

An Apparent Anomaly in Peanut Leaf Conductance

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ABSTRACT

Conductance to gaseous transfer is normally considered to be greater from the abaxial than from the adaxial side of a leaf. Measurements of the conductance to water vapor of peanut leaves (*Arachis hypogaea* L.) under well watered and stress conditions in a controlled environment, however, indicated a 2-fold higher conductance from the adaxial side of the leaf than from the abaxial. Studies of conductance as light level was varied showed an increase in conductance from either surface with increasing light level, but conductance was always greater from the adaxial surface at any given light level. In contrast, measurements of soybean (*Glycine max* [L.] Merr.) and snapbean (*Phaseolus vulgaris* L.) leaf conductance showed an approximate 2-fold greater conductance from the abaxial surface than from the adaxial. Approximately the same number of stomata were present on both peanut leaf surfaces and stomatal size was similar. Electron microscopic examination of peanut leaves did not reveal any major structural differences between stomata on the two surfaces that would account for the differences in conductance. Light microscope studies of leaf sections revealed an extensive network of bundle sheaths with achloroplastic bundle sheath extensions; the lower epidermis was lined with a single layer of large achloroplastic parenchyma cells. Measurements of net photosynthesis made on upper and lower leaf surfaces collectively and individually indicated that two-thirds of the peanut leaf's total net photosynthesis can be attributed to diffusion of CO₂ through the adaxial leaf surface. Possibly the high photosynthetic efficiency of peanut cultivars as compared with certain other C₃ species is associated with the greater conductance of CO₂ through their upper leaf surfaces.

Controlled environmental studies have indicated that certain peanut (*Arachis hypogaea* L.) cultivars have the potential for high rates of photosynthesis relative to certain other C₃ species (17, 24). High photosynthetic efficiencies for peanuts have also been deduced from field studies (18). This report describes an apparent anomaly concerning the anatomy and conductance of peanut leaves that may at least partially explain this plant's high photosynthetic capacity.

MATERIALS AND METHODS

Single plants of peanut, cv. Florigiant or Florunner, soybeans (*Glycine max* [L.] Merr. cv. Bragg or Stoneville No. D-62-7812) and bush beans (*Phaseolus vulgaris* L. cv. Contender) were grown in 1.4-liter containers of a peat-Vermiculite mix (Jiffy Mix)¹ and in square cans containing 45 kg fertilized Tifton loamy sand, a member of the fine loamy siliceous, thermic Plinthic Paleudults.

¹ Trade names and company names are given for the benefit of the reader and do not imply preferential endorsement by the United States Department of Agriculture over similar products.

Soil water was monitored in each container with electrical resistance blocks. The soil-water potential was maintained above -1 bar throughout the growth period by rewatering to -0.05 bar as determined by soil-water desorption curves. Plant-water potential was measured as described in ancillary experiments (16). The plants were grown in water-cooled chambers programmed for 25 C, 60% RH, and 350 $\mu\text{mol CO}_2 \text{ l}^{-1}$ air for 14-h photoperiods, and 20 C, 90% RH and 400 $\mu\text{mol CO}_2 \text{ l}^{-1}$ for 10-h nyctoperiods. The primary light source for growth and experimentation consisted of a bank of VHO cool-white fluorescents supplemented with incandescents with a total light intensity of 1.17 joules $\text{cm}^{-2} \text{ min}^{-1}$ of total radiation at the soil surface of which 49% was IR (340 $\mu\text{E m}^{-2} \text{ s}^{-1}$ PAR). During experimental periods a constant day-night temperature of 25 C was maintained with 60% daytime RH and a 90% nighttime RH.

