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CALCULATING LEAF BOUNDARY LAYER PARAMETERS WITH THE TWO-DIMENSIONAL MODEL 2DLEAF COMPARING TRANSPIRATION RATES OF NORMAL (CV. DÉSIRÉE) AND TRANSGENIC(SUCROSE TRANSPORT ANTISENSE) POTATO PLANTS

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PACHEPSKY L. B., MUSCHAK M., ACOCK B., KOßMANN J., BLECHSCHMIDT-SCHNEIDER S., WILLMITZER L. and FISAHN J. Calculating leaf boundary layer parameters with the two-dimensional model 2DLEAF comparing transpiration rates of normal (cv. Désirée) and transgenic (sucrose tranport antisense) potato plants. BIOTRONICS **27**, 41–52, 1998. The leaf boundary layer, i. e. the layer of air adjacent to a leaf surface in which gas flow is significantly influenced by the leaf, considerably affects leaf gas exchange. Numerous factors, both external conditions and leaf properties, have a strong influence on boundary layer characteristics and the challenge to develop a reliable model of this link in the leaf gas exchange pathway has persisted for decades. Two parameters, the boundary layer thickness, d, and the ratio, B, of the diffusion coefficients of gases in the boundary layer and in the intercellular space, were shown to be sufficient to represent the effect of the boundary layer in a two-dimensional leaf gas exchange model 2DLEAF. An algorithm for calculation of these parameters is described and applied to simulate the transpiration rate of leaves in normal (cv. Désireé) and transgenic (expressing a mRNA antisense construct targeted to the cp-fructose-6-bisphosphate phosphatase) potato plants (*Solanum tuberosum*). For these leaves, both gas exchange and leaf anatomy have been studied. Parameters d and B were different for normal and transgenic leaves, and they expressed real differences in anatomy and surface properties.

Key words: *Solanum tuberosum*; potato; transpiration; leaf boundary layer; diffusion; two-dimensional modeling; transgenic plants.

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INTRODUCTION

Leaf surface significantly affects the air movement. The part of the atmosphere near the leaf surface is referred to as the leaf boundary layer (LBL). The idea of the LBL first appeared in 1953 when Bange (*1*) introduced the concept of a micro "vapor cup" as a region over the stomata where gas flow is different from the surrounding atmosphere. He considered the interactions of these "cups" at very high stomatal frequency. According to a definition by Nobel (*15*), the LBL consists of two sub-layers. The surface region nearest the leaf is "dominated by the shearing stresses originated at some surface in a laminar sub-layer of air where movement is parallel to the leaf surface; air movement is arrested at the surface and has increasing speed at increasing distances" from the leaf surface. Farther from the surface, the second sub-layer is a region of turbulent gas movement.

For decades, LBL has been remarkable challenge for both experimentalists and modelers, often causing incomparable and contradicting results of transpiration measurements (*1*). Beginning with Brown and Escombe's (*3*) experiments conducted in still air, a failure to account for the LBL led to the erroneous conclusion that stomatal aperture had little effect on transpiration. This, in turn, "led to a long and unproductive argument concerning the importance of stomatal control of transpiration" (*11*) until later experiments summarized by Slatyer (*21*) showed that stomata control transpiration. Numerous attempts to relate stomatal conductance to stomatal dimensions were unsuccessful becaeuse the LBL was not accounted for (*19*).

LBL parameters such as thickness, effective coefficients of gas diffusion, and resistance were shown to be strongly dependent on wind speed (*4, 11*), intensity of air stirring within a leaf chamber (*15*), temperature (*20*), relative humidity (*13*), and leaf properties, i. e., leaf size and shape (*15*), stomata size, shape, frequency, and distribution (*1, 11*), stomatal aperture (*18*), and roughness of leaf surface, i. e., how grooved and hairy is it (*12*). Many of these factors, like wind speed or stomatal aperture, are highly variable and difficult to control in experiments. This makes measurement of the LBL extremely difficult. Therefore, modeling of the LBL appears to be necessary.

Different models of the LBL have been considered. Nobel (*15*) introduced resistance of the LBL as one of several resistances in the gas exchange pathway. The coefficient of diffusion for various gases in the LBL in combination with LBL thickness were used by Jones (*9*) and Pachepsky and Acock (*16*). Both approaches are approximations for a complete description of gas flow near the leaf surface. The parameters in both models were found to be dependent on environmental conditions.

