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TRUMPET FILAMENTS IN LAMINARIA DIGITATA AS AN ARTEFACT

J. L. VAN WENT and P. M. L. TAMMES[1]

Afd. Plantkunde, Wageningen
Centrum voor Plantenfysiologisch Onderzoek, Wageningen

SUMMARY

When turgor is lost, the walls of the filaments in the medulla swell and the lumina are constricted to a fraction of their original diameter. The sieve plates of the filaments resist the constriction, giving rise to the double "trumpet" form with a plate in the middle. The original situation was deduced from: *a.* calculation of cell wall and lumen volume, based on K and Na analysis and examination of sections; *b.* perspex replicas from deep-frozen tissue and sections from tissue frozen before fixation; *c.* subsequent loss of volume and recovery of medullary tissue after being cut through; *d.* plasmolysis experiments.

1. INTRODUCTION

The cauloid of *Laminaria digitata* consists of three tissues: an outer layer, the meristoderm, a thick cortex parenchyma, and a central medulla with a meshwork of filaments. In sections of fresh and chemically fixed tissue, trumpet filaments are visible in the medulla. They were discovered by REINKE (1876).

The ultrastructure of the trumpet filaments and their sieve plates was described by ZIEGLER (1963) for *Macrocystis*, and by ZIEGLER & RUCK (1967) for *Laminaria*.

Translocation of photosynthates proceeds through the medulla and the trumpet filaments are often compared with sieve tubes of higher plants. But their structure seems inefficient with thick lateral walls and very thin filamentous lumina. One should expect isodiametric tubes and thin walls as in sieve tubes of higher plants. The question therefore arose whether the "trumpet" form exists in vivo in turgescent tissue or whether it is an artefact, due to local constriction of filaments by the swelling of side walls.

2. MATERIAL AND METHODS

Entire plants were harvested and stored at 2° C.

Microtome sections were made from *a.* material fixed at –2°C in glutaraldelyde and postfixed in $KMnO_4$ or directly in $KMnO_4$. *b.* Whole cauloids were kept for one night in melting sea-ice at –2°C and then deep-frozen in solid CO_2 (–78°C). Small pieces of medullary tissue were cut from the frozen

[1] Guestworker: Nederlands Instituut voor Onderzoek der Zee, Texel.

cauloid and thawed in potassium permanganate fixative in sea water at –2°C for 2 hrs. Fixation and embedding as described earlier (VAN WENT & TAMMES 1972). For comparison hand-cut sections were treated in lactophenol and cotton blue and embedded in glycerol.

The relative volume of cell wall to lumen in dehydrated sections from cortex and medulla of the cauloid was calculated from micrographs. Lumina and walls were traced on transparant paper, cut out and weighed.

For the preparation of Perspex replicas (polymethylacrylate) from the frozen medulla, a solution of 2% Perspex in ethylene dichloride was used. Cauloids were frozen in solid CO_2. They were then left for 2 hours in a deep-freeze room at –20°C and further treated at that temperature. Longitudinal cuts were made from discs of the cauloid through the long axis of the medulla. Then the solution was applied with a glass rod and gently rubbed on the surface to make good contact. After drying the pieces were thawed at room temperature and the dried films lifted off. Usually the prints are poor; but when many were made, some acceptable ones could be selected.

Plasmolysis of tissues in 4 M NaCl to which some eosin was added.

Artificial calcium alginate membranes were made by pouring a 1.5% sodium alginate solution in water in a Petri dish. The dish was left open until the solution became a thick syrup by evaporation. Then a layer of 30% $CaCl_2$ in water was poured on top. Calcium alginate gelatinated and a thick film could be taken out of the dish. This was washed for a night in running tap-water and stored in sea water at 2°C.

3. RESULTS

In the cauloid, the proportion of lumen to cell wall in dehydrated sections was for the cortex 0.76 to 0.24 by area (0.66 to 0.34 by volume) and for the medulla 0.2 to 0.8 (0.09 to 0.91 by volume). This shows the large proportion of medullar cell walls.

