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EFFECT OF ACIDIFIED WATER ON THE TRACHEARY ELEMENTS OF THE FIRST MAIZE (ZEA MAYS L.) INTERNODE AND CONDITIONS DETERMINING ELONGATION OF THIS INTERNODE

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SUMMARY

Conditions for the formation of a long first internode (mesocotyl shoot) of maize (*Zea mays* L.) formed in a relatively short period of time were analyzed. If an inert substrate was used, the growth of such a shoot is mainly determined by the uptake of water. Effect was examined of acidified water (a substitute for acid rain) on morphology and anatomy of first internodes and of roots of young seedlings produced by deeply sown maize grains in an inert substrate under conditions mentioned above.

Light microscopical investigations of transverse sections of first internodes and roots revealed more tracheary elements mainly consisting of a greater number of tracheids, in the mesocotyl of those seedlings cultivated with acidified water. The effect was more distinct if a combination of sulphuric and nitric acid was applied and less distinct if nitric acid, sulphuric acid or hydrochloric acid were applied. Radial endodermis cell walls (cross walls were not measured) in the mesocotyl were up to twice as thick in plants treated with acidified water than in plants of the control with tap water. Possible water stress symptoms caused by the supplied acidified water are discussed.

1. INTRODUCTION

The first internode of the maize embryo is situated between the first and second node. On these nodes arise the scutellum and the coleoptile respectively. It is the only internode of the embryo showing some elongation. In the embryo other internodes are not elongated and the young leaves are still enclosed by the coleoptile. This first internode between scutellum or cotyledon and coleoptile is usually described as rhizome, but mesocotyl shoot would be a more appropriate term. During germination of deeply sown maize grains, this mesocotyl shoot may elongate considerably as a result of the uptake of nutrients from the endosperm and water supplied by the roots. In this way the mesocotyl pushes upwards the coleoptile which encloses the embryonic leaves and the apical meristem to approximately the soil level. The rapid elongation is caused by the activity of

an intercalary meristem located at the top of the internode just below the coleoptilar node, and is different from the other internodes in which the intercalary meristems are located near the base (HAYWARD 1938).

In order to ascertain clearly the effect of applied acidified water on seedlings, it is desirable that a large mesocotyl length is produced in a short period of time. Furthermore, the substrate has to be inert, so that the growth is mainly determined by the uptake of water.

Our study was carried out in connection with investigations of the effects of air pollution on the vegetation, especially acid rain as one of its components. The principal objectives were:

(1) to establish the conditions under which a maximum mesocotyl length will develop;

(2) to determine the effect of acidified water (as a substitute for acid rain) and the effect of its acidity on the structure and length of the stem and roots of seedlings produced by deeply sown maize grains.

2. MATERIALS AND METHODS

The plants used in this study were grown from grains of *Zea mays* L. hybrid strain A188. The caryopses were planted in different substrates at several depths (from 2 to 15 cm), either dry or previously germinated for two days in a moist cloth. The substrates used were either perlite (inert), sterile sand, aerated tap water, or a 2 cm thick layer of garden-mould, compost or sterile sand with on top of which a perlite layer of different thickness. Some plants were grown in the greenhouses of the department of Plant Cytology and Morphology at a temperature of 24 °C and with a day-night rhythm normal for September (12 hours light, 12 hours dark), others were grown constantly in the dark, also at 24 °C. Every day the plants were supplied with double distilled water, tap water or acidified water with a pH range of 4.5 to 2.0. Acids used were hydrochloric acid, nitric acid, sulphuric acid or a combination of the last two. The influence of the grain weight was also tested, using two categories, namely grains of 0.26–0.28 g and those with a weight of 0.28–0.30 g.

The seedlings were harvested after a growing period of 2 to 14 days. The total mesocotyl and main root length were determined before fixation in FAA (formalin, acetic acid and alcohol). Anatomical features were studied by light microscopy (LM) in 10–20 μm thick sections. Staining was with toluidine blue (cytoplasm), sudan III (fatty substances, latex), iodine (starch) or phloroglucin-hydrochloric acid (lignin). All LM sections were embedded in Kaiser's gelatin-glycerin.