As Kramer and Boyer (*11*) pointed out that "molecular genetics is being increasingly used in physiological researches." 2DLEAF has been used with transgenic plants to quantitatively separate the effects of anatomical, biochemical and environmental factors of leaf gas exchange (*17*). Therefore, it was a natural next step to determine LBL parameters describing with the 2DLEAF transpi-

ration rates for normal and transgenic potato (*Solanum tuberosum* L.) leaves with different and well characterized leaf internal structures.

The objectives of this work were (i) to determine the minimum number of LBL parameters needed to explicitly account for the boundary layer effect on leaf gas exchange, (ii) to determine the values of these LBL parameters with data on transpiration and leaf anatomy for normal (cv. Désirée) and transgenic potato plants, and (iii) to formulate an algorithm to quantitatively describe the leaf boundary layer in two-dimensional leaf gas exchange models.

MATERIALS AND METHODS

Plant materials.

The plants used in the experiment have been described in detail by Muschak et al. (*14*). In brief, potato plants, both normal, *Solanum tuberosum* L. cv. Désirée, and transgenic plants were grown in a greenhouse at 100–200 μmol (photons) $m^{-2} s^{-1}$ light intensity, day/night temperature 18/16℃, and 60% relative humidity. Transgenic plants, that expressed a FBPase- antisense mRNA targeted to cp-FBPase, were regenerated as described by Koßmann *et al.* (*10*). Various independently generated lines used in the experiment were characterized by the FBPase activities of 809±130 U m^{-2} (100%, normal) and 103±12 U m^{-2} (12%, transgenic). Three days prior to measurement all plants were transferred to a growth chamber in which conditions were set at 400 μmol (photons) $m^{-2} s^{-1}$, 20℃, 60–70% relative humidity, and photoperiod 15 h. Leaf gas exchange cuvettes were mounted inside the growth chamber.

The custom-designed leaf gas exchange unit provided a multiplexed, computer controlled recording of up to five leaf cuvettes sequentially, and has been described in detail by Muschak et al. (*14*). Gas exchange was measured on 60 leaves from different plants at eight light intensity values. The experiments were performed on leaf five. Over 50 samples for microscopy were taken in the morning. Discs of 4 mm diameter were cut between the first two second-degree vascular bundles and close to the midrib. Quarters of these discs were fixed and embedded as described in Hoffmann-Benning *et al.* (*7*). Thin (1 mm) sections stained with toluidine blue were viewed in a Zeiss Axiphot microscope.

The 2DLEAF model

The 2DLEAF model (*16*) simulates (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) fluxes of water vapor the cell surfaces due to the difference between cellular and intercellular water vapor pressure. Gas transport is considered as a two dimensional flow. The gas flow domain extends through the leaf and the boundary layer. The 2DLEAF model can be used for both amphystomatous (*17*) and hypostomatous leaves (*16*). The hypostmatous version has been chosen for the current study as the stomatal density for the abaxial side for both normal and transgenic plants, was an order of magnitude higher than that on the adaxial side according Bolhàr

-Nordenkampf and Draxler (*2*), and our own measurements (230 stomates per mm^2 on the abaxial versus 10–20 on the adaxial side). Leaf cross-sections used in this study are shown in Fig. 1, and their representations in the model are demonstrated in Fig. 2. Stomatal density of the transgenic plants was 1.3 times

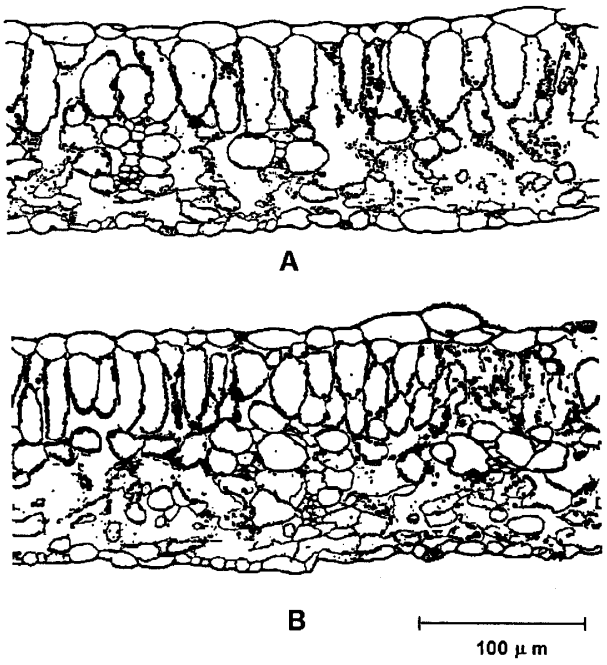

Fig. 1. Leaf cross-sections of the potato plants, A-cv. Désirée, B-transgenic plant.