The potassium content on dry weight basis for isolated tissues from the cauloid was 9.9% in the cortex and 8.0% in the medulla. For sodium these values were 3.4% in the cortex and 5.1% in the medulla.

Perspex replicas from deep-frozen medullary tissue show large lumina and thin walls (*plate 1A*).

In tissue fixed after freezing, ice crystals form. In the medulla the ice crystals are formed in the walls and appear as long slits in the sections. The walls are still thick but the proportion of lumen is fairly high (*plate 1 C* and *D*). When artificial calcium alginate membranes are frozen at –20°C and then thawed, water can be pressed out as from a sponge and a thin fibrous mass is left.

When a cauloid is cut transversely, a little exudate appears on the medullary region and, when this is wiped off, the cut surface looks sunken.

When square rods are cut from the cauloid through the medulla, the result is an immediate decrease in volume of the medulla (*fig. 1A* and *B*).

When a cauloid disc is cut longitudinally through the long axis of the medulla

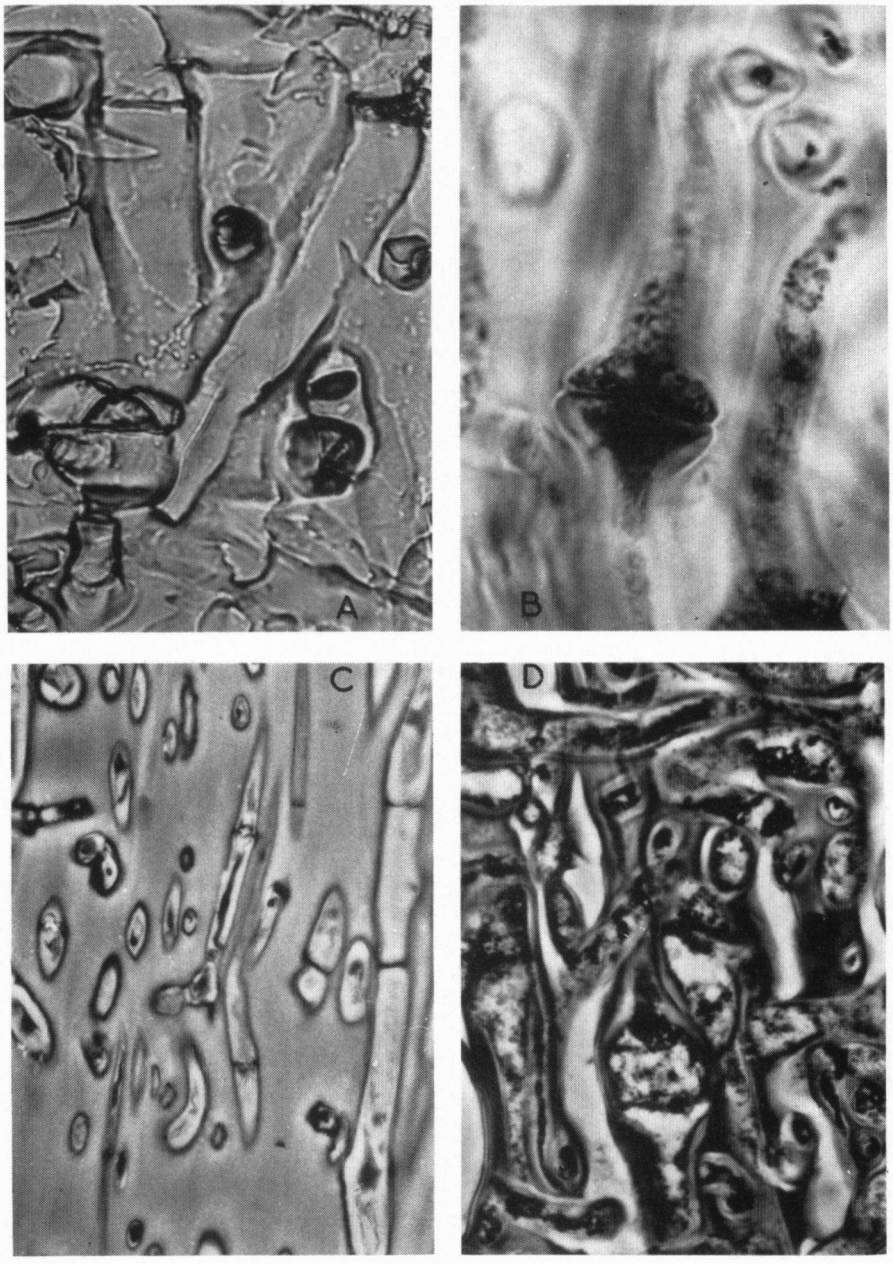

