ANALYSIS OF ABAXIAL AND ADAXIAL STOMATAL REGULATION IN LEAVES OF PIMA COTTON (GOSSYPIUM BARBADENSE L.) USING THE 2DLEAF, TWO-DIMENSIONAL MODEL OF LEAF GAS EXCHANGE

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ANALYSIS OF ABAXIAL AND ADAXIAL STOMATAL REGULATION IN LEAVES OF PIMA COTTON (*GOSSYPIUM BARBADENSE* L.) USING THE 2DLEAF, TWO-DIMENSIONAL MODEL OF LEAF GAS EXCHANGE

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PACHEPSKY L. B., LU Zh. and REDDY V. R. *Analysis of abaxial and adaxial stomatal regulation in leaves of Pima cotton* (Gossypium barbadense *L.*) *using the 2DLEAF, two-dimensional model of Leaf gas exchange.* BIOTRONICS **29**, 79–95, 2000. Leaf photosynthesis and transpiration of eight genotypes of Pima cotton (*Gossypium barbadense* L.) were measured in the field at the Maricopa Agricultural Center in August 1994. Microphotographs of leaf cross-section, and of the abaxial and adaxial surfaces of leaves taken from the same field were scanned and analyzed with the image analysis software. Selection process, as reflected in the sequence of the studied Pima cotton genotypes, did not significantly affect the leaf gas exchange or the leaf anatomical characteristics. Only the oldest variety, P32, had the parameters significantly different from those of the more recent lines.

The data were used to parameterize and validate the 2DLEAF model developed earlier for two-dimensional modeling of leaf gas exchange and accounting for leaf anatomy. The model was used to study the effect on transpiration of the stomatal regulation on the abaxial and adaxial sides. The hypothesis about possible differences presented in several earlier studies with a number of species was confirmed in this study. At low stomatal aperture, the mode of stomatal closure at different leaf sides affects the transpiration rates more strongly than at higher values of stomatal openness. Transpiration rate is more sensitive to the abaxial stomatal closure, but the adaxial stomata play a more important role when the stomata are widely open.

Key words: *Gossypium barbadense* L., cotton, leaf gas exchange, leaf anatomy, diffusion, stomatal regulation, two-dimensional modeling

INTRODUCTION

Temperature is one of the primary factors controlling the rate of cotton plants growth and development (*43, 44*). Pima cotton (*G. barbadense* L.) is grown in the hottest areas of the Southwestern United States (*5*). Fryxel in 1986 (*16*) noted that "in addition to extremes of temperature, attention should be given to fluctuations of temperature, whether diurnal or seasonal." Such

fluctuations may be as high as 20℃ for areas like Arizona and as low as 4–5℃ for the tropical areas. As an environmental factor, temperature cannot be separated from water supply, because transpiration affects the leaf temperature creating differences between leaf and air temperature up to 5–8℃. A linkage between transpiration rate and heat resistance, as a heat avoidance mechanism, has been reported often, see e.g. (*40*). Burke & Upchurch in 1989 (*4*) studied the transpiration of upland cotton as related to its estimated thermal kinetic window (a temperature range that permits normal enzyme functioning in plants) 23.5–32℃, considering the relationship between leaf and air temperatures and plant water uptake. They found that transpirational cooling occurs when leaf temperature exceeds the lower temperature of the thermal kinetic window. The thermal kinetic window has not been determined for Pima cotton, but it is possible to assume that its minimum and maximum are higher than for the upland cotton.

Pima cotton was bred for irrigated production in very hot areas (*42*). New genotypes of Pima cotton respond well to irrigation (*41*). They have extremely high transpiration rates at high temperature, and the corresponding cooling of the leaves provides a protection against the heat damage. Breeding has substantially increased stomatal conductance in this species in the absence of soil water stress. The midday conductance is around 1 mol m^{-2} s^{-1}. Besides high stomatal conductance, a lack of stomatal responsiveness to atmospheric CO_2 concentration and significant decrease in correlation between the photosynthesis rate and the stomatal conductance were found in Pima cotton (*45*). The cotton leaves response to high temperature is quite different from that of the other agricultural species (see, for example, *3*).

Cotton has an amphystomatous leaf. Plants grown in field conditions have about 100–160 stomata on adaxial and 220–330 stomata on abaxial sides (*25, 53*). The importance of the comparative studies of the abaxial and adaxial stomatal regulation has been emphasized in many publications (*35, 38, 52*). Different behavior of the abaxial and adaxial stomata was experimentally observed for several species in a number of studies, Radin *et al.* (*42*) and Cornish *et al.* (*44*) for *Gossypium barbadensa*, Sharpe (*47*), Nagarajah (*26, 27*) for *Gossypium hirsutum*, Terashima and Saeki (*48*) for *Camelia japonica* L., Terashima and Inoue (*49*) for *Spinacea oleracea*, Lu (*0, 21*) for *Triticum aestivum*, Yera *et al.* (*54*) for *Vicia faba* L., Pamedasa (*35, 36*) for *Stachytarpheta indica, Coreopsis grandiflora, Crotalaria retusa, Tridax procumbens*, and *Commelina communis* L., and Aston (*2*) for *Helianthus annuus*. Lu *et al.* (*22*) observed that Pima cotton plants grown in growth chambers had adaxial stomatal conductances that were higher than the abaxial ones, whereas leaves of greenhouse and field-grown plants had higher abaxial conductances. This difference could not be explained by the stomatal frequency differences between the abaxial and adaxial side, because it was always higher on the abaxial side, independently on growth conditions. Lu *et al.* (*22*), working on epidermal peels of Pima cotton leaves, observed different sensitivity of abaxial and adaxial stomata to light quality and explained that by different pigment contents in the guard cells.