For electron microscopy parts of the mesocotyl were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) for 3 hours and postfixed in 1% osmium tetroxide in the same buffer for 5 hours at room temperature. The material was dehydrated in a graded ethanol series and embedded in Epon 812 through propylene oxide. Ultrathin sections were stained with uranyl acetate and lead citrate.

3. RESULTS

The effects of the investigated conditions on the mesocotyl length of maize seedlings are presented in *table 1*.

Using day-night conditions a maximum mesocotyl length of about 5 cm is obtained after 10 days when planted in sterile sand at a depth of 10 cm. In general, with a day-night rhythm, the mesocotyl length is about half the planting depth when planted at depths ranging from 2 to 10 cm. A planting depth from 10 cm to 15 cm results in a mesocotyl length comparable with the one produced at 10 cm.

In the dark the maximum mesocotyl length is reached earlier, after about six days and its length approximately equals the planting depth (8 cm) or is somewhat larger (see *fig. 1*). As in the case with day-night conditions, the greatest mesocotyl and root length was obtained in the dark if one uses a planting medium consisting of a 2 cm thick layer of garden-mould with a layer of perlite on top, as compared to the other substrates. For our purposes, however, an inert substrate is desirable. Thus seedlings raised in the dark and planted in perlite only, at a depth of 8 cm, are most suitable.

The variation of the measured mesocotyl lengths (see *fig. 1*) is rather large. The weight of the maize grains arranged in two categories, had no effect on the standard error of the measured mesocotyl length produced in the dark after six days. Pre-germination of the grains on a wet cloth followed by a selection on the length of the sprout to ensure an equal start, also had little influence on the variability of the observations. Although with pre-germination the maximum mesocotyl length was reached earlier, it required the same amount of time to pre-germinate the grains.

The effect of the applied acidified water on the length of some seedling parts is given in *fig. 2*. In comparison with tap water, acidified water had no significant influence on the produced lengths.

The hypocotyl is very short and remains short during germination and development, its internal structure is root-like. The vascular transition from exarch to endarch primary xylem is episcutellar (-cotyledonary). It occurs mainly in the first internode, but continues during growth to some degree in the second

Table 1. Effects of investigated conditions on the mesocotyl length of maize seedlings (− = no or no significant effect; + = significant positive effect).

Investigated conditions	Effect
weight maize grains	−
pre-germination	−
time (up to 10 days after planting in day-night regime and up to 6 days growing in the dark)	+
darkness (in comparison with a day-night regime)	+
substrate	+
planting depth (up to 10 cm deep)	+
acidity of given water	−

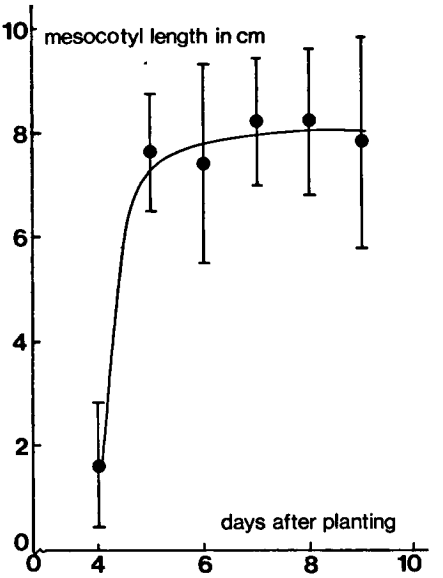

Fig. 1. Relationship between days after planting maize grains (*Zea mays* L.) and the produced mesocotyl length. Maize grains were planted without pre-germination 8 cm deep in perlite, in the dark at 24 °C, and watered with tap water. Each value represents the average ± SE of 12 seperate determinations.

internode and sometimes even in the third or fourth internode. The first internode, mesocotyl, develops into a more or less elongated rhizome in deeply sown grains and consists of the following tissues: an epidermis, one cell wide, with a thin cuticle; a cortex of several cell layers composed of thin-walled parenchyma cells with many intercellular spaces and an endodermis consisting of one cell layer; a vascular cylinder consisting of a pericycle of one cell wide, an annular zone of vascular tissues and a central pith. The annular zone of vascular tissues is interrupted on the side where the scutellum is attached and possesses groups of exarch xylem which regularly alternate with endarch collateral bundles. In these bundles the primary phloem is situated between the primary xylem and the pericycle; it also occurs in alternate radial position in connection with the exarch xylem groups (*fig. 3*). The mesocotyl therefore, exhibits both root- and stem-like characteristics (HAYWARD 1938).