Plate 1. Medulla: A: perspex replica from frozen tissue, tubular structures and horizontal plates, longit. × 636; B: trumpet part, hand section after killing by freezing, lactophenol – cotton blue – glycerol; C: longitud. 2μ section × 254 phase contrast, right side transition to cortex; D: cross section, fixation after freezing, light slits where ice crystals were present, relatively larger proportion of lumen when compared with C, phase contrast × 636.

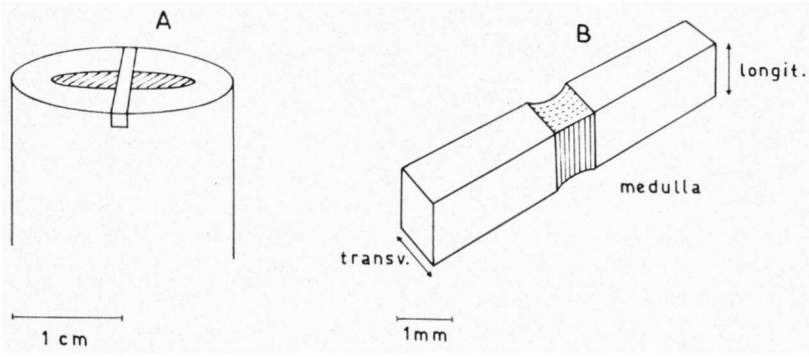

Fig. 1. Decrease in volume of medulla in a square rod cut from the cauloid. (A: situation; B: after cutting).

an immediate curvature appears with the medulla inwards (*fig. 2A* and *B*). The original curvature straightens after 5 minutes and is recovered after 15–20 minutes, and the exudate has been sucked back. When dry acid fuchsin is rubbed on to the wet surface, it is sucked in and extends through the whole medulla after straightening. The uptake of water can be demonstrated by applying a solution of 1% ferrocyanide and 2.5% NaCl in water. The solution is

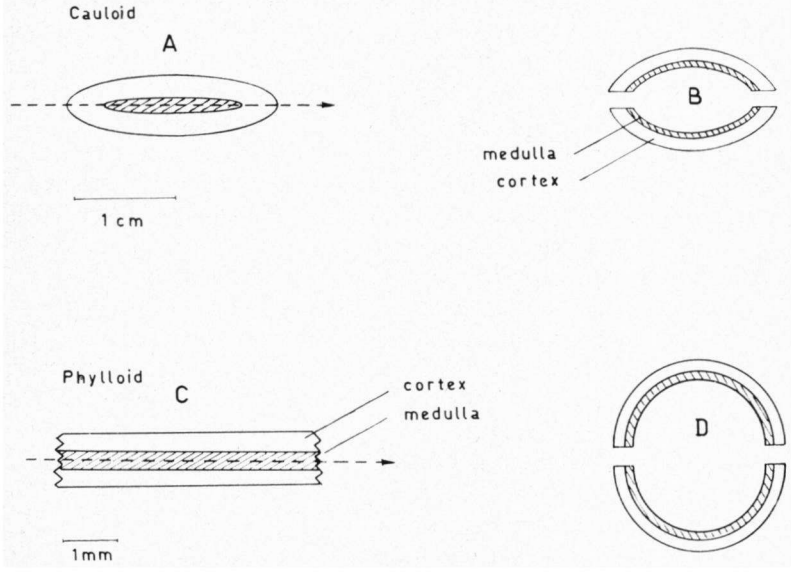

Fig. 2. Curvature of longitudinal section through long axis of cauloid medulla. (A: situation; B: curvature). For phylloid (C: situation; D: curvature).