Abaxial stomata have been shown to adapt to their low light environment via a higher sensitivity to light (*35, 38, 52*). At non-saturated light photon flux densities, this increased sensitivity is expressed as higher conductances or wider apertures in abaxial than adaxial stomata. Adaxial stomata have been reported to open faster with increasing light intensity (*54*, for *Vicia Faba*). In cotton, differences in the light response of abaxial and adaxial stomata from both *G. hirsitum* and *G. barbadense* have been reported (*8, 26, 27*) and some of the differences have been correlated with the growth environment (*6, 47*). Pemadasa (*35, 36*) remarked that the environments of the adaxial and abaxial leaf surfaces differ in many ways, with differences in prevailing light intensity and quality being perhaps the most significant. Lu *et al.* (*22*) pointed out that, in addition to light quality and intensity, there are other important gradients in the leaf affecting the upper and lower surfaces, including anatomical and functional differences in palisade and mesophyll cells, evaporative demands and pathways of water supply inside the leaf. These authors conjectured that these differences are likely to be expressed in different functional demands placed on abaxial and adaxial stomata.

It is not known to-date whether and how the differences in stomatal regulation on abaxial and adaxial sides of leaves affect leaf gas exchange, in particular, transpiration. Using a leaf gas exchange model is an appropriate starting point to begin quantifying effects of those differences on gas exchange. The 2DLEAF model was developed to simulate effects of leaf anatomy and stomatal regulation on leaf gas exchange (*30, 32*). It has been used to estimate anatomical, stomatal, and biochemical components of differences in photosynthesis and transpiration of wild-type and transgenic tobacco leaves (*33*), to analyze contradicting data on possible changes in stomatal density in future high CO_2 atmosphere (*30*), and to calibrate the crop models for different cultivars (*15*). The 2DLEAF model can simulate different patterns of the abaxial and adaxial behavior and their effects on leaf gas exchange.

The objectives of this study were (1) to parameterize and to validate the model 2DLEAF for field grown Pima cotton, (2) to test a hypothesis about the independent stomatal regulation on the adaxial and abaxial leaf surfaces, and (3) to simulate possible effects of different abaxial and adaxial stomatal regulation on Pima cotton leaf transpiration.

MATERIALS AND METHODS

Plant material

Experimental studies were carried out in 1994 at the Maricopa Agricultural Center of the University of Arizona (33.07°N, 111.98°W, elevation 358 m ASL), at an experimental farm occupying about 400 ha in the midst of an irrigated agricultural area. Surrounding fields are planted predominantly with cotton and aflalfa during the summer, with an equal area of fallow land interspersed. Large uncultivated areas surrounding the agricultural belt support Sonorah desert vegetation. Rainfall is usually under 100 mm during the growing season

whereas potential evapotranspiration is about 1,000 mm.

Eight Pima cotton (*Gossypium barbadense* L.) cultivars were studied. These cultivars, Pima 32, Pima S-1, Pima S-2, Pima S-3, Pima S-4, Pima S-5, Pima S-6, and Pima S-7 further will be referred to as P32, PS-1, PS-2, PS-3, PS-4, PS-5, PS -6, and PS-7, correspondingly. The eight lines represent a selection gradient in a breeding program conducted with Pima cotton for the last 50 years (*10-14, 23, 51*). Seeds were planted in plots 13.7 m long and 1 m wide on April 13, 1994 on fine-loamy, mixed, hyperthermic Typic Haplargid soil. After seedling establishment, plants were thinned to a uniform spacing of 15 cm between plants. The air temperature was around 43/25℃ (day/night), relative humidity averaged around 35%, and maximum PAR intensity reached 2,000 μmol $m^{-2} s^{-1}$ during the generative stages. Standard regional management practices were followed for irrigation schedule, fertilization, and insect control (*24*).

Measurements of transpiration and photosynthesis rates and leaf area were made on August 13-16, 1994, during the fruit maturation period. The first fully expanded main stem leaf of 10 individual plants was used for all measurements. Leaf temperature from three individual plants of each cultivar was measured continuously with copper-constantan thermocouples (OMEGA TT-T-40), attached to the lower side of the leaf surface and connected to a CR21 micrologger (Campbell Scientific Inc., Logan, UT, USA). Air temperature was measured with a shaded thermocouple positioned 10-15 cm above the canopy. During the measurements, air temperature was 44/25℃, relative humidity was around 31% and PAR was around 2,000 μmol $m^{-2} s^{-1}$ at noon. The days were clear and sunny. Measurements started 3 days after irrigation with no water stress observed. Photosynthesis rates were measured between 1:00 and 4:00 p. m. with a portable steady-state gas-exchange system (Analytical Development Co., Ltd). Transpiration rates were measured with a Li-Cor steady-state porometer (LI-COR Inc., Lincoln, NE, USA). Leaf area was calculated from the data on leaf length and width. Stomatal density was measured on August 28, 1994, and the same day the first fully expanded leaf was taken for the cross-sectional microscopic analysis.