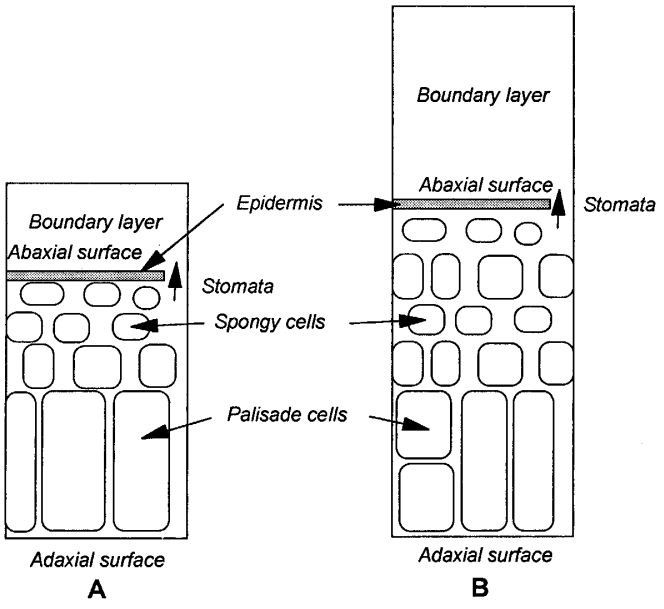

Fig. 2. Domains created to run the 2DLEAF model, A-cv. Désirée, B-transgenic plant.

that of the normal plants.

Assimilation of CO_2 and evaporation of water are simulated on the surfaces of the polygons representing palisade and spongy mesophyll cells. No gas movement and/or assimilation is modeled within cells. Values of CO_2 concentration, $[CO_2]$, at the outer (bottom) edge of the boundary layer are equated to the ambient $[CO_2]$ value. Water vapor pressure at the cell surfaces is set to the saturated value for the leaf temperature.

The system of equations of the model includes three diffusion equations for CO_2, O_2, and water vapor, and five algebraic carbon assimilation equations as boundary conditions for CO_2 transport, according to the carbon dioxide assimilation model based on Rubisco kinetics (*5, 6*). Boundary conditions are defined also by constant values of $[CO_2]$, $[O_2]$, and water vapor pressure at the outer border of the boundary layer. Temperature, air humidity, $[CO_2]$, and light intensity must be known to calculate the coefficients in the system of equations (diffusion coefficients, parameters of the light response curve, respiration rate, Michaelis–Menten constants for carboxylation and oxygenation) and to set the boundary conditions. A complete mathematical description of the system of equations of the 2DLEAF model and the method for its solution were presented in Pachepsky and Acock (*16*). The system of equations was solved in the complex domain representing intercellular space and the boundary layers as shown in Fig. 2. To facilitate this, a two-dimensional spatial grid is superimposed on the leaf intercellular space and the adjacent boundary layer. Gas concentrations are defined at the nodes of this grid. The system of equaions is solved numerically using a Galerkin-type finite element scheme (*8*).

Grid generation and flow domain selection must be completed before the application of 2DLEAF to a particular plant. To schematize the leaf anatomy, the software package SigmaScan is used (a) to calculate an average mesophyll thickness and a distance between stomata to define the domain boundaries, (b) to calculate a nodal spacing that would accommodate stomatal aperture, cell sizes and intercellular spaces to a reasonable approximation, (c) to calculate average width and height of palisade cells, average diameter of spongy cells, and width and depth of sub-stomatal cavities, and (d) to count the numbers of both palisade and spongy cells located in the part of the leaf cross-section corresponding to the width of the flow domain. Eight photographs of the leaf cross-sections from both normal and transgenic plants (Fig. 1) were scanned. Table 1 presents the results of some leaf cross-sections measurements. A cell surface area index was calculated as the ratio of the total cell cross-section perimeter to the width of the cross-section. A service mapping program was used to prepare a data set from these results for the 2DLEAF program replacing palisade and spongy cells by polygons.