Light microscopical investigations of cross sections made of the primary main root and half way the mesocotyl length of plants cultured with tap water or acidified water, revealed differences in the number of tracheary elements in the mesocotyl only (*tables 2* and *3*). These tracheary elements comprise tracheids, protoxylem vessels with annular – or helical thickenings and metaxylem vessels with reticulate – or pitted secondary walls. The differences between the numbers of tracheids only, of tracheids and protoxylem vessels, and of tracheids, proto- and metaxylem vessels combined counted in transverse sections of the acidified water and tap water material, were tested with the Wilcoxon signed rank test

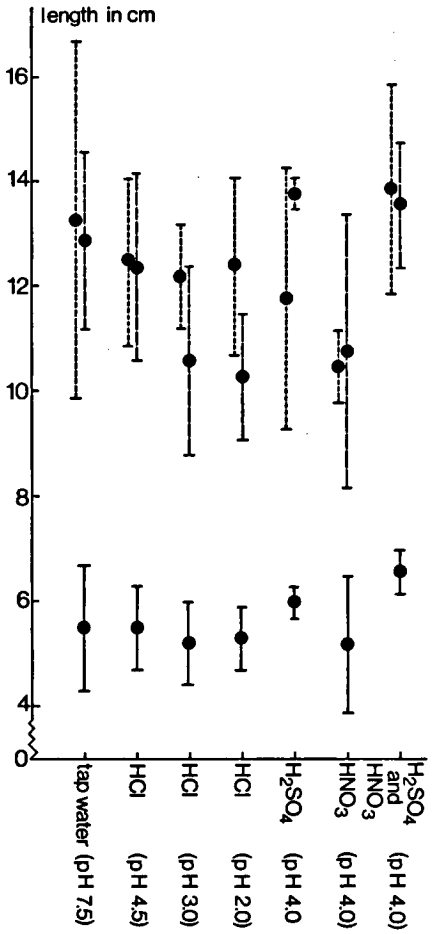

Fig. 2. Effect of tap water and tap water acidified with respectively hydocloric acid, sulphuric acid, nitric acid and a combination of the last two, on the length of the mesocotyl (– –), seedling minus main root (–––) and root (...). Pre-germinated (for 2 days) maize grains were planted 8 cm deep in perlite, in the dark at 24 °C and measured 6 days later. Each value represents the average ± SE of about 25 determinations. In comparison with tap water acidified water had no significant influence on the produced lengths.

with a one sided significance level of 0.05. Although there are always more tracheary elements in the mesocotyl of plants watered with acidified water, the differences are significant only for sulphuric acid, nitric acid and the combination of the two, all with a pH of 4.0. The effects increased in the same sequence as mentioned above. The differences between the treatment with tap water and with hydrochloric acid acidified water with a pH of 4.5 and even 3.0, are not statistically significant. The greater number of tracheary elements in acid environments is due to the greater number of tracheids produced (*table 3, figs 4* and *5*). The number of proto- and metaxylem vessels almost remains the same (*table*

