Three-dimensional representation of leaf anatomy – Application of photon transport

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ABSTRACT: Great progress has been made within the last decade in the modeling of radiation transfer in vegetation canopies and recently in plant leaves. Radiosity or ray tracing models have opened new prospects in the application of remote sensing to agriculture and ecology. At the leaf scale, it is now possible to track a single photon from cell to cell and to derive the optical properties of the entire blade by following the paths of millions of photons. As a detailed description of the three-dimensional leaf internal structure is required, the first part of this paper reviews the methods to obtain that kind of information. The second part is a sensitivity analysis of the Raytran model: the absorption profiles of two leaves with different internal structures are calculated and commented.

RÉSUMÉ : des progrès considérables ont été accomplis au cours de ces dix dernières années dans la modélisation du transfert radiatif, d'abord dans les couverts végétaux, ensuite dans les feuilles. Les modèles de radiosité et de lancer de rayons ont ouvert de nouvelles perspectives en télédétection appliquée à l'agriculture ou à l'écologie. A l'échelle de la feuille, il est maintenant possible de suivre un photon de cellule en cellule, et de déduire les propriétés optiques de la feuille entière en étudiant le trajet de millions de photons. Une description détaillée de la structure tridimensionnelle des feuilles étant nécessaire, ce texte passe en revue dans un premier temps les méthodes permettant d'obtenir ce type d'information. La seconde partie est une étude de sensibilité du modèle Raytran : les profils d'absorption de deux feuilles aux structures internes différentes sont calculés et commentés.

1 INTRODUCTION

Raytran (Govaerts 1996) is a ray tracing model which simulates the optical properties of vegetation from leaf to canopy level. In particular, it has been successfully used to calculate the spectral and bidirectional reflectance of a typical dicotyledon leaf (Govaerts et al. 1996). The current representation of the internal leaf structure was based on observations derived from the literature. To go further, increased precision of leaf structure and composition may be crucial. Many synthetic images of natural objects are beautiful and some of them seem so realistic that they approach real pictures. However, representations can be misleading since visualization algorithms are generally designed to extrapolate properties. For purposes other than pure visualization, for instance the study of light scattering and absorption, the 3-D representation of

leaf internal structure requires validation in terms of physiological processes (biochemical content) and solid mechanics (arrangement of the cells). In contrast to the avalanche of publications related to plant biochemistry and metabolism, ultrastructural studies on plant cells or tissues have been largely ignored or kept in a qualitative field. The reasons are as much historical as technical. With recent progress in genetics and analytical biochemistry, entire traditional disciplines of Botany, such as plant anatomy, are now considered too descriptive and not sufficiently innovative to warrent research support, and so are now in jeopardy. As a result, the biosynthesis, structure, and function of many molecules in the cell are now well known, while the macroscopic description of leaf cells and tissues remains undescribed. Nonetheless, it is well known that natural selection has led to the proliferation of a variety of internal leaf structures whose influence on

the physiology, growth, and development of plant leaves seems evident.

The first part of this article attempts to document different methods used to observe (destructive or non-destructive methods), reconstruct (stereology, geostatistics, computer modeling) and model leaf tissues. In the second part, we tackle remote sensing applications. Besides better understanding of the physiological aspects, studying the photon transport in a plant leaf should provide useful information to improve understanding of canopy reflectance. Present leaf optical properties models, which are mostly based on N-flux equations, can impressively simulate the reflectance and transmittance over the solar spectrum, but they are not sophisticated enough to describe, for instance, the absorption profile of light inside the leaf or the bidirectional reflectance at the blade surface. The Raytran model, originally designed to calculate the light scattering in three-dimensional plant canopies has been adapted for this purpose. The virtual leaf is consequently defined as a three-dimensional object. In this paper, sensitivity analyses of Raytran are performed to investigate the influence of leaf anatomy on photon transport through such a medium, in particular the net radiation flux inside the leaf.