Leaf boundary layer parameterization

In this study an emphasis was placed on modeling the transpiration rates to describe the LBL and on specifying and calculating the values of paramenters that are necessary and sufficient to account for the LBL effect in leaf gas

Table 1. Cell sizes for cv. Désirée and transgenic potato leaves as measured on 8 two-dimensional leaf cross-sections.

Genotype	cv. Désirée				Transgenic			
Cells	Palisade		Spongy		Palisade		Spongy	
Number per 100 μm	6.4		26.3		12.5		37.2	
Parameter	Area	Perimeter	Area	Perimeter	Area	Perimeter	Area	Perimeter
Mean	579.2	117.3	106.6	41.7	289.5	76.06	120.9	44.84
Coefficient of variation	6.05%	3.38%	5.77%	2.79%	5.24%	3.15%	4.30%	2.79%

exchange models. Two parameters, LBL thickness d, defined according to the Noble's definition (*15*), and the ratio of the coefficient of diffusion in the boundary layer and in the intercellular space B, were introduced. Two sets of experimental data on transpiration with normal and transgenic plants were used to determine d and B, and to validate the model. These measurements of transpiration rates were made at 20℃, 60–70% relative humidity (Exp. #1) and 70–80% relative humidity (Exp. #2) for a number of light intensities (Table 2). Calculations with the 2DLEAF model were made for the same conditions. Two experimental points from both Exp. #1 and Exp. #2, the maximal and minimal values of the transpiration rates, were used for model parameterization. All other experimental data were used for modal validation.

The algorithm of calculating the LBL parameters, d and B, consisted of three steps.

Step 1. The range of d and B values was estimated. The d values were varied over the range 300–2000 μm, for both normal and transgenic plants. This range is based on the range of 280–2800 μm given by Nobel (*15*) for leaves 5 cm long. For B it was assumed that the coefficient of diffusion in the boundary layer is higher than in the intercellular space because of convection (*11*). The range for B was varied from 1 to 5.

Step 2. Transpiration rates were calculated by 2DLEAF model for all possible combinations of the values of d and B, with the step equal to 1 for B, and 100 mm for d, at two values of stomatal aperture, maximal, 10 mm and minimal, 1 mm. The corresponding surfaces of transpiration values were plotted for both normal (Fig. 3) and transgenic (Fig. 4) plants.

Step 3. On these surfaces, the values equal to the measured transpiration values have been found, and the corresponding d and B values were the parameters of the LBL for the particular experiment.

RESULT AND DISCUSSION

Figs. 3 and 4 present the dependencies of the transpiration rates on these two parameters for normal and transgenic plants, respectively. The lower

