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Refractive Index of Soybean Leaf Cell Walls

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ABSTRACT

The refractive index of soybean (*Glycine max* [L.] Merr.) leaf cell walls was measured by two methods. The refractive index of fully hydrated walls in the living leaf was about 1.415, while that of dried cell walls was about 1.53. The refractive index of the external surface of the living leaf hair was 1.48.

A leaf reflects light largely because of refractive index discontinuities between air and cell walls. Most of these discontinuities are at the interfaces between intercellular air spaces and wet cell walls. Therefore, knowledge of the refractive index of the wet cell walls is important in theoretical considerations of leaf reflectance. The refractive index of cellulosic materials changes with degree of hydration and consequent swelling, so that assessment of cell wall water status by measurement of refractive index may become possible.

The refractive index of cell walls cannot be measured precisely because growing conditions vary with time and cell location, and because cellulosic materials are birefringent. The two indices of refraction of a piece of wet cellulose typically differ by about .07.

The cell wall refractive index was measured by two different methods: (*a*) measuring the reflectance of leaf pieces whose intercellular spaces had been infiltrated with oils of different refractive indices. The average refractive index of the cell walls was assumed to be the same as that of the oil giving the lowest reflectance; (*b*) observing microscopically the cell walls of leaf pieces immersed in a series of oils of different refractive indices to determine which oil matched the cell wall refractive index. The validity of these methods was tested on regenerated cellulose film, the refractive index of which had been measured with an Abbe refractometer. The external surfaces of the leaf hairs also were observed in the various oils. It was expected that this surface would be at least partly cutinized and would have a refractive index different from that of the internal cell walls.

MATERIALS AND METHODS

Infiltration Method. Nearly fully expanded glasshouse-grown soybean (*Glycine max* [L.] Merr., var. 'Harosoy') leaflets were collected before midmorning (to ensure opened stomata and consequent ease of infiltration). Leaf pieces, 24 × 30 mm, were cut, number coded by edge notches, and floated on water in closed Petri dishes under a lamp. After 1 to 2 hr, each piece was individually removed and blotted, and its reflectance was measured at approximately normal light incidence in a Beckman DK-2A[1] spectroreflectometer. The face (adaxial surface) of the leaf piece was toward the light source and integrating sphere of the spectroreflectometer. The wave length was 800 nm, at which neither the leaf tissue nor the infiltrating oils absorb appreciable amounts of radiation. After this initial reflectance measurement, the leaf pieces were returned to the water for about 1 more hour, then were removed, blotted, and placed in oils. Eight leaf pieces were placed in 20 ml of oil in each of four 50-ml suction flasks, each flask having an oil of a different refractive index. Alternate suction (almost 1 bar for 10 min) and pressure (0.25 bar for 20 min) were applied until the leaf pieces appeared to be completely infiltrated with oil, in that no light colored air pockets could be found when the abaxial side of the leaf was examined with a low powered hand lens. This usually took 2 to 5 cycles, after which suction and pressure were continued for 2 additional cycles. The leaf pieces were removed and blotted, and their reflectances were again measured.

Characteristics of the different oils are shown in Table I. The refractive index measurements (for the standard wave length of 589.3 nm) and the refractive index dispersion compensator settings were obtained with an Abbe refractometer at 25 C. The dispersion compensator settings were used to estimate the refractive indices at 800 nm. The viscosities and surface tensions of the oils are of practical importance in infiltration. A viscosity of between 50 and 400 centistokes is desirable for ease of infiltration and for resistance to leaking out of the stomata of the infiltrated leaf. These oils were used either singly or in mixtures to give the desired refractive indices as measured with the refractometer. They are not all completely miscible with one another, but can be mixed to give any refractive index between 1.402 and 1.556. None of the oils is miscible with water.