2 CHARACTERIZATION OF LEAF ANATOMY

Obtaining information about the three-dimensional structure of leaf tissues is a non-trivial problem (Parkhurst 1982). First, the leaf internal structure is a very complex medium, second, the small size of the studied objects makes direct observations tricky. Ideally, the leaf anatomy should be characterized without destroying the cells and tissues. However, due to technological limitations, current non-destructive methods fail to reach the same resolution as the one issued from destructive ones. Although the relationships between the shape and function of objects or living systems has been exhaustively investigated in the past (Thompson 1992), these questions seem to have experienced a renewal of interest (Prusinkiewicz & Lindenmayer 1990 ; Guyon & Troadec 1994 ; Hildebrandt & Tromba 1995).

2.1 *Nondestructive methods*

Observations of plant tissues using magnetic resonance imaging (MRI) have been recently attempted on roots, stems, fruits, etc. (Brown et al. 1988 ; Veres et al. 1991). The sample is placed in a strong homogeneous electromagnetic field and a radio-frequency field. The magnetic field causes the protons, chiefly those associated with water, to precess synchronously about the field vector at the Larmor frequency. Application of a 90° radio-frequency pulse shifts the protons away from the equilibrium position. In this process, the protons absorb energy which is later reemited. The reemited signal can be spatially localized to create a digital image of the spatial distribution of water in plant tissue (Walter et al. 1989). This non-destructive technique provides plant anatomists and physiologists with a tool for truly *in vivo* microscopy. However, obtaining quality images of plant leaves with MRI has been technically difficult as demonstrated by the scarceness of literature (Walter et al. 1989 ; Veres et al. 1993). The laminar shape of this organ and its small size require a special device − small radiofrequency coil − to obtain a good signal to noise ratio (SNR). The heterogeneity of leaf internal structure also contributes toward this difficulty. Cells present a great variation of shape, size, and arrangement. They are generally closed, that is each cell is sealed off from its neighbours by membrane like faces (Gibson & Ashby 1988) and, in comparison with other cellular solids, they do not fill space; intercellular air spaces occupy a significant volume which varies as a function of plant species, leaf tissue, and also environmental conditions (sun / shade leaves). The different components of leaves have different magnetic susceptibilities so that in the end only few protons are available to provide signal for imaging. In consequence, the attempts to extract slices from leaves have been largely unsuccessful. Nevertheless, improvements in SNR in the future should allow to distinguish between two different tissues or two different cells.

2.2 *Destructive methods*

Destructive methods have been practiced for a long time in botanical research to study the internal leaf structure both at cell and tissue levels. Cross-sections perpendicular to the blade are generally performed in two stages: the leaf sample is first fixed, dehydrated and embedded in paraffin or epoxy resin before it be cut. With light or low power microscopy, one uses sections as thin as possible, but of finite thickness (5-10 μm); in high power

microscopy, the slices are called ultrathin (0.5-1 μm). In a second stage, the section is prepared for microscopy. In each stage some systematic errors may be introduced, for instance due to a compression of the sample when sectioning occurs, or during the preparation, which may result in a loss of valuable anatomical and physiological information. As developed later on, some three-dimensional information can be deduced from one slice only but serial cross-sections are generally necessary to fully access the third dimension.

2.3 Three-dimensional reconstruction

Different techniques for 3-D reconstruction, all based on destructive methods, have existed for nearly 80 years. They may be classified into three groups: stereology, reconstructed media, and computer modeling.

2.3.1 Stereology

Stereology is the three-dimensional interpretation of two-dimensional images (Elias 1971 ; Toth 1982). When a thin section is examined, only two-dimensional structures are seen: spheres become circles, planes lines, and lines points. We can generalize rules as a principle: "a section through an n-dimensional object is, in general, an (n−1)-dimension figure. Conversely, an n-dimensional figure in a section results, in general, from cutting an (n+1)-dimensional object". The second statement is the basis of stereology. Although many structural parameters of cells and tissues can be quantified using this technique, it appears that botanists have made little use of it (Parkhurst 1982). It is a pity because stereology seems suitable for the study of leaf tissues which are highly non-isotropic media. Fundamental properties such as volume fractions, surface areas, or number of particles could be determined. For instance, the amount of cell surface area in contact with air space is a physiologically important variable whose effects on photosynthesis rates have been recently investigated. However, stereology provides only partial information and cannot be used to reconstruct a cellular tissue in three dimensions. The next two methods require serial cross-sections.