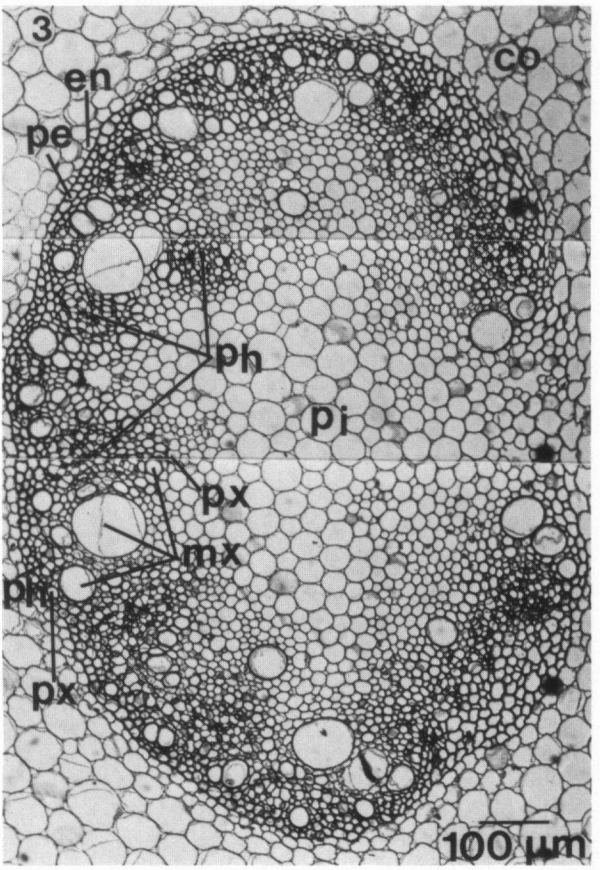

Fig. 3. Transverse section of the first internode (mesocotyl) of *Zea mays* L. Plant supplied with tap water (pH 7.5). Middle right (the interrupted annular zone of vascular tissue) facing the attachment of the scutellum. Both endarch collateral bundles (for instance indicated slightly left from the centre of the photograph), as well as a radial arrangement of exarch xylem (indicated below that area) and primary phloem occur. co, cortex; en, endodermis; mx, metaxylem vessel; pc, parenchyma cell; pe, pericycle; ph, primary phloem; pi, pith; px, protoxylem vessel; tr, tracheids.

3). In cross sections tracheids can be distinguished from protoxylem vessels because they do not possess annular or spiral thickenings and regularly show bordered pit-pairs; they can be distinguished from narrow metaxylem vessels because they are more angular in outline. Furthermore longitudinal sections reveal that metaxylem vessels are almost always much wider than tracheids. The countings of tracheids in cross sections of mesocotyl shoots of plants treated with acidified water with a pH of 2.0, were not analysed because in this case the determination was questionable.

The walls of the metaxylem vessels in plants treated with acidified water, are not thicker than those of the control (*figs 4, 5, 6* and *7*); radial walls of the endodermis cells on the other hand are up to twice as thick when grown with acidified

Table 2. Effects after six days of applied acidified water on the mesocotyl of germinating maize grains (− = no or no significant effect; + = significant effect).

Mesocotyl characters	Effect
number of tracheids (water acidified with hydrochloric acid)	−
number of tracheids (water acidified with sulphuric acid)	+
number of tracheids (water acidified with nitric acid)	+
number of tracheids (water acidified with sulphuric and nitric acid)	+
number of protoxylem vessels	−
number of metaxylem vessels	−
wall thickness tracheary elements	−
diameter tracheary elements	−
radial wall thickness endodermis cells	+
all other mesocotyl elements (like those in the root)	−

water (measurements were made with light microscopy and on electron microscopic photographs, *figs 8 and 9*; thickness of cross walls were not measured). Significant differences could not be found between the diameters of the tracheary elements of the control and those of the treatments.

The results in summary are:

- a well developed mesocotyl length is achieved six days after planting the maize grain in the dark, 8 cm deep, in perlite at 24 °C.
- within the pH range from 2.0 to 4.5 the mesocotyl and root length is not significantly influenced by the acidity of the water supplied and the type of acid used.
- mesocotyl shoots of seedlings cultivated with acidified water produce more tracheids (as a water conducting system subsidiary to vessels), especially when a combination of sulphuric and nitric acid is used.
- radial endodermis cell walls in the mesocotyl are thicker in plants supplied with acidified water, than in plants of the controle with tap water. Wall thickness of tracheids and vessels remain almost the same.

Table 3. Average number with standard deviation (of about 25 determinations) of metaxylem vessels, protoxylem vessels and tracheids counted in the total transverse section areas of the middle part of the first internode (mesocotyl) of *Zea mays* L., in relation to treatment. The number of tracheids increases in the series of presented treatments, whereas the number of vessels remains almost constant.

given water		number of		
		metaxylem vessels	protoxylem vessels	tracheids
tap water only	(pH 7.5)	60.3 ± 12.1	38.7 ± 11.7	117.7 ± 38.2
tap water + HCl	(pH 4.5)	55.4 ± 5.5	40.0 ± 7.4	142.0 ± 37.7
tap water + HCl	(pH 3.0)	55.8 ± 1.5	42.2 ± 14.1	158.3 ± 6.1
tap water + H_2SO_4	(pH 4.0)	55.8 ± 9.8	51.5 ± 10.3	169.0 ± 12.9
tap water + HNO_3	(pH 4.0)	55.5 ± 14.9	43.5 ± 7.1	185.0 ± 17.2
tap water + H_2SO_4 and HNO_3	(pH 4.0)	49.2 ± 6.2	43.6 ± 11.9	222.2 ± 28.1