The results of the infiltration experiments are shown in Figure 1. The minimum reflectance was at a refractive index of about 1.415 at a wave length of 800 nm. Consideration of the dispersion of water suggests that the 800-nm refractive index of the wet wall would be about 0.002 lower than that at 589.3 nm, a difference too small to be detected by this method. The average refractive index of the wet cell walls exposed to air in the mesophyll of the soybean leaf appeared to be between 1.41 and 1.425.

These values cannot be reconciled with the value of 1.48 which I previously reported for the wet mesophyll cell wall (2), probably because of the polyethylene glycol used in the previous work. This polyethylene glycol could have penetrated

[1] Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the named products by the United States Department of Agriculture or University of Illinois.

the cell wall or may have partially dehydrated the wall. The report of 1.48 is incorrect.

Microscopic Observation Method. Small pieces (about 5 × 10 mm) from areas between the main veins of almost fully expanded soybean leaves were cut diagonally as shown diagramatically in Figure 2. About 10 sec later, the pieces were placed in oil under cover slips on microscope slides. The truncated, vertically oriented, antidermal walls of the abaxial epidermis were observed as the microscope was focused up and down. The optics of the system are such that, with a narrow cone of illumination, the microscopist can tell whether a narrow vertical object has a higher or lower refractive index than that of the surrounding medium (1). If the object (cell wall) has a higher refractive index than that of the medium (oil), the object will appear bright when the microscope is focused slightly above the object plane, and dark when the focus is lower than the object plane. This effect is reversed if the object has a refractive index lower than that of the medium. With complex shapes, the optical effects become hard to interpret, but vertically oriented, truncated cell walls surrounded by oil present a system that can be readily evaluated. The aqueous cell contents present some difficulty, but this material partially evaporates and the remainder pulls back to the uncut cells after the oil is applied. The microscopist can see where the cell contents are intact and can observe the adjacent cells from which the contents have just retreated, thus being sure that he is observing fully hydrated walls. This method requires some experience and is not completely objective, but the refractive indices of all fully hydrated walls appeared to be higher than 1.400 and lower than 1.420. The great majority fell between 1.405 and 1.415.

The same microscopic technique was used with oven-dried leaves, except that the dried leaf pieces were crushed on the microscope slide before oil was applied, and fragments of epidermis having the desired orientation were found by search among the broken leaf pieces. The refractive index of the antidermal walls of the epidermal cells of dried soybean leaves was between 1.525 and 1.545.

The external boundary of any object tends to disappear when that object is immersed in a medium having the same refractive index as this external surface. This effect showed that the external surface of living leaf hairs (hairs in which protoplasmic streaming could be seen) had a refractive index between 1.47 and 1.49. The dried leaf hair external surface had a refractive index of about 1.53, except for the extreme tip of the hair, where the refractive index was 1.48, the same as in the living hair.

Regenerated Cellulose Film. Regenerated cellulose dialysis film (Union Carbide Corp.) was used as a model to test the possibility of interaction between the oil and the cell wall. Regenerated cellulose film is similar to the cell wall in that it is laminar in shape, is a cellulosic material, and swells upon imbibition of water to about the same degree as does the cell wall. The refractive index of the film can be checked in other ways than by immersion. Pieces of this film were washed overnight in deionized water to remove the glycerine with which they had been impregnated by the manufacturer. They were lightly blotted and placed in an Abbe refractometer. Although this refractometer is designed for liquid samples, it can be used for films if the sample film is pressed firmly against the measuring prism. The determination is less accurate with films than with liquids.

The refractive index of fully hydrated, regenerated cellulose film was between 1.41 and 1.425 as measured by the refractometer. Small pieces of the same films, observed microscopically in oils, had refractive indices between 1.41 and 1.42.