2.3.2 Reconstructed media

This method has been used in Earth Science to statistically characterize porous media (Adler 1992). Unlike an accurate representation of the leaf tissue as described thereafter, the three-dimensional random medium generated here is a statistical representation with the average properties of the leaf (Figure 1). The porosity and the correlation function of the pore space measured on leaf cross-sections are used to derive geometrical properties of the simulated material. Such a method should apply to characterize the spongy mesophyll with its complex network of cells and air spaces.
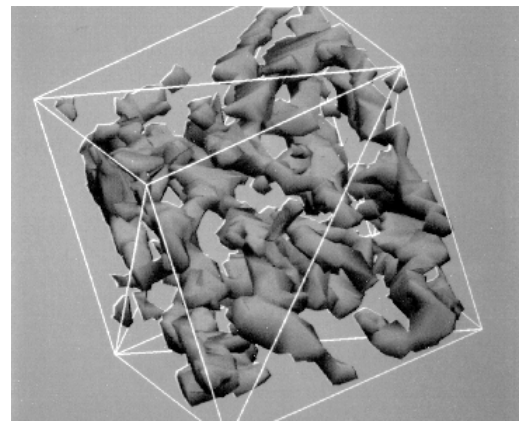

Figure 1. A three dimensional reconstructed porous medium (after Adler 1992).

2.3.3 Exact reconstruction

The problem is here to create three-dimensional models from a set of serial cross-sections. For a long time, stacking transparent slides sequentially was the only way to imagine the 3-D structure of plant organs (Banchoff 1996). In the 80's, the full view of such a structure was made possible with the advent of computer graphics systems (Delozier et al. 1987). Nowadays, two kinds of methods are classically used: surface and volume reconstruction (Geiger 1993).
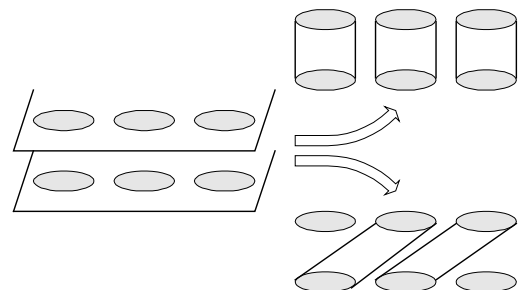

Figure 2. A set of 6 contours, pairwise superimposed in two horizontal, parallel planes shows two possible connections (after Geiger 1993).

1. In surface reconstruction, the problem consists in building a surface between contours in adjacent cross-sections. A polyhedral representation with triangular faces is well adapted to ray-tracing methods;
2. In volume reconstruction such as the Delaunay reconstruction, volume elements consist of tetrahedra that are adapted to the object shape.

2.4 *Modeling*

The modeling of leaf anatomical properties in two dimensions is much simpler than in three. When 3-D models are used for purpose other than pure visualization, which only cares about surface properties, the precision becomes crucial. To perform calculations on a system as complex as cells bonded to other cells, a number of assumptions are required about the shape and size of the cells, and the connections between cells. This implies making many decisions which are necessarily an abstract and simplified version of reality. The cell shapes of vegetable parenchyma have been classically modeled as polyhedra. The rhombic dodecahedron (10 parallelogram faces) or the tetrakaidekahedron (8 hexagonal faces and 6 square faces) which conveniently fill space when assembled (Figure 3) were initially proposed in the first half of this century (Lewis 1923 ; Marcior & Matzke 1951).
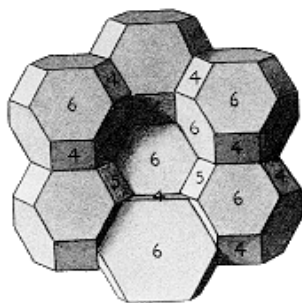

Figure 3. A group of orthic tetrakaidecahedra (after Lewis 1923).

Hexagonal and cubic cells also provide a good representation of plant epidermis to understand and simulate its growth (Korn 1974). Unlike some simple and homogeneous plant tissues, leaf tissues are nevertheless differentiated, not compact, and anisotropic. Working on the assumption that polyhedra with many faces can assimilate to sphere-like volumes, the Raytran model (Govaerts et al. 1996) considers cells to be primitive objects (spheres, ellipsoids, cylinders, etc.) which are carved

out and assembled using Constructive Solid Geometry techniques to define more complex objects (Mortenson 1985).

