PHOTOBIO: Modeling the Stomatal and Biochemical Control of Plant Gas Exchange

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ABSTRACT

Simulation models are increasingly being used to describe physiological processes in the plant sciences. These models, while useful for research purposes, also offer tremendous potential for demonstrating a wide array of scientific concepts to students. We have developed an educational software package that illustrates the stomatal and biochemical control of transpiration and photosynthesis. The simulation program uses a biochemical model of C assimilation that, when coupled to an empirical submodel describing stomatal conductance, can be solved iteratively for leaf photosynthesis, stomatal conductance, and transpiration. Graphic and tabular presentations, combined with on-screen requests for student input, serve to effectively convey the basic fundamentals of plant gas-exchange, and the diurnal patterns of photosynthesis and transpiration in response to fluctuating environmental conditions. More advanced topics focus on the biochemical limitations imposed on photosynthesis by Rubisco activity, electron transport capacity, and the regeneration of inorganic P. Also included is an exercise that challenges students to use the lessons learned to optimize C assimilation, while minimizing water losses, over a 3-d simulation period. Application of the program can assist instructors in illustrating important concepts regarding stomatal and biochemical control of plant gas-exchange.

SUPPLEMENTING classroom lectures and assigned readings with hands-on experiences can effectively stimulate a student's desire to learn and may aid in the retention of information (Buhr and Knauft, 1984). Use of such experiential techniques provides students with an opportunity to discover potentially relevant concepts for themselves and encourages students to visualize how individual components govern the functioning of more complex processes. Unfortunately, conventional wisdom and financial constraints often preclude the incorporation of hands-on exercises into crop science courses, particularly in the area of stomatal behavior and leaf photosynthesis, where instructors may be reluctant to place high-priced instruments into student hands.

In an attempt to bridge the gap between classroom instruction and hands-on experiences in plant gas-exchange processes, we have developed an educational software package that enables students to address fundamental, as well as more advanced, topics of stomatal behavior and C assimilation. Our primary interest in developing this simulation model was to incorporate current knowledge of stomatal conductance, photosynthesis, and transpiration into a user-friendly program that would encourage students to examine the physiological basis for C and water vapor exchange. Specific objectives of this article are (i) to briefly describe the model's development, (ii) to discuss user-selectable input and output options, and (iii) to illustrate some of the many educational applications of this software package.

MODEL DESCRIPTION

Because the processes of leaf photosynthesis and stomatal conductance are highly interdependent, neither can be solved independently of the other. Therefore, modeling plant gas-exchange requires that equations describing both the supply of CO_2 through the stomata and the demand of CO_2 by photosynthesis be solved simultaneously. For clarity, we will address the stomatal conductance model first, then discuss the biochemical model of C assimilation, and finally combine the two,

along with considerations of transpiration, into an integrated simulation model.

Empirical Model of Stomatal Conductance

An adequate description of stomatal behavior must account for the coordinated response of stomata to light, humidity, and temperature. Ball et al. (1987) observed that the interactive effects of several environmental factors on stomatal conductance could be summarized by the empirical formula

$$g_s = GFAC \ (Ah_s/C_s) \qquad [1]$$

where g_s is stomatal conductance; A is carbon assimilation rate; h_s and C_s are relative humidity and CO_2 partial pressure at the leaf surface, respectively; and GFAC is an empirical constant representing stomatal sensitivity to these factors (Tenhunen et al., 1990). Thus, stomatal conductance is dependent on assimilation, while assimilation is dependent on g_s through an effect on internal CO_2 concentration (C_i). Given these interdependencies, Eq. [1] must be combined with a model of photosynthesis and iteratively solved for a common C_i according to the equation

$$C_i = C_a - 1.56 \ (A/g_s) \qquad [2]$$

where C_a is the partial pressure of CO_2 external to the leaf, and the factor 1.56 corrects for the differing diffusivities of water vapor and CO_2 in air (Nobel, 1986).

