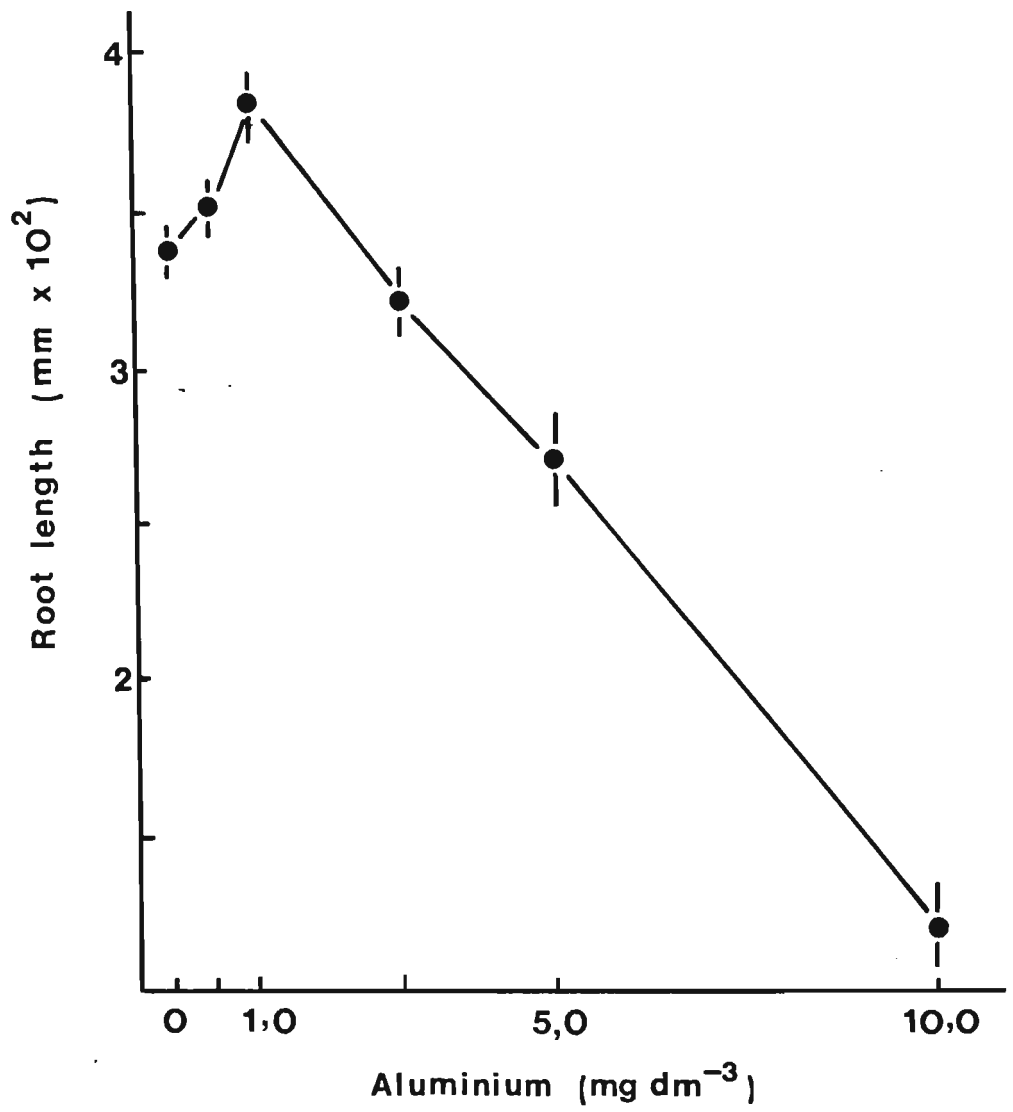Error bars represent the range for two experiments.

Figure 6   The effect of different Al concentrations on the number
            and location of amyloplasts present in the root cap.
            Increasing Al concentration caused a decrease in
            amyloplast numbers found in cap cells over
            29 hr growth period.   Error bars indicate the S.E.
            of the mean, where amyloplast counts on a minimum of
            5 roots were made in determining each point.