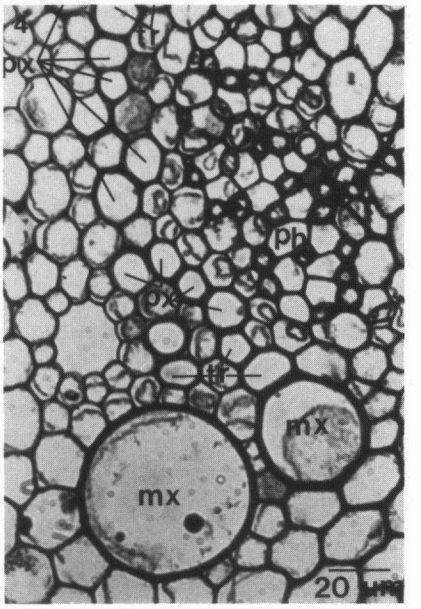

Fig. 4. Magnification of a part of *fig. 3*, slightly above middle right. Plant given tap water (pH 7.5); legends see *fig. 3*.

– there are no significant differences between the diameters of the tracheary elements of the control and those of the treatments.

4. DISCUSSION

BISSING (1982) suggests that in dicotyledenous woods the relative abundance of vascular tracheids is largely influenced by the level of moisture in the habitat at the time of wood formation. This evidence supports CARLQUIST's (1975, 1980, 1982, 1985) hypothesis (concerning the ecological significance of vascular tracheids) that vascular tracheids are elements adapted to 'safe' transport during times of water stress. Similarly, the large number of tracheids (as a safer water conductive system subsidiary to vessels) produced in the mesocotyl shoots from acid environments, may also be the result of water stress. However, one has to consider that the above mentioned literature, like BAAS *et al.* (1983) mentioned already, is partly speculative and deals with adaptations to external circumstances in combination with genotypical adaptations during evolution. These effects may possibly not be compared with experimentally induced adaptations.

The thicker radial endodermis cell walls of the mesocotyl of plants grown with acidified water might be the result of an intensive control of the apoplastic water transport from soil, epidermis and cortex into the central cylinder. Eventual differences in chemical composition of these walls were not investigated.

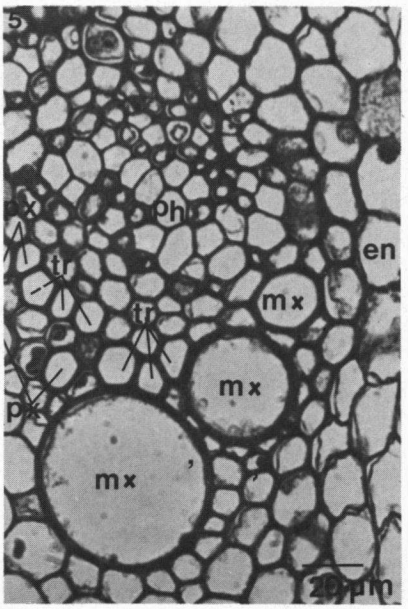

Fig. 5. The corresponding area of *fig. 4*, but from a plant given water acidified with sulphuric- and nitric acid (pH 4.0); legends see *fig. 3*. Compared with *fig. 4*, here a greater number of tracheids is present.

According to YEO *et al.* (1977) salt stress produced in mature roots a strikingly increased wall thickness of a very loose fibrillar texture in the areas of the half-bordered pits between vessels and xylem parenchyma cells. Under acid stress conditions, however, we could not demonstrate such thickenings with the electron microscope (walls were equally thick as in the control).

Light microscopical examination of transverse sections from half way along the main root did not reveal any difference between either the roots of the control or treated groups. Differences in numbers of mitochondria in paratracheal parenchyma cells of the mesocotyl could not be detected using electron microscopy.

Tracheid dimensions in pine could be used to measure anatomical changes caused by environmental conditions (GOLDA *et al.* 1983), though the effect of environment on these cell properties is believed to be primarily indirect (LARSON 1964; HERDI 1984). In young red pines drought periods resulted in the production of narrow tracheids and diminished wall thickness (LARSON 1964). In sunflower, tracheal cell walls were thinner than in the control in all treatments with MCPA (2-methyl-4-chloro-phenoxyacetic acid), a herbicide with hormonal effect used in cereal crops (HERDI 1984).