Biochemical Model of Photosynthesis

Carbon assimilation was calculated using the mechanistic model proposed by Farquhar et al. (1980) and later modified by Sharkey (1985). Primary assumptions underlying the model are that photosynthesis is regulated by (i) the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), (ii) the rate of ribulose-1,5-bisphosphate (RuBP) regeneration via electron transport, and (iii) the availability of inorganic P and the rate of triose phosphate utilization.

Photosynthesis is determined by the "minimum" of these three limitations according to the expression

$$A = min \ (W_c, \ W_j, \ W_p) - R_d \qquad [3]$$

where W_c, W_j, and W_p are limitations imposed on C assimilation by Rubisco activity, RuBP regeneration, and regeneration of inorganic P, respectively. Day respiration is designated R_d and refers to CO_2 release in the light by processes other than photorespiration (Brooks and Farquhar, 1985).

When Rubisco activity is limiting carboxylation, C assimilation can be described by

$$W_c = (Vc_{max} \ C)/[C + K_c \ (1 + O/K_o)] \qquad [4]$$

where Vc_{max} is the maximum carboxylation velocity of fully activated Rubisco, C is the CO_2 concentration in the stroma, O is the O_2 concentration in the stroma, and K_c and K_o are the Michaelis constants of Rubisco for

CO_2 and oxygen, respectively. Rubisco activity is likely to limit assimilation under conditions of low C_i and high irradiance.

When photosynthetic electron transport limits the regeneration of RuBP, assimilation rate can be expressed by

$$W_j = J/[4.5 + 10.5 \ (\Gamma^*/C)] \qquad [5]$$

where J is the potential rate of electron transport and Γ^* is the CO_2 compensation point (Farquhar and von Caemmerer, 1982). Ribulose-1,5-bisphosphate regeneration is likely to be limiting when irradiance is low and/or C_i is high.

When regeneration of inorganic P restricts C assimilation, the limiting condition can be described by

$$W_p = (3 \times TPU)/[1 - (\Gamma^*/C)] \qquad [6]$$

where TPU is the rate of triose phosphate use and reflects the general availability of inorganic P for the Calvin cycle (Sharkey, 1985). Regeneration of inorganic P does not often limit assimilation, but may be important at high C_i and high irradiance.

The biochemical model presented above has great utility in defining the limitations imposed on leaf photosynthesis (Sage, 1990). However, additional information on C assimilation can also be gained by using a modified version of the above model as proposed by Harley et al. (1986). According to these modifications, the limitation imposed on C assimilation by electron transport can also be described by the expression

$$W_j = W_c \ [P_m/(W_c + W_o)] \qquad [7]$$

where W_c is as described in Eq. [4], P_m is maximum photosynthesis and, hence, a measure of RuBP regeneration, and the expression $[P_m/(W_c + W_o)]$ approximates the limitation imposed on photosynthesis by RuBP regeneration relative to its consumption. If the ratio $P_m/(W_c + W_o)$ is less than one, the availability of RuBP is limiting C assimilation.

The RuBP-saturated rate of oxygenation is given by an expression analogous to that for carboxylation, such that

$$W_o = (Vo_{max} \ O)/[O + K_o(1 + C/K_c)] \qquad [8]$$

where Vo_{max} is the maximum oxygenation velocity of Rubisco.

Integrated Model of Plant Gas-Exchange

The preceding discussion illustrates how Rubisco activity, electron transport, and the regeneration of inorganic P interact to determine photosynthesis. By combining the biochemical model of C assimilation with a submodel describing stomatal conductance, the unknown quantities of A, g_s, and C_i can be determined. Furthermore, once g_s is known, transpiration can be calculated by the expression

$$E = g_s(e_s - e_a)/P \qquad [9]$$

where E is transpiration, g_s is stomatal conductance, e_s is the saturated vapor pressure at leaf temperature, e_a is the air water vapor pressure, and P is atmospheric pressure. We currently ignore important considerations of boundary layers and wind speed in our analyses of water-vapor exchange. Those wishing an expanded discussion on the role of these factors in controlling transpiration are referred to Jarvis and McNaughton (1986).