The 2DLEAF model was described in detail by Pachepsky and Acock for hypostomatous (*30*) and by Ferreyra *et al.* (*15*) for amphystomatous leaves. The model simulates the transport of three gases: water vapor, carbon dioxide, and oxygen, as a two-dimensional flow in a domain that extends through the leaf cross-section and the leaf boundary layer on both the abaxial and adaxial sides. The processes described in the model are: (a) transport of CO_2 and water vapor in the intercellular spaces and in the boundary layer adjacent to a leaf, (b) fluxes of CO_2 across cell surfaces due to assimilation, and (c) water vapor fluxes from the cell surfaces due to the difference between atmospheric and intercellular water vapor pressure.

Fig. 1 presents an algorithm of constructing the two-dimensional domain in which the system of the partial derivatives equations of the 2DLEAF model was solved numerically on the two-dimensional spatial grid superimposed on the leaf intercellular space and the adjacent leaf boundary layers. The photographs of

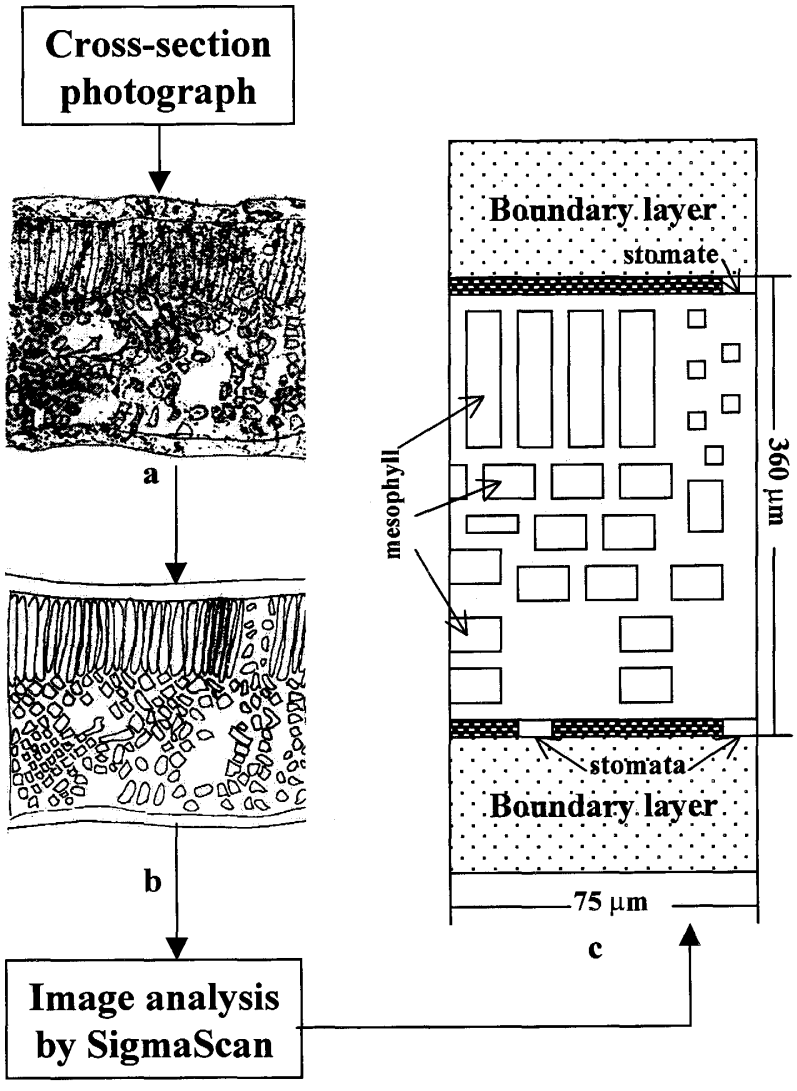

Fig. 1. Schematization of the internal Pima cotton leaf structure, ***a***–step 1, ***b***–step 2, ***c***–step 3, domain for the 2DLEAF model based on the analysis and measurements of images presented in Fig. 2a and the analogous ones for other replicates.

leaf cross-sections are scanned and analyzed by SigmaScan software package. The following data are obtained as a result of this image analysis: (a) a leaf thickness and a distance between stomata to define the domain left and right boundaries, (b) a node space (or a grid size) that would accommodate stomatal aperture, cell sizes, and intercellular spaces to a reasonable approximation, (c) average width and height of palisade cells, average diameter of spongy cells, and width and depth of substomatal cavities, and (d) a number of both palisade and spongy cells located in the part of the leaf cross-section corresponding to the width of the flow domain. A cell surface area index (CAI) is calculated as a ratio of the total cell cross-section perimeter to the width of the cross-section.

A mapping program uses the results of these measurements to prepare a data set for the 2DLEAF code replacing palisade and spongy cells with polygons.

Evaporation of water and assimilation of CO_2 occur on the sides of the polygons representing the surfaces of palisade and spongy mesophyll cells. These surfaces are a part of a complex domain boundary for gas flow, and corresponding boundary conditions are to be set on the surfaces for both water vapor and CO_2. Internal spaces of plant cells are not a part of the domain. Water vapor concentration at the cell surfaces is set equal to the saturation value at the leaf temperature. Gas diffusion coefficient depends on temperature. Gas concentrations on the outer edges of the boundary layers are set equal to the atmospheric values. Carbon dioxide assimilation by mesophyll cells (boundary conditions on the cell surfaces) was described by Farquhar's model (*9*) in which the parameters also depend on temperature. A linear decrease of irradiance inside the leaf is assumed.