rapidly sucked in by the medullary tissue and not by the cortex. When sections are fixed in 1% ferrichloride in ethanol, a deposit of Prussian blue is found in the cell walls of the medulla.

A cross section of a cauloid too sucks in the ferrocyanide in the medulla up to 0.5 cm. When killed by being placed in boiling water for 3 minutes, there is no suction and only some diffusion in both cortex and medulla over a short distance. There is neither curvature nor exudate when the medulla is cut.

Phylloids cut into 0.5 cm ribbons can be split parallel to their surface with a pocket knive. Both halves curve inwards (*Fig. 2 C* and *D*).

After using a 4 M NaCl solution with some added eosin the following was observed for *Laminaria* cauloid tissue. Plasmolysis is not possible in the meristoderm. The lumina decrease in size but the protoplast does not retract from the walls. In the inner cortex plasmolysis with globular vacuoles is very clear. In the filaments of the medulla there is no plasmolysis, except sometimes a slight retraction from the lateral walls in the trumpet part.

4. DISCUSSION

The "trumpet" filaments in Laminariales have been known for almost a century (REINKE 1876). They are very clear in sections from fresh tissue in sea water or from tissue embedded in glycerol after passage through a solution of lactophenol and cotton blue (*plate 1 B*). In sections from dehydrated and embedded material their form is less pronounced (*plate 1 C*). For fixed and embedded cauloid material, the average relative volume of cell wall to cell lumen can be calculated from relative area of cell wall to cell lumen as estimates in sections. For the cauloid cortex the average relative volume is 0.5 and for the cauloid medulla 10. For fresh material the relative volume of cell wall to cell lumen can be approached from the results of K and Na analysis, since K is present only in the cell lumen and Na in the cell walls, (e.g. the exudate analysis of PARKER 1966) and by assuming that the K concentration in the lumen is the same in cortex and medulla. It was found that in fresh material the relative volume was for the cauloid cortex 0.34 and for the cauloid medulla 0.64. Thus there is about twice the volume of cell walls in the medulla as in the cortex. This is a factor 2 instead of 20 found after measuring sections. In vivo the walls are probably less voluminous.

Perspex replicas from deep-frozen medullary tissue show a large lumen and thin walls (*plate 1A*). They are probably closer to the situation in vivo.

Freezing causes ice crystals to form in the walls. In sections they are visible as white slits. When fixation took place after freezing, the relative area of lumen was larger than in unfrozen tissue (*plate 1 D*). Freezing of artificial calcium alginate membranes also caused freezing out of water. After thawing, water could be pressed out and a fibrous mass resulted.

In a review ROELOFSEN (1959) stated that alginic acid (like pectin in higher plants) occurs mainly in the middle lamella and primary wall, which are very markedly thickened. The secondary layer contains cellulose.

Electron microscopy shows a thin secondary layer with cellulose fibres in the medulla (see plates by ZIEGLER 1963 for *Macrocystis* and VAN WENT & TAMMES 1972 for *Laminaria*). Alginates have a strong swelling capacity.

There is a remarkable loss of medullary volume when a cauloid is cut through. It can be demonstrated in various ways (*fig. 1* and *2*). The filaments are drained near a wound (VAN WENT & TAMMES 1972). This is accompanied by small amounts of exudate from the medulla and occurs also in very short cauloid discs, e.g. 0.5 cm long. Exudation in these discs must be due to tissue tension as the medullary volume decreases. The inner tissue of the pneumatocyst of the related *Nereocystis* consists of medullary filaments. When RIGG (1925) isolated strips from this tissue, he observed a contraction which was often more than 15%.