Assimilation and Conductance Measurements. Most plants studied ranged from 1 to 2 months in age. Some diffusive resistance measurements were made simultaneously with the leaf- and stem-water potential measurements already reported (16) and others were made simultaneously with Pn². All measurements were made on recently fully expanded unshaded leaves. Pn measurements were made in a semiclosed compensating system where air was circulated through a Plexiglas water-cooled leaf chamber at 42 cm s^{-1} . The chamber air temperature and VPD were controlled by bringing humidified air to the desired dewpoint temperature and reheating it before it entered the leaf chamber. The CO₂ content of the chamber was monitored by a model 315 NDIR Beckman IR CO₂ analyzer and held at steady-state $300 \pm 2 \mu\text{mol CO}_2/\text{l}$ by the compensating system. Air VPD was monitored continuously by a model 880 thermoelectric dewpoint hygrometer and leaf and air temperatures were measured by small thermocouples. Pn was first measured for 1 standard day (conditions similar to those during plant growth) and then Pn and leaf resistance were measured for several days at differing light intensities. The effect of varying light intensity on Pn and leaf resistance was measured only during those hours of the day when the rates of Pn showed the least fluctuation due to endogenous control, i.e. 0900–1500 h. At any light level other than 340 $\mu\text{E m}^{-2} \text{ s}^{-1}$ PAR the radiant energy was applied for 15-min intervals. Light was normally increased in at least four steps (i.e., 340–570, 570–920, 920–1150, and 1,150–1,400 $\mu\text{E m}^{-2} \text{ s}^{-1}$ PAR) and then decreased in reverse order in experiments both before and after noon. Since neither tungsten filament spot nor quartz iodide lamps at close range provided evenly distributed high light intensity over the test plants, several light sources were used in combination at distances of 1 m from plants: (a) the standard fluorescent-incandescent light bank used for routine plant growth; (b) 625- and 1,500-w quartz iodine lamps; and (c) a carbon arc burning high intensity photo 88 rods which simulates the spectral composition of sunlight. Stomatal resistance was measured with a Lambda diffusion porometer calibrated according to Kanemasu *et al.* (14). Conductance was

² Abbreviations: Pn: net photosynthesis; VPD: vapor pressure deficit.

computed as the reciprocal of resistance.

An attempt was made to resolve the importance to leaf photosynthesis of the differences in conductance of the two epidermi of peanut leaves. After Pn measurements indicated photosynthesis had achieved steady state, the leaf chamber was momentarily opened, a lanolin paste was brushed over one surface of a leaf, the chamber was closed, and steady-state photosynthesis was re-achieved. After values for the new steady-state of the single epidermis were obtained, the leaf chamber was again momentarily opened, the other surface of the leaf was brushed with lanolin, and Pn was monitored to ensure that the paste had sealed the leaf to gaseous exchange.

Morphological and Anatomical Measurements. Stomatal densities and size were assessed by the silicon rubber technique. Peanut leaf anatomy was assessed by taking small leaf sections from both young and recently expanded leaves. For both electron and light microscopy, the sections were fixed in a mixture of 2% glutaraldehyde and 1% depolymerized paraformaldehyde in 50 mM phosphate buffer (pH 7.2) for 2 h at room temperature and rinsed several times in barbital buffer (pH 7.2). The sections were then treated with 1% OsO_4 in 40 mM barbital buffer (pH 7.2) with 40 mM sucrose for 1–2 h in an ice bath. Tissues were washed in several 25-min rinses of cold, distilled H_2O . Post stain consisted of immersion for 1 h in a cold, 1% uranyl acetate solution followed by two rinses in distilled H_2O , dehydration in an alcohol series and embedding in a Spurr epoxy resin mixture. Sections were cut approximately $0.5 \mu\text{m}$ thick. For light microscopy they were placed at low heat on a hot plate and stained with equal parts of Paragon multiple stain and 2% sodium borate solution.

RESULTS

The response of peanut leaf conductance to increasing and decreasing light level is summarized in Figure 1. Conductance increased up to the highest light level tested. Pn rates show essentially the same curvilinear relationship as conductance to light intensity. Conductance was almost 2-fold greater from the adaxial than from the abaxial surface of the peanut leaf. Figures 2 and 3 show data collected in a similar manner from soybeans and snapbeans.

The higher potential for conductance from the adaxial side of peanut leaves than from the abaxial did not change with soil-water stress and concomitant lowering of plant-water potential (Table I). However, conductance from both surfaces decreased with the lowering of plant-water potential. Cycling in the con-