The system of equations of the 2DLEAF model consists of three diffusion equations for all three gases with the boundary conditions in a form of Farquhar's equations and constant values of CO_2, O_2, and water vapor concentrations at the outer edges of the boundary layers and saturated water vapor pressure on the cell surfaces. To solve the system in the domain, a two-dimensional spatial grid is superimposed. Gas concentrations are defined at the nodes of this grid. The governing model equation is solved numerically using a Galerkin-type finite element scheme (*18*). Approximating the spatial derivatives results in a linear system of algebraic equations for the water vapor pressure and in a nonlinear system of algebraic equations for $[CO_2]$, the non-linearity being caused by the nonlinear boundary conditions on the cells' surfaces. The Newton-Raphson method for non-linear systems of equations is applied (*39*). Differences between the total fluxes of CO_2 from the atmosphere into the flow domain and entering the cells are used to check the convergence. The details of the solution were presented by Pachepsky and Acock (*30*).

Statistical analysis

Statistical analysis of the data was made by SigmaScan image analysis software (Jandel Scientific). Student-Neuman-Keuls method with a level of confidence $P<0.05$ (*7*) was used to compare the genotypes' leaves. To assess the performance of the model, a set of the statistical tests proposed for the model of photosynthesis by Pachepsky *et al* (*31*). Significance of differences between the variability of prediction errors and the experimental variability, that is the quantitative adequacy of the model, were evaluated by F-test. Qualitative assessment was done by the analysis of residuals and autocorrelation. F-test compares variability of predictions with variability of the data (*7, 37*). If these variabilities are statistically indistinguishable, then the model is considered to be quantitatively adequate. Autocorrelation test compares a shape of the calculated and measured output curves. Residuals should be randomly distributed around the predicted values. Systematic deviation from randomness indicates that the model is not good enough qualitatively.

RESULTS AND DISCUSSION

Leaf and gas exchange parameters

Leaf characteristics for the studied eight cultivars are shown in Table 1. Both leaf width and length varied among genotypes but in only 7% of comparison cases was the difference statistically significant. For leaf thickness, both in the morning and in the afternoon, the genotypical differences were insignificant. A number of stomata on the abaxial side of the P32 leaf was significantly less than that on the same side of PS–1, ..., PS–7 lines, but such difference was not found for these genotypes. On the adaxial side, PS–7 had significantly more stomata than other genotypes (Table 1). On average, Pima cotton leaf for these varieties had a leaf 13.4 cm wide and 16 cm long with the leaf area about 200 cm^2. Leaf thickness varied between 300 and 350 mm. Stomatal density on the abaxial side was 2.5 fold of that on the adaxial side and ranged from 400 to 450 stomata per mm^2 (Table 1).

We also compared the photosynthesis and transpiration rates of various genotypes, but only within one day of measurements, when the environmental conditions (temperature, CO_2 concentration in air, PAR, and VPA) varied within 2% to 5% (Tables 2 and 3), which was very close to the variability of the external conditions in the laboratory (controlled) conditions. A significant difference was found between the environmental conditions on August 13 and August 16, 1994, therefore, a comparison using simultaneously all the experimental data was not possible. Within one particular day, no significant differences for photosynthesis and transpiration between the genotypes was detected.

Parameters of the 2DLEAF model

Parameters of the 2DLEAF model are listed in Table 4. The first essential

Table 1. Characteristics of the leaves of Pima cotton (*Gossypium barbadense* L.) and their internal structure. Samples taken in the fields of Maricopa, Arizona, USA, ***n***–number of replicates.

Genotypes	Leaf size, samples taken on Aug 16, 1994, ***n***=15			Leaf thickness, μm, taken in July 1994, ***n***=20		Stomatal density per mm^2, ***n***= 20, 28 Aug 1994	
	Width, cm	Length, cm	Area, cm^2	Morning	Afternoon	Abaxial side	Adaxial side (% of abaxial)
P32	13.4±0.09	17.2±1.2	211.27±14.8	304±21.3	299±21.0	382±27	137±7(35.9)
PS–1	12.6±1.3	16.1±1.3	189.4±13.2	318±22.3	344±24.0	431±34	141±8(32.7)
PS–2	13.9±.97	16.0±1.8	205.91±14.4	331±23.2	349±24.4	448±31	140±6(31.3)
PS–3	16.0±1.76	17.5±1.2	252.07±17.6	302±21.1	344±24.1	427±47	119±12(27.9)
PS–4	14.2±1.14	15.7±1.4	205.13±14.4	317±22.2	335±26.8	419±34	146±7(34.8)
PS–5	14.1±0.99	15.8±1.26	203.9±14.27	290±20.0	322±29.0	436±44	172±5(39.5)
PS–6	11.7±1.05	14.6±1.5	165.06±11.6	306±21.4	306±21.4	455±22	177±8(38.9)
PS–7	11.4±0.8	14.3±1.0	159.29±11.0	296±20.7	301±24.1	433±39	200±10(46.2)
Mean values	13.4±0.95	16.03±1.12	199.0±13.9	304±21.4	325±23.0	429±35	154±8(35.9)

Table 2. Photosynthesis rates, P_n, μmol $m^{-2} s^{-1}$, for eight genotypes of Pima cotton (*Gossypium barbadense* L.), measured in the field in Maricopa, Arizona on Aug 13 1994 between 1:00 and 3:00 PM. Environmental variables measured simultaneously are: T_l and T_a, temperature of leaf and atmosphere, respectively, ℃; [CO_2], atmospheric CO_2 concentration, μmol m^{-3}, ***PAR***, photosynthetically active radiation, μmol (photons) $m^{-2} s^{-1}$. All values are mean ones for 10–15 measurements. Coefficients of variation are in a range 2–22%.