            The frequency distribution indicates the proportion (%)
            of amyloplasts observed to be present in the proximal
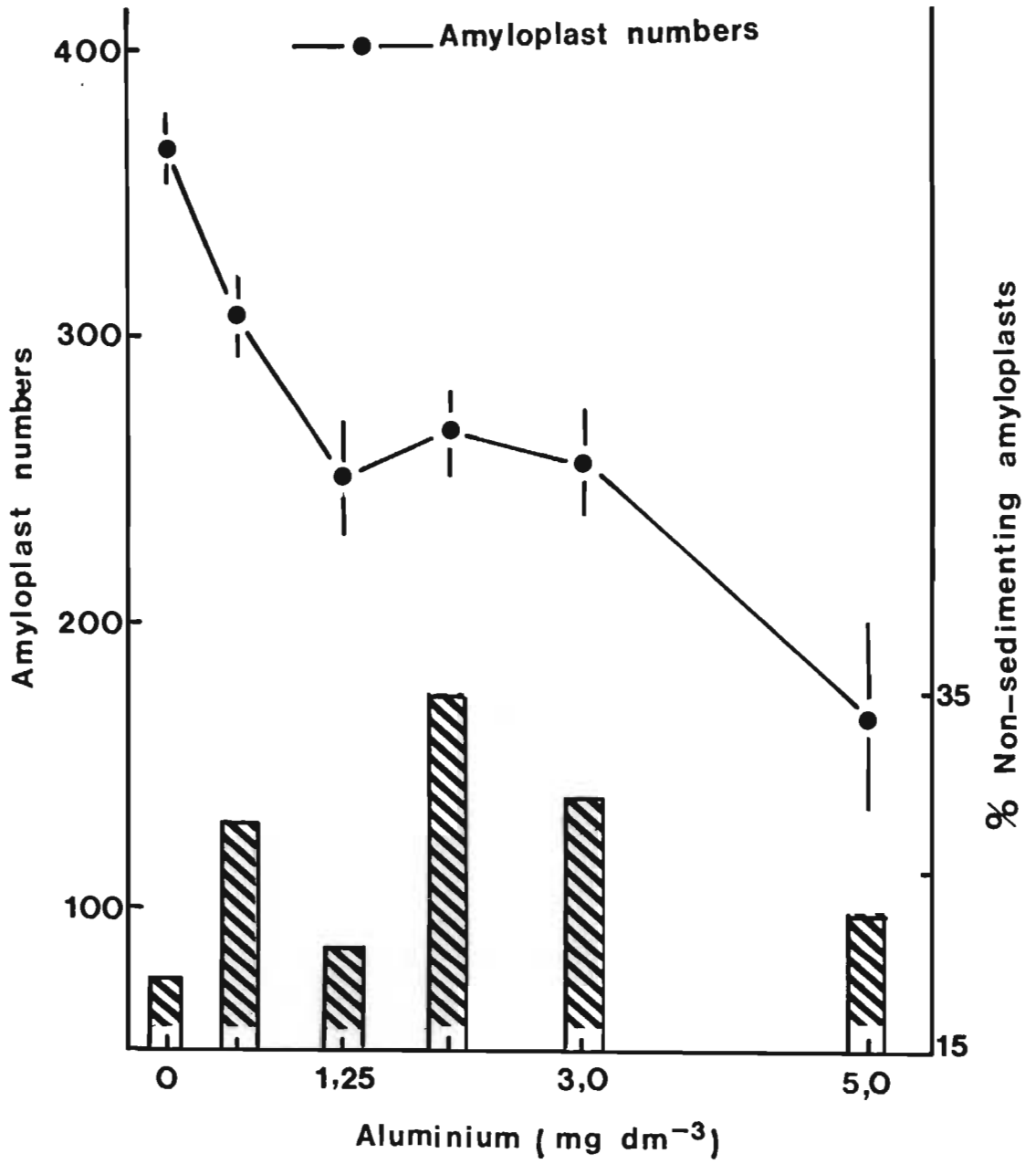   half of central cap column cells.