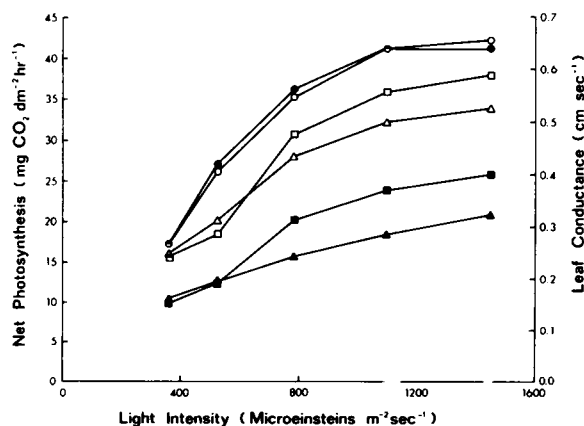


FIG. 1. Effects of light intensity on peanut leaf conductance to water vapor (morning: adaxial surface Δ and abaxial surface \blacktriangle ; afternoon: adaxial surface \square and abaxial surface \blacksquare) and net photosynthesis (morning: \circ and afternoon \bullet) at 25 C leaf temperature, 9.8 mm Hg VPD, and $300 \mu\text{l CO}_2 \text{l}^{-1}$ air.

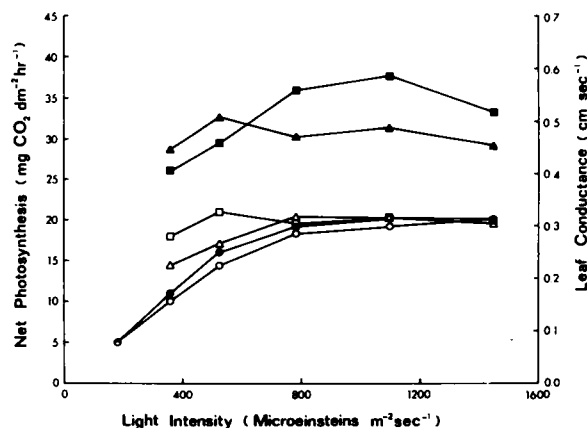


FIG. 2. Effects of light intensity on soybean leaf conductance to water vapor (morning: adaxial surface Δ and abaxial surface \blacktriangle ; afternoon: adaxial surface \square and abaxial surface \blacksquare) and net photosynthesis (morning: \circ and afternoon \bullet) at 25 C leaf temperature, 9.8 mm Hg VPD, and $300 \mu\text{l CO}_2 \text{l}^{-1}$ air.

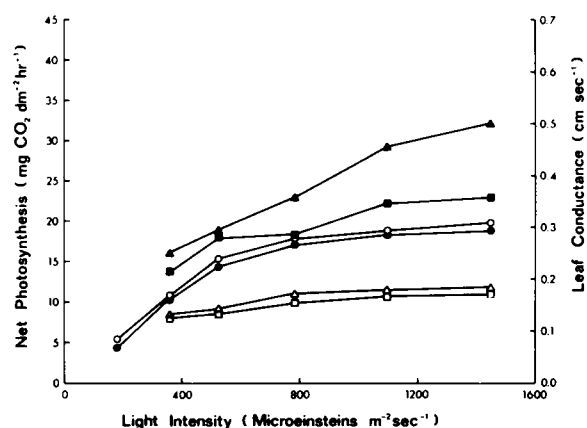


FIG. 3. Effects of light intensity on snapbean leaf conductance to water vapor (morning: adaxial surface Δ and abaxial surface \blacktriangle ; afternoon: adaxial surface \square and abaxial surface \blacksquare) and net photosynthesis (morning: \circ and afternoon \bullet) at 25 C leaf temperature, 9.8 mm Hg VPD, and $300 \mu\text{l CO}_2 \text{l}^{-1}$ air.

ductance of peanut leaves is evident at the 3-bar level (Table I). Such cycling is associated with cycling in plant-water potential (16). Contrastingly, in soybeans, higher conductance was maintained by the abaxial than by the adaxial side of leaves under plant-water stress (Table I). Measurements of net photosynthesis from adaxial and abaxial leaf surfaces singularly indicated that approximately two-thirds of the peanut leaf total net photosynthesis could be attributed to diffusion of CO_2 through the adaxial leaf surface.

Stomatal counts made on the two peanut cultivars indicated equal numbers of stomata on the adaxial and abaxial leaf surfaces, whereas on soybeans and snapbeans numbers of stomata were 3- and 5-fold greater, respectively, on the abaxial than on the adaxial surface (Table II). Similar counts for peanuts (5) and soybeans (8) have been reported. The size of peanut stomata were also identical on both surfaces.