SOFTWARE PROGRAM OPTIONS

Students can select from the following menu of gas-exchange topics:

1. General illustrations
2. Stomatal control of gas-exchange
3. Biochemical control of photosynthesis
4. Integrated control of plant gas-exchange
5. Gas-exchange challenge

Each lesson begins with an on-screen explanation of the topic being addressed, followed by an opportunity for students to either accept or modify model estimates before proceeding. Although the sequence in which these topics are addressed is not critical, the concepts presented in each session become more complex as students progress from one session to the next. Therefore, instructors wanting to slowly introduce these topics should have students work progressively through the exercises, but advanced students could pursue topics at a level of their choice.

General Illustrations

Students needing to reacquaint themselves with the basic fundamentals of plant gas-exchange can view a series of illustrations that demonstrate the response of photosynthesis to irradiance, temperature, and stomatal conductance. Graphs highlight concepts of light compensation point, quantum-use efficiency, species differences in these characteristics, photorespiration, and differences in the response of C3 and C4 plants to temperature. Contrasts between the stimulation of C assimilation by CO_2 concentration, and its inhibition by O_2, convey the competitive nature of these two gases in determining photosynthesis; hence, prepare students for later enzymatic concepts associated with the function of Rubisco. Effects of stomatal conductance and vapor pressure deficit on transpiration are also illustrated.

Stomatal Control of Gas-Exchange

In this section, students focus on those environmental factors that influence stomatal conductance and examine the interactive role of C assimilation in determining stomatal control of plant gas-exchange processes. Because stomatal conductance is dependent on assimilation, illustrations attempt to highlight the manner in which one process affects the other. For example, as presented in Eq. [1], the expression Ah_s/C_s is a linear function of stomatal conductance with the slope (GFAC) indicating
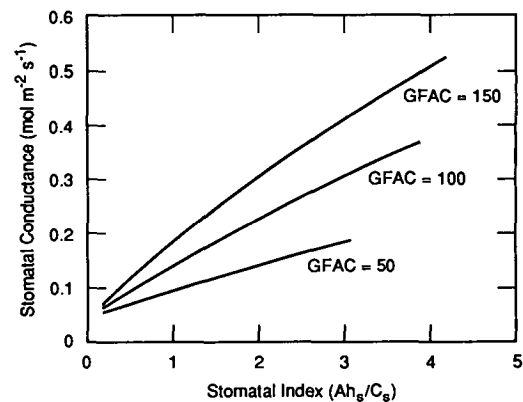

Fig. 1. Modeled response of stomatal conductance and the expression Ah_s/C_s to changes in the stomatal sensitivity factor (GFAC).

the collective sensitivity of stomata to these variables. Students can examine this relationship for several environmental scenarios by altering GFAC, but otherwise leaving h_s and C_s unaltered (Fig. 1). Modeled results indicate that as GFAC increases, stomatal conductance also increases. Students should conclude from this example that because h_s and C_s were held constant between the two simulations, assimilation also increases with increasing stomatal sensitivity. Although this may not seem surprising, establishing a connection between GFAC and assimilation is essential for later use when students are asked to select those plant attributes that will maximize C assimilation and minimize transpiration in response to a fluctuating environment. Selecting too high a value of GFAC will increase C gain, but will also lead to increased transpiration due to an increase in stomatal conductance.

Other exercises illustrate how stomatal conductance influences photosynthesis, transpiration, water-use efficiency, and the ratio of internal to ambient CO_2 concentrations. The influence of irradiance on stomatal conductance is also demonstrated.