Generating cells is a first step, binding them together is another. The differentiation of cells follows precise genetically controled rules; their arrangement in space is a consequence of mitosis. Although the mechanics of morphogenesis is still at issue, simple tissues have been generated by computers in two (Abbott & Lindenmayer 1981) and three dimensions (Lindenmayer 1984). In order to produce more realistic scenes, variability has to be introduced. At this stage, all the difficulty rests in the introduction of alternative growth processes which may happen at random. In plain language, how to integrate misfires of Nature? Better knowledge of the statistical properties of leaf anatomy would be helpful to improve the construction of our virtual leaves or tissues, but because of the experimental limitations mentioned above, we are obliged to make many assumptions and simplifications.

3 MODELING LEAF OPTICAL PROPERTIES

3.1 *Construction of the leaf*

A typical cell is defined as a concentric object filled by three different media: cell wall material (cellulose + hemicellulose + lignin), chlorophyll, and water in order of appearance. Since they do not contain chloroplasts, epidermal cells are somewhat different and defined by two media only. Each medium is homogeneous, *i.e.*, the physical and biochemical properties at each point are equal and isotropic. It is characterized by its thickness, its refractive index n, and its absorption coefficient k.
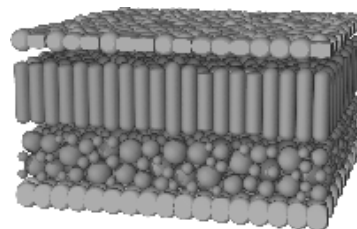

Figure 4. Perspective view of a virtual dicotyledon leaf. The size of the target is 300 μm × 170 μm (after Govaerts et al. 1996)

The construction of the entire leaf consists in connecting cells together to produce tissues, and in assembling tissues together to represent the blade. Figure 4 shows the first model used with Raytran to simulate the photon transport in a typical

dicotyledon leaf. One can recognize the palisade parenchyma and spongy mesophyll sandwiched between two layers of epidermal cells.

3.2 The Raytran model

Photon transport in a virtual three-dimensional leaf can be computed with Raytran, a Monte Carlo ray tracing code primarily designed to investigate radiation transfer problems in various terrestrial environments, and successfully applied at the leaf scale (Govaerts 1996 ; Govaerts et al. 1996). Photons are generated either in a direct and/or diffuse mode in the forward direction. After a first interaction with the leaf surface, they are tracked from collision to collision throughout the blade until the ray is absorbed or escapes from the leaf. The Fresnel formulæ using the refractive index differences between two media, and Beer's law to set the probability that a ray is absorbed by a medium, are equal to computing the bidirectional reflectance and transmittance factors, as well as the light extinction profile inside the leaf. The refractive index n and the absorption coefficient k of the different media that the ray passes through are required. To measure the vertical fluxes in the virtual leaf, twenty horizontal detectors were regularly positioned along a vertical axis. Each time a ray reaches the upper surface of the sensor, the downward flux counter is incremented by one; and conversely, the upward flux counter is incremented when a ray reaches its lower surface. Rays collected in this manner are then divided by the total number of emitted rays to provide relative energy fluxes. For each sensor, the relative net flux is calculated as the difference between the downward and upward fluxes. Finally, to simulate a horizontally infinite leaf, the sampling area is assumed to be surrounded by an equivalent target.

3.3 Sensitivity analysis

Simulations in this article are built on the basis of a real study case: in southeastern Washington State, a unique characteristics of a plantation of hybrid cottonwood trees bred for the paper industry is that all trees are clones, *i.e.*, genetically identical. We contrast two clones at the site, one with small-vertical leaves (*Populus deltoides × Populus nigra*) and another with large-horizontal leaves (*Populus trichocarpa × Populus deltoides*). In addition to their canopy architectural features, differences in leaf anatomy have been observed: leaves of the vertical

clone show two palisade layers on one side and dense spongy mesophyll on the other side, while leaves of the second clone have much more open space in the spongy mesophyll and only one layer of palisade parenchyma cells. It would be interesting to compare the optical properties of each leaf, and try to relate them to the actual internal structure. In this paper however, only absorption profiles calculated for a leaf similar to the second clone will be presented. We used the virtual leaf described in Figure 4 again. The Raytran model was run to quantify the effects of blade position on light absorption. Four absorption profiles corresponding to the following four configurations were simulated with a sun zenith angle θ of 15°:

1. vertical leaf with light incident on palisade parenchyma,
2. vertical leaf with light incident on spongy mesophyll,
3. horizontal leaf with ligh incident on palisade parenchyma,
4. horizontal leaf with light incident on spongy mesophyll.
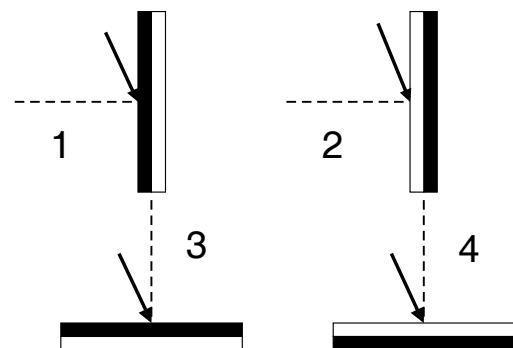
Figure 5 illustrates these four configurations.

Figure 5. Four situations selected to simulate the absorption profiles of a dorsiventral leaf. The black box represents the palisade parenchyma, the white box the spongy mesophyll.

In terms of calculation, these configurations amount to keeping the leaf horizontal and changing the illumination angle: 65° (case 1), 115° (case 2), 25° (case 3), and 155° (case 3). Light gradients simulated at 675 nm perpendicular to the leaf surface are gathered in Figures 6 and 7. The distribution of light shows several notable features in accordance with experimental results obtained using a fibre-optic probe (Vogelmann et al. 1989). For instance, one can see small variations in the profiles when a transition between two different tissues occurs. The

attenuation of transmitted light is exponential, indicating significant amounts of absorption − 80% to 100% of the incident flux is absorbed in the palisade
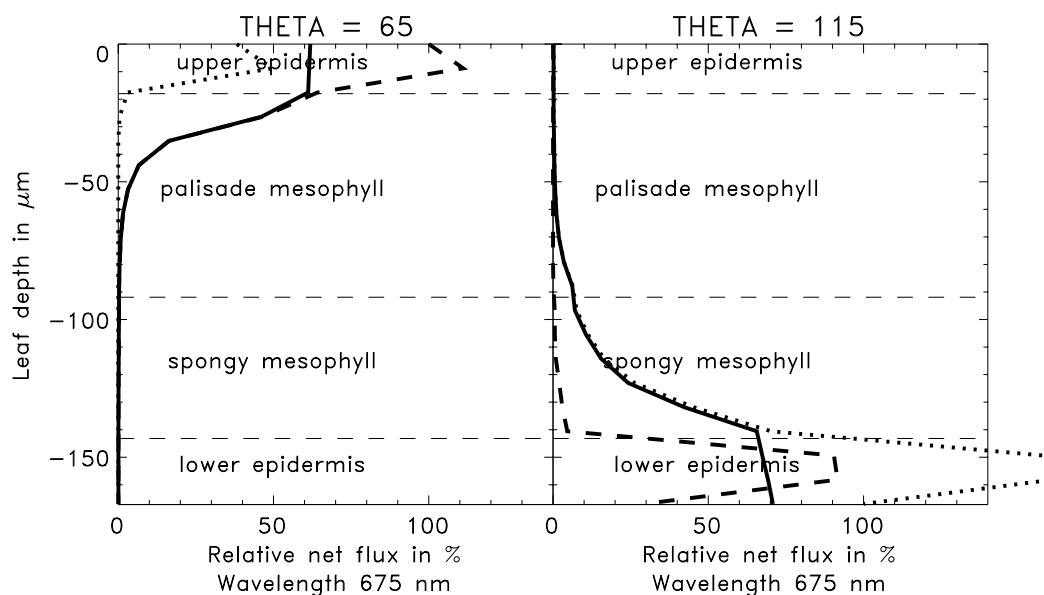