Table I. *Characteristics (at 25 C) of Oils Used for Infiltration and in Refractive Index Standards*

	Refractive Index at 589.3 nm	Estimated Refractive Index at 800 nm	Viscosity	Specific Gravity	Surface Tension	Composition
			centistokes		*dynes/cm*	
Dow-Corning 200 fluid	1.402	1.399	100	0.96	23	Dimethylsiloxane
General Electric SF-1153 fluid	1.420	1.416	81	0.98	23	Dimethyldiphenylsiloxane
CVC Products "Octoil-S"	1.449	1.446	40	0.91	31	Dioctyl sebacate
Walgreen baby oil	1.461	1.458	50	0.84	30	Light mineral oil (with traces of lanolin and perfume)
American Oil Co. white oil No. 31 U.S.P. Heavy	1.477	1.473	150	0.88	34	Heavy mineral oil
Cargille Type A microscope immersion oil	1.515	1.511	150	1.03	34	Unknown
Dow-Corning 704 fluid	1.556	1.548	39	1.07	32	Tetramethyltetraphenyltrisiloxane

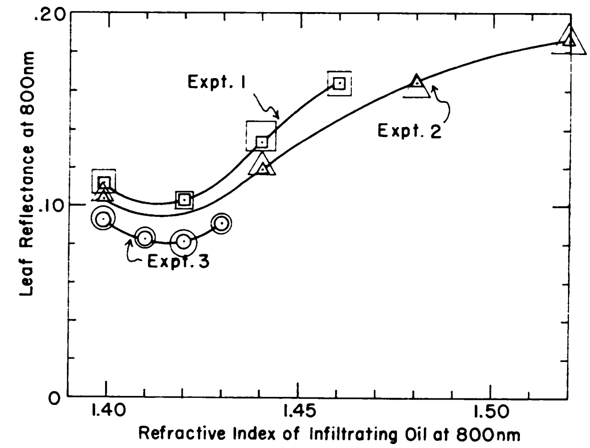

FIG. 1. Reflectance of leaves infiltrated with oils of various refractive indices. Each set of points is a single experiment involving 32 leaf pieces. The size and position of the external symbol at each point represents the total range in reflectance shown by the eight leaf samples represented by that point. The only justification for the dip in the curve for experiment 2 is the shape of the curves for experiments 1 and 3. The average fresh reflectances were: experiment 1, 0.44; experiment 2, 0.43; experiment 3, 0.47.

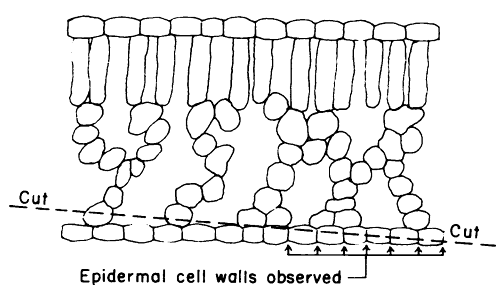

FIG. 2. Sketch showing razor blade cut made for observation of the antidermal walls of the abaxial epidermis.

Dried regenerated cellulose film had a refractive index range of 1.535 to 1.555 by either method.

The correspondence of the refractive indices obtained by the two methods indicates that the oils do not penetrate or otherwise interact with the cellulose so as to invalidate the refractive index measurements. Despite the fact that the refractive index of the regenerated cellulose is slightly higher than that of the cell walls, both wet and dry, the regenerated cellulose seems to be a good model for the cell wall.

CONCLUSIONS

In growing Harosoy soybean leaves, the average refractive index of the hydrated mesophyll wall exposed to the intercellular space is between 1.41 and 1.425.

The refractive index of the antidermal walls of the abaxial epidermis is between 1.405 and 1.415 in the fully hydrated living leaf, and between 1.525 and 1.545 in the oven-dried leaf.

The refractive index of the external surface of the leaf hair is about 1.48 in the hydrated living hair with cyclosis occurring. (The exernal cutinized wall of the leaf epidermis may have a refractive index similar to this.) The oven-dried hair has a refractive index of about 1.53 except at the extreme tip, where the refractive index is about 1.48.

LITERATURE CITED

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