However, after some time the loss of volume is restored by suction of water into the cell walls in *Laminaria*. This process takes 5–20 minutes. The water comes from sucking back of exudate or from the nearby cortex. In a transversely

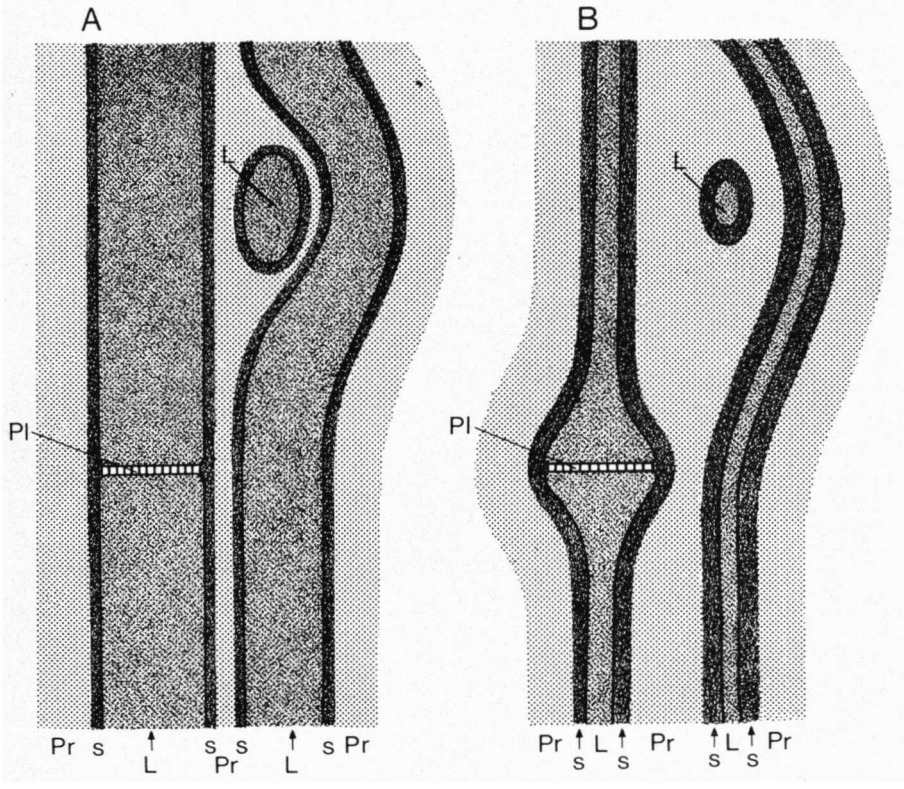

Fig. 3. Schematic representation of constriction. (A: original situation, B: after injury and swollen walls). Pr: primary wall and middle lamella; s: secondary wall, L: lumen of filaments, Pl: plate.

cut cauloid a solution of potassium ferrocyanide is sucked into the medulla and not into the cortex over a distance up to 0.5 cm. When sections are made and fixed in ethanolic ferrichloride, a deposit of Prussian blue was found only in the walls. It seems that after drainage the loss of volume is restored by swelling of the walls in the medulla by water uptake.

KOTTE (1915) mentioned that the exact estimation of osmotic pressure in many marine algae is impossible because of swelling of the cell walls. The protoplasm only retracts from the walls at very high concentrations. WALTER (1923) described for *Bangia* that the (secondary) walls have such a strong swelling capacity that protoplasm does not retract from the walls even at the highest concentrations of plasmolytic agent. The pressure of the cells is balanced by the swelling pressure of the walls.

Plasmolysis in 4 M NaCl was proved possible in the inner cortex of the cauloid. In the meristoderm the lumen decreases in size but walls swell and the protoplasm does not retract from them. Likewise, in the filaments of the medulla no retraction from the walls was observed, except that sometimes there is a slight retraction in the sides of the trumpet parts. The fact that no plasmolysis with retraction from the walls is observed in the long filaments of the medulla shows that the walls swell after loss of turgor.

