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Communication

Water Is Allocated Differently to Chloroplasts in Sun and Shade Leaves[1]

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ABSTRACT

Hydrogen-1 nuclear magnetic resonance spectroscopy was used to study water allocation in cell compartments of sun and shade leaves. NMR spectra of *Acer platanoides* were resolved into two peaks that were assigned to chloroplast and nonchloroplast water. Sun leaves contained 1.7 times more water per unit area of surface than shade leaves, and the water was allocated differently. Chloroplasts in sun leaves contained 17% of the total leaf water *versus* 47% in shade leaves. Comparing equal leaf surface areas, the chloroplasts in shade leaves contained 60% more water than those in sun leaves.

Plant leaves that develop in full sunlight often are quite different from those that grow in the shade. Sun leaves exhibit greater rates of transpiration, respiration, and photosynthesis than shade leaves (2, 3), and they dissipate heat more effectively (12). Sun leaves also are thicker in cross-section than shade leaves (3, 14), are they contain more water per unit area of leaf. These features were adaptations that improve the efficiency of sun leaves in hot, bright environments. To understand fully the adaptive advantage of additional water in sun leaves, we need to know how sun and shade leaves differ in the way that water is distributed among the cell compartments.

Perhaps the most obvious way to study water allocation is to combine separate measurements of water-compartment volumes and water concentrations. However, there are experimental difficulties with this approach. Electron microscopy can be used to measure compartment volumes in plant leaves, but special techniques are necessary (11). Only a few such studies have been published; they show that chloroplasts occupy a substantial fraction of the total leaf volume. In wheat, for example, chloroplasts occupy about 20% of the total volume, not including air space (5). Water concentrations also are difficult to measure (13). We are not aware of any quantitative volume and concentration measurements that have been combined to study water allocation to chloroplasts in leaves.

Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) may be the best method for studying water allocation in leaf tissue (7–9); its advantages include rapid measurement and simplified analysis. Signal intensity in the NMR spectrum arises almost entirely from ^1H_2O; other ^{1}H nuclei in the leaf produce either extremely broad peaks (because they are located in biopolymers or membranes) or much less intense peaks (because they correspond to small molecules in much lower concentration than water). The spectrum of a leaf is more complex than that of pure water (which shows a single, narrow peak) because internal structures in the leaf distort the applied magnetic field. When the thylakoids are aligned preferentially with respect to the leaf surface, then the peak from chloroplast water is displaced from that of water in other leaf compartments (8). In some species, the chloroplast and nonchloroplast water peaks are well resolved (9).

MATERIALS AND METHODS

Sun leaves were harvested from branches extending from the south side of a Norway maple tree (*Acer platanoides*, var 'Emerald Queen'). Shade leaves were obtained from the interior of the crown where direct sunlight almost never penetrates.

SEM[2] images were obtained from leaf disks quenched at −216°C and lyophilized at −40°C. Strips from the disks were mounted on stubs and coated with 10 nm of gold-palladium before viewing with a JEOL 35-C SEM operated at 10 kV.

^{1}H NMR spectra were obtained using a Bruker WM-270 NMR spectrometer with 4 mm diameter leaf disks in a sample holder designed to ensure magnetic field homogeneity and to orient the leaf with its surface perpendicular to the applied magnetic field (7, 9). All spectra were obtained within 3 min after the disk was excised from the leaf.

Activities of the soluble enzyme ribulose bisP carboxylase (RuBPCase, EC 4.1.1.39) in palisade cells were measured by the protocol given in Outlaw *et al.* (10) using a first reagent containing 50 mM Tris-HCl (pH 8.1), 5 mM $MgCl_2$, 1 mM reduced glutathione, 77 mM $NaHCO_3$, 0.02% BSA, and 0.6 mM ribulose bisphosphate for 15 min followed by a second reagent containing 100 mM imidazole (pH 6.7), 0.27 mM ATP, 0.033 mM NADH, 0.027 mM NaCl, 0.135 mM EDTA, 0.065 mg/ml dialyzed glyceraldehyde phosphoglycerate kinase (EC 2.7.23) and 0.065 mg/ml dialyzed glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) for 45 min.

RESULTS AND DISCUSSION

Figure 1 illustrates the structural differences between the sun and shade leaves. Sun leaves were thicker (134 ± 3 μm; Fig. 1a)

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[2] Abbreviations: SEM, scanning electron microscope; RuBPCase, ribulose bisphosphate carboxylase.