Figure 7   Electron micrograph illustrating the effect of various
            Al treatments on Golgi apparatus activity.
            A control (0 Al), dictyosome from peripheral cap showing the
            well-ordered cisternal architecture and characteristic
            granular secretory product.   Membrane structure of the
            secretory vesicles (SV) is intact.
            B control (0 Al), Golgi apparatus activity in the peripheral
            cap indicating the accumulation of Golgi apparatus-
            derived material (GM) exterior to the plasmalemma.
            Dictyosome (D).
            C The effect of 0.5 mg dm^{-3} Al (29 hr) on Golgi apparatus
            secretory activity is indicated by the disruption
            of the dictyosome (D) and notable change in the
            appearance of vesicular material and Golgi apparatus-
            derived material (GM).
            D 2.0 mg dm^{-3} Al.   Severe disruption of cisternal structure,
            vesicular membranes (unlabelled arrows) and the appearance
            of vesicle contents is evident in peripheral cap dictyosomes
            after 29 hr treatment.
            E 2.0 mg dm^{-3} Al.   In contrast to D, cisternal
            morphology of dictyosome located in the central cap is
            little affected after 29hr treatment.
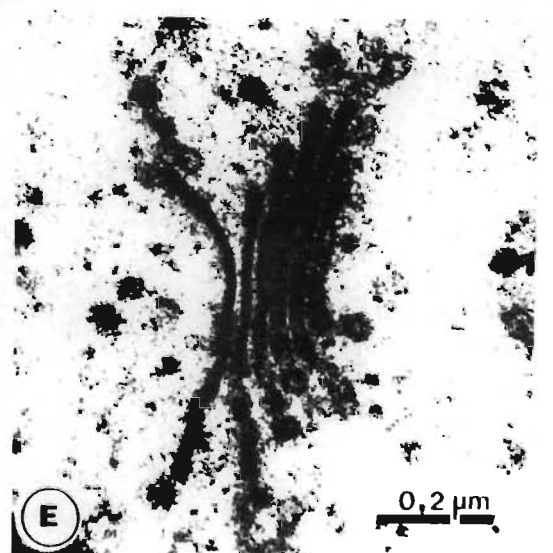   Note smaller size of secretory vesicles.

A SV 0,2 μm

B GM D 0,5 μm

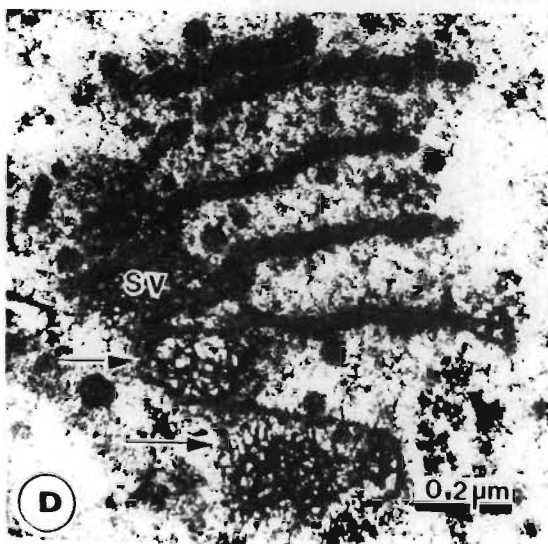C GM D 0,5 μm

D SV 0,2 μm
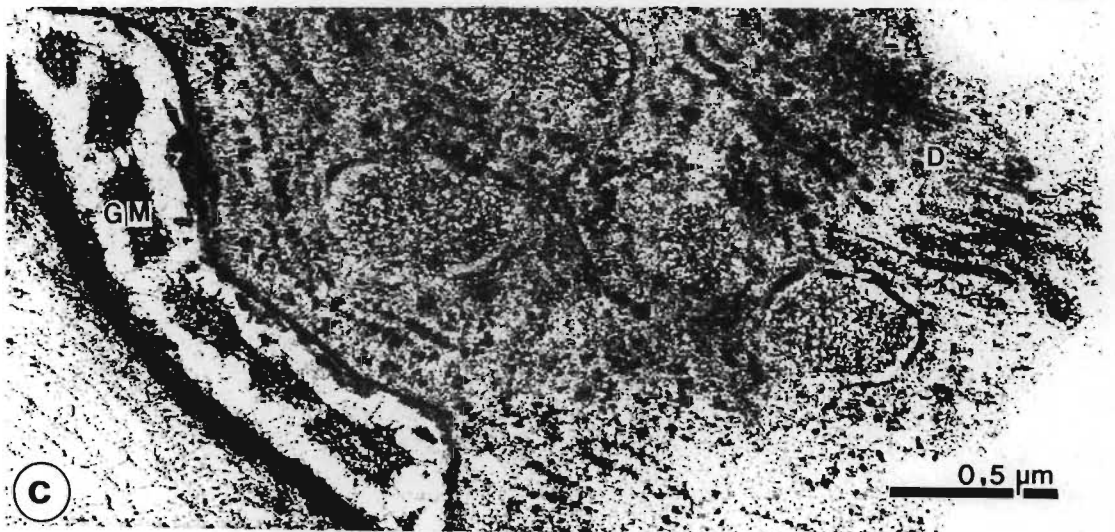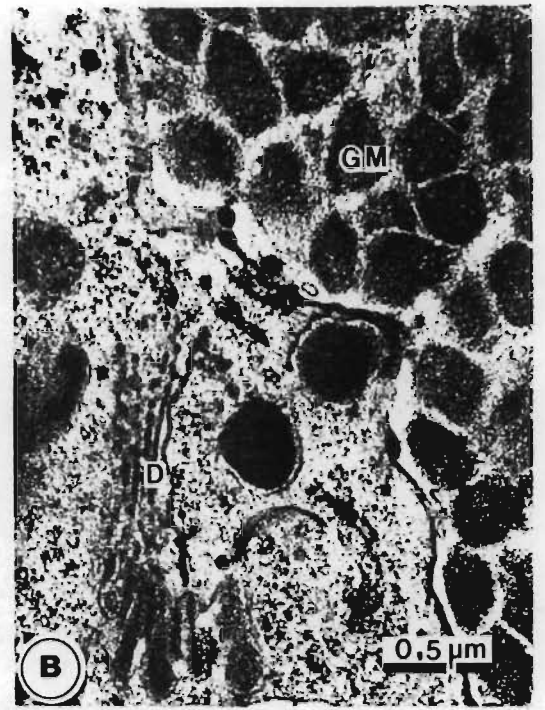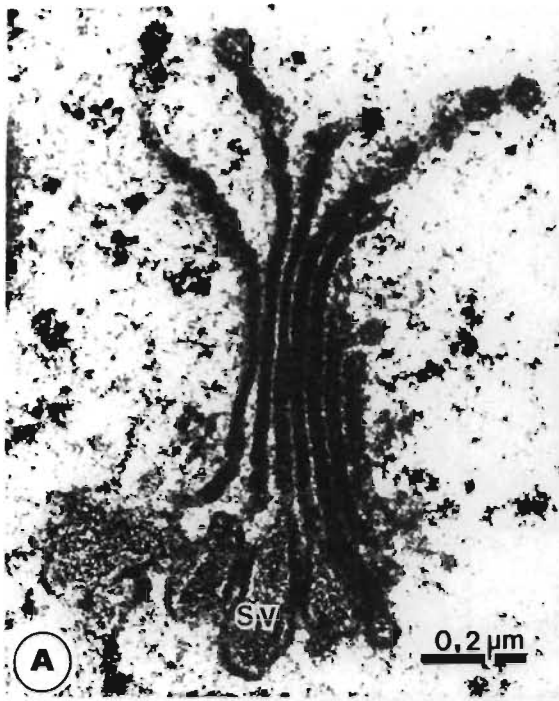
E 0,2 μm

Figure 8   Electron micrograph illustrating the response of cells
             of the cap meristem and cap periphery to Al.