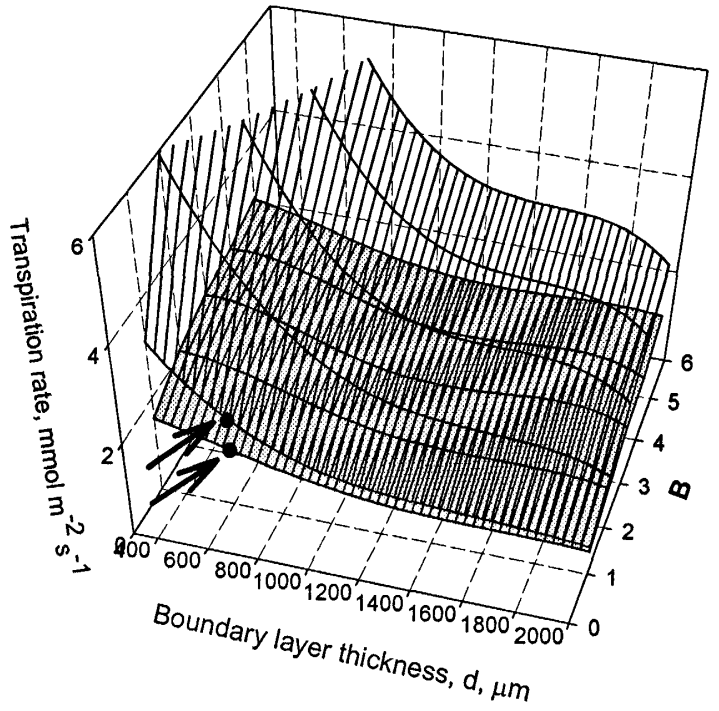

Fig. 3. Transpiration rates calculated for various values of the boundary layer thickness, d, and the ratio of the diffusion coefficients in the boundary layer and in the intercellular space, B, at stomatal aperture values of 1 μm (lower) and 10 μm (upper surface), 70% relative humidity, 20° C temperature for a potato leaf, cv. Désirée. Arrows show the parameter values and transpiration rates that correspond to the measured transpiration rates, $d=600\,\mu$m, $B=1$.

surface presents this dependence at stomatal aperture $ap=1\,\mu$m, and the transparent upper surface gives this dependence at $ap=10$ mm. A comparison of model results (Figs. 3 and 4) with the measured transpiration rates (Table 2) shows that increasing the d value above 2000 μm would only increase the discrepancy between measured and calculated transpiration rates. At a value of B equal to 5 the calculated transpiration rates were already much higher than the measured ones.

For both the normal and transgenic plants, there was only one pair of transpiration values (shown by arrows in Figs. 3 and 4) that corresponded to the maximal and minimal measured values at the same d and B. For the normal leaf: $d=600\,\mu$m and $B=1$, and for the transgenic leaf: $d=1600\,\mu$m and $B=3$.

Both sets of experimental data for transpiration rate, Exp. #1 and Exp. #2 (Table 2) were used for the validation of the model. Since only 2 points from Exp. #1 have been used for parameterization, the other 6 points could be used for validation.

The next series of 2DLEAF model runs for normal and transgenic leaves used the d and B values already determined, to calculate the dependence of transpiration rate on stomatal aperture (open circles and lines in Fig. 5). Then the 6 measured points available for validation were plotted (closed circles). For

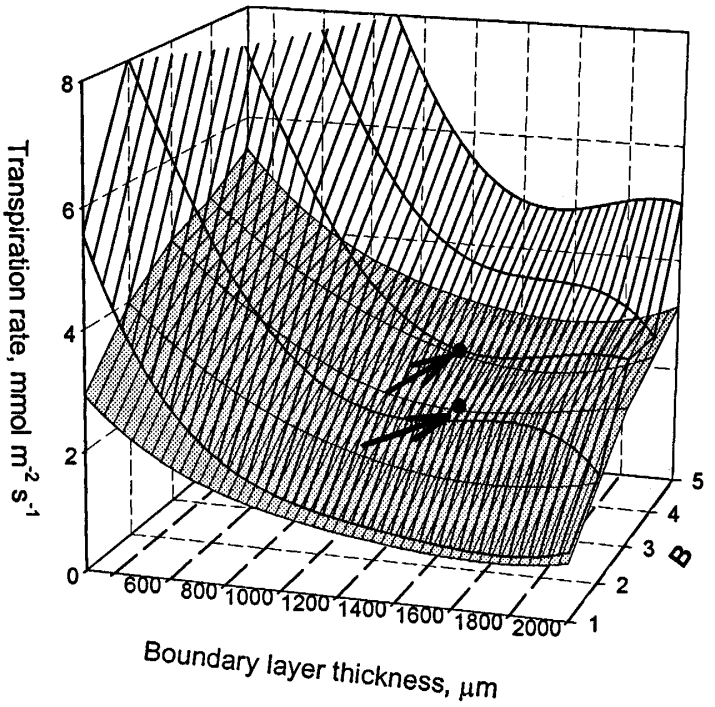

Fig. 4. Transpiration rates calculated for various values of the boundary layer thickness, d, and the ratio of the diffusion coefficients in the boundary layer and in the intercellular space, B, at stomatal aperture values of 1 μm (lower) and 10 μm (upper surface), 70% relative humidity, 20° C temperature for a transgenic potato leaf. Arrows show the parameter values and transpiration rates that correspond to the measured transpiration rates, $d = 1600\,\mu$m, $B = 3$.