Genotype	P_n	T_l	T_a	[CO_2]	***PAR***
P32	10.85	37.43	38.05	0.01467	1,400
PS-1	11.47	37.56	37.84	0.01498	1,350
PS-2	12.13	37.60	37.65	0.01540	1,380
PS-3	12.85	34.10	38.99	0.01661	1,352
PS-4	11.20	38.46	38.58	0.01512	1,450
PS-5	12.18	37.92	37.52	0.01475	1,455
PS-6	13.16	37.05	38.14	0.01674	1,418
PS-7	11.48	37.69	37.79	0.01471	1,470
Mean	11.92	37.13	38.07	0.01537	1,409
Variation coefficient, %	6.2	4	1.3	5.5	3.3

Table 3. Transpiration rates, ***Tr***, and stomatal conductance G_s, both in mol $m^{-2} s^{-1}$, for eight genotypes of Pima cotton measured in Maricopa, Arizona on Aug 16, 1994 between 2:00 and 4:00 PM. Environmental variables measured simultaneously are: T_l and T_a, temperature of leaf and atmosphere, respectively, ℃, ***VPA***, water vapor concentration in atmosphere and ***VPI***, in the leaf intercellular space (calculated as a saturated concentration for the given leaf temperature), both in mol m^{-3}. All data are the mean values for 10–15 measurements, coefficients of variation are in a range between 1 and 19%.

Genotype	***Tr***	G_s	T_l	T_a	***VPA***	***VPI***
P32	14.47	0.5982	34.20	35.40	1.14080	2.14000
PS-1	16.48	0.6536	34.73	36.08	1.17232	2.21506
PS-2	17.97	0.7527	33.33	34.98	1.10347	2.03044
PS-3	15.65	0.6303	34.02	35.40	1.12800	2.12000
PS-4	19.72	0.8102	34.27	36.10	1.17400	2.14368
PS-5	13.34	0.8410	34.20	36.00	1.16721	2.11072
PS-6	17.01	0.7760	32.96	35.26	1.1213	1.99600
PS-7	18.66	0.8418	32.98	35.16	1.1218	1.99600
Mean	16.66	0.7379	33.85	35.55	1.1411	2.09300
Variation coefficient, %	12.8	13.2	1.9	1.25	2.4	3.8

set of parameters for 2DLEAF model characterizes leaf anatomy and is determined by the analysis of images of leaf cross-sections and leaf surfaces (Fig. 2). Gas flow domain for Pima cotton (*Gossypium barbadense* L.) leaves was created using leaf cross-section microphotographs presented in Fig. 1. Relative sizes of spongy cells and the width of palisade cells in both Pima and upland cotton were small compared to the leaf thickness, and it was difficult to get an image clear enough for the automatic computer analysis and measurements. Boundaries between cells and the intercellular spaces were often fuzzy (Fig. 2) and distinguishable only by the human eye. Therefore, boundaries between cells

Table 4. Parameters of the 2DLEAF model determined with the experimental data and used for simulating a gas exchange of the Pima cotton (*Gossypium barbadese* L.) leaf; D_{CO2}, D_{O2}, and D_{H2O} are the corresponding gas molecular diffusion coefficients in air at 760 mmHg atmospheric pressure and 273.15°K, k_D is a constant the corresponding diffusion coefficient is to be multiplied by, to calculate a coefficient of diffusion in the boundary layers; a is a parameter ranging from 1.75 to 2 (*1*); Γ is the CO_2 compensation point; $V_{c\,max}$ is the rate of RuBP carboxylation; b is a portion of assimilated CO_2 lost to respiration, K_c and K_o are Michaelis-Menten constants for carboxylation and oxygenation, respectively; TPU is the rate of triose phosphate utilization; P_{ml} is the rate of photosynthesis that occurs at CO_2- and light saturation; α is the quantum use efficiency; SD is the stomatal density; l is the leaf thickness; k is a constant to calculate the assimilation rate per unit of cell surface; Δ is the thickness of the boundary layer. Parameters marked with stars were determined by fitting the experimental data using as an initial estimate the value from the source shown in the table.

Parameter	Unit	Value	Source
D_{CO2}	$m^2\ s^{-1}$	0.139×10^{-4}	American Institute of Physics Handbook, 1972, *1*
D_{O2}	$m^2\ s^{-1}$	0.634×10^{-4}	
D_{H2O}	$m^2\ s^{-1}$	0.239×10^{-4}	
k_D	no	0.7	
a	no	2.0	Harley and Tenhunen, 1991, *17*
Γ	$mol\ m^{-2}$	0.180×10^{-2}	
$V_{c\,max}$	$mol\ m^{-2}\ s^{-1}$	0.346×10^{-3}	
b^*	$mol\ m^{-2}\ s^{-1}$	0.021	
K_c	$mol\ m^{-3}$	0.014	
K_o	$mol\ m^{-3}$	6.474	
TPU	$mol\ m^{-2}\ s^{-1}$	2.25×10^{-6}	
P_{ml}^*	$mol\ m^{-2}\ s^{-1}$	0.657×10^{-4}	
α	$mol\ m^{-2}\ s^{-1}$	0.009	Ticha, 1982, *50*
SD	mm^{-2}	428-ab; 214-ad	Jones, 1992, *19* Our data, Fig. 1.
d	μm	20	
l	μm	300	
k	$mol\ s^{-1}$	0.7×10^{-8}	Nobel, 1991, *28*
Δ	μm	1,500	