However, if acidified water induces water stress in maize, it did not effect tracheid diameter and wall thickness. Since vessel diameters did neither change, these characters could not be used as indicators for a possible adaptation to xeric conditions of plants grown in acid environments as suggested by CARLQUIST (1975, 1982), BAAS *et al.* (1983) and HUBER (1984).

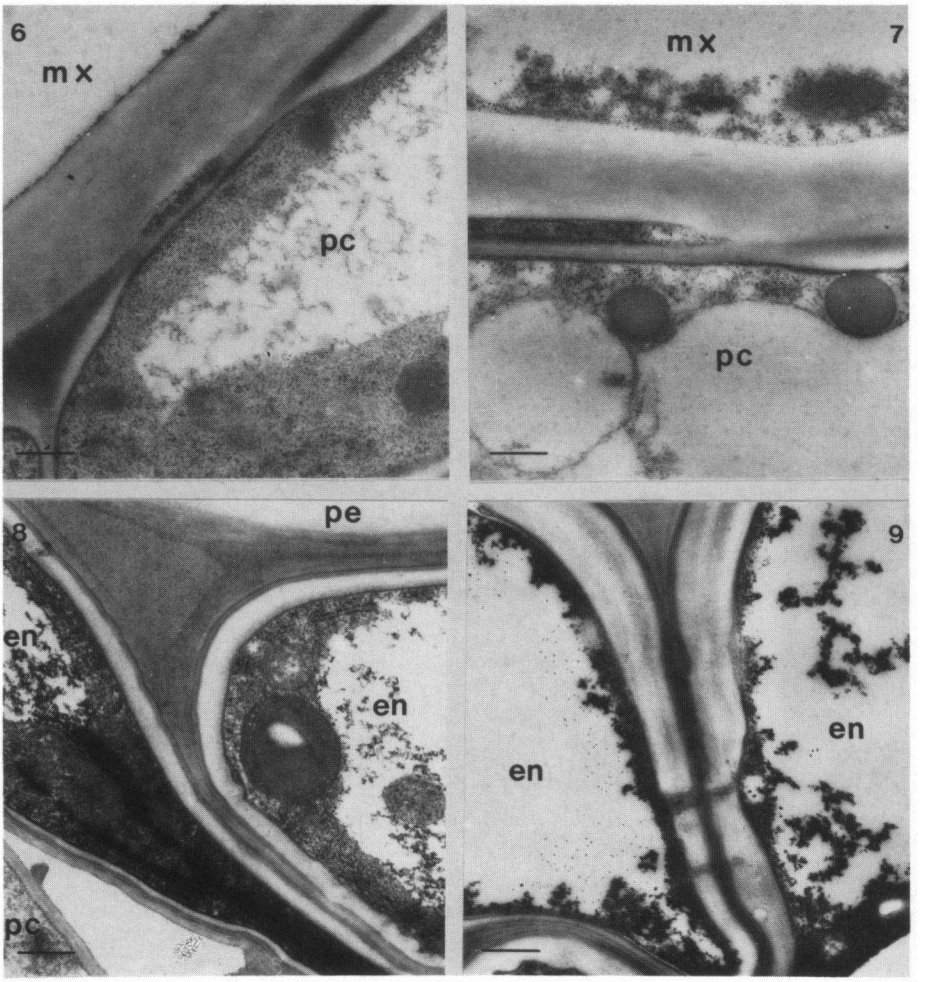

Fig. 6–9. Electron micrographs of a part of the transverse section of the mesocotyl of *Zea mays* L. Bar in all figures 0.5 μm; legends see *fig. 3*. Fig. 6 and 7, walls between a metaxylem vessel and a parenchyma cell; fig. 8 and 9, radial endodermis cell walls. Fig. 6 and 8, plants given tap water (pH 7.5), fig. 7 and 9 plants given water acidified with sulphuric- and nitric acid (pH 4.0). The radial endodermis cell walls are almost twice as thick in plants given acidified water, compared with those grown with tap water. The wall thickness of the metaxylem vessels remains about the same.

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