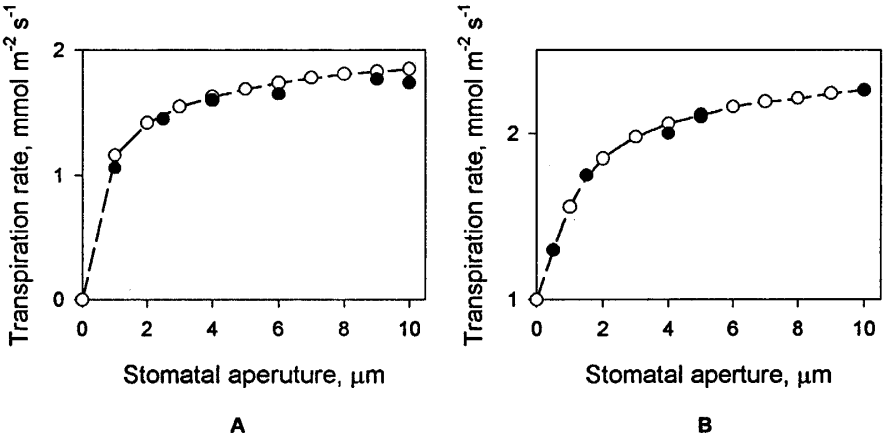

Fig. 5. Verification of the model with the data of Exp. #1, A–cv. Désirée, B–transgenic leaves. Open circles represent calculated and closed circles present the measured transpiration rates.

every point corresponding to a measured transpiration rate the light intensity was known, and it was possible to calculate the stomatal aperture value. This

Table 2. Measured transpiration rates, mmol $m^{-2} s^{-1}$, at various PPFD μmol $m^{-2} s^{-1}$. Experiment #1 was held at 20°C and 70–80% relative air humidity, Experiment #2–at 20°C and 60–70% relative humidity.

#	Light	Désirée	Transgenic
Exp. #1			
3	200	1.05	1.20
4	400	1.45	1.75
5	600	1.60	2.00
6	800	1.65	2.10
7	1000	1.74	2.30
Exp. #2			
1	200	0.60	0.65
2	400	0.97	0.99
3	600	1.30	1.33
4	800	1.40	1.45
5	1000	1.75	1.80

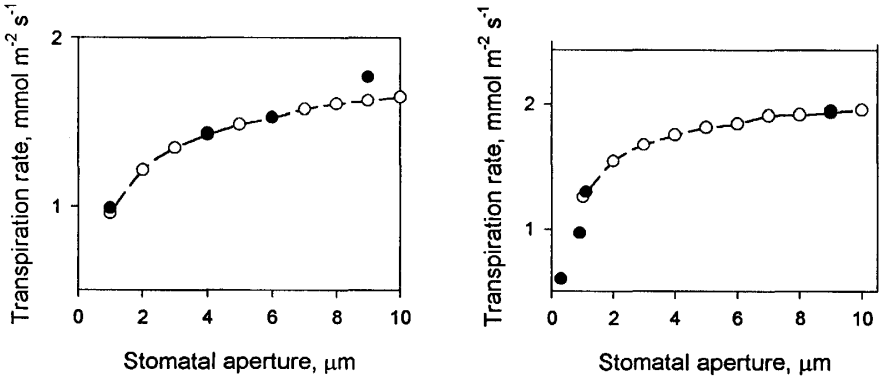

Fig. 6. Verification of the model with the data of Exp. #2, A–cv. Désirée, B–transgenic leaves. Open circles represent calculated transpiration rates, closed circles represent measured transpiration rates.

calculated dependence of stomatal aperture on light intensity is shown in Fig. 7. In a similar way we validated the model with the date from Exp. #2 (Fig. 6) and determined the dependence of stomatal aperture on light intensity (Fig. 7).