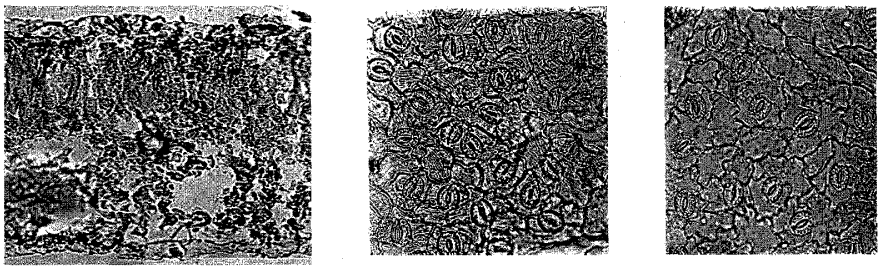

Fig. 2. Leaf cross-section (***a***) of Pima cotton (*Gossypium barbadense* L.), its abaxial (***b***) and adaxial (***c***) leaf surface. Samples were taken on August 28, 1994 in the fields of Maricopa, Arizona, USA.

and intercellular spaces were drawn by hand (step 1 in Fig. 1), and then the image was made containing only a drawing which was easy to analyze and measure (step 2 in Fig. 1) the way it was done for other species' leaf cross-sections (step 3 in Fig. 1), as described, e.g. in (*29*). The number of grid nodes was about 6,000–7,000. To save a run time and a computer memory, different vertical distances between nodes were used for the intercellular space and for the boundary layers, the former was 10 times greater than the latter.

Stomatal densities were measured on the images like **b** and **c** in Fig. 2 and compared with the data on upland cotton from (*19, 46, 50, 53*). Stomatal densities for the Pima cotton did not differ significantly from those for the upland cotton.

Since no other Pima cotton leaf cross-sections were found in the literature, we compared the cross-sections of this work with leaf cross-sections published by Van Volkenburgh and Davies (*53*) for the Upland cotton (*Gossypium hirsutum* L.), that allowed us to make a comparison. Leaf anatomy for these two cotton species seems to be quite similar.

For thick leaves of cotton, we had to take into account a decrease of light intensity inside the leaf. Leaves of the Pima cotton remain stationary at an angle of about 90° to the main axis of the plant. Therefore, non-shaded adaxial surfaces are exposed to very high photon flux densities of solar radiation (up to 2,000 μmol photons $m^{-2} s^{-1}$). On the other hand, abaxial surfaces are shaded by the mesophyll and upper epidermis and usually receive only about 10% of the photon flux densities incident on the adaxial surface (*22*). Thus, the adaxial epidermis of non-shaded leaves develops and functions in a typical sun environment, while the abaxial epidermis is exposed to the environment typical of the shade plants. In the 2DLEAF version developed in this study for the cotton, the assumption was made that the light intensity linearly decreases and the irradiance on the lower leaf surface is equal to 10% of the irradiance at the upper leaf surface.

Temperature, air humidity, $[CO_2]$ in air, and light intensity must be accounted for to calculate the model parameters and to set the boundary conditions. The parameters of the boundary layers were determined as

described in (*34*). Stomatal aperture was simulated separately (*30*) and considered as an input variable to the mapping program.

The volume of the experimental data allowed us to perform parameterization and validation with independent parts of the data set for the relatively wide range of leaf temperature and [CO_2], 13% of the data were used for parameterization and 87% were accounted for in the validation procedure. Table 4 presents the whole set of 2DLEAF parameters for the cotton. Stomatal density on the abaxial surface was assumed only 2 fold greater than that on the adaxial side, although the data showed a bigger difference (Table 1). It is theoretically possible to account for any ratio of the stomatal densities, but if it is not an integer number, the domain becomes several times bigger, and a run takes an unreasonable amount of time, if even possible, with PC. Parameter P_{ml} (Table 4) was estimated with the photosynthesis data at light saturation. The first approximations for the biochemical parameters were taken from work of Harley and Tenhunen (*17*).

The 2DLEAF model upgraded and parameterized for cotton was tested with the experimental data on the transpiration and photosynthesis rates of genotypes PS-2-PS-7 and P32 measured on August 13 and 16. Mean values of light intensity, leaf temperature, [CO_2], and water vapor gradients were calculated for each day and each genotype. Then the model was run for each set of environmental conditions with the parameter values given in Table 4.

Simulation results

Calculated and measured (mean values over replicates for a given genotype and day) values of photosynthesis and transpiration rates are compared in Fig. 3. Calculated values appear in a reasonable agreement with the measured ones. Results of model performance evaluation analysis are shown in Table 5. Table 5 shows that simulating both photosynthesis and transpiration rates, the 2DLEAF model for cotton was adequate both quantitatively and qualitatively in the range of the considered environmental conditions.

Fig. 3 shows that leaf temperature strongly affected the transpiration rate. The difference in 3℃ (37℃ on 13 and 34℃ on 16 of August) decreased the transpiration rate by 23%. There was also a difference in the humidity gradient, mostly related to the leaf temperature, because of the related change in the internal humidity. This difference in temperature increased the photosynthesis rate by almost 40%.