Electron microscope studies did not reveal any anatomical or morphological differences between peanut guard cells of the adaxial and abaxial leaf surfaces. Thin cross-sections of leaves prepared for electron microscopy were further studied with the light microscope for cellular arrangement. In these studies, deviations from what was considered norm for row-cropped, dicotyledonous

Table I. Water Vapor Conductance of the Adaxial and Abaxial Epidermis of Peanut and Soybean Leaves as Affected by Plant-Water Potential

The conditions were: 25 C Leaf Temperature, 9.8 mm Hg VPD, 300 $\mu\text{l CO}_2 \text{ l}^{-1}$ Air and 340 $\mu\text{E m}^{-2}$ PAR

Peanut			Soybeans		
Time	Conductance		Time	Conductance	
	Adaxial	Abaxial		Adaxial	Abaxial
	<i>cm/s</i>			<i>cm/s</i>	
	<i>bars</i>			<i>bars</i>	
0907	0.39	0.18	1415		0.23
0909			1417		0.21
0911	0.36		1419		0.21
0914		0.16	1421	0.06	
0927	0.12		1424	0.05	
0930		0.03	1426	0.06	
0933	0.16			\bar{x} 0.06	\bar{x} 0.22
0936		0.03		SD 0.01	SD 0.01
	\bar{x} 0.24	\bar{x} 0.10			
	SD 0.13	SD 0.08	1605		0.11
			1609	0.03	
0915		0.04	1611		0.10
0921		0.04	1615	0.02	
0926		0.03	1618		0.09
0928	0.08		1622	0.02	
0931	0.13			\bar{x} 0.02	\bar{x} 0.10
0934	0.10			SD 0.01	SD 0.01
	\bar{x} 0.10	\bar{x} 0.04			
	SD 0.03	SD 0.01			

Table II. Number of Stomata on the Adaxial and Abaxial Surfaces of Peanut, Soybean, and Snapbean Leaves

Species and Cultivar	Adaxial	Abaxial
<i>No. cm⁻² × 10³</i>		
Peanut		
Florunner	15	14
Florigiant	15	14
Soybean		
Stoneville	10	30
Bragg	7	18
Snapbean		
Contender	8	40

mesophytes were noted. In peanut leaves the palisade layer, which lies directly below the epidermis, is composed of two to four cells loosely stacked in columnar fashion (Fig. 4). The vascular bundles of the peanut leaf are surrounded by bundle sheath parenchyma cells. Cells similar to those of the bundle sheath (bundle sheath extension cells) extend from the bundle sheath to the upper epidermis. These cells appear to contain very little cytoplasm and no chloroplasts. Contrastingly, the cells at the base of the bundle sheath are directly in contact with a single layer of large achloroplastic parenchyma cells which lie directly above the lower epidermis. These large cells are continuous among themselves paradermally except directly above guard cells, where their absence allows for a substomatal chamber.

DISCUSSION AND CONCLUSIONS

The 2-fold higher conductance from the adaxial side of peanut leaves than from the abaxial held at all light intensities tested. Further, the conductance of water vapor was greater from the adaxial side of the leaf than from the abaxial whether the plant

was under water stress or not. Under field conditions (2, 19), conductance was also found to be greater from the adaxial side of leaves of nonstressed or slightly water-stressed peanut plants. Under very high water stress, conductance was reduced several-fold, but was nearly equal from both surfaces (19).

The conductometric responses of snapbean and soybean leaves were quite different from those of peanut. First, conductance for both snapbeans and soybeans was usually twice as great from the abaxial side of the leaf as from the adaxial. Second, with soybeans, the plot of conductance becomes asymptotic to the x axis starting at intermediate light levels (Fig. 2). With snapbeans, conductance increased little with increasing light intensity, except for morning measurements of the lower epidermis (Fig. 3). Most workers have presented similar data showing that the adaxial leaf surface has a lower conductance for water loss than the abaxial surface (1, 3, 6, 9–11, 13, 21, 23, 25). Several reports indicate similar conductances for some species from both surfaces (1, 3, 10, 21, 23). In only two species of C_3 crop plants, wheat (20) and sunflower (10), is there evidence that the conductance of the upper surface may exceed that of the lower surface at any time.