Experiments reviewed in Kramer and Boyer (*11*) have shown that LBL qualities differ greatly for different species. There are several reasons: leaf size and shape, leaf surface quality, stomatal density and distribution, stomatal size and shape, and their mode of opening and closing. Only stomatal density and

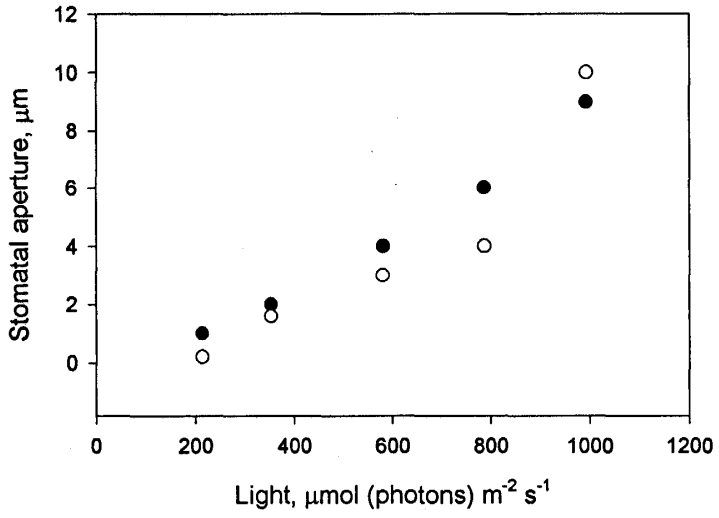

Fig. 7. Dependence of the calculated stomatal aperture on light intensity, closed circles–cv. Désirée, open circles–transgenic leaf.

leaf size have been taken into consideration in the models of the LBL so far (*1, 15*). Consideration of this problem using genetically transformed plants provided an opportunity to account quantitatively for all these factors. Transgenic plants have many properties identical to those of the normal plants, with some well defined differences. Changes occurring on the leaf surface have been noted in many publications (e. g., *22, 23*). Leaf surfaces of the normal and transgenic potato plants used in this study were only slightly different (*10*). Stomatal density of the transgenic plants was 1.3 times that of the normal leaves. This was the major reason for the differences in the LBL parameter values between the normal and transgenic plants.

These differing qualities of leaf surfaces could be accounted for in the model when two-dimensional gas flow was considered, and two parameters for the leaf boundary layer were introduced. The results show that this approach is sufficient to reproduce transpiration rates in a wide range of light intensities and, consequently, at different stomatal apertures, with a high accuracy (Figs. 5 and 6). Therefore, the algorithm for calculating the LBL parameters described above can be used to determine LBL qualities for a wide variety of plant species, at least for those with flat leaves.

As can be seen in Figs. 3 and 4, the dependence of transpiration rate on the two LBL parameters shows reasonable behavior. Transpiration rate increases with increasing B value, i. e., increasing diffusion in the boudary layer, and it decreases with increasing d value, i. e., thickness of the LBL. It is also evident from these figures that transpiration rate is quite sensitive to both of these parameters.

The calculated LBL thickness, d, was 2.5 fold and the ratio of the diffusion coefficients, B, was threefold greater for the transgenic leaf than for Désirée. These differences certainly reflect different properties of the surfaces of the leaves, mostly, difference in stomatal density which was 1.3 fold greater for the

transgenic than for the normal plants. At the same time, no other significant differences between the transgenic and the normal leaf surfaces have been found by microscopic investigations. It was shown in several early works reviewed by Bange (*1*) that transpiration fluxes from stomata interfere contributing into the BL formation, and the level of this interference, and consequently the thickness of the air disturbed by this interference, depends on the stomatal frequency. Some experimental evidence of the important role of the stomata in BL characteristics are reviewed by Kramer and Boyer (*11*). Parameters d and B are sufficient to quantitatively account for the LBL, but direct conclusions about physical properties of the leaf boundary layer cannot be drawn from these parameter values.

The dependence of stomatal aperture on light intensity calculated for Désirée and transgenic leaves (Fig. 7) demonstrated a reasonable behavior. Stomatal aperture increased with increasing light. At light intensities under 1000 μmol (photons) $m^{-2}s^{-1}$, stomatal aperture for the transgenic leaves was consistently smaller than for leaves of Désirée, and it increased rapidly at higher light intensity (Fig. 7). This could be caused by a difference in carbohydrate content (*14*) between Désirée and transgenic leaves, that, in turn, could affect some guard cell properties.

Therefore, two parameters, the boundary layer thickness, d, and the ratio of the diffusion coefficients in the boundary layer and in the intercellular space, B, being incorporated into two-dimensional model of leaf gas exchange, are sufficient for a complete and sufficiently precise description of the leaf boundary layer. They can be determined (as described above) provided that experimental data on transpiration rates at various light intensities, data on leaf internal anatomy, and data on stomatal density are available.

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