When calculating transpiration rates for the real environmental conditions, we could reproduce the measured transpiration rates only when assuming that the stomatal closure had different courses on the abaxial and adaxial sides, if stomata were closing simultaneously, the correspondence between the calculated and measured values was very poor (Fig. 4). This is another evidence for the existence of different ways of the abaxial and adaxial stomatal regulation

The 2DLEAF model allows us to examine the effect of the different behavior of the abaxial and adaxial stomata. A numerical experiment was designed and carried out for the transpiration rates. It was shown earlier (*29*) that stomatal

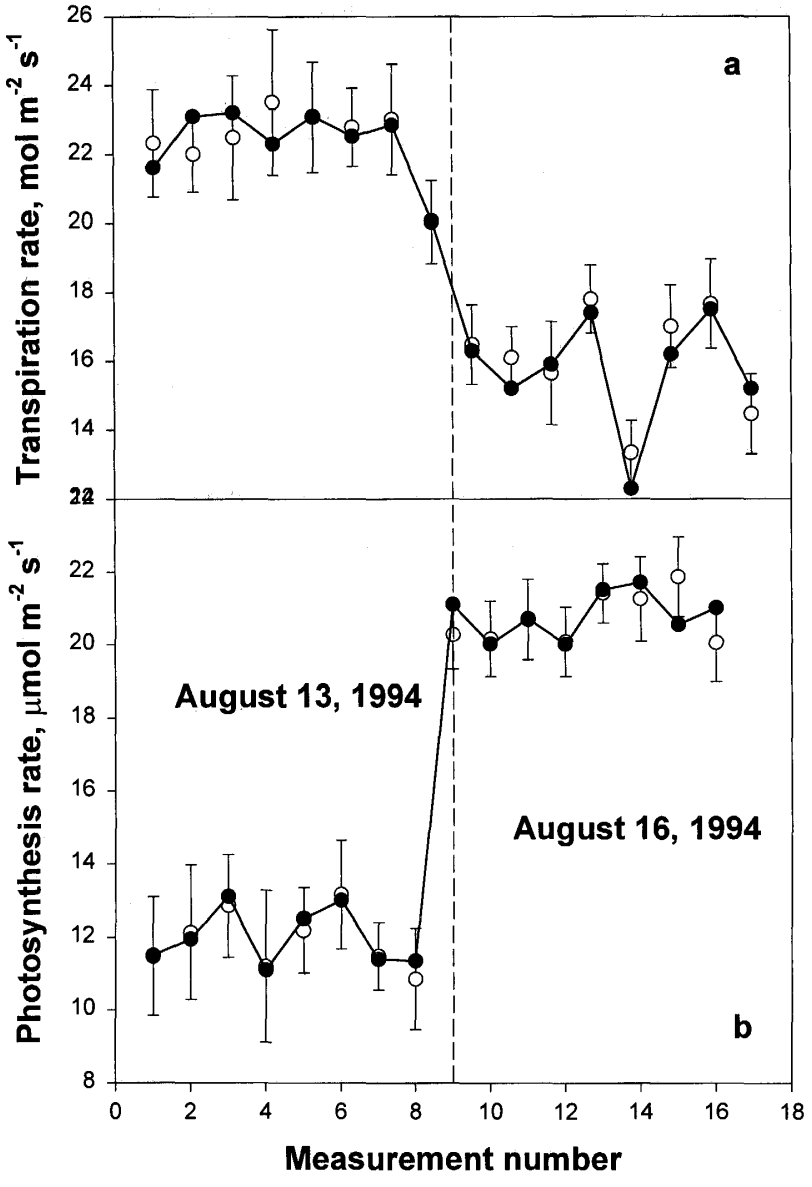

Fig. 3. Calculated, closed circles, and measured on 13 and 16 Aug 1994, open circles, values of transpiration (*a*) and photosynthesis (*b*) rates.

Table 5. Statistical characteristics of the 2DLEAF model for cotton performance estimated by *F*-test (F_{cr} and F_{calc}) and by autocorrelation coefficient (r_{cr} and r_{calc}) criteria (*31*).

Model output	F_{cr}	F_{calc}	r_{cr}	r_{calc}
Photosynthesis rate	2.31	1.23	0.446	0.444
Transpiration rate	2.17	2.01	0.402	0.400

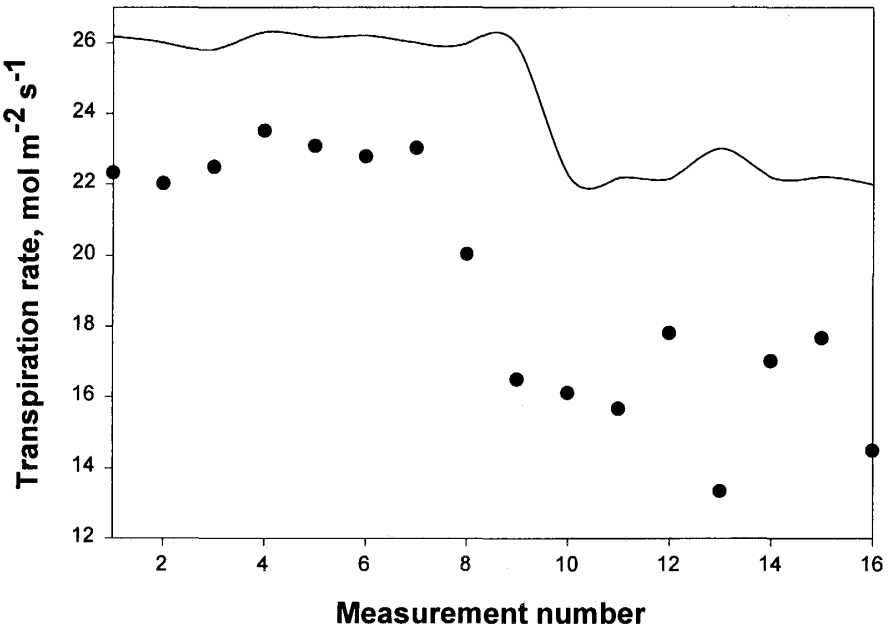

Fig. 4. Measured, points, and calculated with stomata opening simultaneously, a line, transpiration rates.