The Pn rate of peanut shows essentially the same curvilinear relationship as conductance to light intensity (Fig. 1). Snapbean and soybean leaves did not show such a correlation of Pn with conductance. Hofstra and Hesketh (12) also reported poor correlation of soybean Pn with stomatal resistance. If we consider full sunlight to be 2,000 $\mu\text{E}^{-2}\text{s}^{-1}$, then both snapbean and soybean photosynthesis appeared to saturate at a light value of less than one-half of full sunlight (Figs. 2 and 3), whereas peanut, as was found in previous studies (17, 24), did not photosaturate at the highest light intensity used.

Several investigators have provided evidence that stomatal frequency and conductance (15, 25) or CO_2 uptake are correlated (4, 27, 28). The numbers of stomata were about equal as well as their size on the adaxial and abaxial surfaces of peanut leaves (Table II and ref. 5). In peanut leaves then, the higher stomatal conductance to water vapor and higher CO_2 uptake rate through the adaxial surface is not due to more stomata, however the difference appears to have a common denominator which is associated with both water vapor and CO_2 diffusion. Since measurements of the light transmission of the peanut leaf has shown approximately 80% attenuation (18), possibly the differences in conductance between the two surfaces is brought about by the higher light intensities which impinge upon adaxial stomata. Higher light intensities could bring about greater photoactive stomatal opening and allow for greater conductance from adaxial than abaxial stomata.

The subepidermal layer of large water-storing parenchyma cells may help compensate for water loss in any development of leaf-water stress. By their distal position in the leaf structure, the large parenchyma cells would only lessen light transmittance to abaxial guard cells. Vein extensions have long been identified as primary channels for water movement (7, 26) and supply water to tissues in contact with them, both mesophyll and epidermal (22). The system of water transfer in the peanut is evidently very susceptible to perturbation set up by soil-plant water stress which causes oscillations in leaf conductance (Table I) and plant-water potential (16). I have found that the maintenance of optimum soil water availability is an absolute necessity to maintain the high photosynthetic rates we have found in certain peanut cultivars.

Possibly then, greater photoactive opening and thus lesser diffusive resistance of adaxial peanut stomata offers an explanation for the 2-fold greater conductance for both water vapor and CO_2 from the adaxial than from the abaxial surface of their leaves. Coupled with the capacity for a well hydrated cellular environment for carbon fixation inside the leaf, the high photosynthetic efficiency of certain peanut cultivars may be realized.

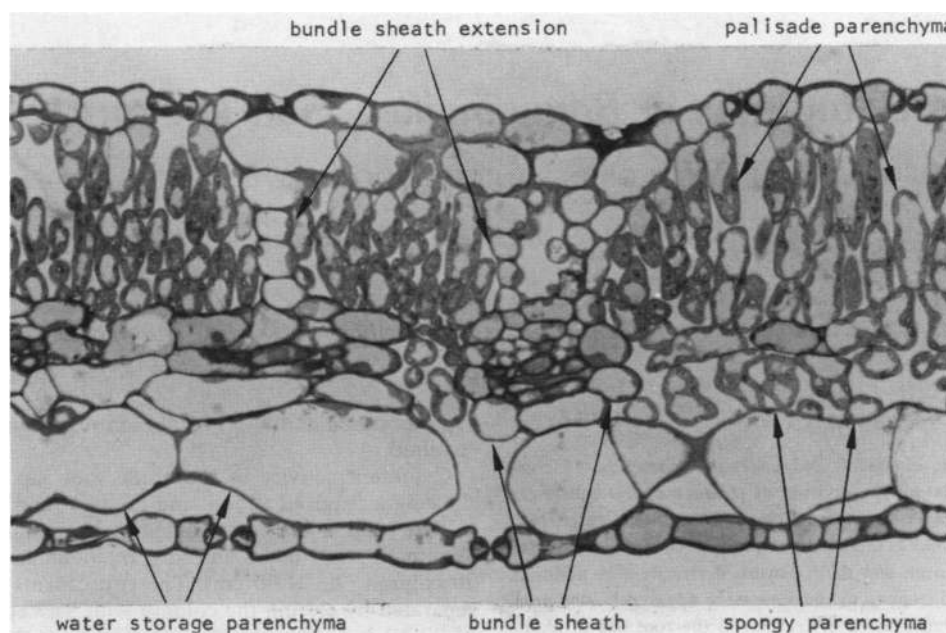


FIG. 4. Cross-section of a peanut leaf showing the distribution of cell types important to photosynthesis and water storage and conduction.

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