Biochemical Control of Photosynthesis

Three general processes of photosynthetic biochemistry are proposed in Eq. [3] to limit C assimilation. Students can examine these limitations by altering several components of the biochemical model and observing the response of photosynthesis to either irradiance or internal CO_2 concentration (Fig. 2). Using the default model estimates, simulations indicate that at high irradiance (PFD = 1800 μmol m^{-2} s^{-1}), Rubisco activity limits assimilation at low CO_2 concentrations, electron transport limits assimilation at intermediate CO_2 concentrations, and the regeneration of inorganic P limits assimilation at elevated CO_2 concentrations (Fig. 2a). In contrast, at low irradiance (PFD = 700 μmol m^{-2} s^{-1}), electron transport limits C assimilation at nearly all CO_2 concentrations, with limitations imposed by Rubisco activity and the regeneration of inorganic P being of little or no significance (Fig. 2b). These relationships are emphasized further by illustrating that Rubisco limits C assimilation when the ratio of RuBP regeneration to consumption increases above one, indicating that the recycling of RuBP
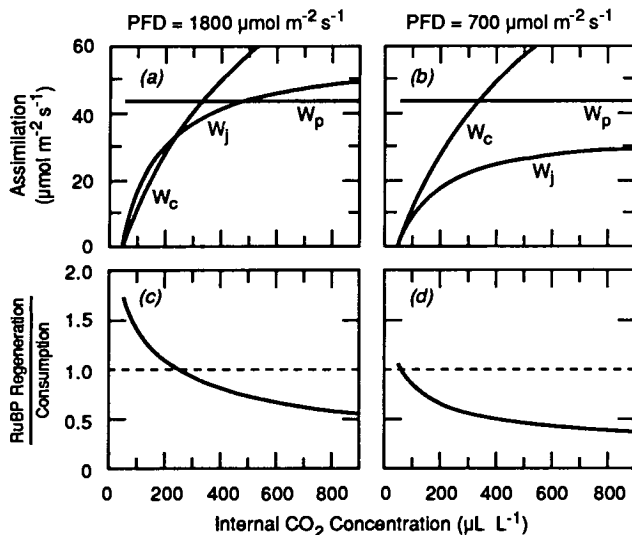
PFD = 1800 µmol m^{-2} s^{-1} PFD = 700 µmol m^{-2} s^{-1}

Fig. 2. Simulated response of carbon assimilation to limitations imposed by Rubisco activity (W_c), electron transport (W_j), or the regeneration of inorganic P (W_p), and the ratio of the capacity of RuBP regeneration to the capacity of RuBP consumption. Responses were modeled at a photon flux density (PFD) of either 1800 µmol m^{-2} s^{-1} (a and c) or 700 µmol s^{-1} (b and d).

via the Calvin cycle exceeds its use in carbxylation reactions (Fig. 2c and 2d).

Integrated Control of Plant Gas-Exchange

Having examined separately the stomatal and biochemical models of gas-exchange, these are now combined to collectively address C assimilation, transpiration, and stomatal conductance. In this section, students can observe how gas-exchange processes change diurnally and how model estimates potentially influence this behavior. For example, at ambient CO_2 concentrations (355 µL L^{-1}), photosynthesis and transpiration exhibit similar patterns, with both increasing to a maximum late in the morning and maintaining this rate until late in the day (Fig. 3). However, at elevated CO_2 concentrations (655 µL L^{-1}), midday photosynthesis is stimulated by over 50% (Fig. 3a) while transpiration is somewhat suppressed (Fig. 3b). This reduction in transpiration is mediated by the CO_2 concentration at the leaf surface (i.e., C_s in Eq. [1]), which decreases stomatal conductance and, hence, limits water-vapor exchange. As a result, the balance of C assimilation to water loss, a measure of water-use efficiency, increases almost twofold at the higher CO_2 concentration.

Each simulation is accompanied by a summary of daily C gained, water lost, and an indication of water-use efficiency. In this manner, students can begin to evaluate the effectiveness of changing model estimates and how various combinations of estimates influence C assimilation and transpiration.