             A cap meristem (control, 0 Al).
             B cap meristem 5.0 mg dm^{-3} Al (29 hr), nuclear
             structure shows little response to Al, cells are, however,
             increasingly vacuolate and while many proplastids are
             present, there is no evidence of starch accumulation
             being initiated.
             C Peripheral cap cells 5.0 mg dm^{-3} Al (29 hr), many cells are
             highly vacuolate and disorganisation of the cytoplasm is
             clearly evident.
             Accumulation of Golgi material (unlabelled arrows) is
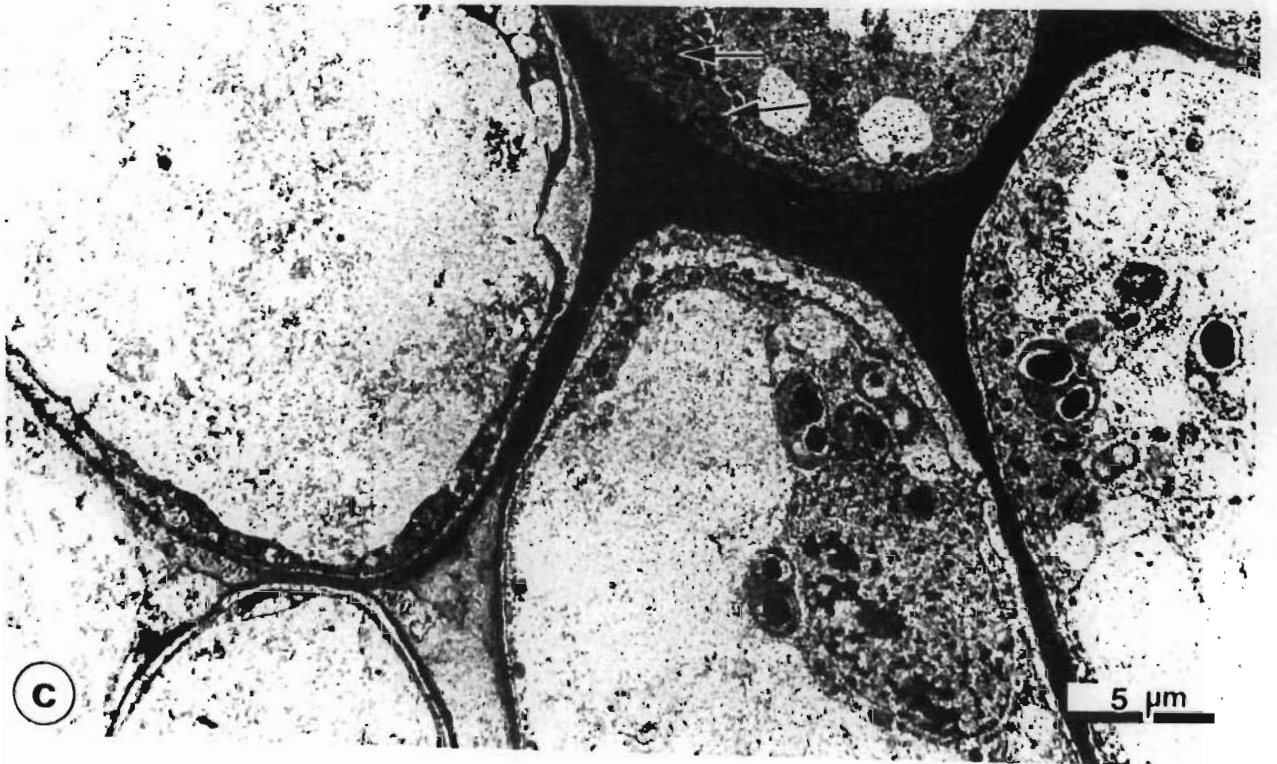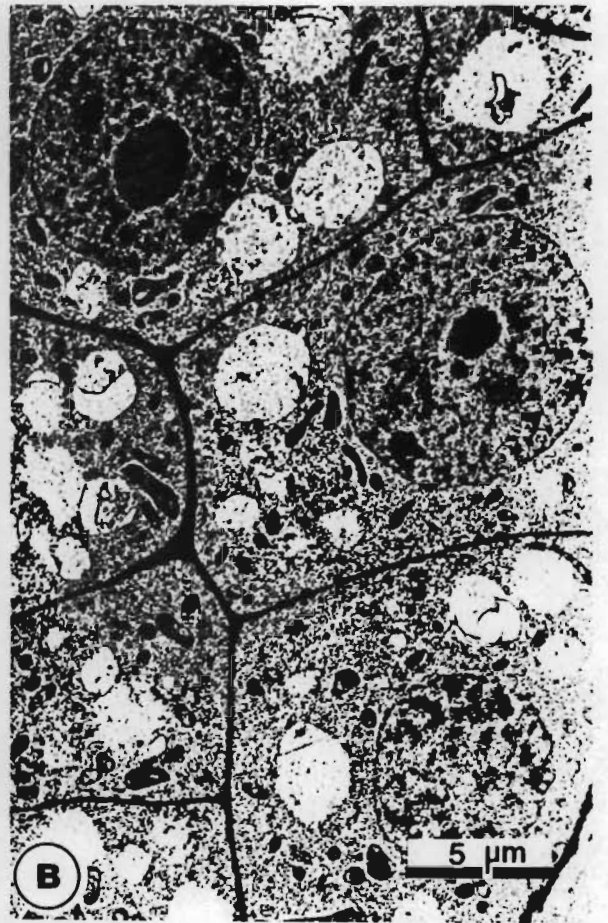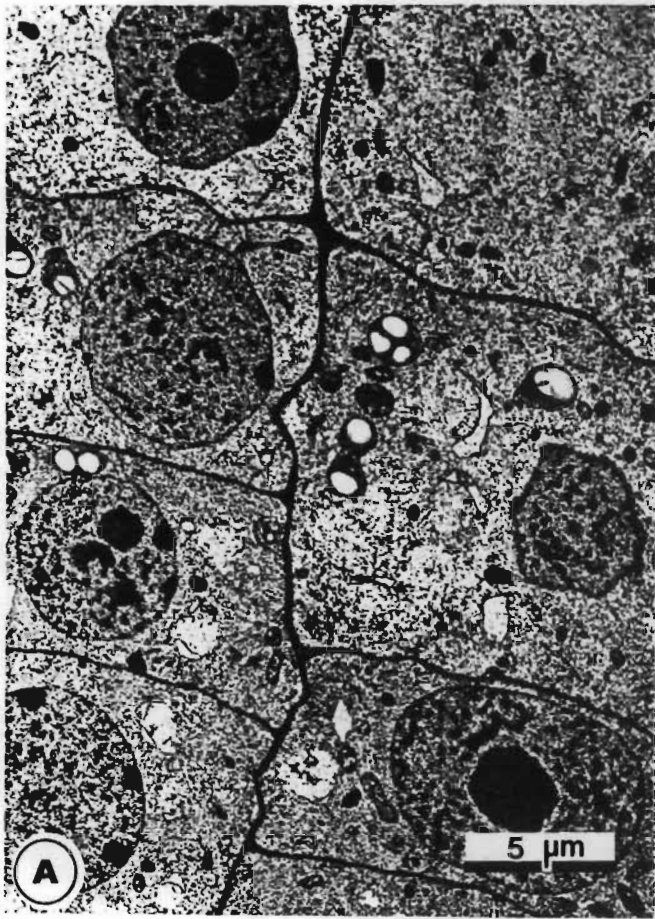   limited to the inner cell.

Figure 9   The effect of different Al concentrations in initiating
            H^+ efflux and cell enlargement.

            H^+ efflux (μg H^+ g^{-1} root dry mass) was determined over a 7 hr
            treatment time.   The experiment was performed in duplicate and
            points represent mean values.
            Error bars give the experimental range for 2 trials.
            Cell enlargement is expressed as the relative
            (% of control) change in root diameter measured
            1 mm from the root apex.
            Each point was determined from the measurements taken
            from 5 roots.