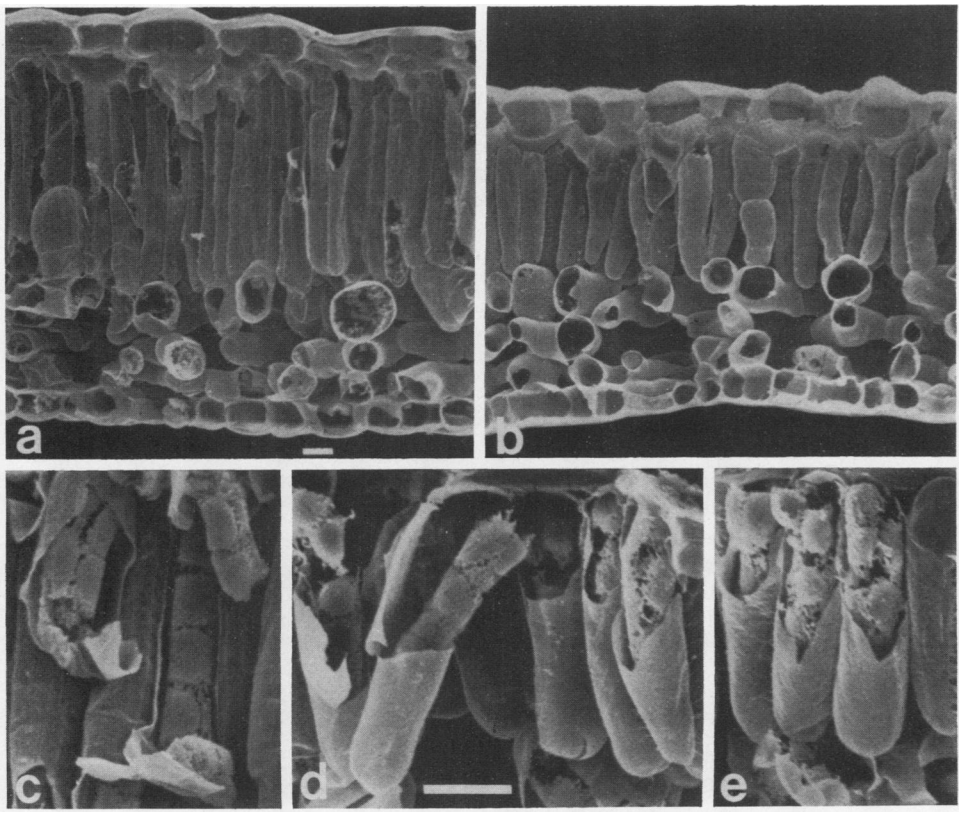

FIG. 1. Scanning electron micrographs from sun and shade leaves of Norway maple, *A. platanoides*, show that their internal organization is similar. Bar lengths are 10 μm. The upper and lower leaf surfaces consist of single layers of epidermal cells, while the interior mesophyll is made up of two cell types, palisade and spongy parenchyma. a, Transectional view of a sun leaf with closely packed palisade cells above irregularly shaped cells of the spongy parenchyma; b, palisade cells in the shade leaf transection are shorter and broader than those in sun leaves, but the size and packing of the remaining cell types are about the same; c, fractured palisade cells from a sun leaf show prominent chloroplasts; d, e, chloroplasts in the palisade cells of shade leaves are slightly smaller and not as flattened as those from sun leaves.