Gas-Exchange Challenge

Through the previous examples, students begin to appreciate how C assimilation and transpiration are affected
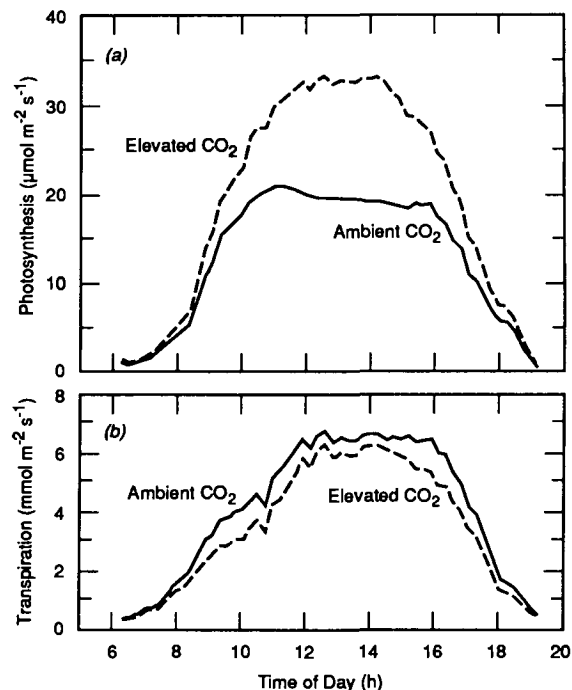

Fig. 3. Diurnal patterns of photosynthesis (a) and transpiration (b) in response to ambient (355 µL L^{-1}) and elevated (655 µL L^{-1}) CO_2 concentration as predicted from the integrated model of stomatal conductance, C assimilation, and transpiration.

by stomatal conductance and how stomatal behavior is regulated in response to environmental conditions. In this section, students must call upon the lessons of these examples and modify a list of plant attributes that will most likely maximize C assimilation while minimizing water losses due to transpiration. Once students have entered their selections, the program calculates daily C gains and water losses over a 3-d simulation period. The environmental conditions that dictate the diurnal pattern of stomatal conductance, assimilation, and transpiration over these 3 d range from warm, dry, and sunny to cool, humid, and cloudy. Thus, the optimum combination of attributes will reflect those that are applicable across a varied environment.

The outcome of this exercise is not easily predicted, and instructors may be surprised by the ingenuity of their students. However, we suspect that those students who succeed in maximizing C assimilation will do so at the expense of increasing transpiration, while those students who minimize transpiration will do so at the expense of decreasing C assimilation. Furthermore, certain combinations of model estimates may be optimal for a sunny day, but not for a cloudy day. Only students who select model estimates that sufficiently balance these two fluxes over the entire 3-d period will have met the gas-exchange challenge.

ALTERNATIVE USES OF THIS PROGRAM

We designed this simulation program to assist instructors in illustrating important determinants of plant gas-exchange and, thus, our discussions have largely dealt

with topics of stomatal behavior and biochemistry. However, application of this software to disciplines outside the plant sciences can also be envisioned. For example, instructors interested in micrometeorology might request that students optimize daily environmental conditions (i.e., irradiance, temperature, and relative humidity) such that given a plant's stomatal and biochemical characteristics, C assimilation is maximized while water loss is minimized. This would of course require some software modification, but depending on the particular circumstances we would be more than willing to work with instructors in making these program changes.

CONCLUSION

We believe that the stomatal and biochemical detail incorporated into this software package makes it a useful tool for illustrating plant gas-exchange processes and stomatal behavior. Students who work progressively through the program will examine a minimum of 25 illustrations and tables, while those who fully utilize the interactive menus could increase this number into the hundreds. Because the model simulates photosynthesis and transpiration in response to several environmental scenarios, it also provides an excellent opportunity to highlight the relationships between plant physiology and microclimate. We hope that instructors will find this software package a useful supplement to classroom discussion and assigned reading.

SOFTWARE SPECIFICATIONS

Program PHOTOBIO was written in Microsoft Quick-BASIC and consists of a compiled file, PHOTO-BIO.EXE, totaling 110 kB for use with DOS 2.0 or higher, on an XT, AT, or other IBM-PC compatible computer. Graphic routines require a monitor resolution of 720 × 348 pixels (Hercules Graphics Card). Twelve ascii files are also included that are necessary for the program's execution. PHOTOBIO is public domain software and will be supplied free, along with documentation, upon receipt of a blank 5 1/4 inch diskette and a postpaid mailer. We request that the program be used for educational purposes only. Send requests to S.D. Wullschleger, Environmental Sciences Division, P.O. Box 2008, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6034.

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