The experiments indicate that when turgor is lost by cutting or fixing of the medulla, the walls swell by taking up water and the lumen of the tubes is constricted to thin filaments with thicker walls. The plates resist the constriction

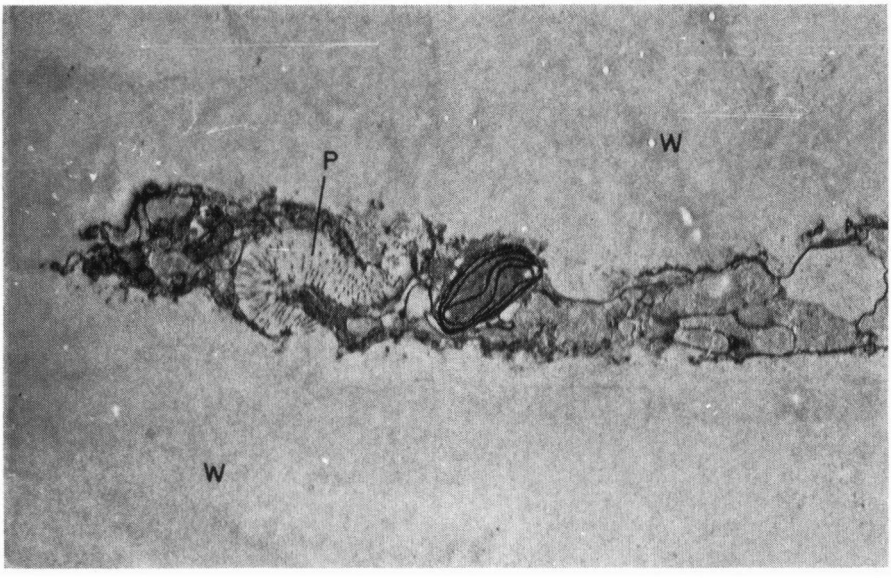

Plate 2. Electron micrograph of trumpet filament. The constriction by the walls w. has also crushed the sieve plate P. Glutaraldelyde – $KMnO_4$ × 6500.

and the double "trumpets" arise with a plate in the middle (*fig. 3*). Sometimes even the sieve plate is crushed (*plate 2*). The deduced situation in vivo seems more efficient for a translocating system. The swelling of the walls may have a pressure-buffering capacity along the translocation path.

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REFERENCES

KOTTE, H. (1915): Turgor und Membranquellung bei Meeresalgen. *Wiss. Meeres-Untersuch.* N 7: 115–167.

PARKER, B. C. (1966): Translocation in Macrocystis. III. Composition of sieve tube exudate and identification of the major ^{14}C labeled products. *J. Phycol.* **2**: 38–41.

REINKE, J. (1876): Beiträge zur Kenntnis der Tange. *Jahrb. Wiss. Bot.* **10**: 317–382.

RIGG, G. B. (1925): Some physiology of the sieve tubes of Nereocystis. *Publ. Pudget Sound Biol. Stat.* **3**: 311–329.

ROELOFSEN, P. A. (1959): The plant cell wall. *Encycl. Plant Anatomy* **3**. p **4**: Berlin.

WALTER, H. (1923): Protoplasma und Membranquellung bei Plasmolyse. *Jahrb. Wiss. Bot.* **62**: 145–213.

WENT, J. L. VAN, & P. M. L. TAMMES (1972): Experimental fluid flow through plasmodesmata of Laminaria digitata. *Acta Bot. Neerl.* **21**: 321–326.

ZIEGLER, H. (1963): Untersuchungen über die Feinstruktur des Phloems. II. Die Siebplatten der Braunalge Macrocystis pyrifera L. *Protoplasma* **57**: 786–799.

— & J. RUCK (1967): Untersuchungen über die Feinstruktur des Phloems. III. Die Trompetenzellen von Laminaria-Arten. *Planta* **73**: 62–73.