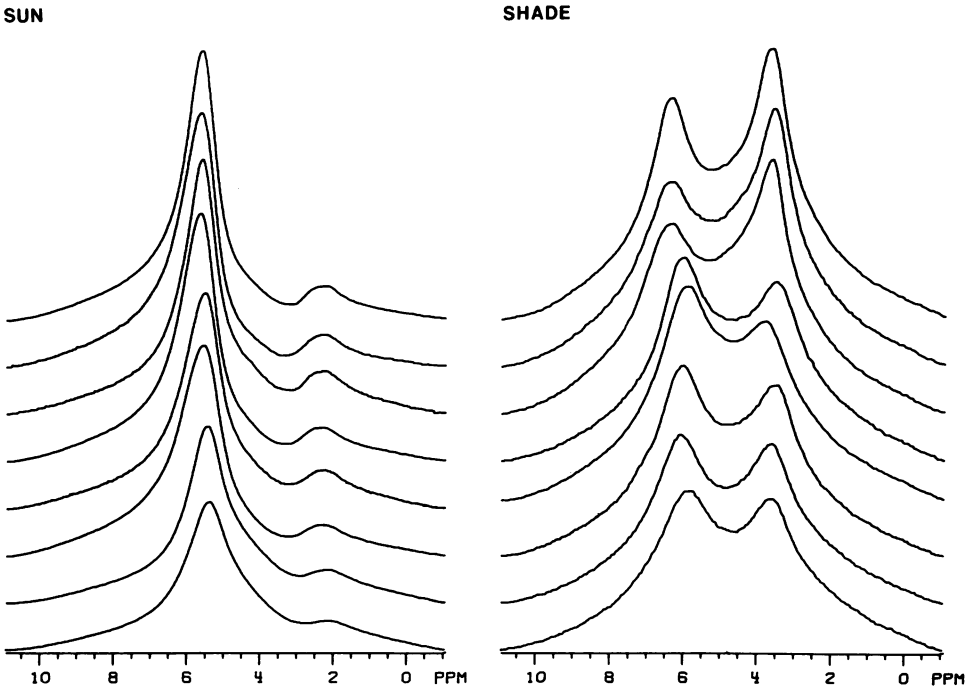

FIG. 2. ^{1}H NMR spectra from eight sun leaves and eight shade leaves of *A. platanoides*. Each trace was recorded using a different leaf sample. The scale on the horizontal axis is graduated in parts per million (ppm) of magnetic field; its origin is arbitrary because the sample contains no chemical shift reference.

than shade leaves (99 ± 3 μm; Fig. 1b) primarily because of differences in the thickness of the palisade cell layer. Chloroplasts, observed in fractured cells, were slightly longer in sun leaves (maximum dimension 6.2 ± 0.5 μm; Fig. 1c) than in shade leaves (4.7 ± 0.3 μm; Fig. 1, d and e), but the sun-leaf plastids were flatter. This result is somewhat unusual; in most species, shade leaves have the larger chloroplasts (3).

Figure 2 compares NMR spectra obtained from individual sun and shade leaves. It shows that NMR, like SEM, detects marked differences between the sun and shade leaves, and that the differences are much greater than the range of individual variation within each type of leaf. Spectra shown in the figure were obtained on a single day, but essentially identical results were obtained on many other occasions during the middle of the growing season.

Using the orientation dependence of the spectra (8), we have

assigned the peaks in Figure 2 to different water compartments. The peak on the right (in both sun and shade leaves) is the signal from chloroplast water; water in all other leaf compartments contributes to the peak on the left. The well resolved chloroplast peaks indicate a high degree of order in the thylakoids of both sun and shade leaves.

Water allocation to the chloroplasts was measured by comparing areas under the chloroplast peaks to total integrated signal intensities. Upon averaging results from 88 sun-leaf and 88 shade-leaf spectra, we found that chloroplasts in sun leaves contained 17±4% and chloroplasts in shade leaves contained 47±5% of the total leaf water. Integrated signal intensities revealed that sun leaves have about 1.7 times more water per unit area than shade leaves. Together, the two measurements showed that shade-leaf chloroplasts contain about 1.6 times more water per unit area of leaf surface than sun-leaf chloroplasts (*i.e.* 47% *versus* $1.7 \times 17\% = 29\%$).

We measured RuBPCase activities of 1.3 mol (kg min)$^{-1}$ in sun leaves and 0.3 mol (kg min)$^{-1}$ in shade leaves (expressed in terms of total leaf water content). Combined with water allocation data, these results show that RuBPCase is 12 times more active in sun-leaf than in shade-leaf chloroplasts based on equal quantities of chloroplast water (*i.e.* 1.3÷17% *versus* 0.3÷47%).

It is well known that sun leaves contain more RuBPCase than shade leaves (3); indeed, in typical sun-leaf chloroplasts, the concentration of RuBPCase is high enough (about 0.5 mM or 0.36 g/ml) to reduce the water concentration significantly (6). We have not measured absolute RuBPCase concentrations, but our data indicate that water concentrations are much lower in sun-leaf than in shade-leaf chloroplasts.

The chloroplast water fractions reported here are unusually high, and should not be regarded as typical of most plant species. *A. platanoides* was chosen for these studies in part because of its large chloroplast water fraction; the NMR method works best in such cases. We found much lower chloroplast water fractions in most of the species that we have studied (9). However, our high results are not unprecedented. Fagerberg (4) found that chloroplasts occupy 31% of the volume of sunflower palisade cells, or 36% of the volume if solids are excluded. Berlin *et al.* (1) reported that chloroplasts make up 42.6% of the volume of cotton palisade cells, or 44% if cell walls are excluded. Of course, such measurements are not directly comparable with ours, but they do suggest that chloroplasts could contain as much as half the water in a leaf.

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