Maintenance of Air in Intercellular Spaces of Plants

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ABSTRACT

Although air-filled intercellular spaces are necessary and ubiquitous in higher plants, little attention has been paid to the possible mechanisms by which these spaces are kept from being flooded. The most likely mechanism is that the living plant cell may maintain a hydrophobic monolayer on the surfaces of adjacent intercellular spaces. The existence of 'apparent free space' in cell walls and the fact that detergent solutions do not enter the intercellular spaces argue against this hypothesis. It is concluded that the mechanism by which these important air spaces are maintained is still unknown.

Over 90 years ago, Devaux (3) demonstrated the ubiquity of interconnected air-filled intercellular spaces in thick plant tissues. In fact, such spaces are present in almost all plant tissues, thick and thin, yet they have received remarkably little attention in textbooks or in research. Except for recognition that continuous air space in the leaf is necessary for photosynthesis and that large lacunae exist in many submerged roots, modern plant physiology textbooks usually ignore intercellular spaces. Lenticels and porous portions of the epidermis are recognized in elementary botanical textbooks, with the implication that such structures function in the aeration of tissues, but little experimental work has been published. The question of how these spaces might be maintained has scarcely been approached. The purpose of this present work is more to call the attention of the scientific community to the fact that a problem exists than to give a definite solution, but some experimental results are presented and a tentative solution is suggested.

Air-filled intercellular spaces have many important functions in higher plants. Interconnected air spaces are necessary for the respiration of all massive plant tissues, such as potato tubers and large fruits, at least during growth and development (3, 9). In addition to their function as pathways for diffusion of CO_2 for photosynthesis, air-filled intercellular spaces (with adjacent cells) in the leaf provide refractive index discontinuities which cause the leaf to scatter much of the incoming radiation (12). Flotation of aquatic plants or plant parts is possible because of air-filled intercellular spaces. Respiration of crop plant roots submerged in flooded soils to a distance of several centimeters from an air source is possible because of diffusion of air in longitudinally interconnected air-filled intercellular spaces (6, 11).

The thickness at which a plant tissue needs air spaces to facilitate respiration can be calculated approximately, using small maize roots as examples. The intercellular spaces of small roots are not interconnected radially, and therefore do not function as a major path in the radial movement of O_2, but such roots, when in aerated soil, can obtain their O_2 through radial movement in the root tissue. Woolley (10) found an apparent diffusion coefficient (dif-

fusion aided by protoplasmic streaming) for tritiated water in small maize roots to be about one-fifth of the diffusion coefficient of tritiated water in free water. It is unlikely that O_2 diffusion would be faster than tritiated water diffusion in such a system, so the maximum effective diffusion coefficient for radial movement of O_2 into a small root might be taken to be about 4×10^{-2} m^2 s^{-1}, one-fifth of its free water value. This, with the solubility of O_2 in water and the volume concentration of O_2 in air, can be used with the formulas of Philip (8) to determine the limiting size of a maize root that can be supported at a given respiration rate. From this calculation, it appears that a maize root respiring at a typical rate of 8×10^{-5} volumes O_2/volume root/s could have a maximum diameter of about 1.5 mm without radially connected air spaces. Calculation from the curves given by Luxmoore, Stolzy, and Letey (6) gives a slightly larger value, about 2 mm. It is doubtful that any rapidly growing or metabolizing higher plant tissue larger than 3 mm in minimum diameter could survive without radially continuous air spaces.

Although intercellular air spaces are necessary, their existence poses a problem that has not been widely recognized by plant physiologists. That is, how can air spaces be maintained in a matrix consisting primarily of water? Even roots cultured in nutrient solutions maintain air in their intercellular spaces. These spaces are separated from the nutrient solution by living cells, it is true, but at equilibrium the spaces would be flooded. Indeed, when a small root in nutrient solution is killed by heat or anoxia, water immediately floods the intercellular spaces, giving the root a translucent or 'water-soaked' appearance, quite different from that of a healthy root. Thus the intercellular spaces are obviously wettable, at least in the dead root. A wettable intercellular space with a diameter of 2 μm would have a capillary potential of about -1.5×10^5 newtons m^{-2}, while typical nutrient solutions have osmotic potentials in the range of -5×10^4 newtons m^{-2}, so that as a cell approaches equilibrium with the nutrient solution the cell water potential exceeds the capillary potential of the intercellular space, so (given sufficient time) the water should flow into that space. This problem of maintenance of air in intercellular spaces contrary to apparent equilibrium conditions appears to be the inverse of the classical problem of the maintenance of water columns in the xylem of tall trees. The solutions of the two problems may be related.