Figure 6. Normal distribution of light inside a dicotyledon leaf for a sun zenith angle of 15°: net flux (solid line), downward flux (dotted line), and upward flux (dashed line). The leaf is vertical with ligth incident on a) the palissade parenchyma b) the spongy mesophyll.
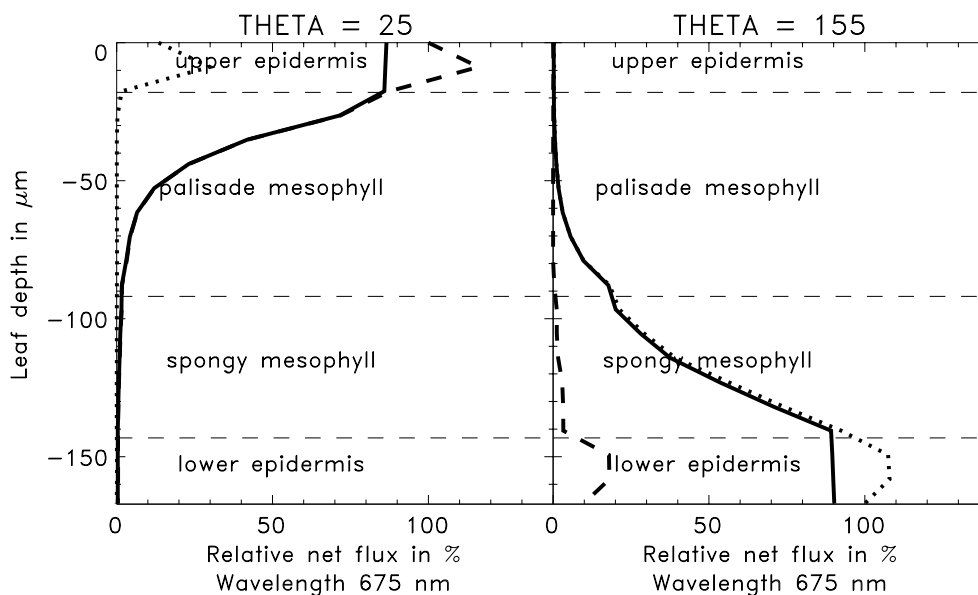

Figure 7. Idem. The leaf is horizontal with ligth incident on a) the palissade parenchyma b) the spongy mesophyll.

parenchyma, *i.e.*, within the initial 90 μm or so of the leaf. The reason why the relative downward flux in the upper epidermis can be higher than 100% is that the same ray may be scattered several times inside an epidermal cell and then be counted more than once by a detector. Let first consider the influence of the illumination zenith angle on the absorption profiles: Figures 6a and 7a (light incident on palisade parenchyma) and Figures 6b and 7b (light incident on spongy mesophyll) clearly show that, in both case, the penetration of light within the leaf decreases when the illumination zenith angle increases. It confirms previous simulations of the bidirectional reflectance (Govaerts et al. 1996) where a strong specular effect was produced in the same conditions. If we now consider Figures 6a and 6b (vertical leaves) and Figures 7a and 7b (horizontal leaves), the light is more efficiently

absorbed when the palisade parenchyma is illuminated. Finally, the third configuration which is the present situation in the field produces the best result in terms of absorption of photosynthetically active radiation.

4 CONCLUSION

As highlighted by a recent NASA report (Wickland & Smith 1995), remote sensing studies at leaf level are of great importance to better understand the scaling properties of observable electromagnetic features as we move from the elementary leaf to the plant canopy or larger. For instance, we remain at the early stages in relating the BRDF of leaves to deterministic factors, and in quantifying its effects on canopy bidirectional reflectance.

This paper was partly motivated by the use of the Raytran model to produce new simulations of photon transport within plant leaves, and to test its behavior in various conditions. Another purpose was to help us better understand the relationships between leaf anatomical structure and some physiological processes such as light interception. A field experiment which primarily aimed at investigating the way light interacts with a canopy of *Populus* clones, both at leaf and tree levels, served us as a basis for the simulations. We showed that an horizontal dorsiventral leaf with a palisade parenchyma located on the upper side of the blade was the best compromise to efficiently absorb visible light.

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