aperture affects much stronger the transpiration than photosynthesis rates, therefore we concentrated on simulating transpiration rates. Five different modes of stomatal closure were examined: 1–stomata close simultaneously on both sides of a leaf; 2–abaxial stomata stay fully open, adaxial stomata close; 3 –adaxial stomata stay fully open and abaxial close; 4–both sides stomata close but the adaxial stomata close faster, they stay open 2 μm less; and 5–both sides stomata close but abaxial close faster, they are open 2 μm less than the adaxial ones. Stomatal aperture had the values 0, 2, 4, 6, 8, and 10 μm. Transpiration rates were calculated for every of these 5 modes and for every value of stomatal apertures (Fig. 5) at the same environmental conditions, leaf temperature equal to 33.05℃, and the gradient of water vapor concentration between leaf interior and atmosphere equal to 0.938 mol m^{-3}.

Transpiration decreased with stomatal aperture reduction most rapidly when stomata were closing on both sides simultaneously (Fig. 5). The highest transpiration rates were observed for mode 3, that is when the adaxial stomata were fully open and only the abaxial ones were closing. The lowest transpiration rates were obtained for mode 5, when the lower surface stomatal closure was delayed with respect to the upper ones. Maximal difference between modes 3 and 5 equal to 2.01 mol $m^{-2} s^{-1}$ (about 8% of the maximum) occurred at the stomatal aperture equal to 2 μm. Modes 2 and 4 produced very similar intermediate values of transpiration rates (Fig. 5).

At the aperture values under 2 μm, the results are different. The highest transpiration rates were observed for mode 2, when only upper stomata were closed. The minimal value of the transpiration rate was obtained for mode 4,

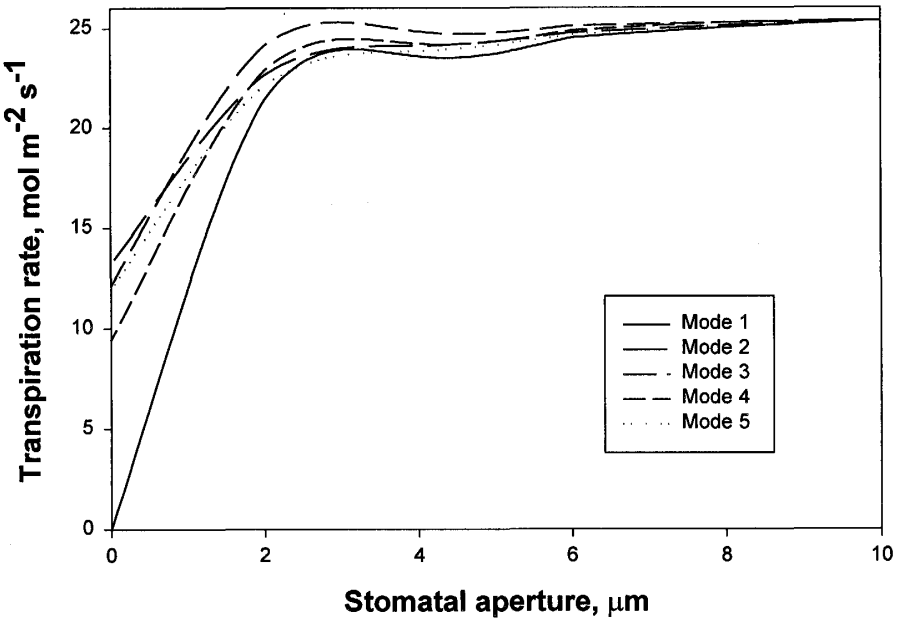

Fig. 5. Transpiration rates calculated for various modes of stomatal opening at leaf temperature 36.05℃ and water vapor concentration gradient equal to 0.983 mol m^{-3} between leaf interior and atmosphere. Values of stomatal aperture correspond to the side of the leaf on which stomata are closing or in which they are more closed. Mode 1–stomata close simultaneously on both sides of a leaf; Mode 2–abaxial stomata stay fully open, adaxial stomata close; Mode 3–adaxial stomata stay fully open and abaxial close; Mode 4–both sides stomata close but the adaxial stomata close faster, they stay always open 2 μm less; and Mode 5–both sides stomata close but abaxial close faster, they always are open 2 mm less than the adaxial ones.

when the adaxial stomata closure was delayed. The difference in transpiration rates at low apertures was higher and equal to 3.78 mol $m^{-2} s^{-1}$ of the maximal value. The results for modes 3 and 5 at low apertures almost coincided.

Therefore, at low values of stomatal aperture the mode of stomatal closure becomes very important. It appears that transpiration rates are more sensitive to the abaxial stomatal closure. At higher values of stomatal aperture the adaxial stomata seem to play a more important role. At all modes of stomatal closure, the dependence of transpiration rates on the stomatal aperture is strictly non-linear, and the non-linearity for the amphystomatous leaves is much more pronounced than for the hypostomatous plants (e.g., *32*).

Regulating stomatal aperture on two sides of the leaf independently, Pima cotton can better regulate its temperature regime. When water is in unlimited supply, it can transpire as much as needed through widely open adaxial stomata and efficiently reduce leaf temperature. But when leaf temperature is below the damaging values, it can transpire through the abaxial stomata keeping the adaxial ones almost closed. This may lead to a substantial savings of water in non-irrigated conditions or between irrigations.

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