Maintenance of Air in Leaves. At most times, the leaf water potential is more negative than -1×10^5 newtons m^{-2}, so that the intercellular spaces remain free of liquid water. When transpirational stress is low, and especially during prolonged abundance of water, little water enters the leaf from the immediate environment because of the relatively impermeable cuticle and because of the hydrophobic surface of the epidermis and guard cells. Under such conditions, however, root pressure may force water through the xylem into the leaf, threatening to flood the intercellular spaces. In most leaves this flooding is confined to the intercellular spaces of a specialized tissue, the epithem (4). The epithem is typically surrounded by endodermis or by a layer of cells having obviously hydrophobic surfaces, so that the water can flow through the

epithem and out the hydathode without flooding the main portion of the leaf mesophyll.

Maintenance of Air in Roots and Tubers. Burström (2) thought that the intercellular spaces of roots were kept free of water by CO_2 pressure. This theory is untenable because of the high solubility of CO_2 in aqueous solutions and because the CO_2 concentration in the air in root intercellular spaces ranges around 5% to 10% (5).

When a plant tissue such as a potato tuber (Fig. 1) or a small root is cut, water enters the intercellular spaces to a depth of about one cell diameter (9), but does not penetrate farther. Even if the tissue is cut while submerged in water, so that the water supply is unlimited, the water penetrates only this same distance. This capillary entry of water demonstrates that the cell walls adjacent to the intercellular spaces are wettable. There is no obvious reason why the water should stop. In very small pieces of tissue the pressure built up by entrapped air might stop the advance of the water, but the water stops at the same depth regardless of the size of the tissue.

It may be that a root can use water faster than the water can enter the root. That is, the root cells might excrete solutes, followed osmotically by water, to the xylem elements faster than the water can enter, thus keeping the root water potential low enough that equilibrium never occurs. In an intact plant, this would give rise to root pressure. If this mechanism were operative in a cut root segment submerged in water, it would cause a flow of water out of the cut ends of the xylem elements, compensating for the inward flow in the intercellular spaces. I have placed 2-cm maize root segments in neutral red or methylene blue solutions and have observed them microscopically, hoping to see streams of undyed water emerging from the cut xylem elements, but have seen no such streams. Water or dye solution enters the intercellular spaces rapidly, but the advancing front stops in a few seconds. Water obviously does not emerge from the cut xylem fast enough to compensate for a continued flow into the intercellular spaces at the initial rate. I therefore conclude that this is not the mechanism by which the spaces are kept free of water.

When the menisci between air and water in the intercellular spaces of potato tuber tissue are examined microscopocally, some
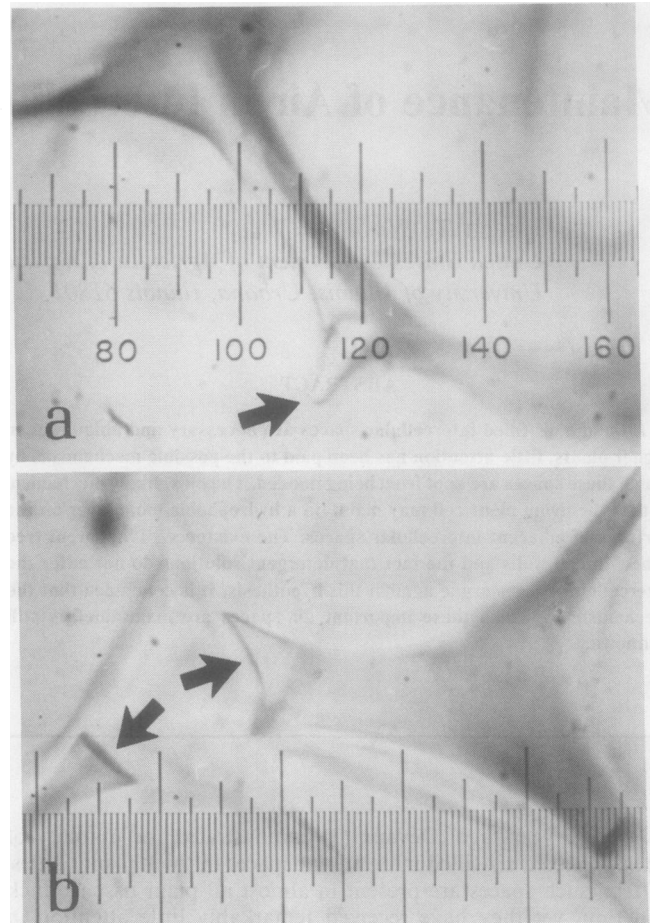
FIG. 2. Menisci between air and water in intercellular spaces of potato tuber. One division on the scale is equivalent to 0.8 μm. a, Meniscus apparently bulging toward water. b, Menisci apparently bulging toward air.

menisci appear to bulge toward the water, while others bulge toward the air (Fig. 2). In some cases these menisci appear to form at the narrowest parts of the intercellular spaces, but in other cases they appear at relatively wide places. In root segments, too, the menisci bulge sometimes toward the water, sometimes toward the air, giving no conclusive evidence as to the wettability of the surfaces. Such a result might be expected because of the optical and mechanical complexity of the plant and because the microscope must be focused below the surface layer of cells to observe intercellular spaces, but this result is also compatible with a change in the wettability of the cell surface at a given point.

I postulate that the cell wall surfaces within the intercellular spaces are normally hydrophobic. This non-wettability might be conferred by a layer of oriented molecules maintained in position by the adjacent living cell. Upon the death of the cell, this layer could become disrupted, leaving the hydrophylic cell wall exposed to the intercellular space. This hypothesis would explain the penetration of water in cut tissue only to the depth of about one cell layer, but would require that the living protoplasm exert an influence through the thickness of the cell wall. This protoplasmic influence would cease immediately upon the death of the cell. Such an influence is seemingly incompatible with the cell wall's being the site of the 'apparent free space' of the plant (1, 7).

In attempts to test this hypothesis, I have tried to force oils through intact potato tubers. Such attempts have failed. Even with oils having a lower viscosity than that of water, I have been unable to force a significant amount of the oil through the tubers,
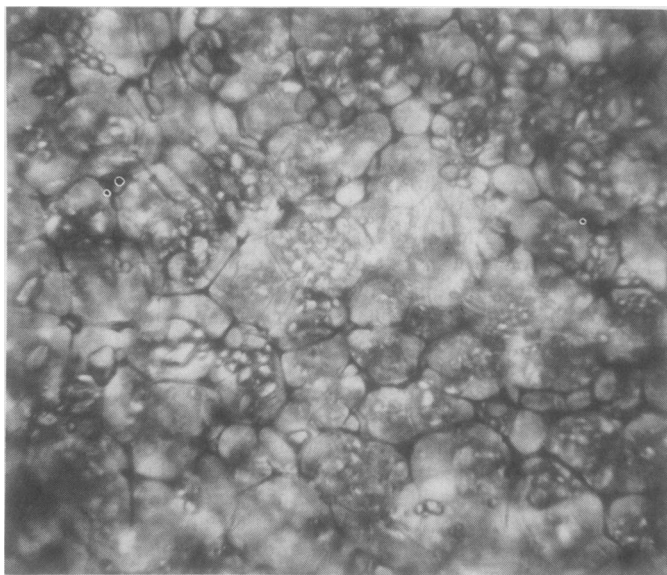

FIG. 1. Network of air-filled intercellular spaces in potato tuber tissue. The air-filled spaces appear black in the photograph because of their low refractive index. The definition is hazy because the microscope is focused 0.1 mm below the surface of the specimen. The oval objects are starch grains.

although I have forced water or air through them (9). This may be because high-viscosity oils flow too slowly and low-viscosity oils have low enough mol wt to be toxic to the cells, killing them and allowing the oil pressure to collapse portions of the tissue.

In further attempts to test the hypothesis, I have cut maize roots and potato tubers while the tissues were submerged in surfactant solutions (1% solutions of Vel, sodium cholate, sodium deoxycholate, Tween 20 and X-100). These solutions entered the intercellular spaces to no greater extent than did pure water, casting some doubt upon wall non-wettability as the mechanism for water exclusion. Furthermore, the effects of wettability or non-wettability of intercellular surfaces can be significant only if the intercellular space is small enough that surface tension effects are equivalent to significant pressures. Thus larger systems, such as submerged seaweed air bladders with smallest dimensions of a few millimeters or more, cannot be explained on a basis of wettability alone. In fact, the problem of the maintenance of seaweed air bladders is quite different from the problem of air in intercellular spaces of crop roots, although neither seems to be understood. Air bladders must be kept inflated by positive gas pressure. Otherwise they will either collapse or become water-filled. They cannot be connected to the atmosphere. In contrast, root and tuber intercellular spaces are connected to the external atmosphere, and their structural integrity is maintained by the framework of cell walls. It may be that the osmotic potential of sea water (about -2×10^6 newtons m^{-2} or -20 atm) has some significance in the maintenance of seaweed air bladders.

I conclude that the mechanism by which intercellular spaces are kept free of water is still unknown, but that the most likely explanation in most roots and tubers is non-wettability of the cell walls adjacent to living cells, with those cell walls becoming hydrophylic upon the death of the adjacent cells.

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